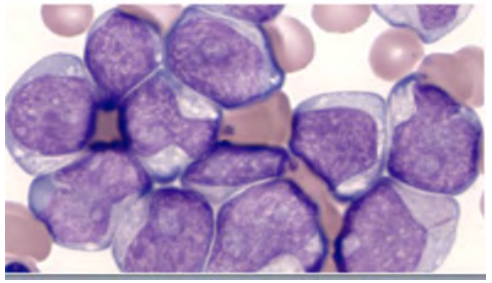
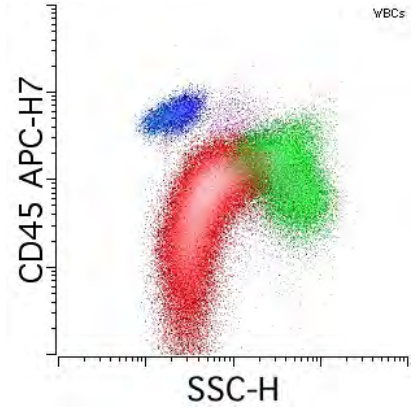


Case studies in Acute Leukemia



Indonesian Cytometry Association Online Workshop May 25-27 2021

Sindhu Cherian

University of Washington, Seattle, WA, USA

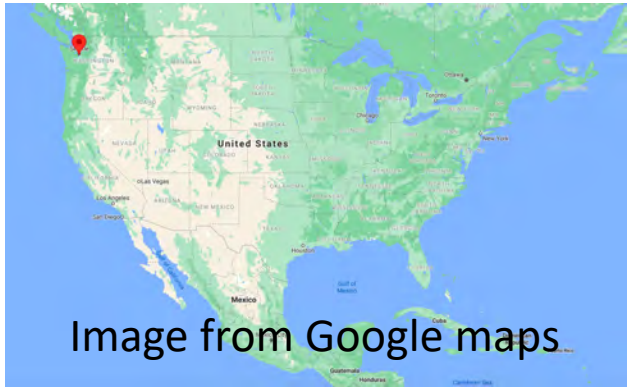
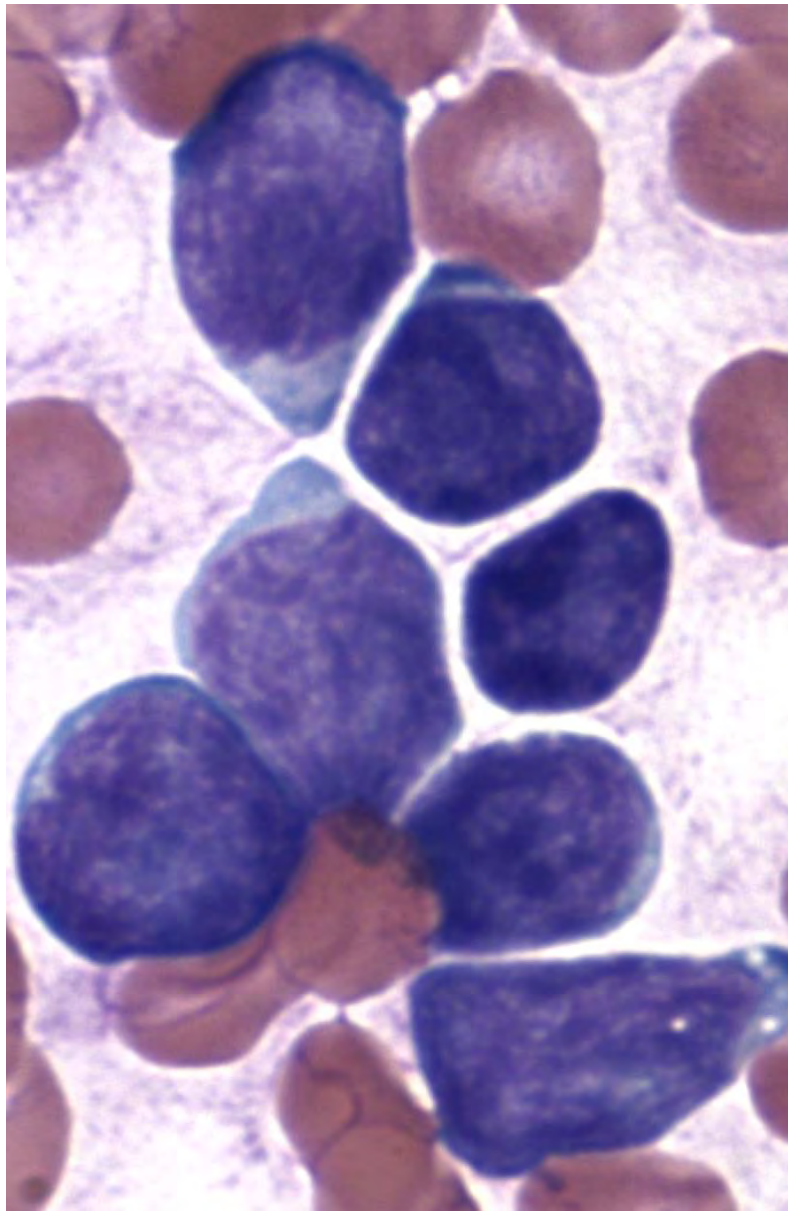


Image from Google maps

cherians@uw.edu
Twitter:
@ICCS_Education
@sindhucherian

*** I have no disclosures to report**

Flow cytometry plays a critical role in characterizing blasts and is a cornerstone of acute leukemia diagnosis



Flow cytometry can…

- Confirm your morphologic impression that an abnormal population represents blasts/progenitor or blast equivalent
  - Progenitor markers
    - These may include CD34, TdT, CD117

- Immunophenotype blasts
  - Define lineage of the blasts
  - Provide additional clues for WHO Classification
  - Determine how different a blast population is from its from normal counterpart

Lineage and WHO classification

Acute myeloid leukemia (AML) and related precursor neoplasms

- AML
  - Recurrent genetic abn
  - MDS related changes
  - Therapy related
  - NOS
- Myeloid sarcoma
- Myeloid proliferations associated with Down Syndrome

Precursor Lymphoid Neoplasms

- B-lymphoblastic leukemia/lymphoma (B-LL)
  - NOS
  - Recurrent genetic abn
- T-lymphoblastic leukemia/lymphoma (T-LL)
- NK-lymphoblastic leukemia/lymphoma

Acute leukemias of ambiguous lineage

- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia (MPAL)
  - NOS
  - Gene rearrangements

Blastic plasmacytoid dendritic cell neoplasm

Flow cytometry cannot…

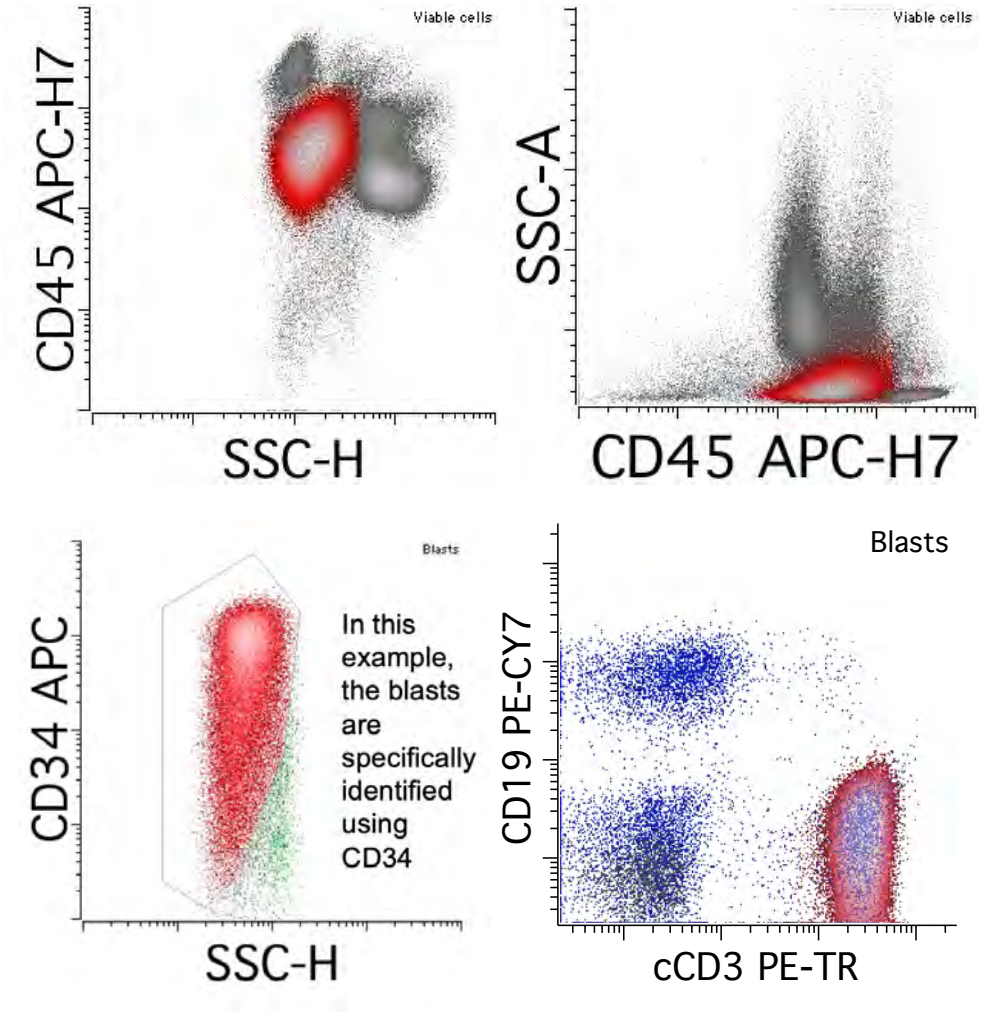
- Accurately enumerate blasts in the **marrow**
  - Hemodilution
  - Partial lysis of nucleated erythroid forms

Morphology is the gold standard for blast enumeration in the marrow

Blast enumeration by flow cytometry in a fresh peripheral blood specimen will be more accurate than in the marrow

What should I include in my acute leukemia panel?

- CD45
  - CD45 versus SSC gating is often a first step
- Markers to confirm that what you think is a blast is really a blast
  - Progenitor markers
    - CD34 is one example
- Sufficient markers to
  - Assign lineage
  - Determine if your blast population is abnormal



Sample Panel for the work up of a new acute leukemia

Laser BD LSRII	405 Violet	488 Blue					594 Yellow	633 Red		
Fluorochromes	**PB or V450**	**FITC**	**PE**	**PETR**	**PECy5, PECy5.5, or PERCP Cy5.5**	**PECy7**	**A594**	**APC**	**APC A700**	**APCCy7 or APCH7**
B cell tube	CD20	Kappa	Lambda		CD5	CD19	CD38	CD10		CD45
T cell tube	CD8	CD2	CD5	CD34	CD56	CD3	CD4	CD7	CD30	CD45
Myeloid 1 tube	HLA-DR	CD15	CD33	CD19	CD117	CD13	CD38	CD34	CD71	CD45
Myeloid 2 tube	HLA-DR	CD64	CD123	CD4	CD14	CD13	CD38	CD34	CD16	CD45

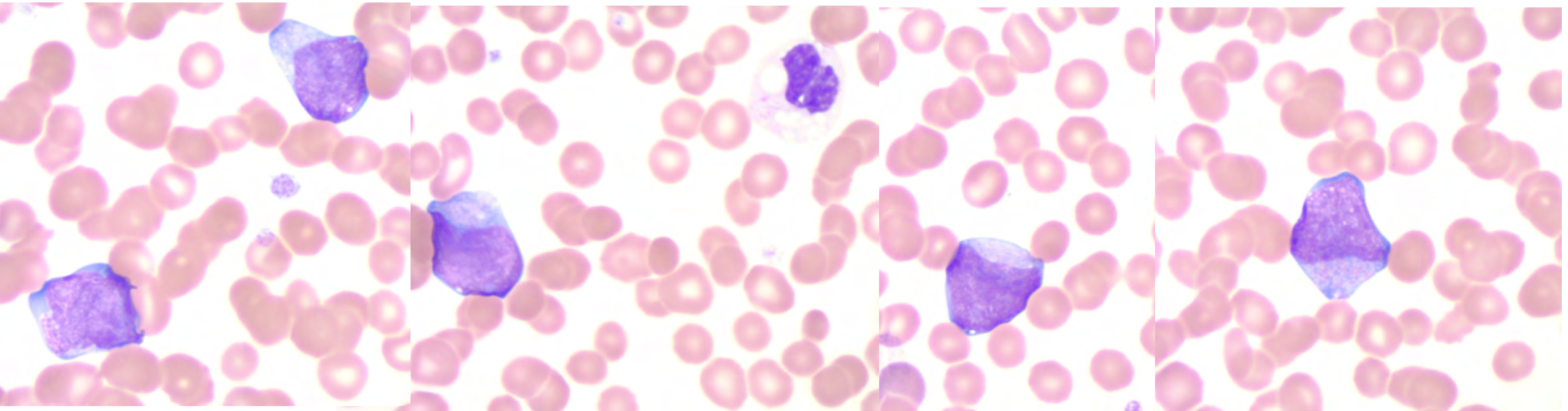
Lineage tube: cytoplasmic CD3, CD79a, MPO

Additional markers are evaluated on an as needed basis:
Potential ALL additions: TdT, CD1a, CD22
Megakaryocytic or erythroid lineage: CD41, CD61, CD235a

Case 1

- A 34 year old female presents to the ED with fatigue and easy bruising
- She is found to have a leukocytosis with circulating blasts
- A portion of the peripheral blood is submitted for flow cytometry

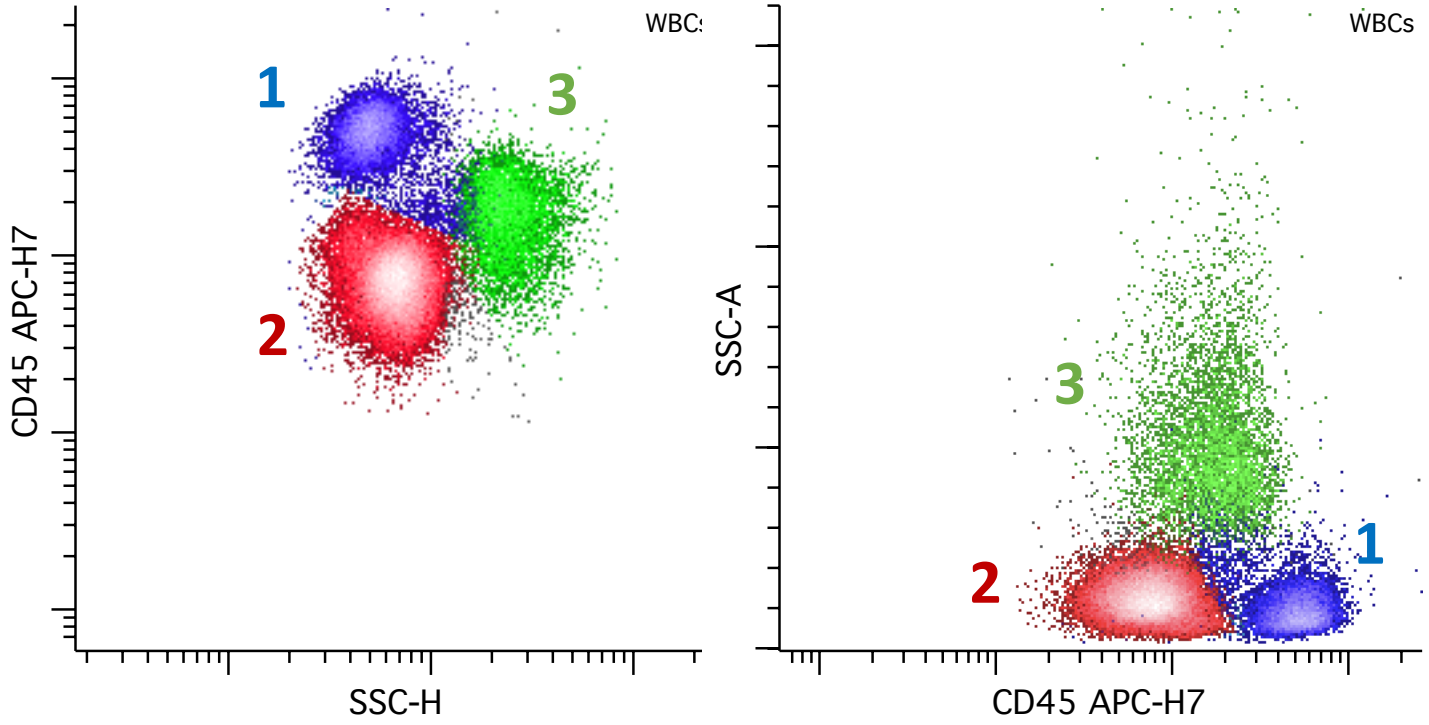
Circulating blasts! Concern for acute leukemia…

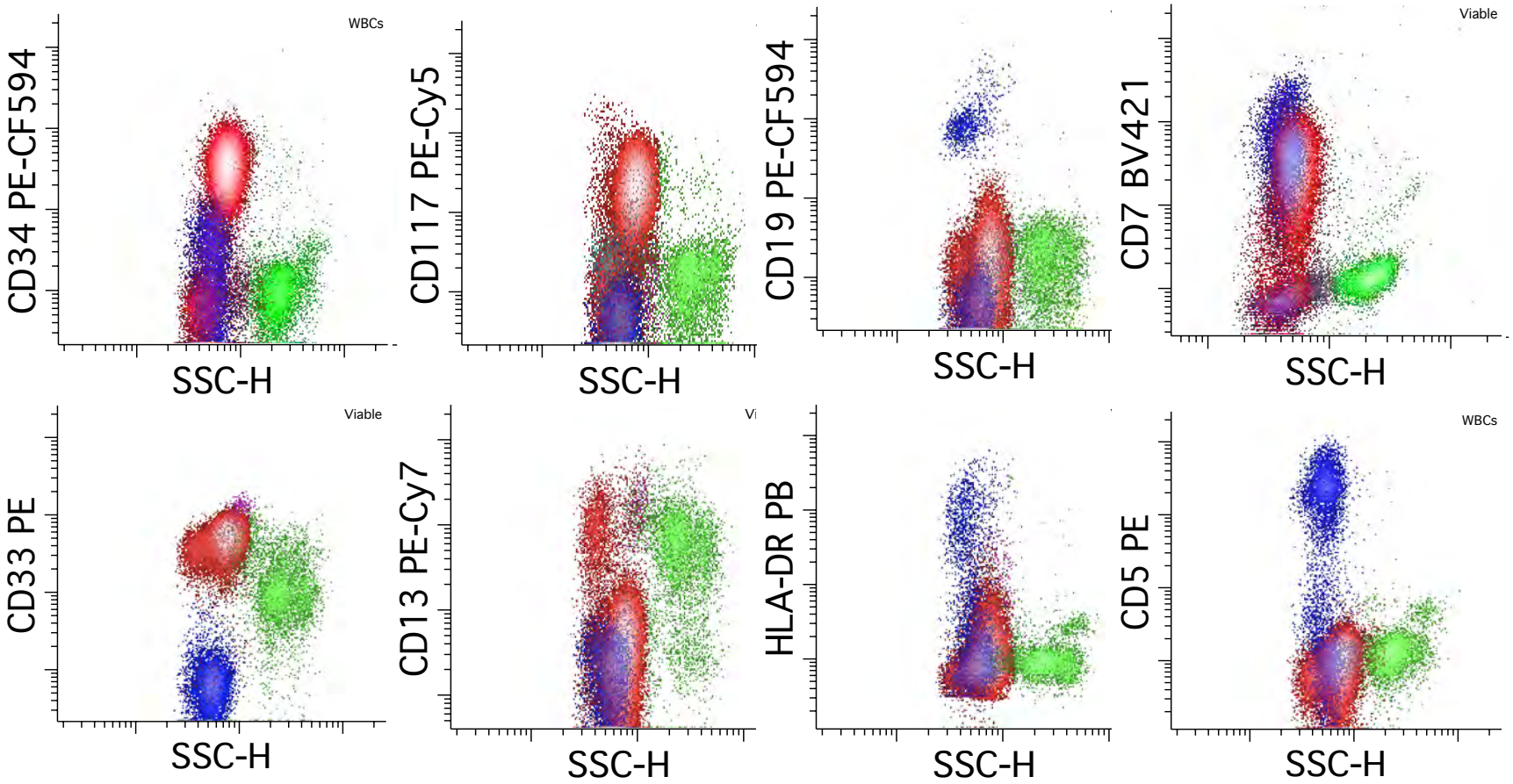


Case 1

What population do you want to focus on?

A. Population 1 15% of WBC
B. Population 2 75% of WBC
C. Population 3 10% of WBC





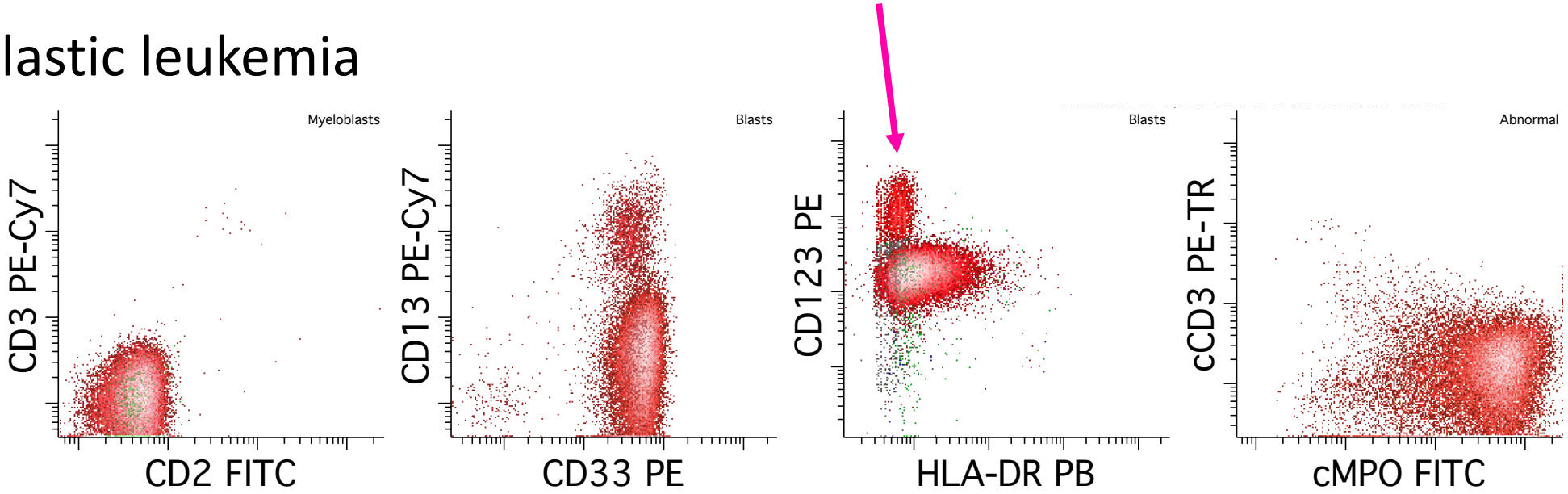
What is your diagnosis?

A. This is NOT acute leukemia
B. AML
C. B lymphoblastic leukemia
D. T lymphoblastic leukemia
E. Need more information

What is your final diagnosis?

A. AML

B. T lymphoblastic leukemia



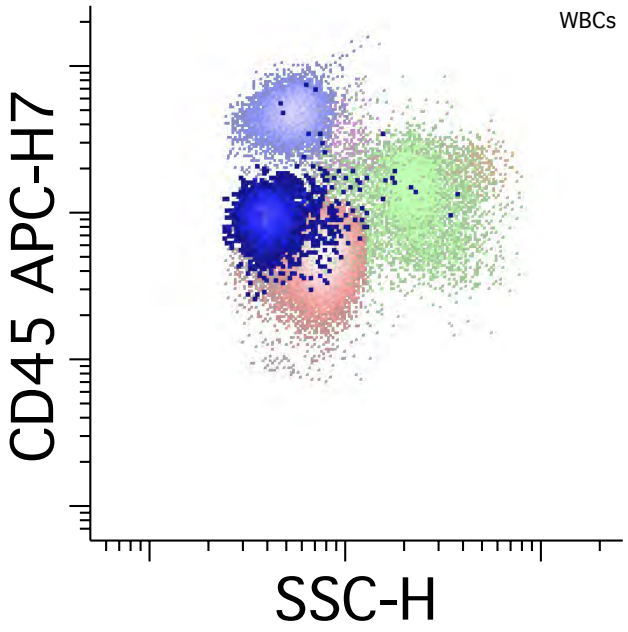
What is the population highlighted by the arrow?

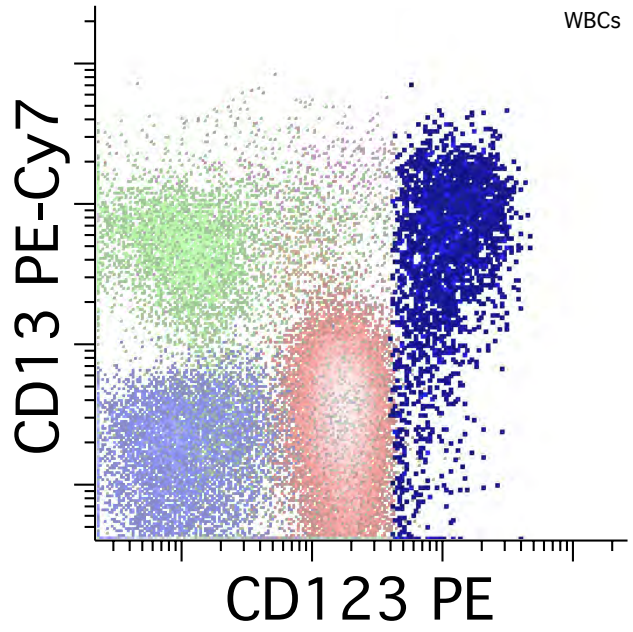
A. A subset of the blasts

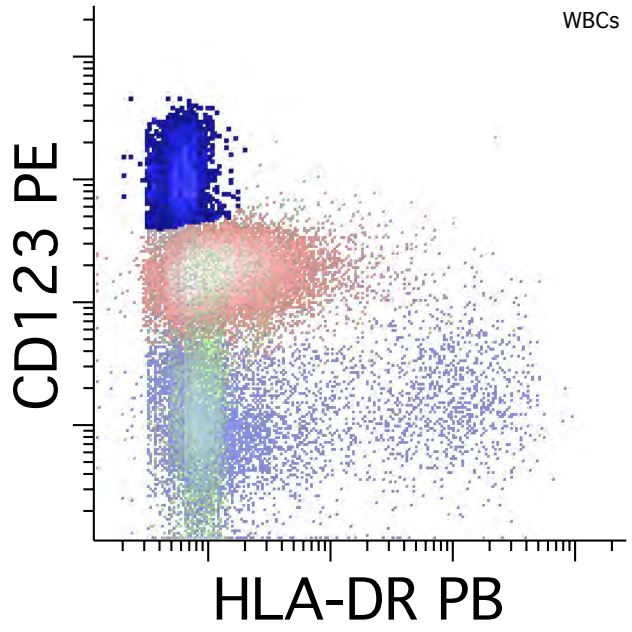
B. I am not sure, need more information

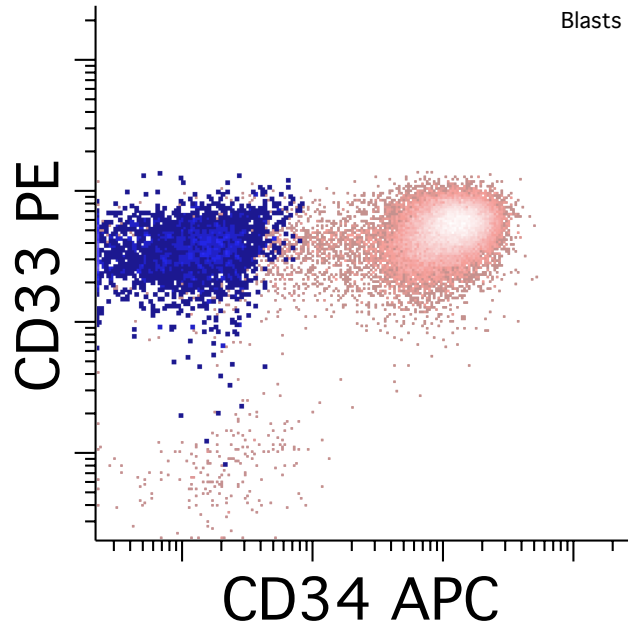
C. Basophils

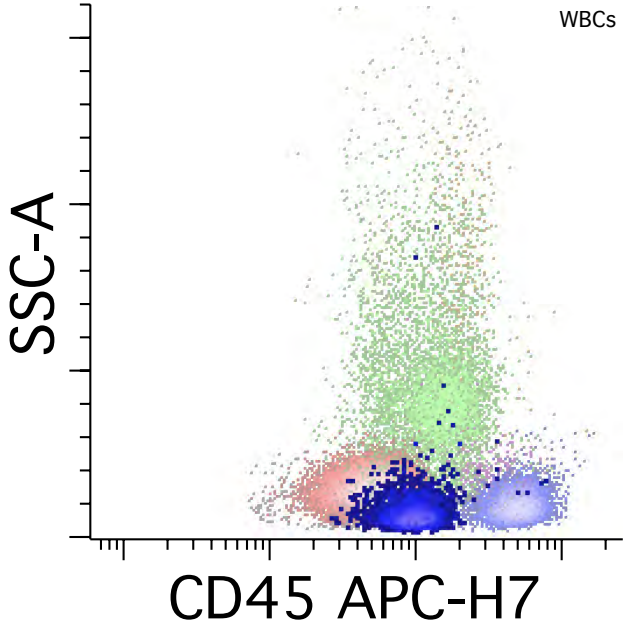
D. Plasmacytoid dendritic cells











Negative for CD117 as well

These are basophils

- Note, they can occupy a traditional CD45 vs SSC defined blast gate
- Basophils express CD123 without HLA-DR and express CD13, CD33, and CD38 without CD34 or CD117

Case 1 First take home message:

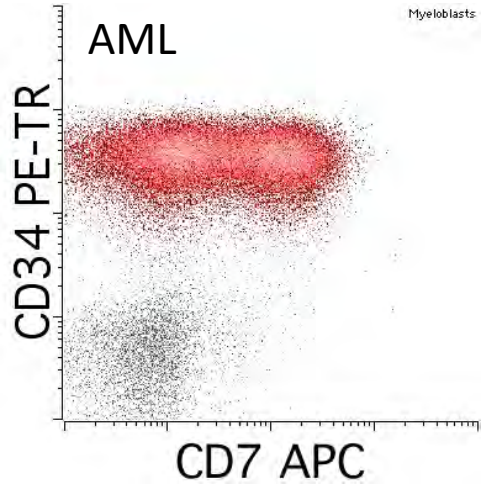
Accurate Lineage assignment requires that you take a few things into consideration…

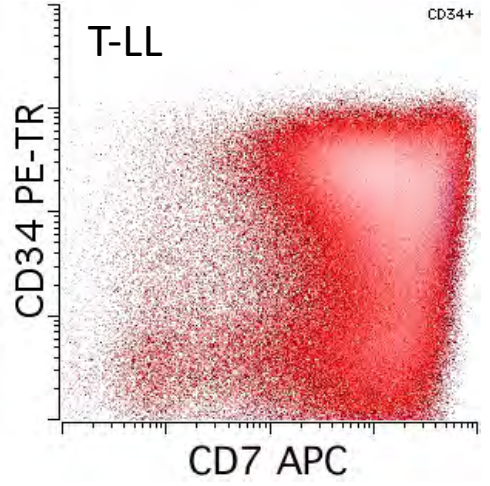
- Accurate lineage assignment requires evaluation of a ***thorough panel*** of antigens
  - B cell: **CD19**; CD20, CD22, cCD79a
  - T cell: **CD3 (surface and cytoplasmic);** CD2, CD5, CD7
  - Myeloid: **MPO**; CD117, CD13, CD33
  - Monocytic: CD14, CD64

- Abnormal blast may aberrantly express an antigen typically associated with a different lineage

Abnormal blasts may show aberrant expression of antigens associated with a different lineage

- Abnormal blasts can aberrantly express markers of another lineage.
  - CD2, CD5, CD7, CD19, and CD56 may be aberrantly expressed in AML
  - CD13, CD33, CD15 may be aberrantly expressed in ALL
- As a general rule, the closer antigen expression is to that of normal cells of a lineage, the more likely expression of an antigen is to reflect lineage differentiation.
  - ***BEWARE—exceptions apply***
- Strong expression of multiple antigens of a lineage is more likely to reflect lineage differentiation than weak expression of a single antigen
- WHO classification has strict criteria for when to consider Mixed phenotype acute leukemia



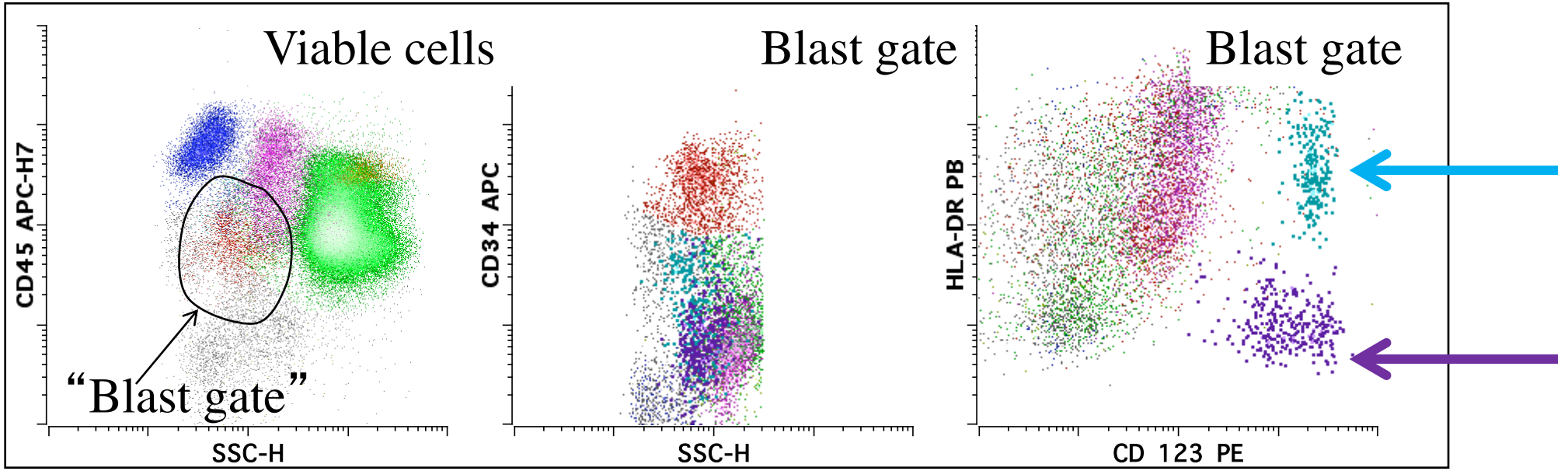


More on this later…

Case 1 second take home message:

Not all cells in the “blast gate” are blast equivalents

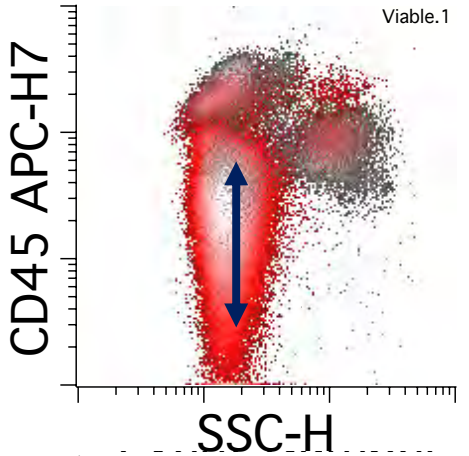
- The following may be present in the “blast gate”
  - **Basophils**, plasmacytoid dendritic cells (PDC), hypogranular myeloid cells, early monocytic cells, plasma cells
- Specific identification of blasts using a progenitor marker is ideal
  - For instance CD34, CD117, TdT

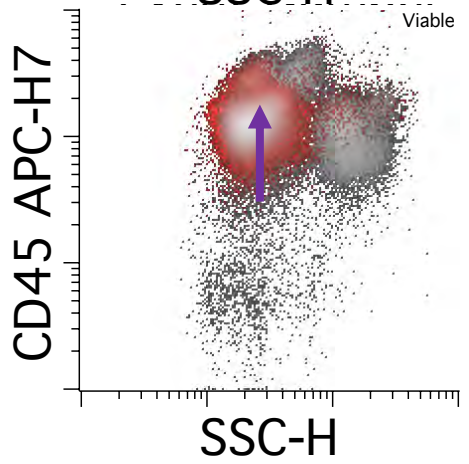


On the flip side… Not all blasts occupy the CD45 vs SSC defined blast gate

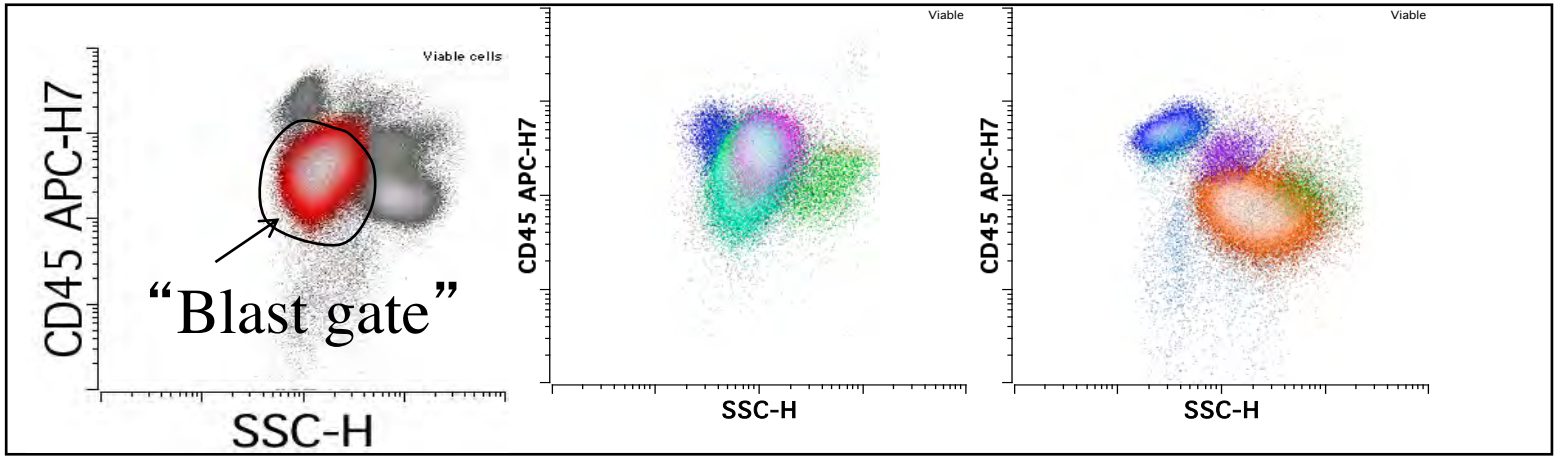
- Immature monocytic cells (promonocytes, monoblasts)
  - Higher CD45, overlap with monocytes
- Abnormal promyelocytes (acute promyelocytic leukemia)
  - Higher side scatter, overlap with granulocytic region
- Abnormal lymphoblasts (B or T cell lymphoblastic leukemia)
  - CD45 expression may range from high to low (and can even be absent
  - Make sure to not exclude CD45 negative populations; TdT can be very helpful

Abnormal lymphoid blasts in ALL



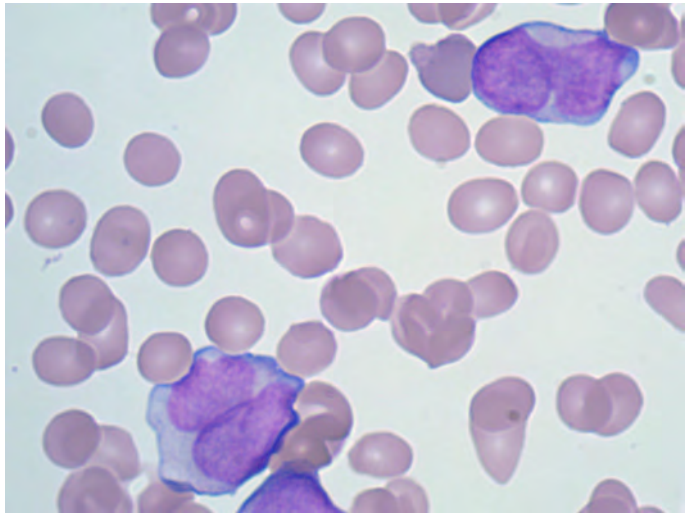


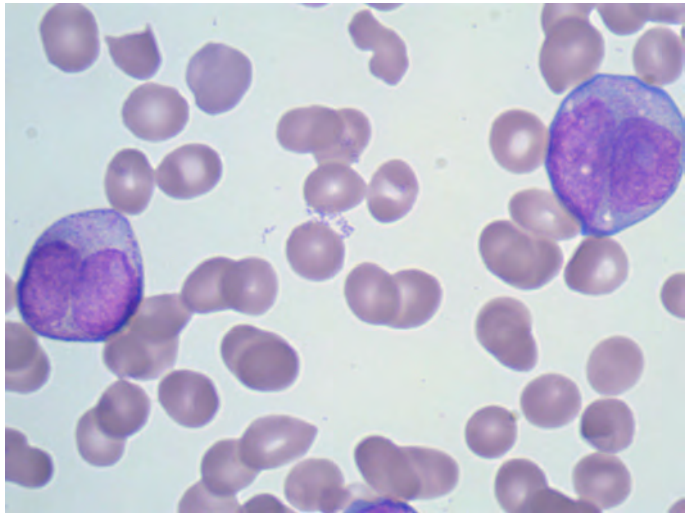
Standard AML | Immature monocytic cells | Abnormal promyelocytes



Case 2:

- 42 year old male presents with 3 days of swelling in his leg initially thought to be infection.
- Upon admission he is noted to have a WBC count of 35K with many abnormal circulating WBC
- Coagulation studies reveal that the patient is in DIC

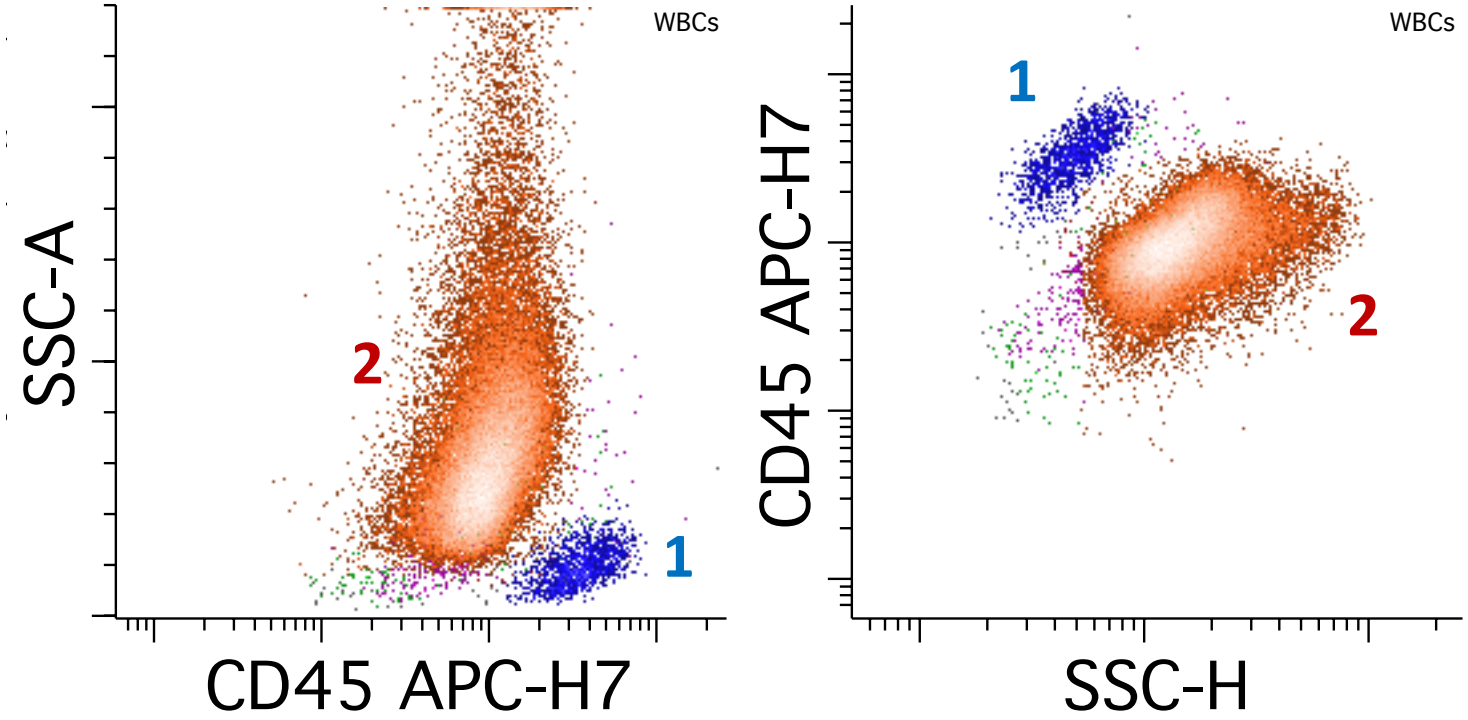




Case 2

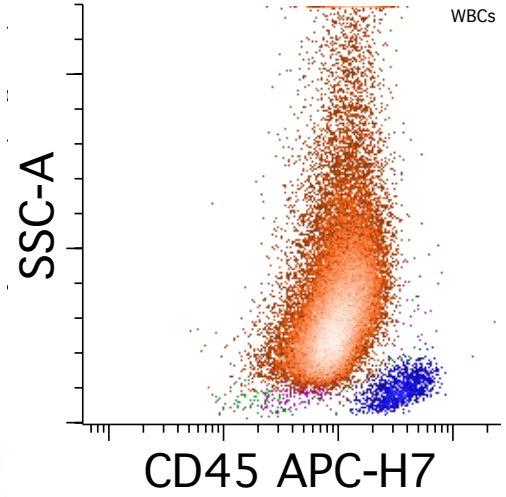
What population do you want to focus on?

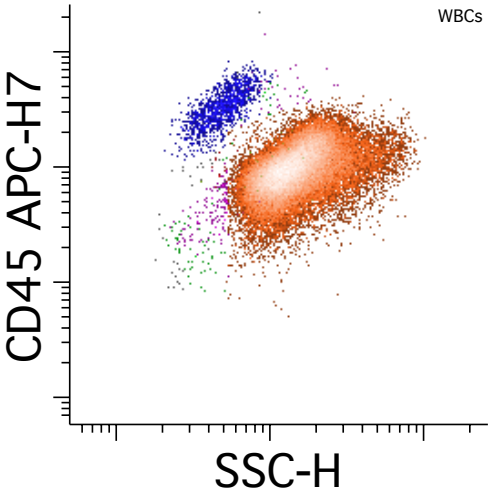
A. Population **1** 5% of WBC
B. Population **2** 95% of WBC

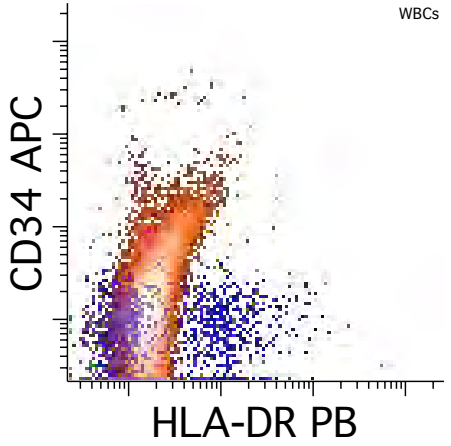


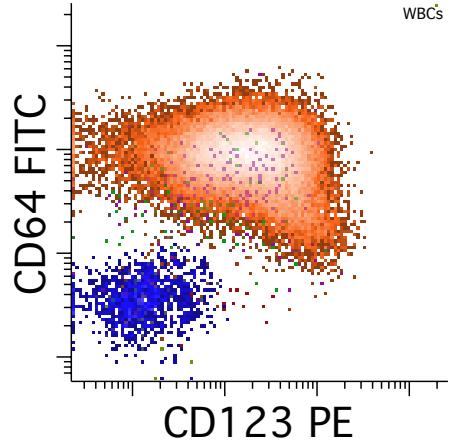
What is your favored diagnosis?

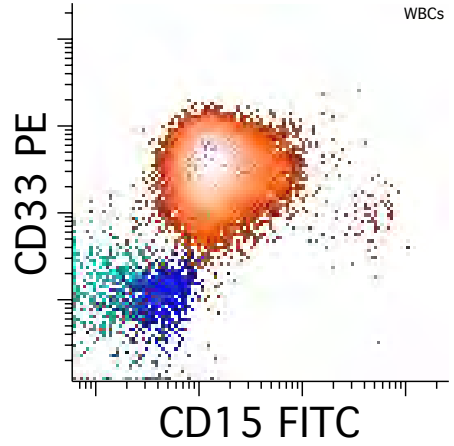
A. Lymphoblastic leukemia
B. Acute promyelocytic leukemia
C. Acute monocytic leukemia

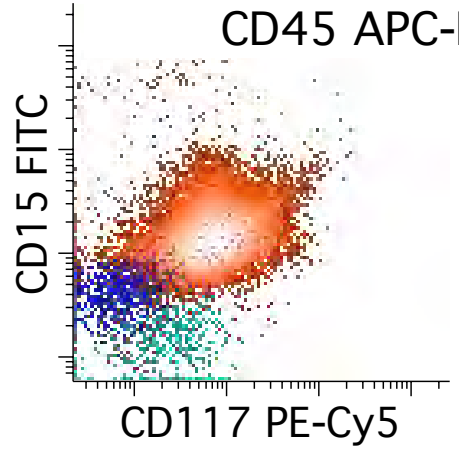


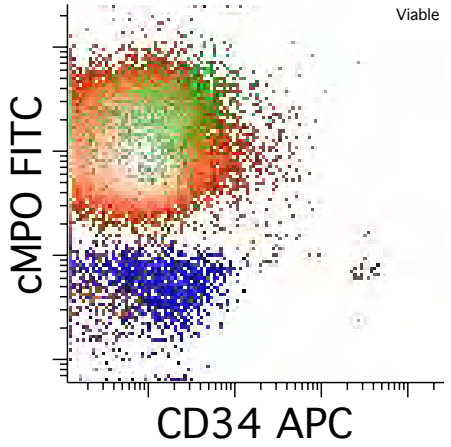


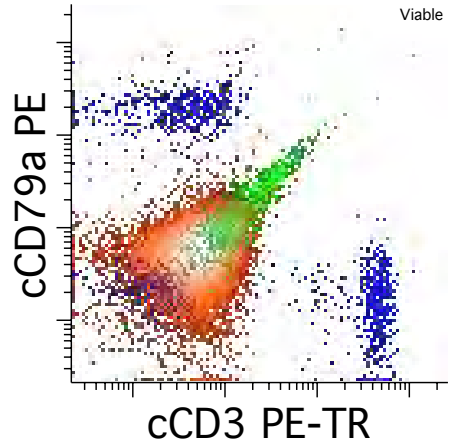


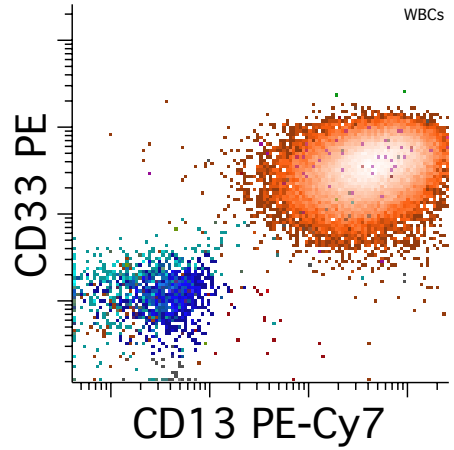


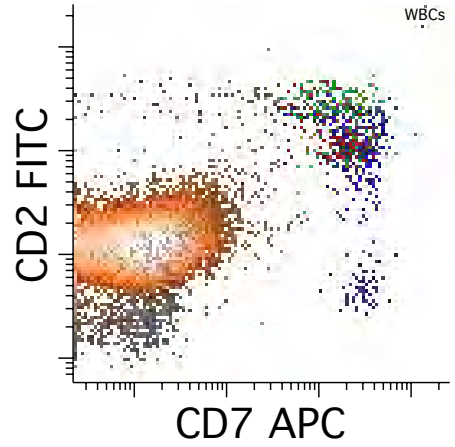




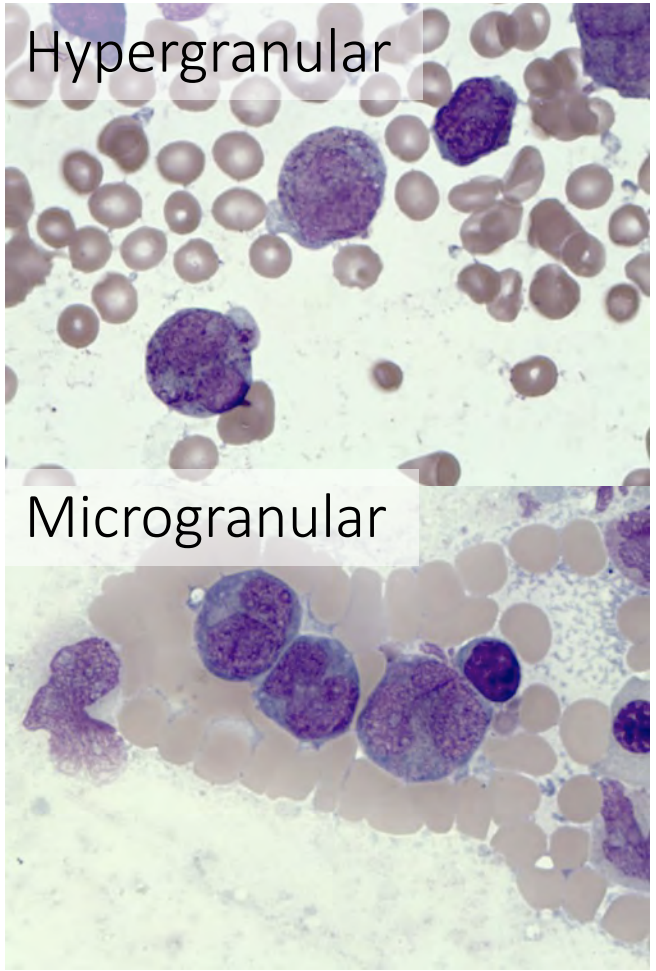








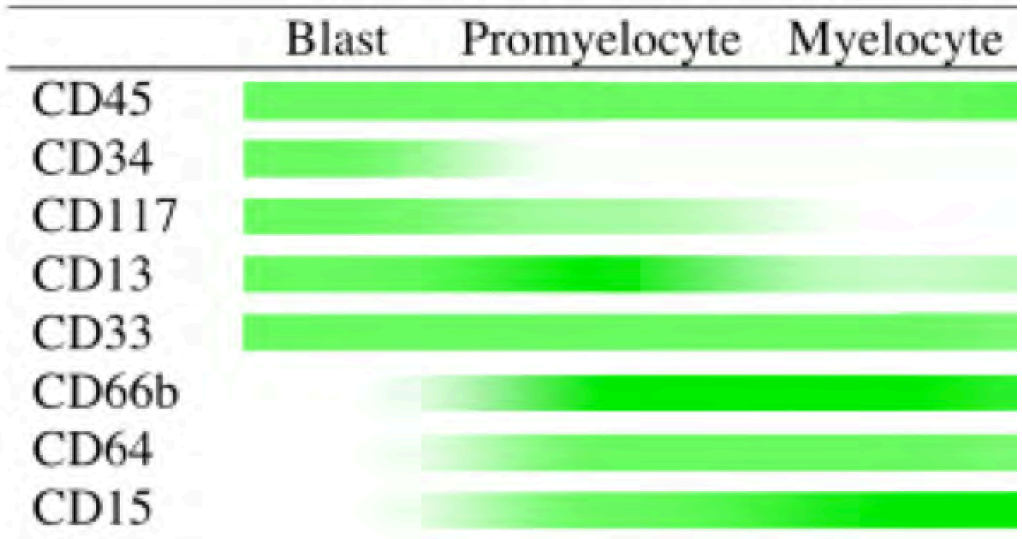
Case 2 take home message: It is important to recognize acute promyelocytic leukemia



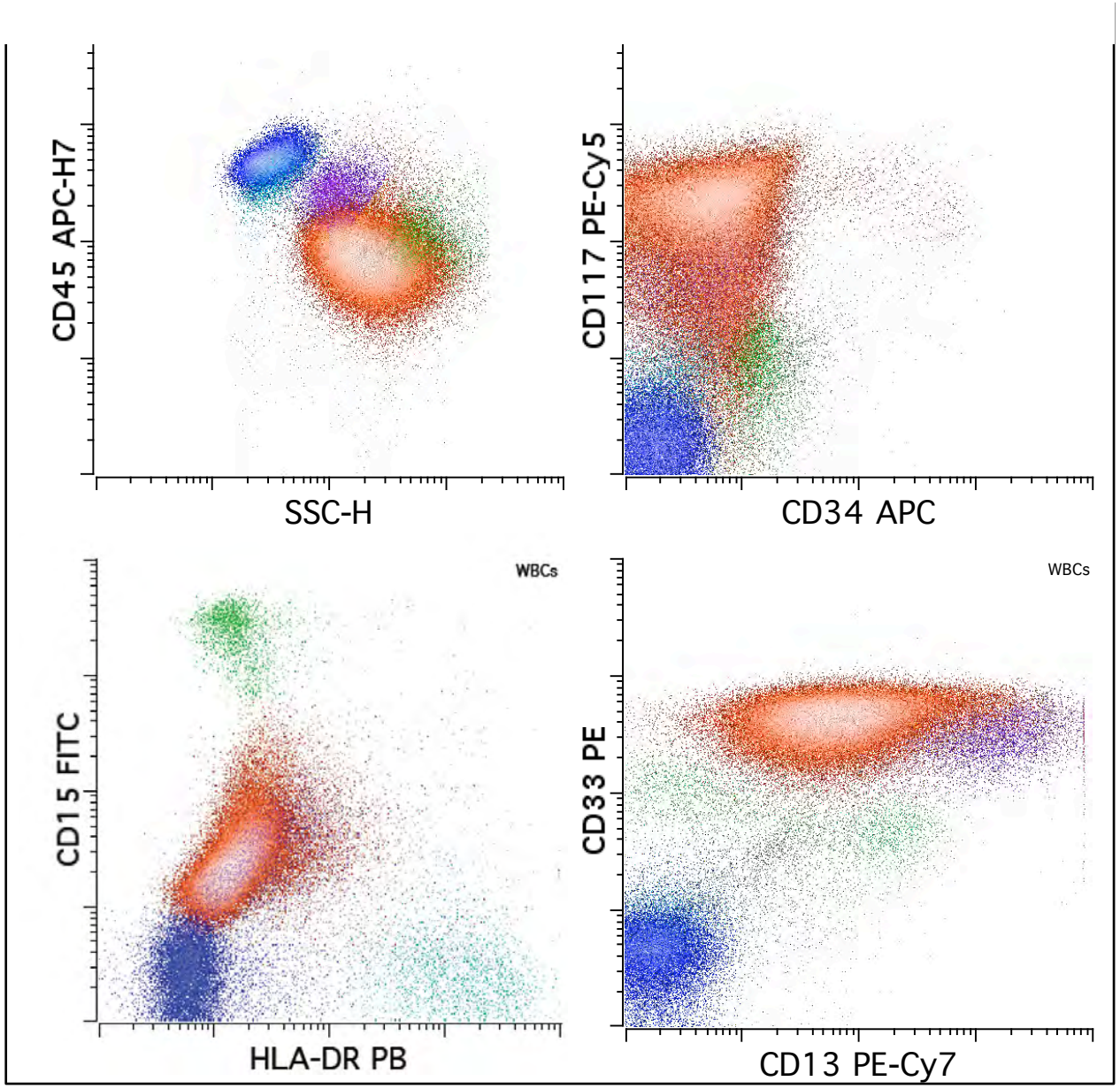
Clinical presentation: Life threatening DIC
Treatment is specific!! (often ATRA + Arsenic)

- Typical immunophenotype
  - CD117+, MPO+
  - CD33 (bright, uniform), CD13 (heterogeneous)
  - **Negative for CD34 and HLA-DR**
  - Low to absent CD15 *** This is in contrast to normal promyelocytes**
  - May express CD56
- Micrograniular variant
  - More likely to express CD2 and variable CD34
- **Flow cytometry is characteristic; however, it is not entirely specific and correlation with genetics (FISH or PCR) is required for definitive diagnosis**
  - For instance absence of or decreased CD34 and HLA-DR may be seen in AML with NPM1 and/or FLT3 mutations.

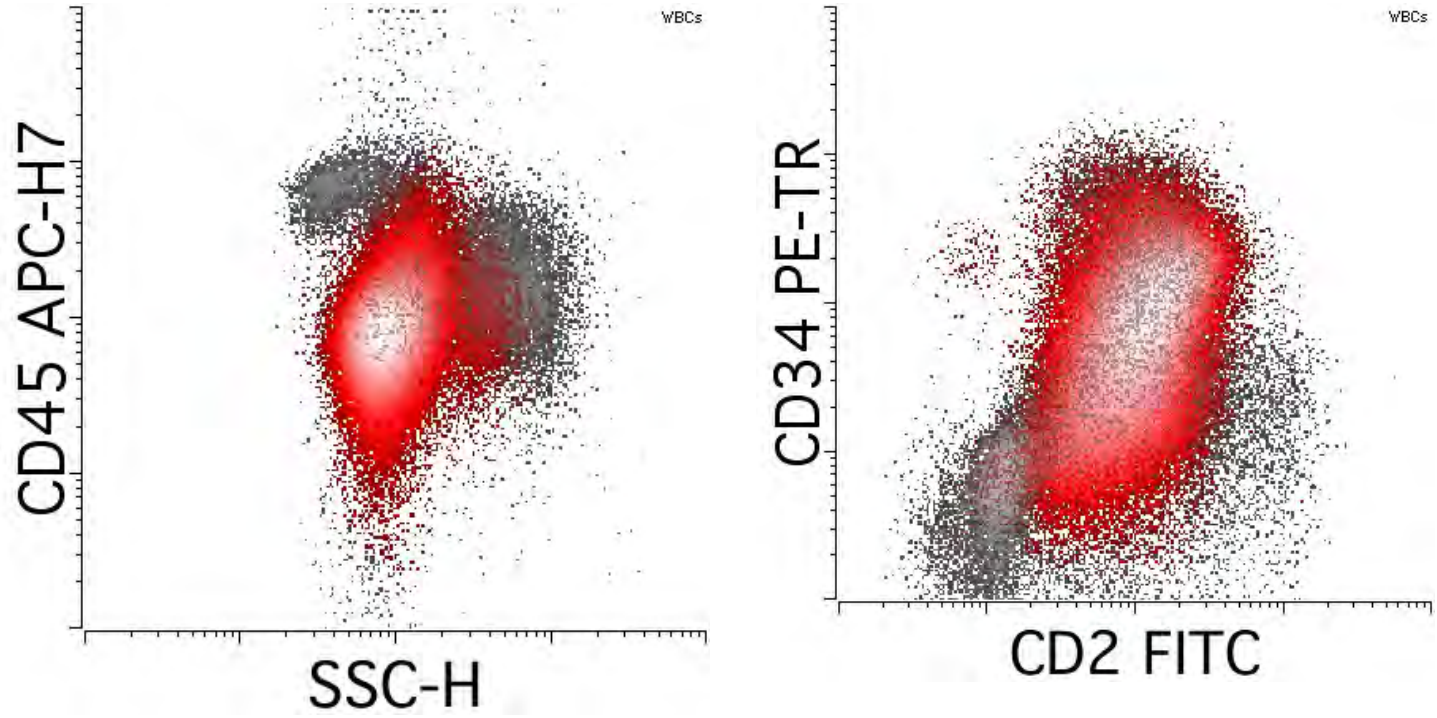
AML with t(15;17); typical hypergranular variant



Wood, Cherian, and Borowitz (2017) Henry's Laboratory Methods



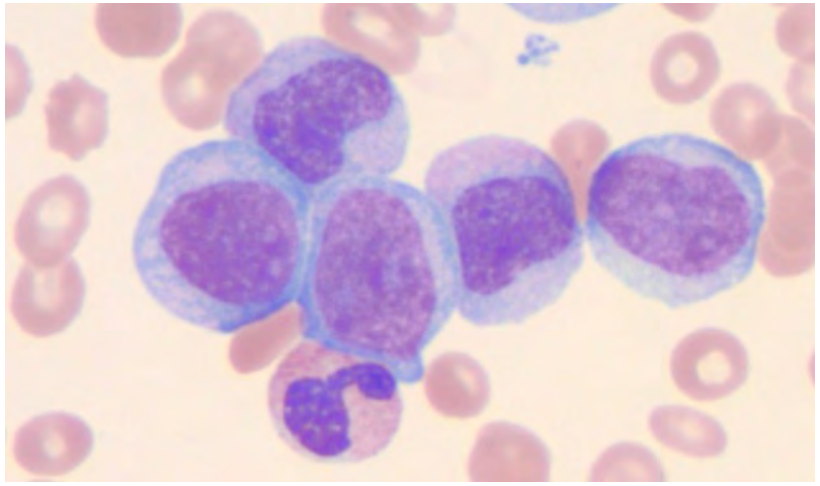
APL microgranular variant

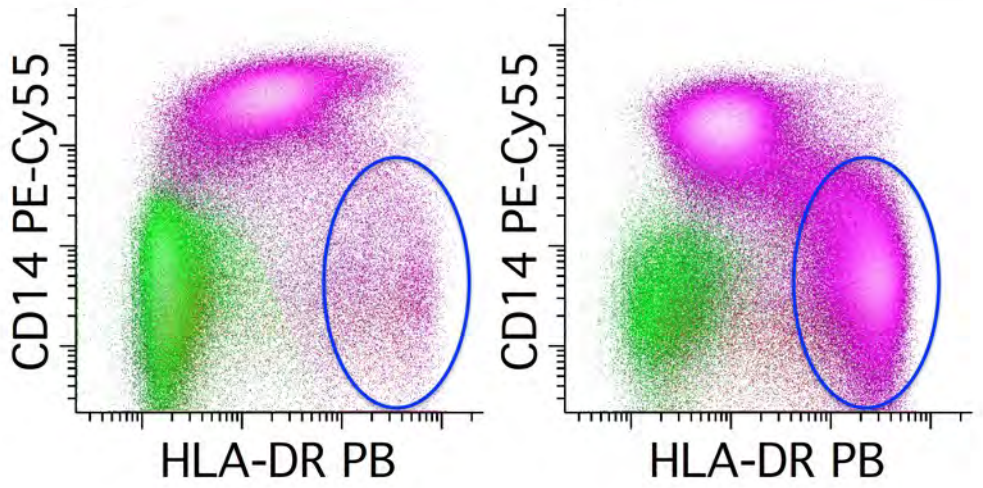


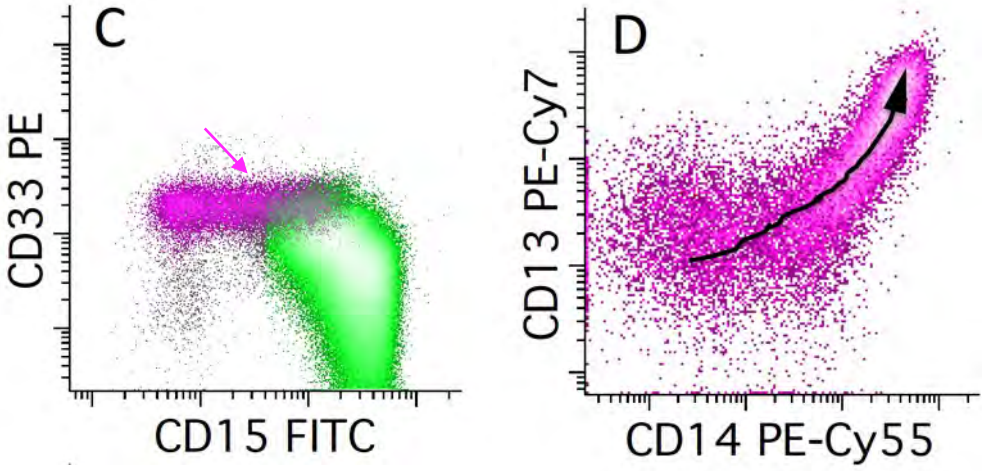
- Relatively lower side scatter
- Increased CD34
- CD2 may be positive

Monocytic lineage

- Bright CD33 CD4, CD64
- HLA-DR
- CD11c, CD15
- CD13, **CD14**
- Aberrant CD56





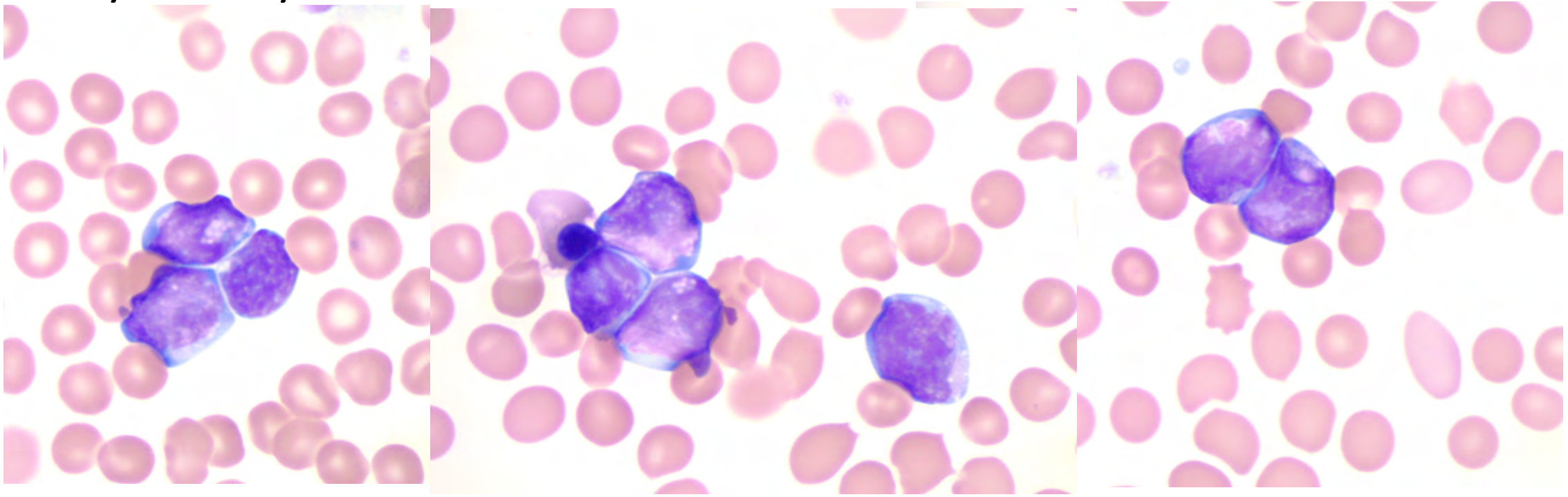


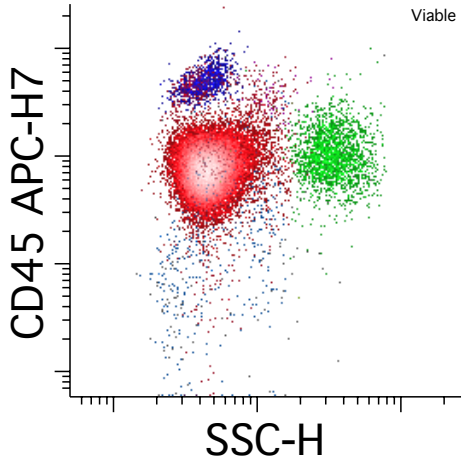
May see an increase in PDCs

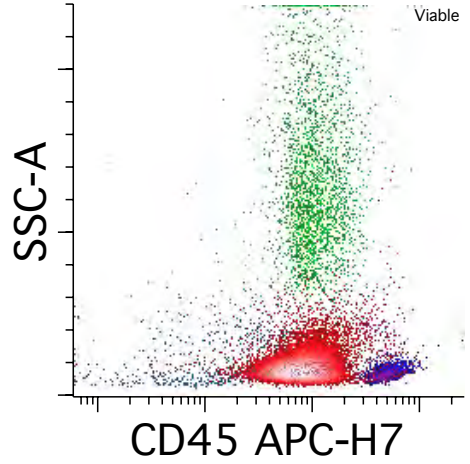
- *CD123 and HLA-DR positive, CD56 negative*

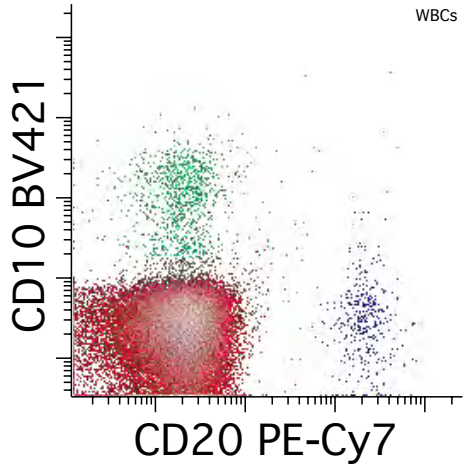
Case 3:

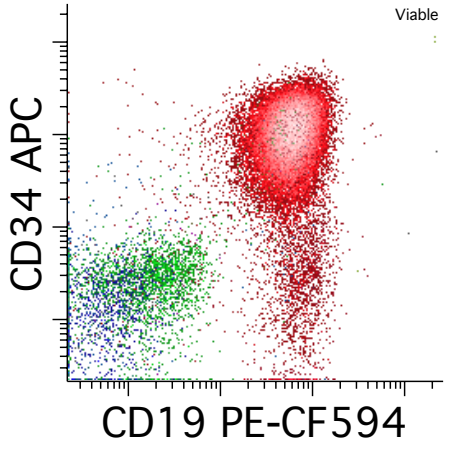
- 7 month old infant presents with failure to thrive. She is found to have a leukocytosis.
- A bone marrow aspirate is performed and is submitted for flow cytometry

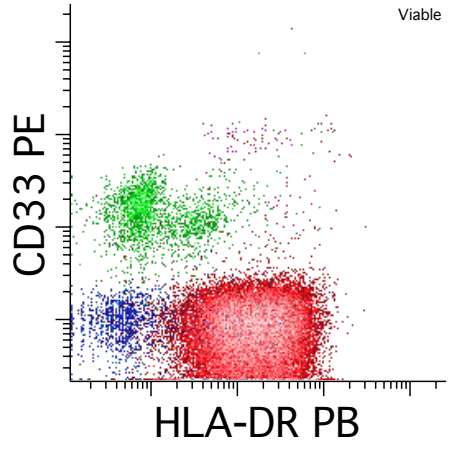


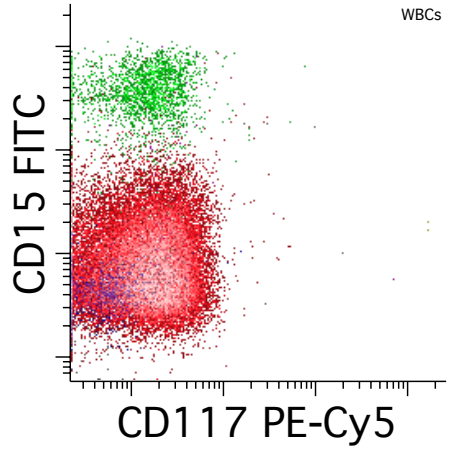


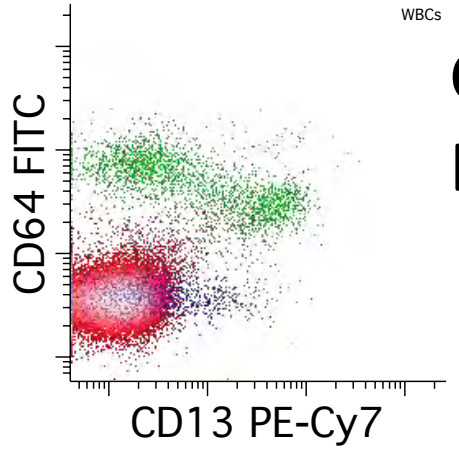


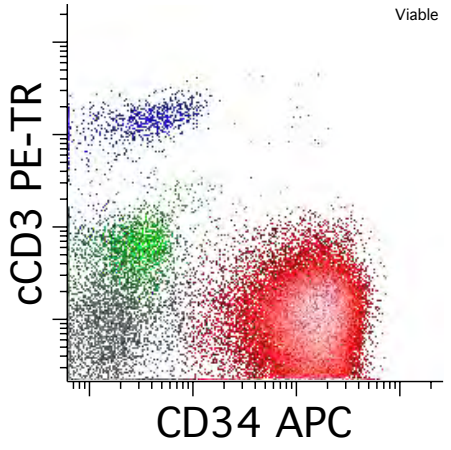


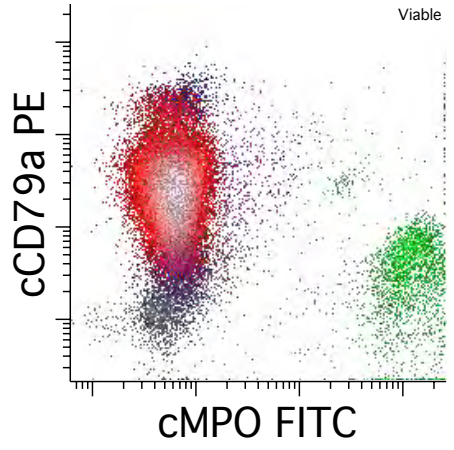


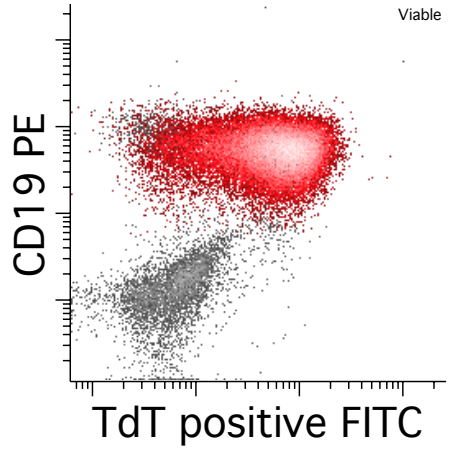


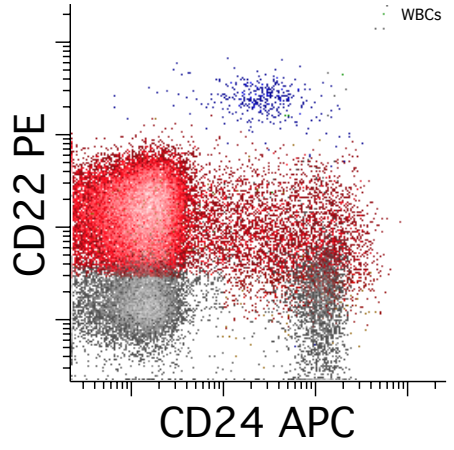










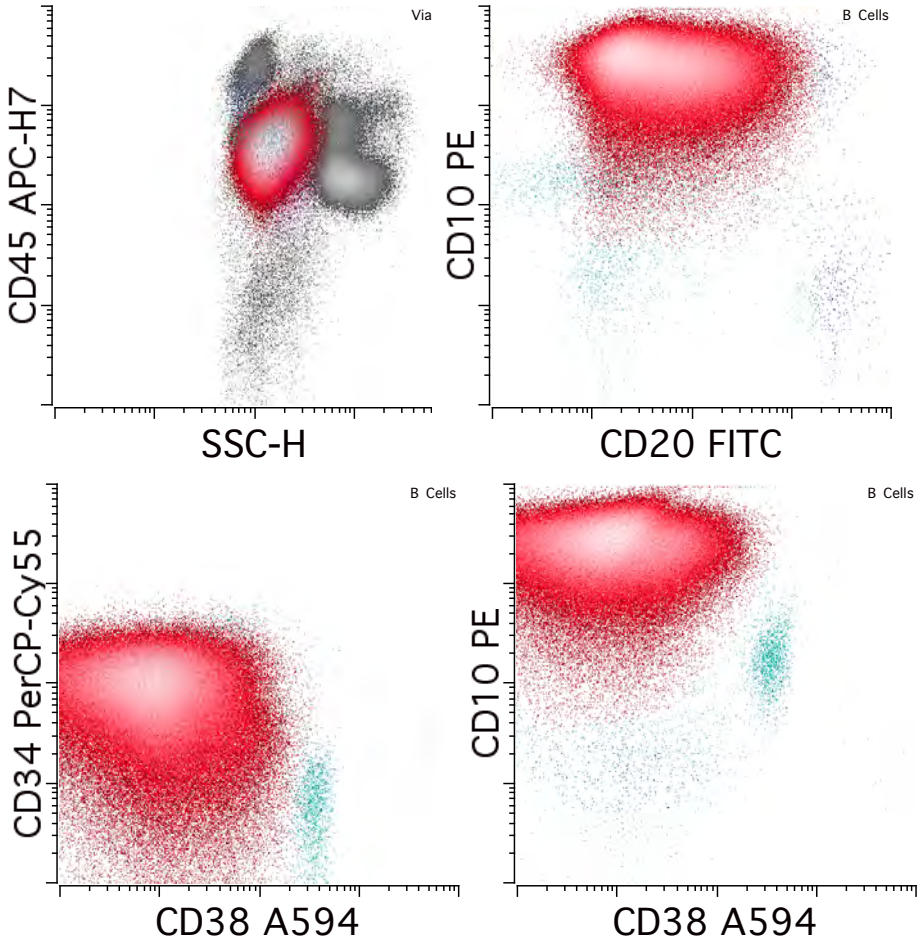


What is your diagnosis?

A. AML
B. Undifferentiated acute leukemia
C. B-LL
D. I am not sure, I need more information

Case 3 take home messages:
Some tips for evaluating B-LL by flow cytometry

- Classic B lymphoblastic leukemia (B-LL) has the immunophenotype to the right.
- There is no one immunophenotype for B-LL
  - Evidence of B cell lineage
    - CD19, CD22, CD79a
  - Evidence of immaturity
    - Lack of surface light chains
    - Decreased: CD20, CD45
    - Increased: CD10
    - Markers of immaturity: TdT, CD34
- Some immunophenotype genotype correlations are described.



2017 WHO classification, B-LL

- B-LL, NOS
- B-LL with recurrent genetic abnormalities
  - t(9;22); BCR-ABL1
  - t(v;11q23.3); KMT2A-rearranged
  - t(12;21); ETV6-RUNX1
  - Hyperdiploidy
  - Hypodiploidy
  - t(5;14); IGH/IL3
  - t(1;19); TCF3-PBX1
- BCR-ABL1 like
- iAMP21

Positive: CD10, CD19, TdT
Frequent CD13, CD33
CD25

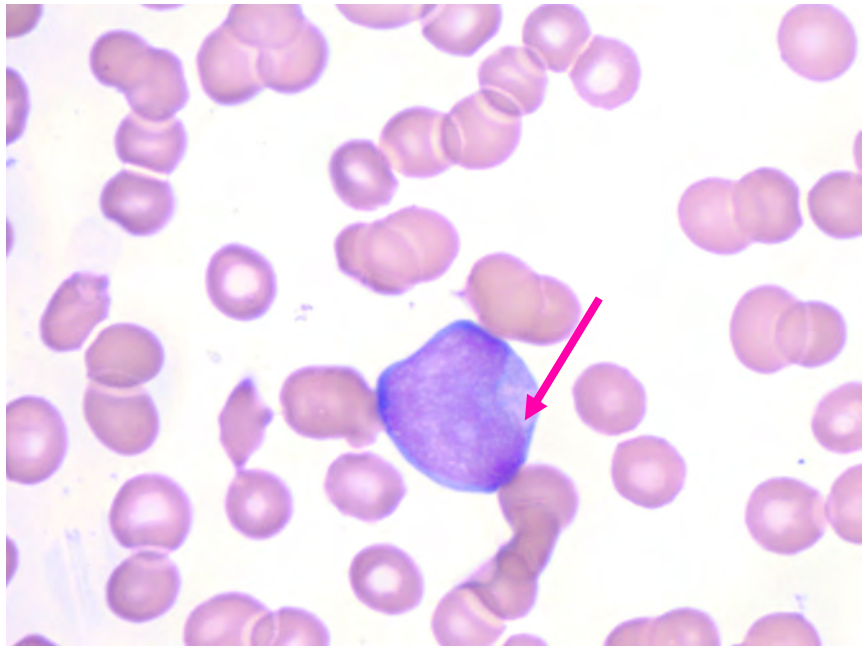
CD19+
Negative for CD10 and CD24
CD15 positive

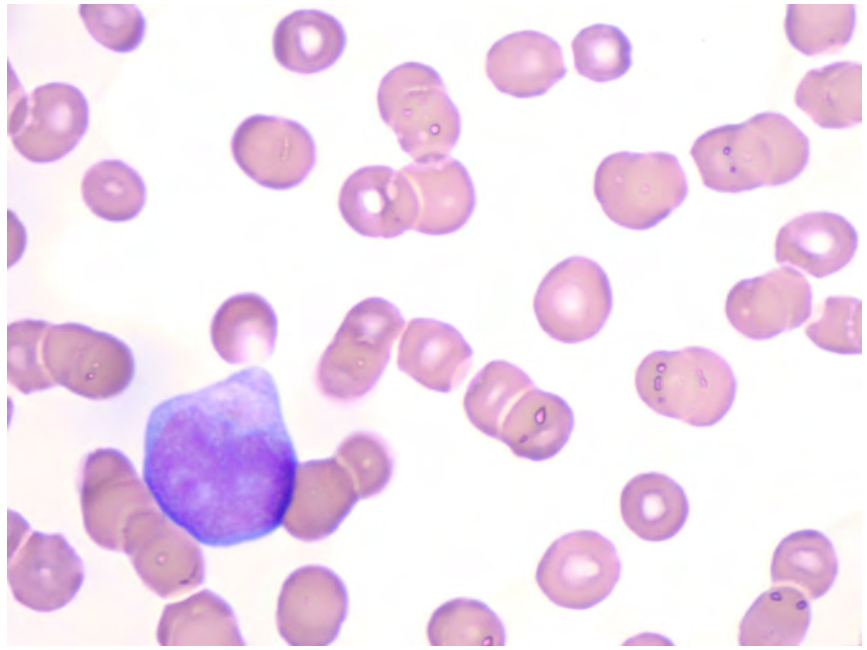
Positive: CD19, CD10
Usually CD34+
Negative or low CD9, CD20, CD66c
CD13 expression is common

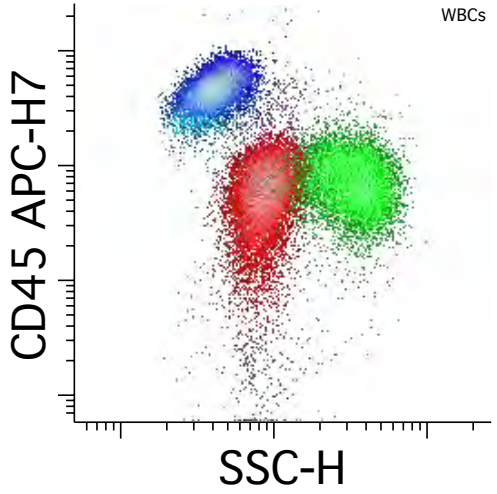
Positive: CD19, CD10, cyMU heavy chain
Usually strong CD9
Negative or limited CD34

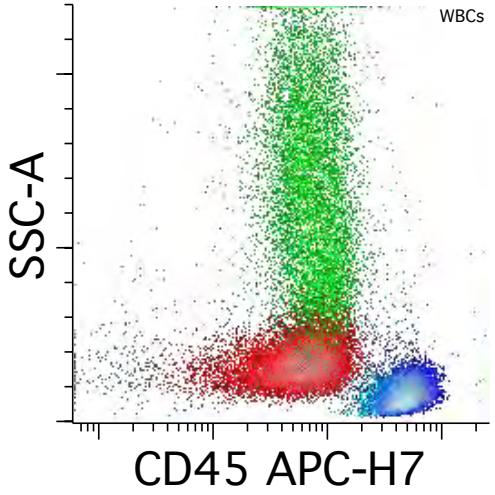
Case 4

- 49 year old female presents with fevers and a toothache
- The patient is presumed to have an infection but… A CBC is performed and shows ~15% “unclassified cells”
- A peripheral blood smear is shown below…





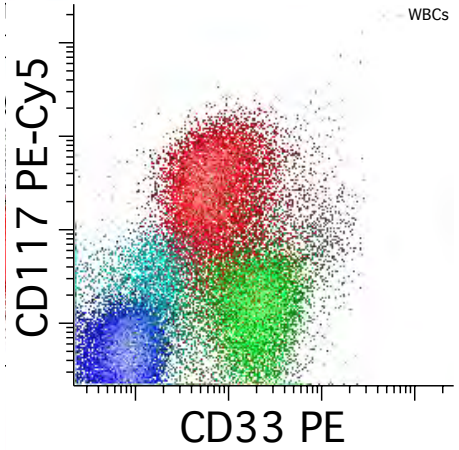


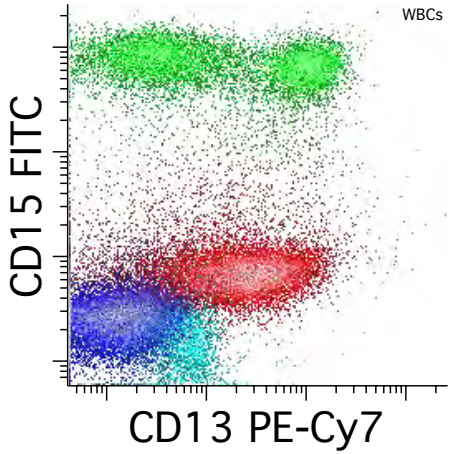


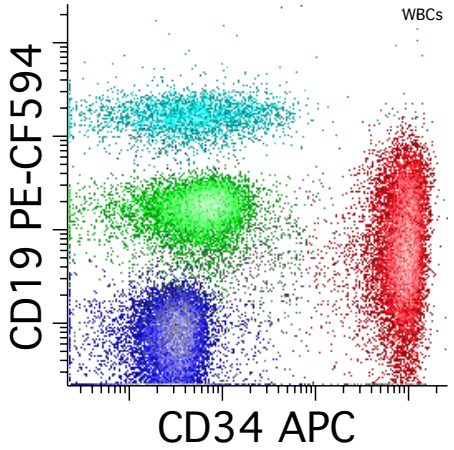
Additional data:
Negative for cCD3, CD10, CD20, CD22

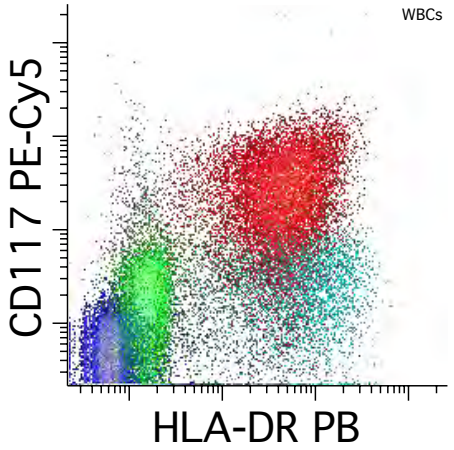
What is your diagnosis?

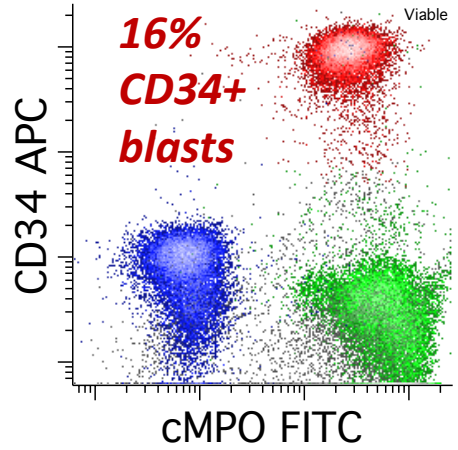
A. AML
B. B-LL
C. MPAL B/Myeloid
D. I am not sure, I need more information
E. The blast percentage is <20%, this is not acute leukemia!
F. Need a marrow for diagnosis

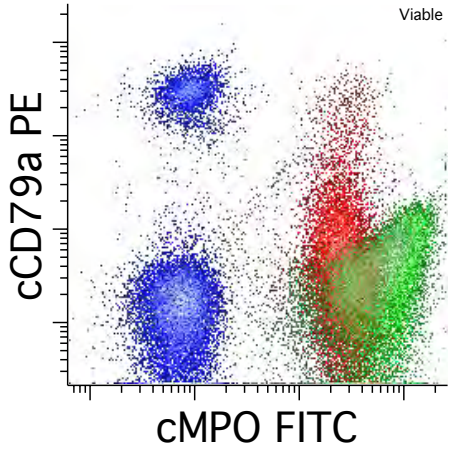


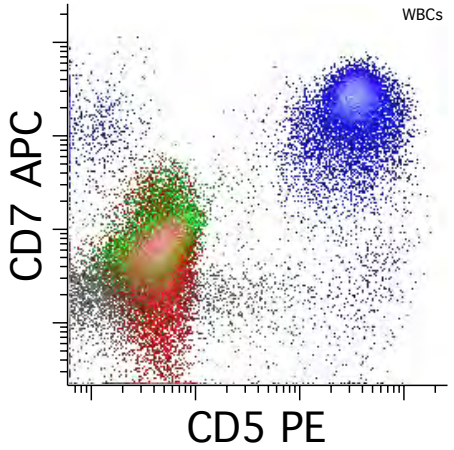


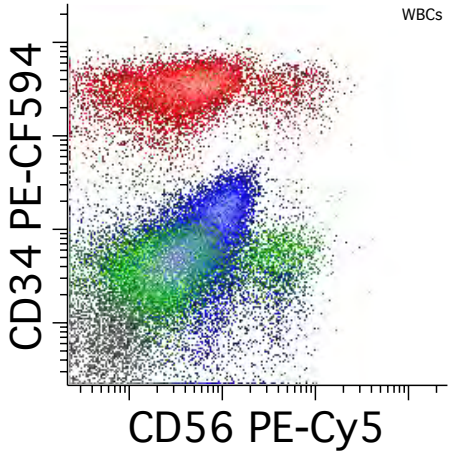












MPAL (mixed phenotype acute leukemia) Diagnostic criteria

1. **There are 2 or more distinct populations of leukemic cells**
   - The blast populations together account for ≥20%
   - One blast population meets immunophenotypic criteria for AML and the other independently meets criteria for B or T cell lymphoblastic leukemia

2. **A single population of blasts is identified expressing myeloid/monocytic markers and B or T lineage markers**
   - WHO specifies strict criteria for lineage assignment in this scenario

In standard cases of AML or ALL, the more strict MPAL criteria need not be met to assign lineage

MPAL: lineage assignment

- **T cell**
  - **Strong** cytoplasmic CD3 (epsilon chain)
  - Surface CD3
- **B cell**
  - **Strong** CD19 and 1 additional marker or weak CD19 and 2 additional markers
  - Additional markers may include **strong** expression of CD79a, cCD22, CD10
- **Myeloid**
  - MPO
    - Flow cytometry, immunohistochemistry, cytochemistry
  - Monocytic differentiation
    - At least 2: NSE cytochemistry, CD11c, CD14, CD64, lysozyme

Applies primarily in cases where a single blast population is present

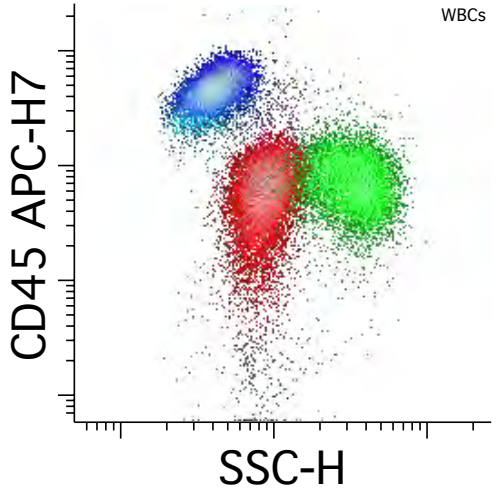
In standard cases of AML or ALL, the more strict MPAL criteria need not be met to assign lineage

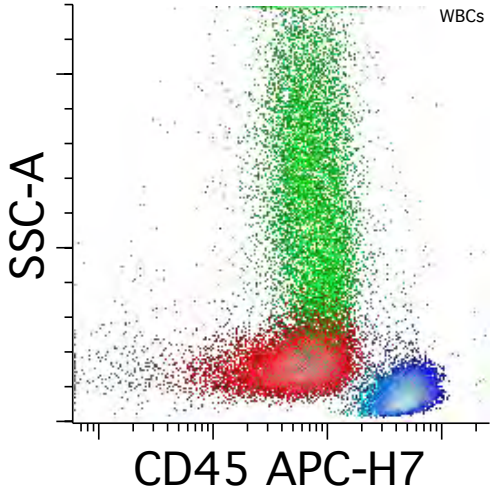
Strong = Expression as bright or nearly as bright as normal background B or T cells on at least a subset of the blasts

Swerdlow, S. H., Campo, E., Pileri, S. A., Harris, N. L., Stein, H., Siebert, R., Advani, R., Ghielmini, M., Salles, G. A., Zelenetz, A. D., & Jaffe, E. S. (2016). The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood, 127(20), 2375-2390.

MPAL: Exclusions

- Don’t make this diagnosis if a different WHO category fits better…
  - AML with recurrent chromosomal abnormalities
    - ***For example, t(8;21) AML***
  - CML blast crisis
  - Acute leukemia with FGFR1 mutations
  - Therapy related myeloid neoplasms and AML with MDS like changes should be classified as such
    - Include a description of the immunophenotype and secondary notation of the mixed lineage
    - AML with MDS related cytogenetic changes would be included here
- ***Complete clinical and genetic data should be taken into account when considering a diagnosis of MPAL***





Additional data:
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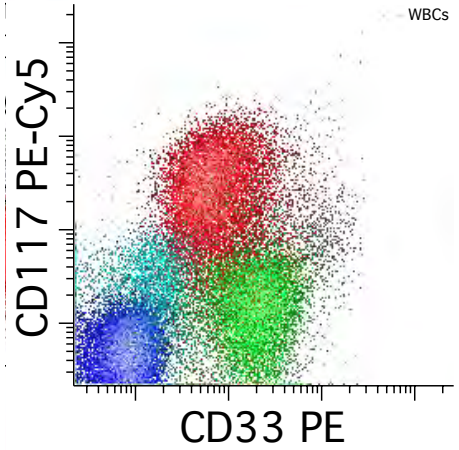
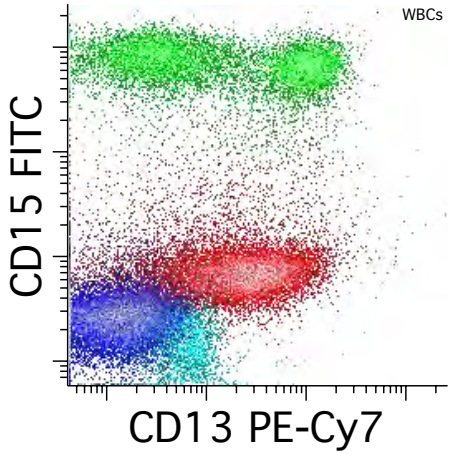
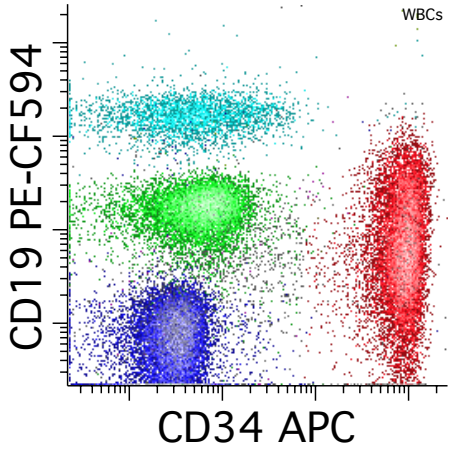
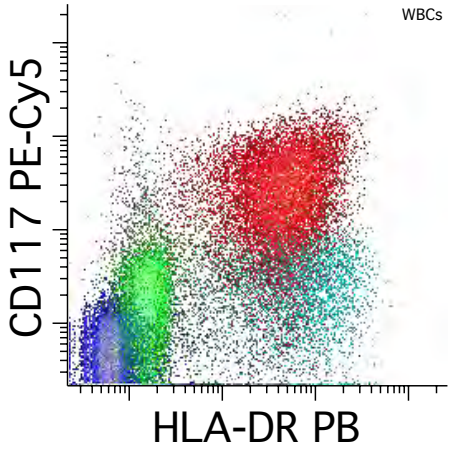
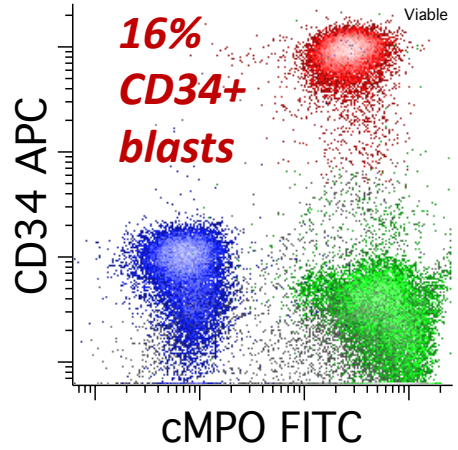
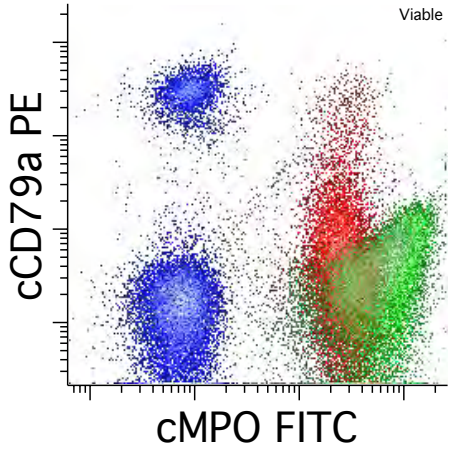


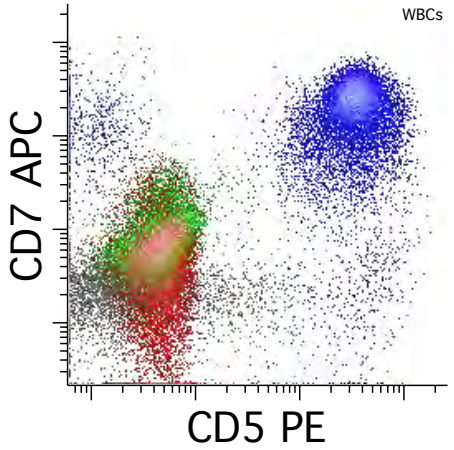


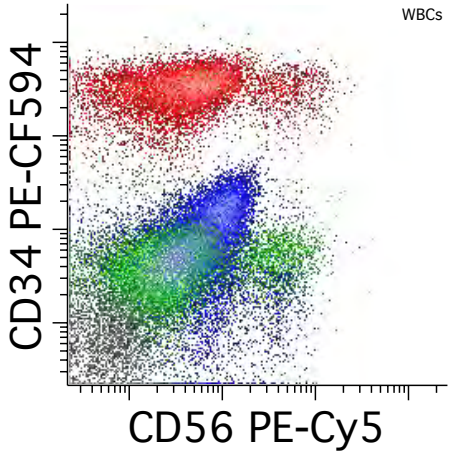












Additional data

- Cytogenetics demonstrated the following abnormal karyotype:
  - t(8;21)(q22;q22)

- Diagnosis

  AML with a t(8;21)(q22;q22)

Case 4 take home message:
Sometimes flow cytometry can give you a clue to classification

- Immunophenotypic features can help to predict genotype or immunophenotype in some cases

Aberrant expression of an antigen of a different lineage may provide a clue to genetics or prognosis

AML defining translocation

t(8;21) AML

Expression of B cell markers (CD19, CD79a) and CD56

B-LL with 11q23 (*MLL/KMT2A*) rearrangement

Expression of CD15 (also often CD10-, sometimes CD34-)

Early Thymocyte Precursor (ETP) T-LL

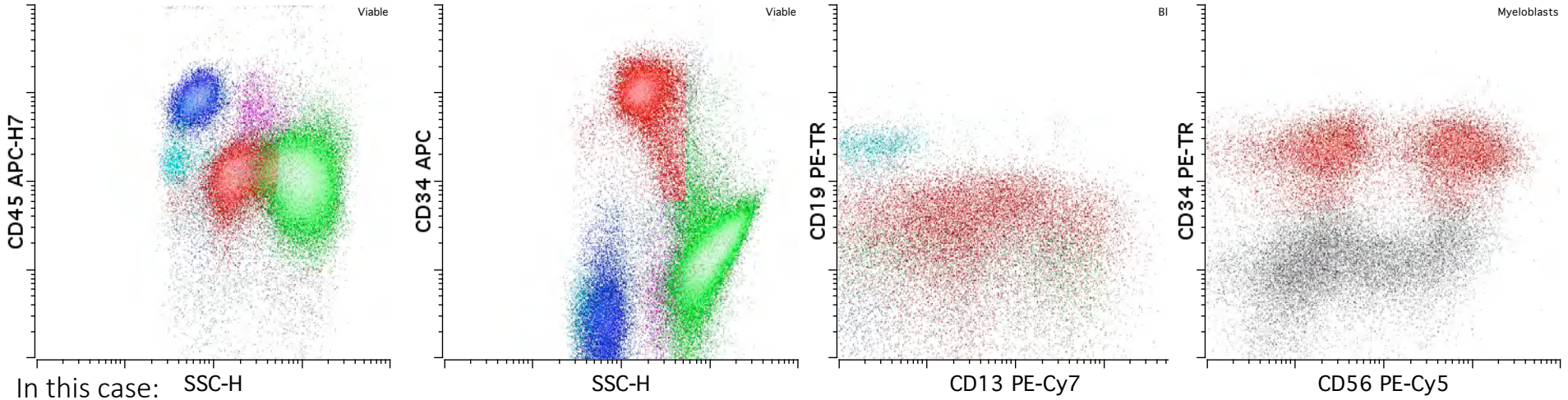
Expression of myeloid/stem cell markers with absence of CD1a and CD8 and low to absent CD5

AML with t(8;21)

t(8;21) is an AML DEFINING TRANSLOCATION

- Immunophenotype
  - Myeloid markers (CD13, CD33, CD117, MPO)
  - CD34 and HLA-DR (strong)
  - Frequent co-expression of CD19 (and other B cell markers including CD79a by flow cytometry and PAX5 by immunohistochemistry) in a subset of blasts
  - CD56 may be positive

CD56 expression has been associated with a poor prognosis →



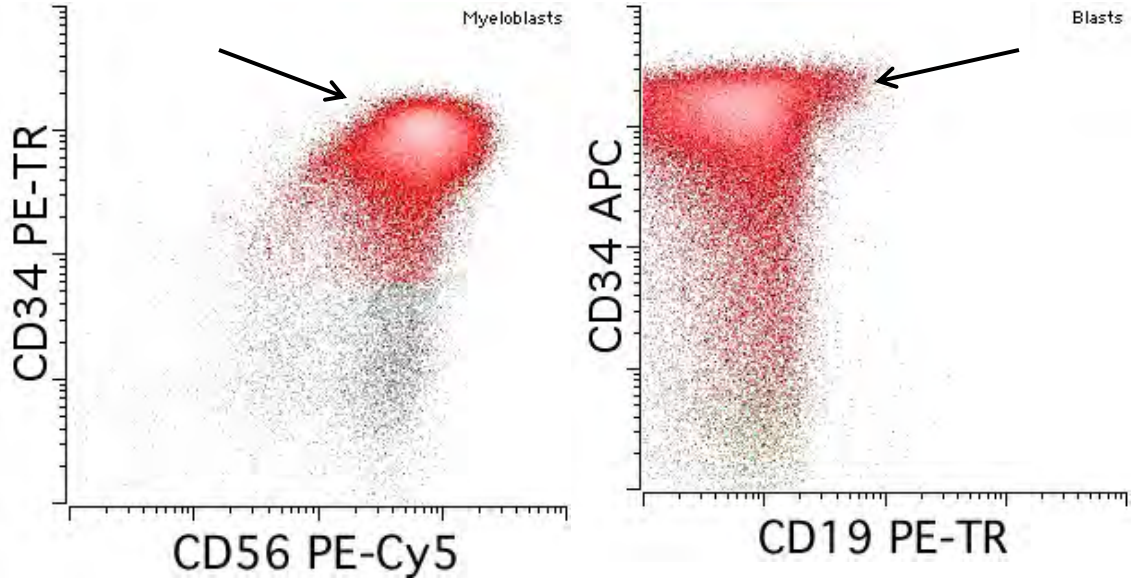
In this case:
20.6% CD34+/CD117+ blasts by flow
17.3% blasts by morphology
t(8;21)+ by FISH

Immunophenoytype can provide a clue that this is AML and not high grade MDS

KIT mutations in t(8;21) AML

- KIT mutations are seen in 15-50% of CBF AML
  - AML with a t(8;21) or AML with an inv(16)
- Some studies suggest a poorer overall prognosis in this subtype of AML that usually has a good prognosis
  - Somewhat controversial, better established in t(8;21)
- KIT activating mutations are associated with relatively lower levels of CD19 expression in t(8;21) AML and with strong CD56 expression

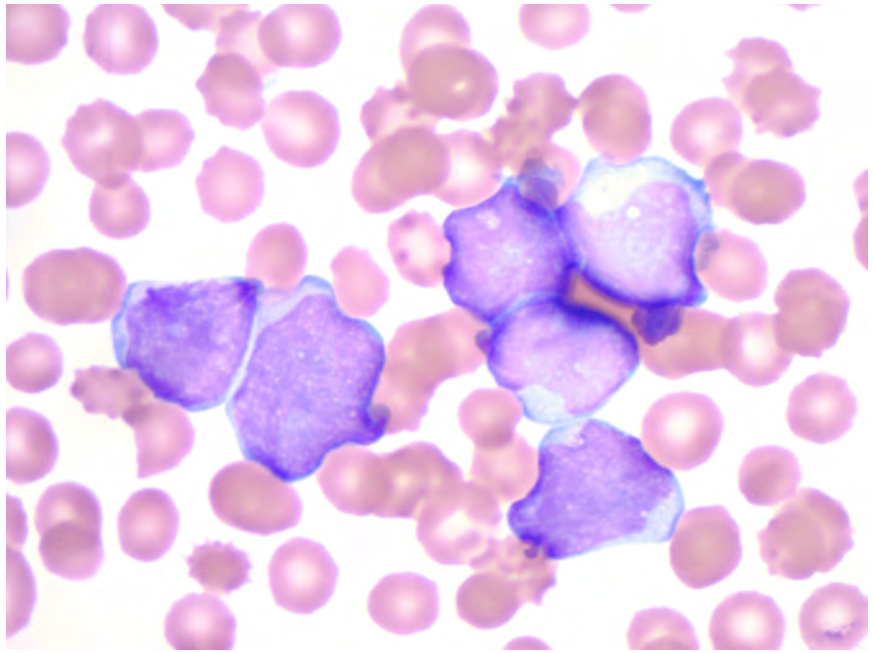
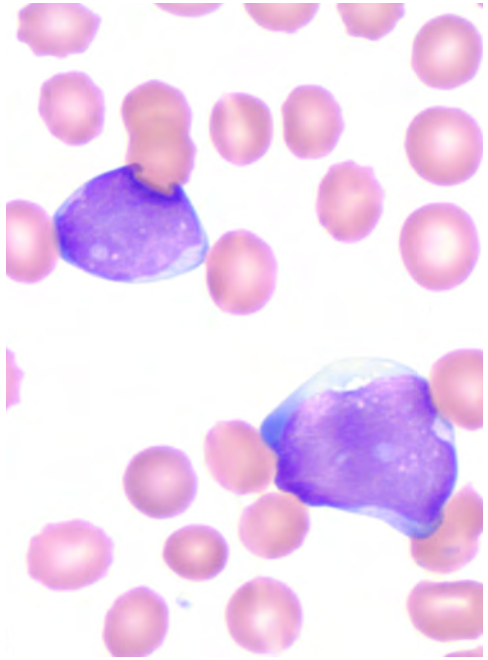
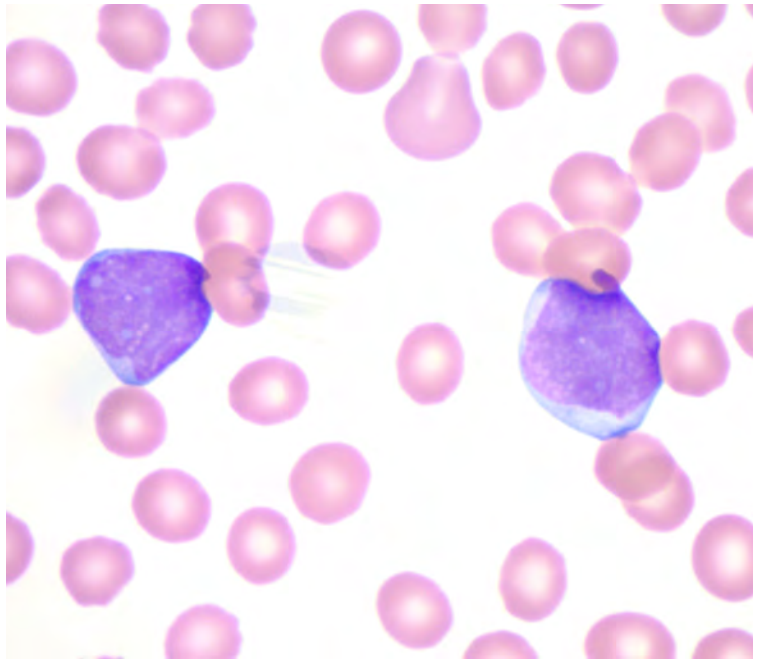
The images to the right are from a patient with t(8;21) AML harboring a KIT mutation

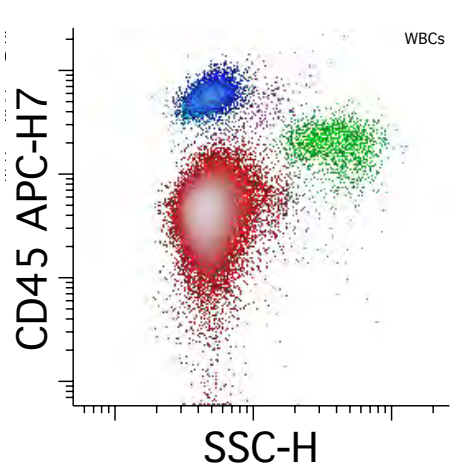


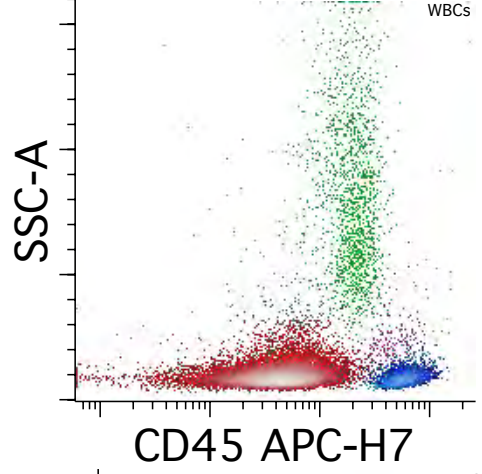
Our patient had no evidence of a KIT mutation on molecular testing

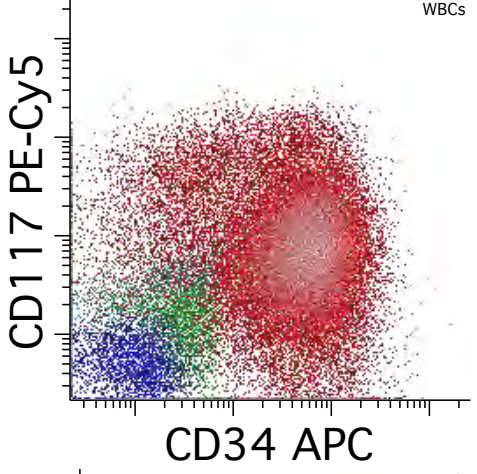
Case 4

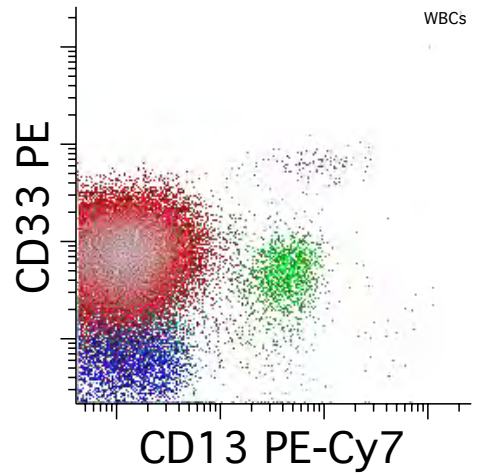
- A 42 year old female presents to the ED with blurry vision and proptosis and is found to have lacrimal gland swelling.
- A CBC performed on admission demonstrate leukocytosis.

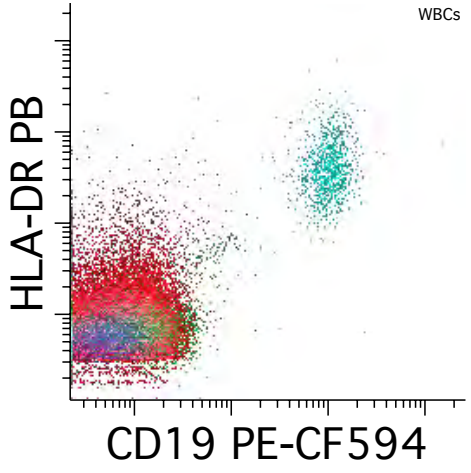




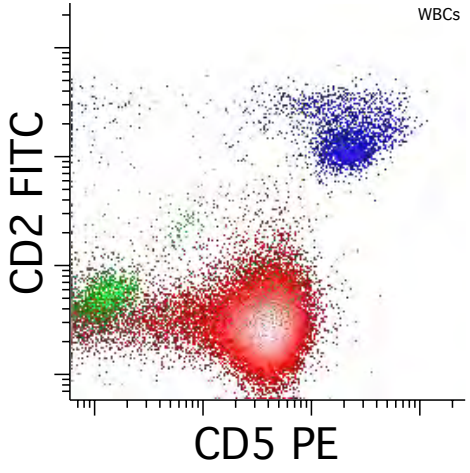


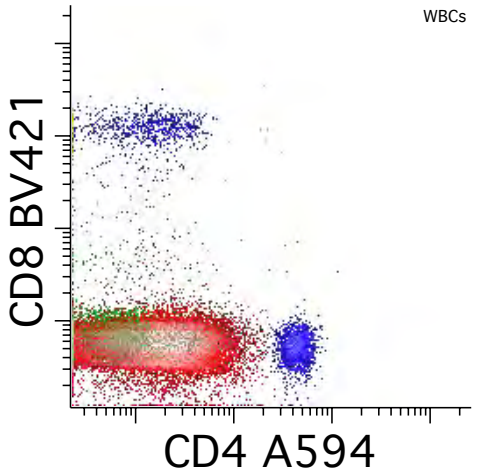


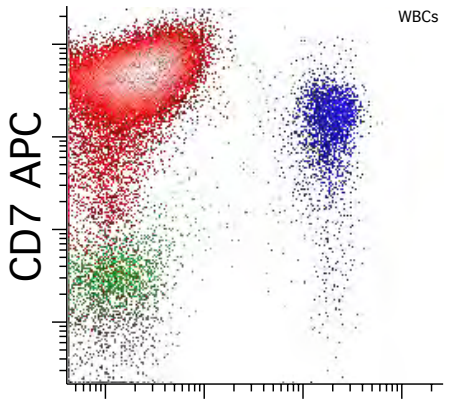


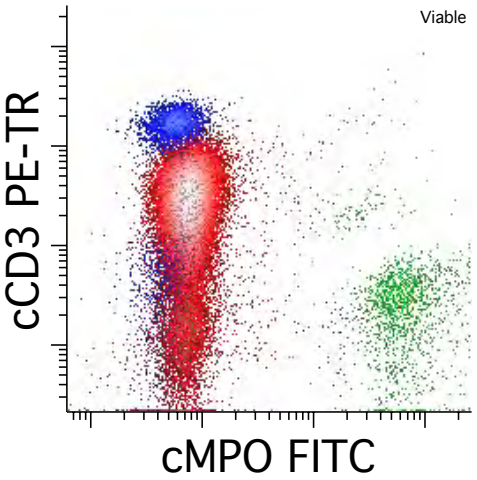


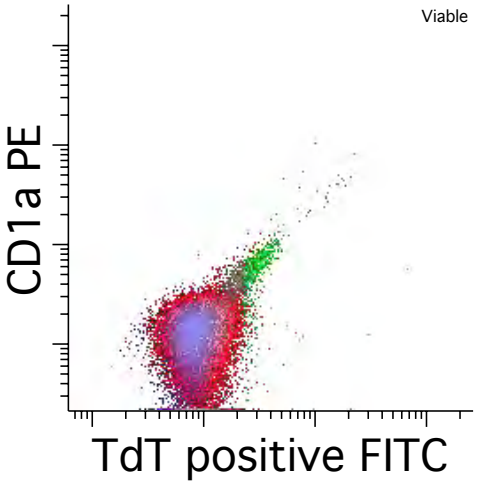












What is your diagnosis?

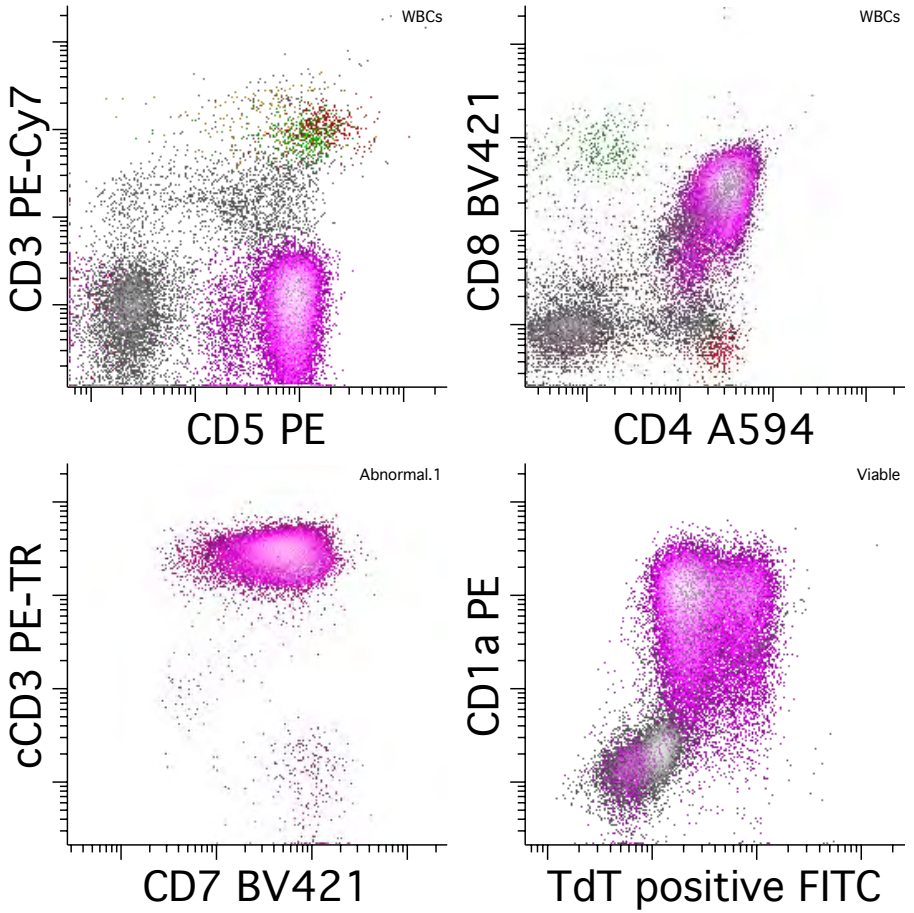
A. AML
B. Undifferentiated acute leukemia
C. B-LL
D. T-LL
E. MPAL T/Myeloid

Case 5 take home message:
Tips for assessing T lymphoblastic leukemia/lymphoma

T lymphoblastic leukemia

- Neoplasm of precursor T cells committed to the T cell lineage
- Immunophenotype
  - Immature
    - TdT*, CD34, CD1a
    - Surface CD3 is often negative
  - T lymphoid
    - cCD3, CD7 are most often positive
    - CD2, CD5
    - CD4, CD8
  - May see aberrant expression of myeloid antigens
    - CD13, CD33, CD117

Classic T-LL



**** TdT is reported positive at diagnosis in 90-95% of cases***

Early thymocyte precursor T-LL

- Early thymocyte precursor (ETP) T-LL (10-15% of T-LL)
- Derived from an early T cell precursor and shows T cell differentiation but retains expression of some myeloid and/or stem cell antigens Coustan-Smith et al. Lancet Oncology 2008;10:147-56.
- Definition
  - T cells: CD7, cCD3 *cCD3 has been described as dim/weak in some cases by IHC Shelly et al. J Clin Diagn Res. 2017 Jul; 11(7): EL01–EL02.
  - Lack CD1a, CD8
  - CD5* is negative or decreased (<75% expression)
  - Expression of 1 or more myeloid/stem cell antigens:
    - Expression on ≥25% of blasts
    - CD34, CD117, HLA-DR, CD13, CD33, CD11b, or CD65

A subset of cases deemed "near ETP" meet these criteria but show increased CD5 expression with otherwise similar features compared to typical ETP-T-LL

Wu et al. Science Translational Medicine 2012;134(4):134ra63.

Alternative criteria

proposed by Zuurbier et al. Haematologica 2014;99(1):94-102.

Negative: CD1a, CD4, CD8

Positive: CD34 and/or CD13 or CD33

CD5 expression removed

Better correlation with ETP gene expression signature which is underestimated using traditional criteria

Genetics in ETP T-LL

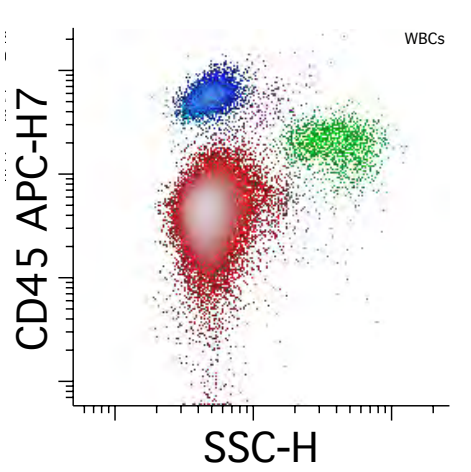
- Frequent myeloid associated gene mutations
  - *FLT3* (~35%)
    - Associated with expression of CD13 and CD117 without CD33
  - *DNMT3A* (16% in adults)
- Less frequent more typical T-LL gene mutations
  - *Notch 1* mutations (15%)
  - Clonal T cell receptor gene rearrangement (59%)

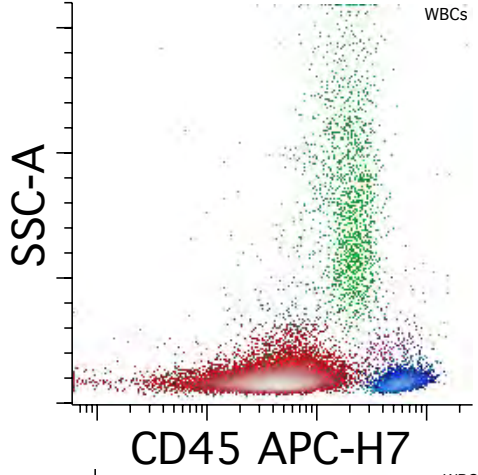
Neumann et al. Blood 2013 121(23):4749-52.
Neumann et al. PLOS ONE 2013;8(1):e53190.
Wu et al. Science Translational Medicine 2012;134(4):134ra63.

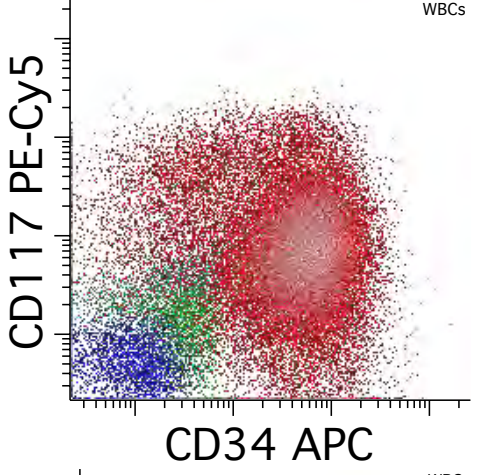
Prognosis in ETP T-LL

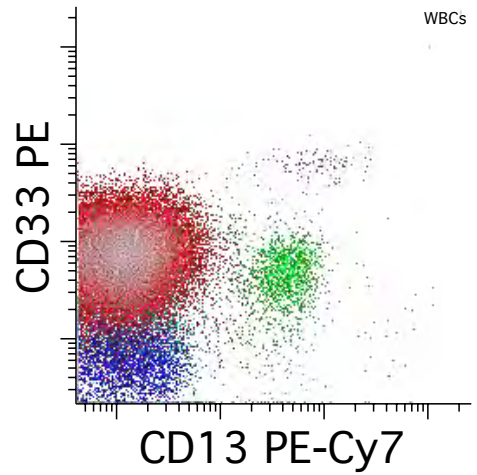
- Many studies show poorer prognosis for T-LL with an ETP immunophenotype
  - Pediatric: Coustan-Smith et al. Lancet Oncology 2008;10:147-56.
  - Adolescents and adults: Jain et al. Blood 2016;127(15):1863-9.
  - Adults: * Genesca et al. Haematologica 2020;105:e295 * .
- Has not been borne out in all studies with some recent pediatric studies demonstrating no significant differences
  - COG: Wood et al. Blood, 2014 124(21), 1. (abstract)
    - (n=1144) Higher rate of induction failure * for both ETP and near ETP but 5-year EFS and OS that were not statistically different
  - COG: Dunsmore et al. *Journal of Clinical Oncology* 2020;38(28);3282-3293.
    - (n-1125) ETP status did not have a stastically significant impact on DFS
  - DCOG and COALL* Zuurbier et al. Haematologica 2014;99(1):94-102.
- ***High rate of induction failure has been noted for ETP subtype****
- Additional studies will be needed to characterize the prognostic impact of this subtype and to determine the optimal course of therapy in patients presenting with this immunophenotype

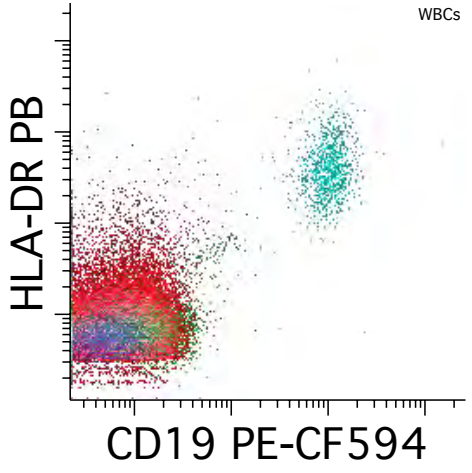
*** Studies used definition proposed by Zuurbier et al:**
Neg for CD4
CD5 not considered

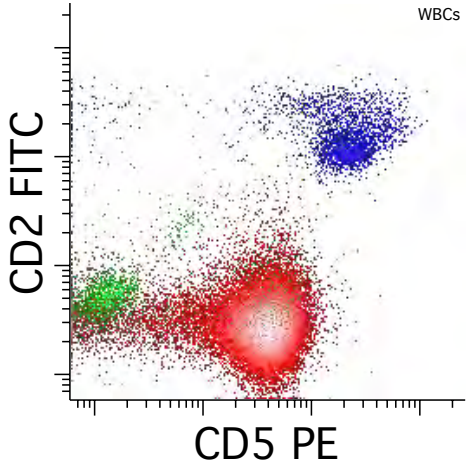


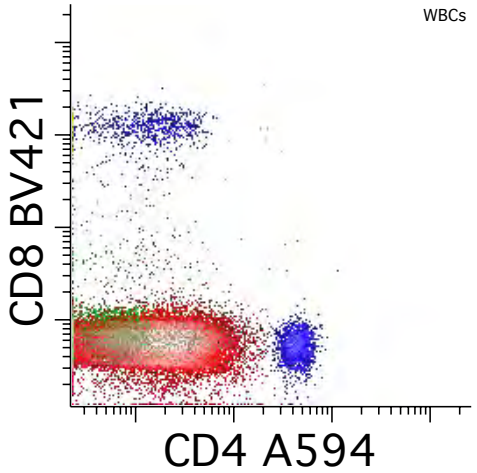


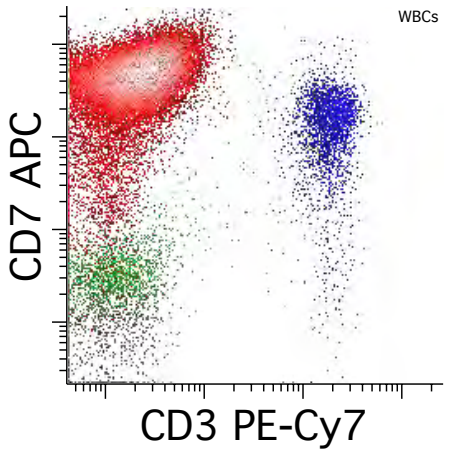


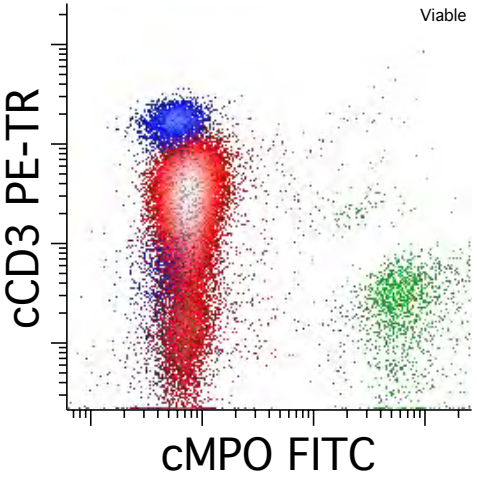


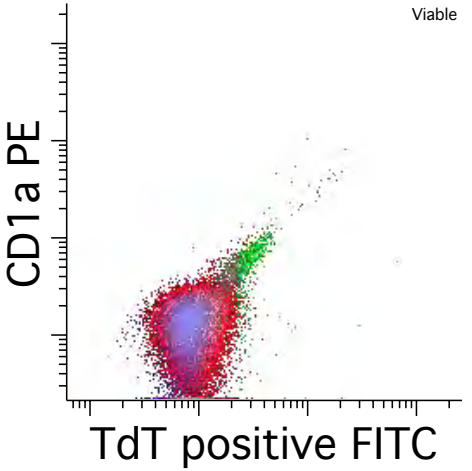










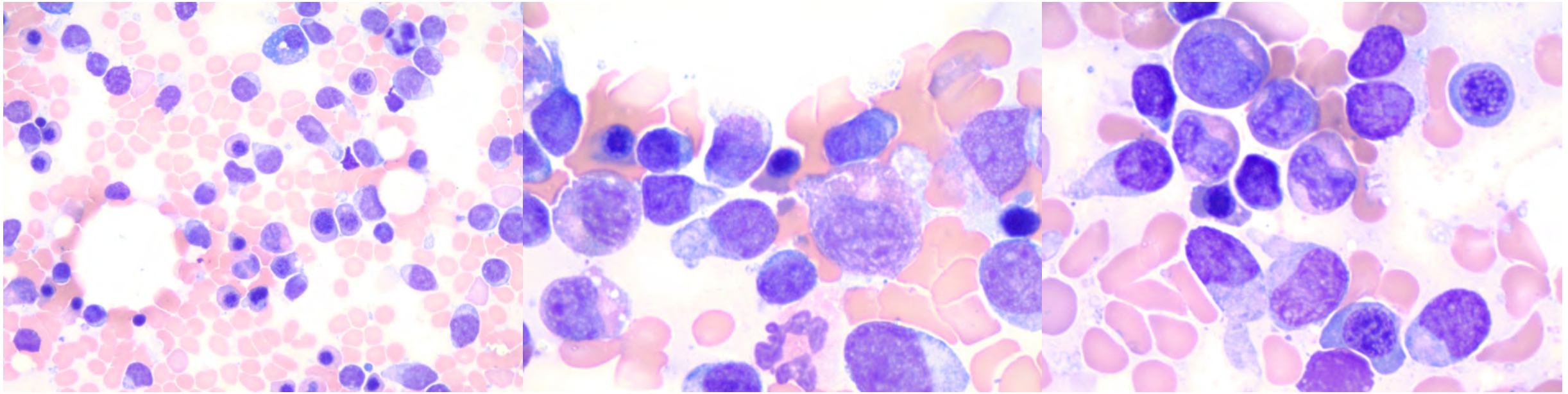


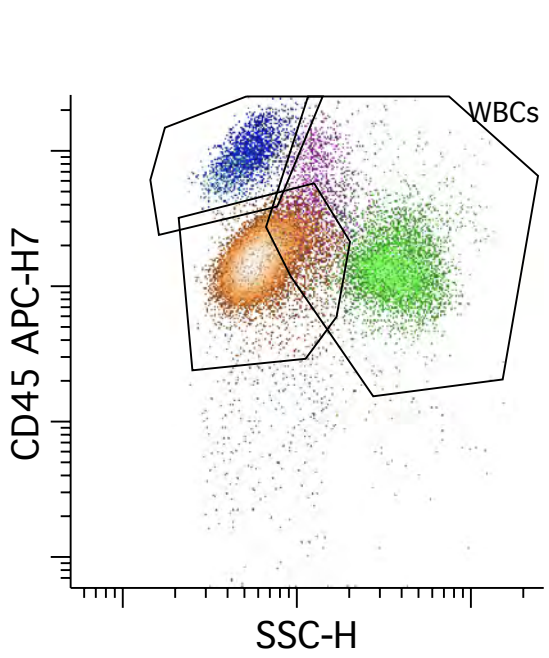
Question 6: Is this case ETP T-LL?

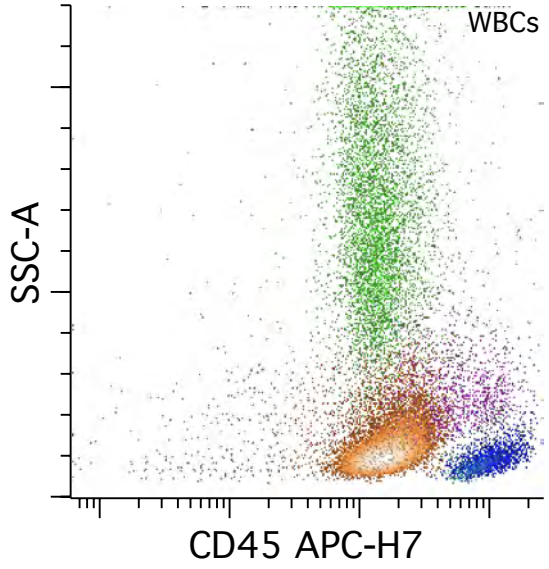
A. Yes, ETP using traditional criteria
B. Yes using criterial proposed by Zuurbier et al.
C. No
D. No but it could be considered near ETP-TLL
E. I'm not sure

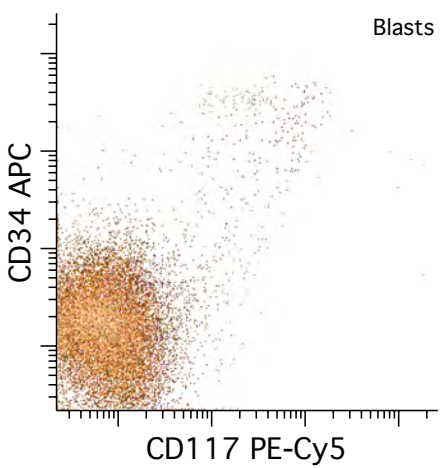
Case 5

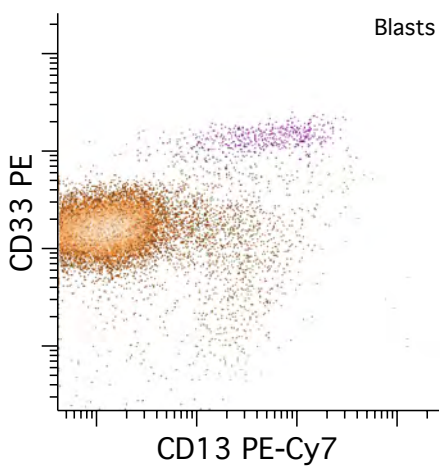
- 63 year old male with skin lesions, lymphadenopathy, and thrombocytopenia.
- A bone marrow aspirate is performed and a portion is submitted for flow cytometry.

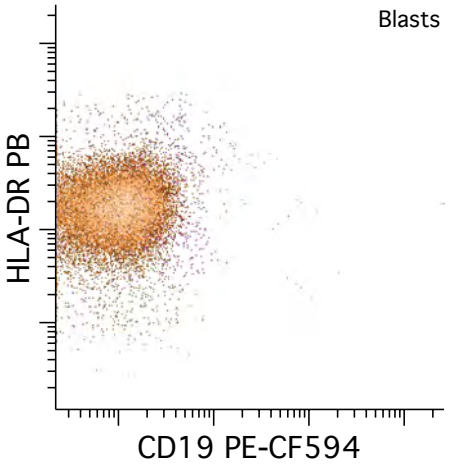


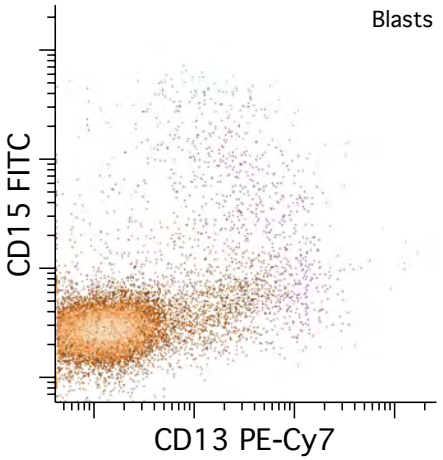


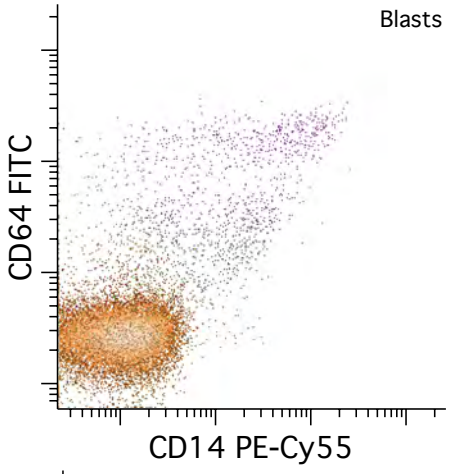


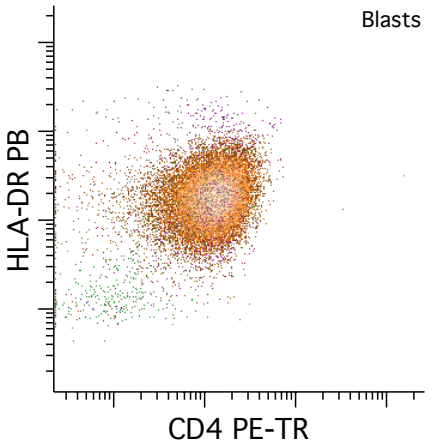


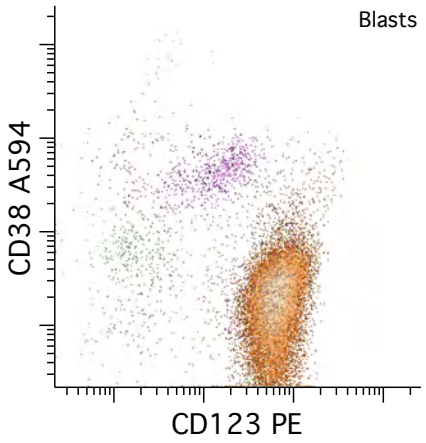


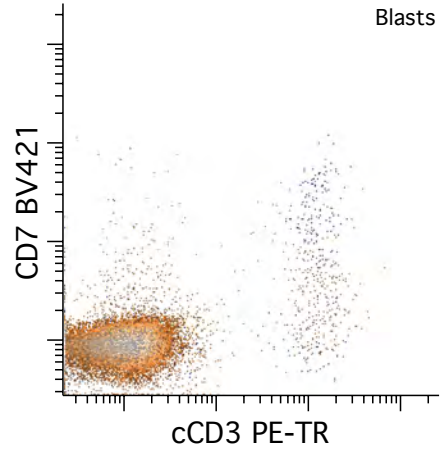


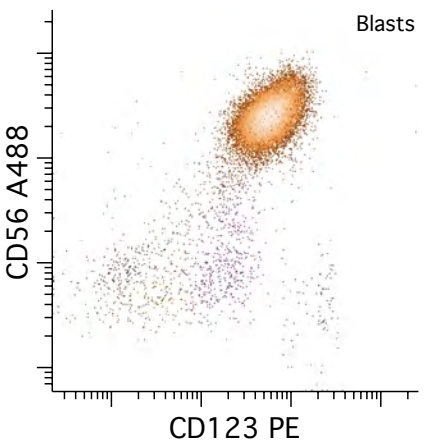










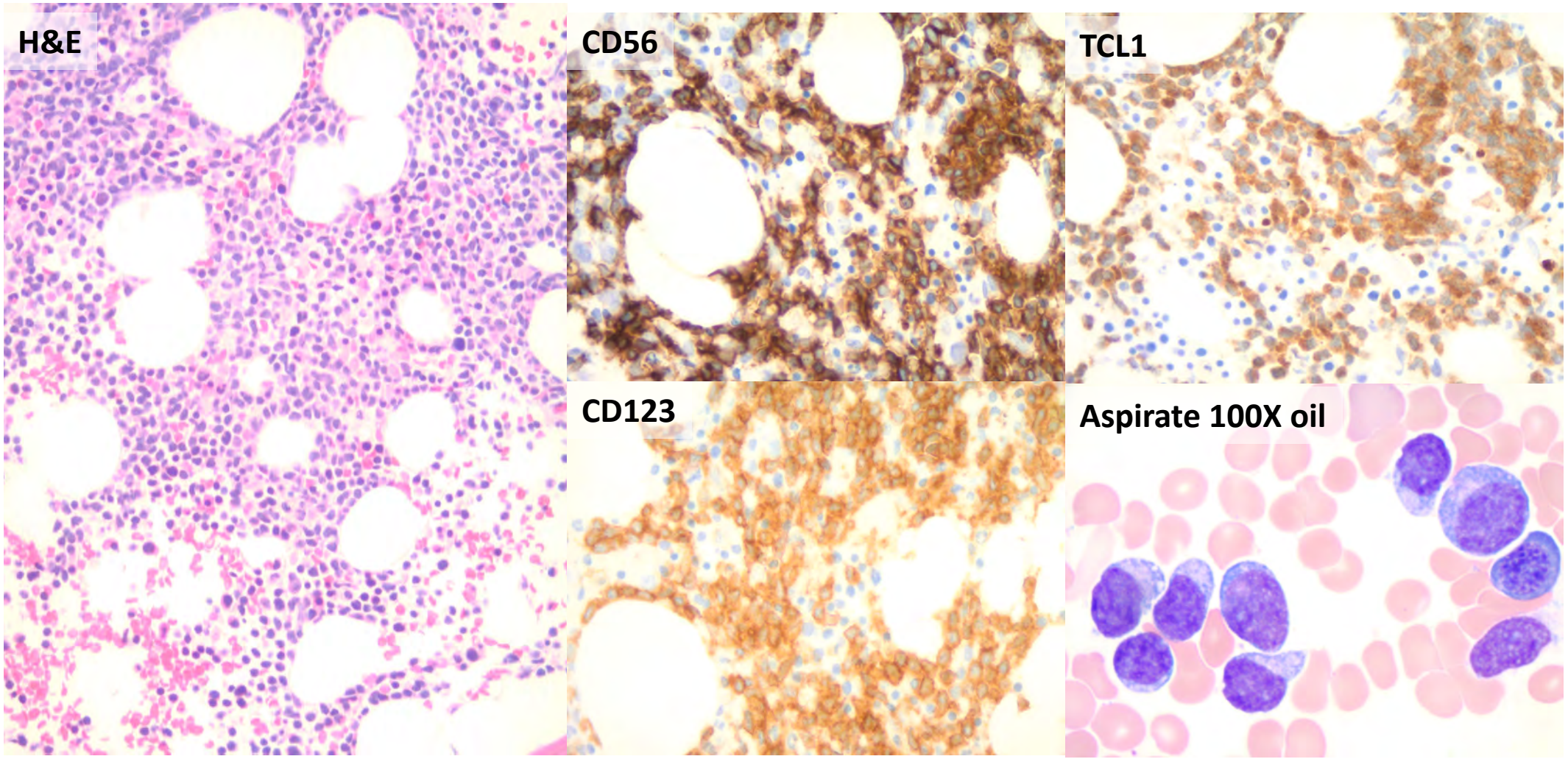


Other data:

Negative:
CD5
CD1a
TdT
CD10
CD20
CD79a

Diagnosis?

Blastic plasmacytoid dendritic cell neoplasm



Case 5 take home message:
Don’t forget about plasmacytoid dendritic cells

- Blastic plasmacytoid dendritic cell neoplasm (BPDCN) are rare but aggressive tumor of plasmacytoid dendritic cell precursors
- Cutaneous and marrow involvement
- Differential diagnosis:
  - ❖Acute leukemia (T-LL, monocytic leukemia), T or NK cell neoplasms
- Characteristic immunophenotype
  - ❖Positive: CD4, CD123, HLA-DR, CD43, CD56, TCL1, CD303
  - ❖Often expressed: CD7, CD33
  - ❖Negative: CD3, CD13, CD16, CD19, MPO, and lysozyme
- Note: PDCs which are CD56 negative may occasionally be increased (and sometimes for cohesive clusters) in myeloid neoplasms with monocytic differentiation such as CMML.
  - ❖This would not represent a BPDCN

- For more information, cases, and resources, check out the following offerings from ICCS:
  - Resource center: https://www.cytometry.org/Resource-Center/
  - Online educational resources: https://www.cytometry.org/web/education-public.php
- Also, Follow ICCS on Twitter

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

cherians@uw.edu

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