CSI case

“Buddy” Franklin Fuda

Sharon Germans

Mingyi Chen

Rolando Garcia

Clinical Presentation

- A 28-year-old man presents to his family physician complaining of fever, night sweats and rapid weight loss over the past 2 months. He claims that he can only eat a little amount of food before feeling full.

- A physical examination reveals generalized lymphadenopathy and massive splenomegaly.

- A complete blood cell count reveals mild normocytic, normochromic anemia, thrombocytopenia and leukocytosis of 165×10^9/L.

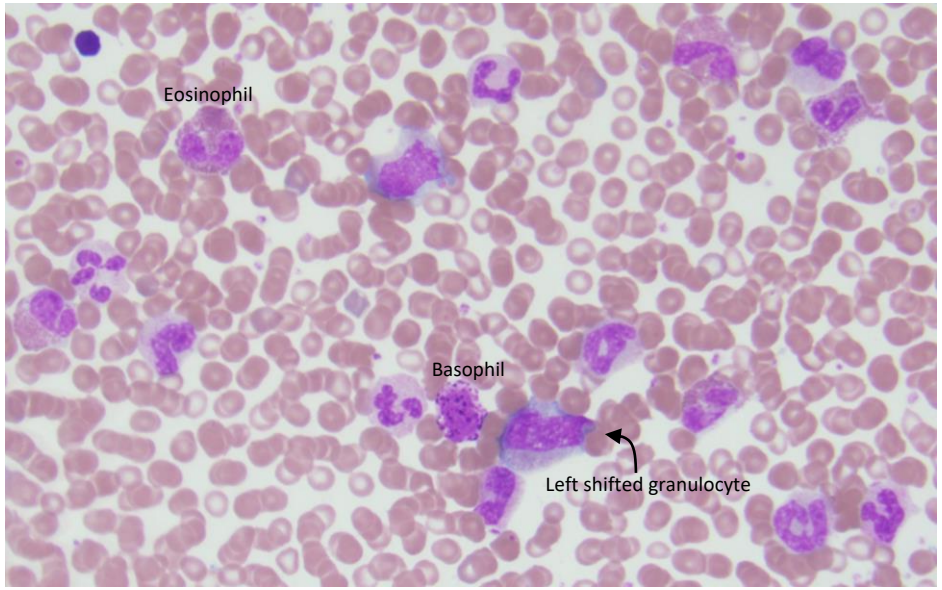
- A peripheral blood smear reveals marked absolute neutrophilia with a shift toward immaturity (i.e., left shift), eosinophilia, and basophilia.

Laboratory Evaluation

- Peripheral blood was sent for flow cytometry

- Initial triage includes examination of two morphologic preparations:
  - Smear
  - Touch Prep

- On triage, a left shift in the granulocytes was noted with a relative increase in eosinophils and basophils.



- Therefore, additional tubes beyond a peripheral blood screening panel were added up front to assess myeloid cells in more detail.
- In addition, CD34 was dropped into the typically empty APC channel on the PB B-cell screening tube

Laboratory Evaluation

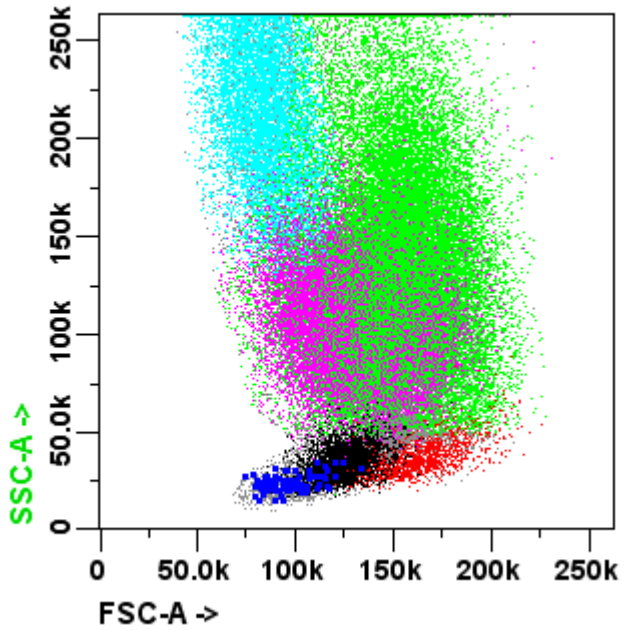
- Instrumentation and Analysis Software
  - 10 color BD FACSCanto
  - Cytopaint Classic
- Initial screening tubes included the following:

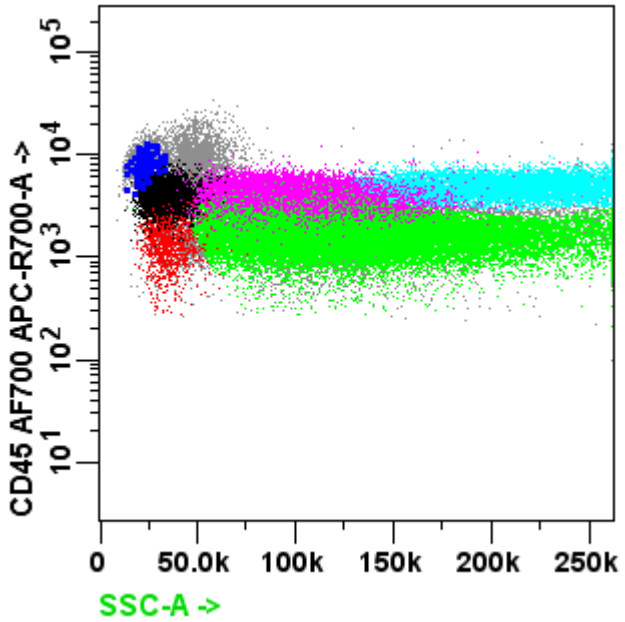
TUBE	FITC	PE	PerCp5.5	PE-Cy7	APC	APC-R700	APC-H7	BV421	V500c	BV605
PB T-cell Tube	**CD2**	**CD3**	**CD5**	**CD56**	**CD4**	**CD64**	**CD8**	**CD14**	**CD45**	**CD7**
PB B-cell Tube	**mLambda**	**CD22**	**CD20**	**CD19**	**CD34 (DROP IN)**	**CD45 AF700**	**CD38**	**CD10**	**CD5 BV480**	**mKappa**
Additional Tube 1	**CD36**	**CD34**	**CD16**	**CD38**	**CD11b**	**CD64**	**CD7**	**CD56**	**CD45**	**CD13**
Additional tube 2	**CD15**	**CD33**	**---**	**---**	**CD34**	**---**	**HLA-DR**	**CD117**	**CD45**	**CD123**

Peripheral Blood
B-cell Tube

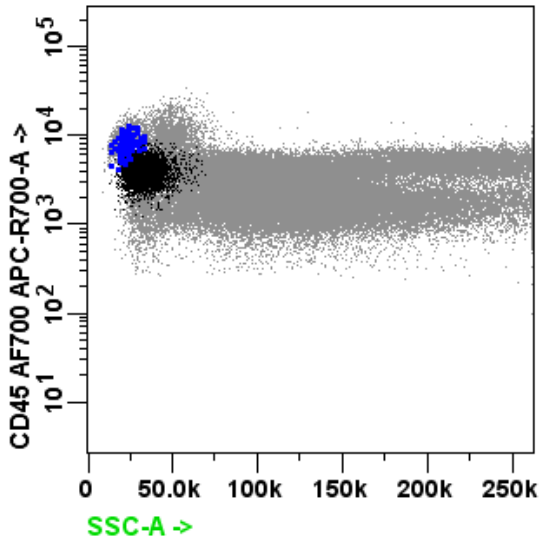
TUBE	FITC	PE	PerCp5.5	PE-Cy7	APC	APC-R700	APC-H7	BV421	V500c	BV605
PB B-cell Tube	mLambda	CD22	CD20	CD19	CD34 (DROP IN)	CD45 AF700	CD38	CD10	CD5 BV480	mKappa

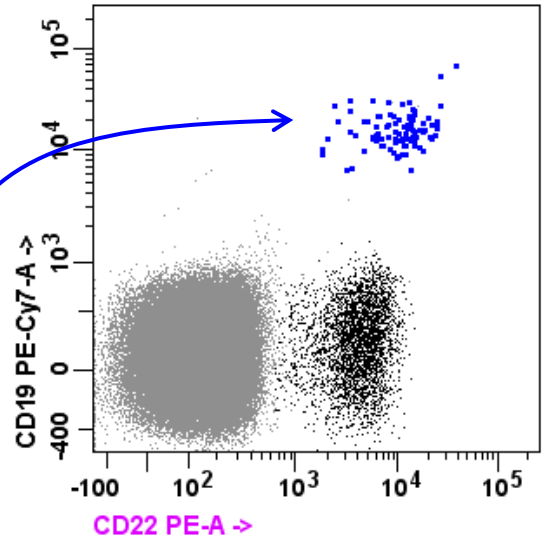
- Several interesting findings can be seen on the B-cell tube

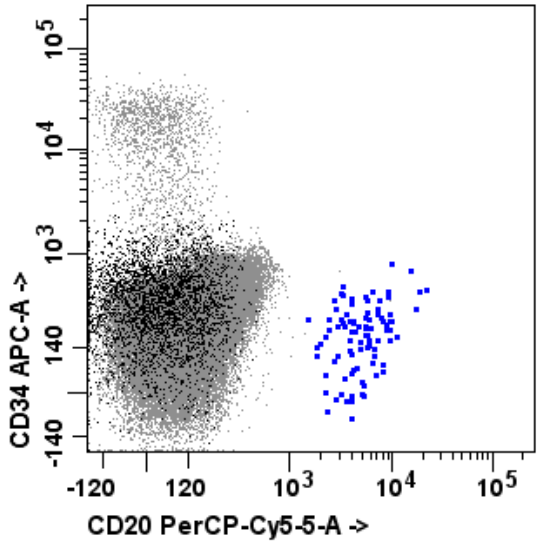


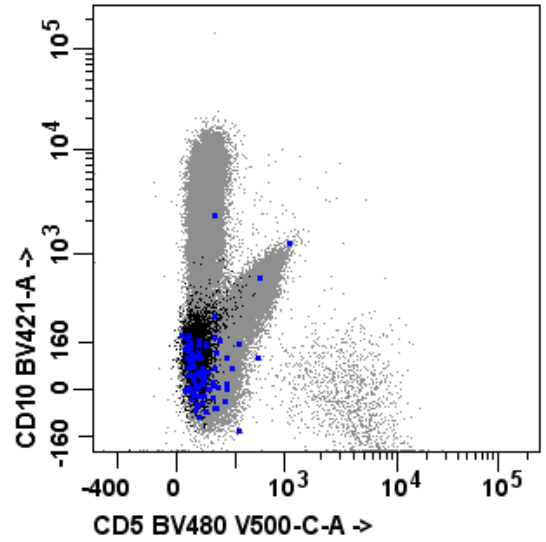


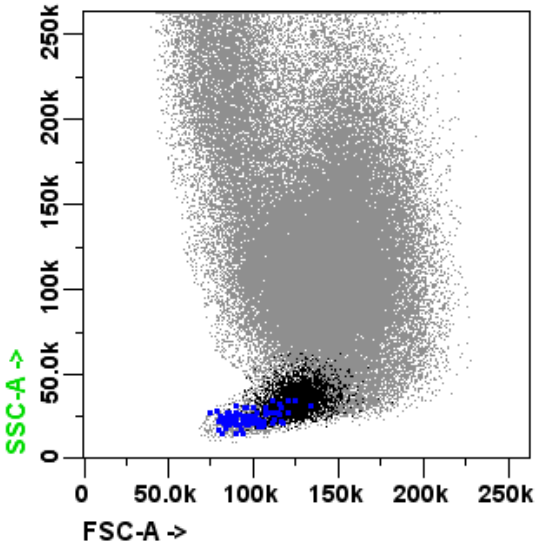
B-cell tube



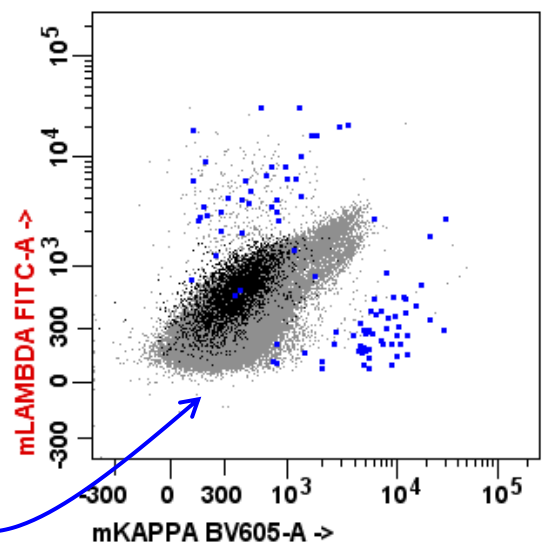


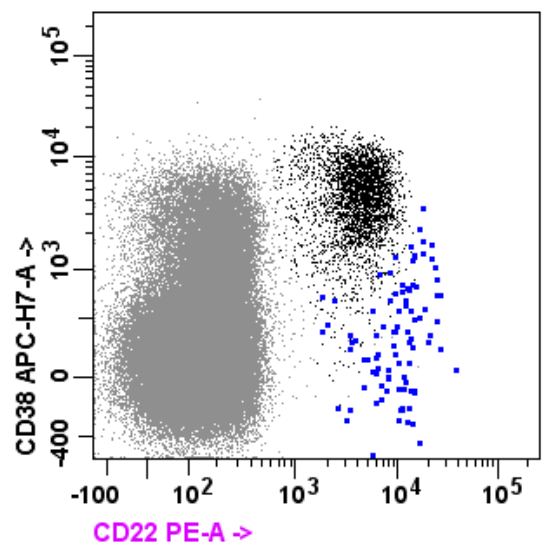




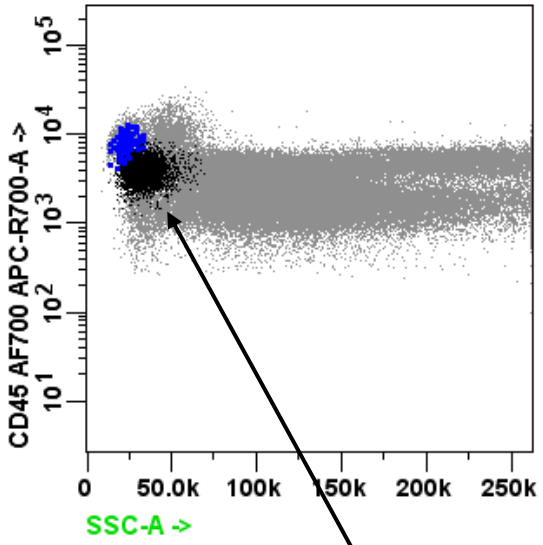


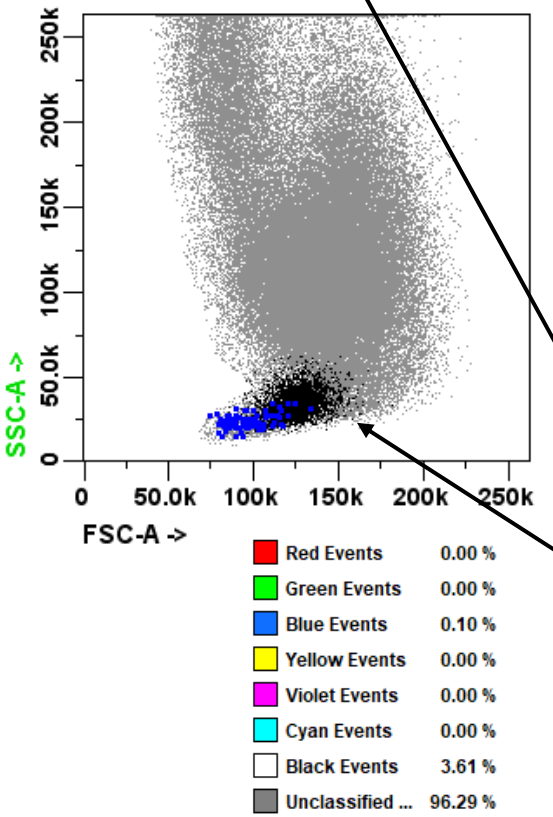
Blue: Mature B-cells are polytypic

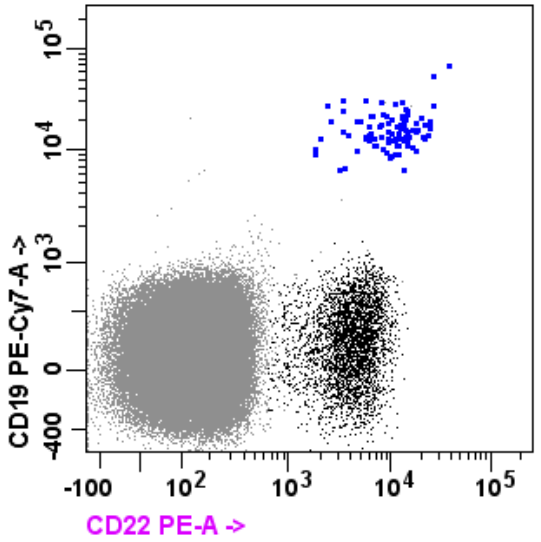


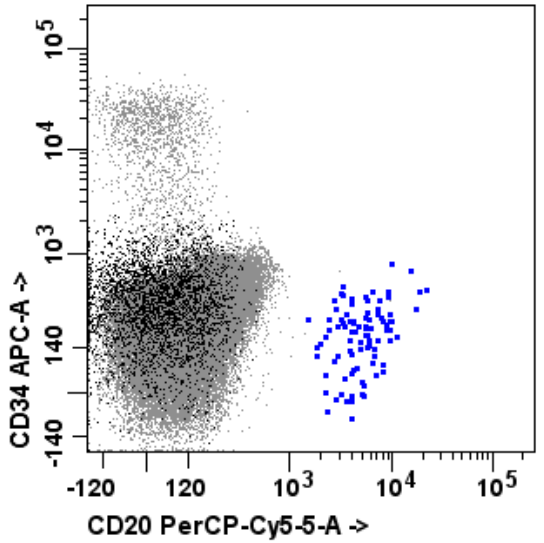


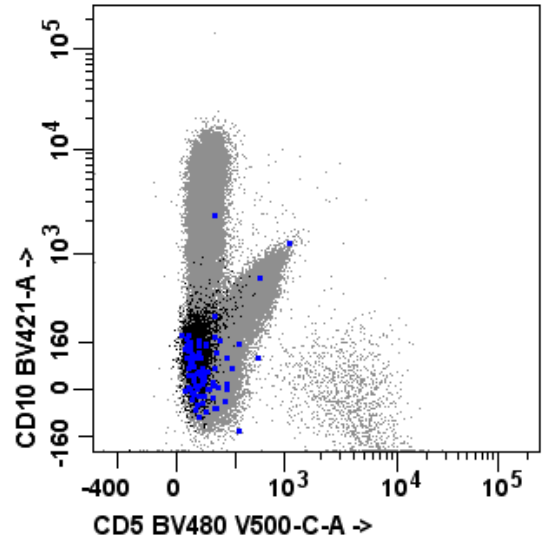
B-cell tube





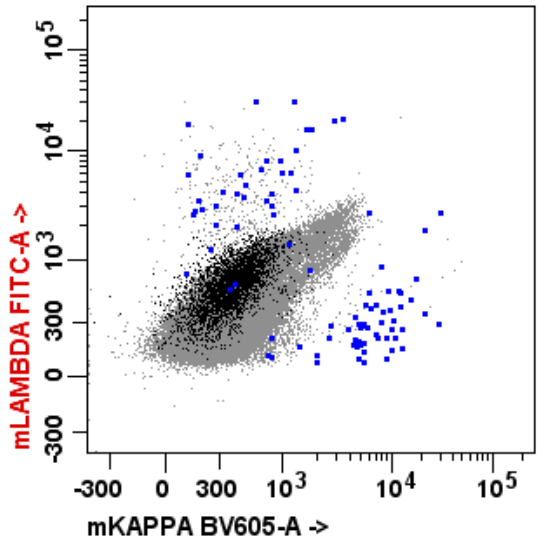


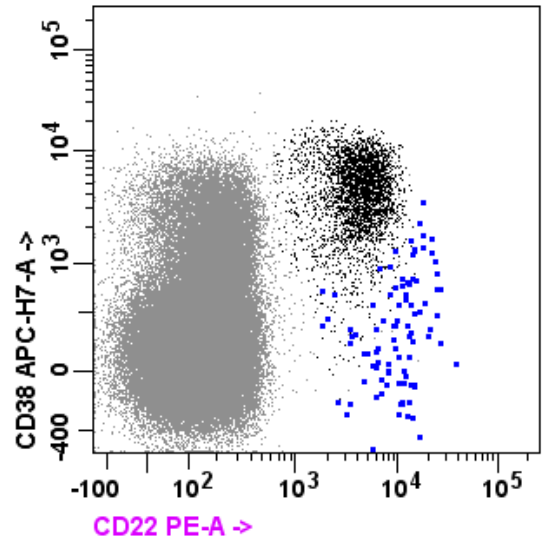




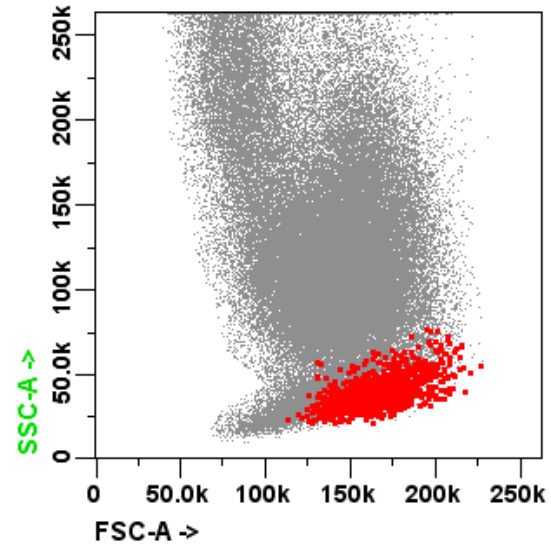
Black:

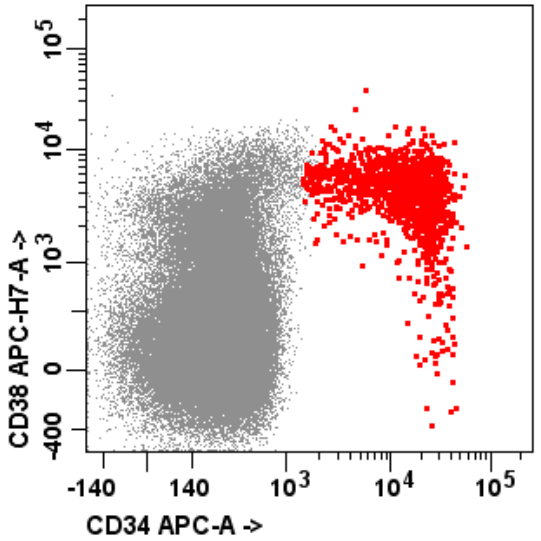
CD22 (+) cells that lack other B-cell associated markers
CD22 antibody clone S-HCL-1 also stains plasmacytoid dendritic cells and basophils that sit in this location on SSC vs CD45 plot
Moderate forward light scatter and low to moderate side scatter favor a predominance of basophils

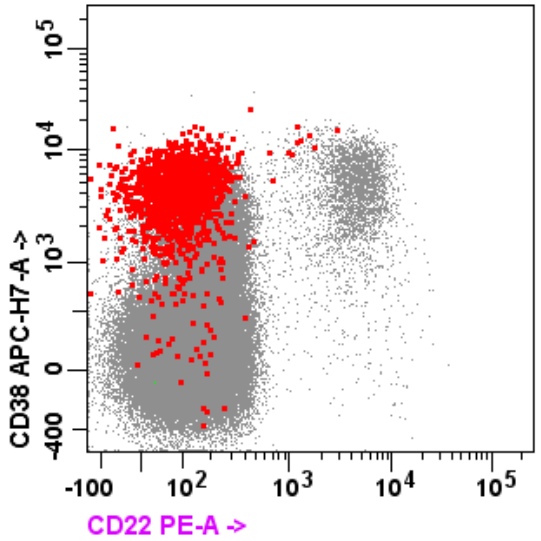


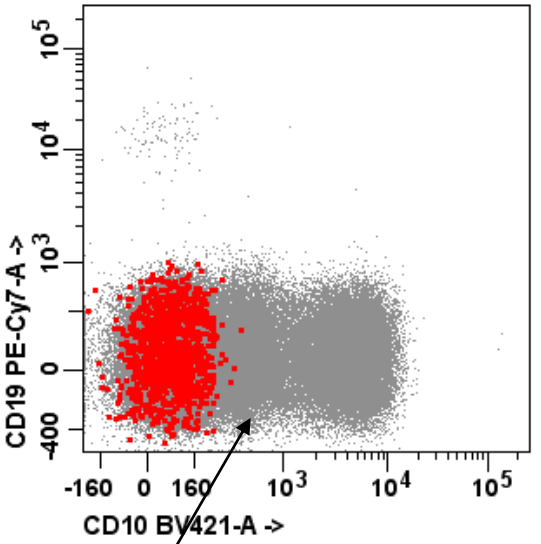


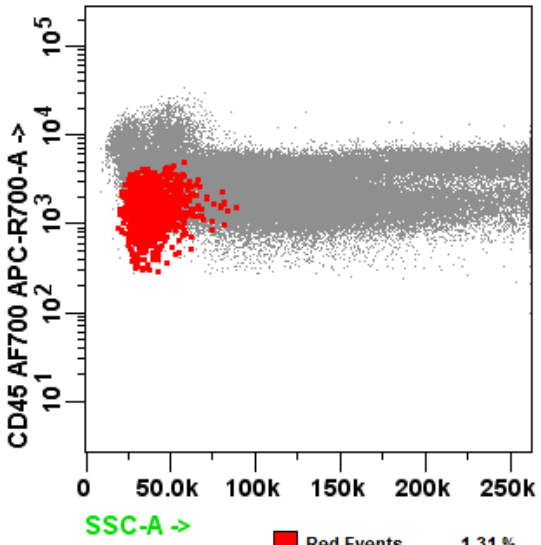
B-cell tube

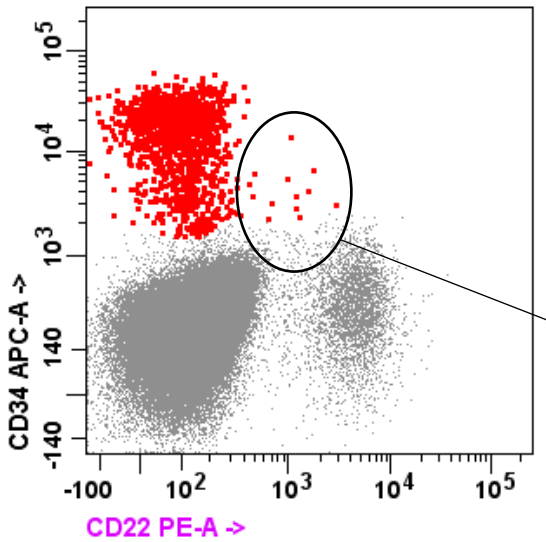


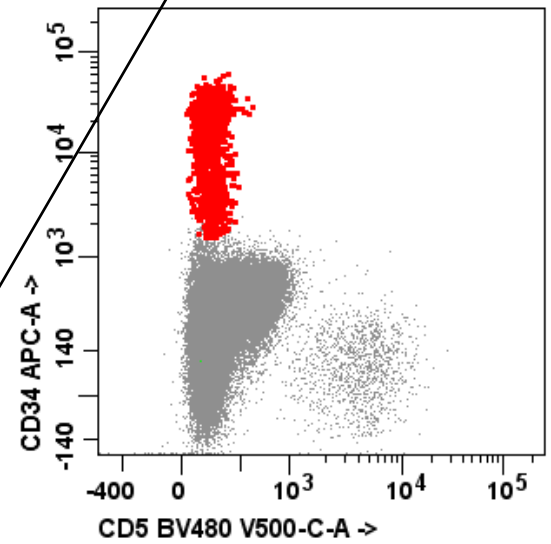












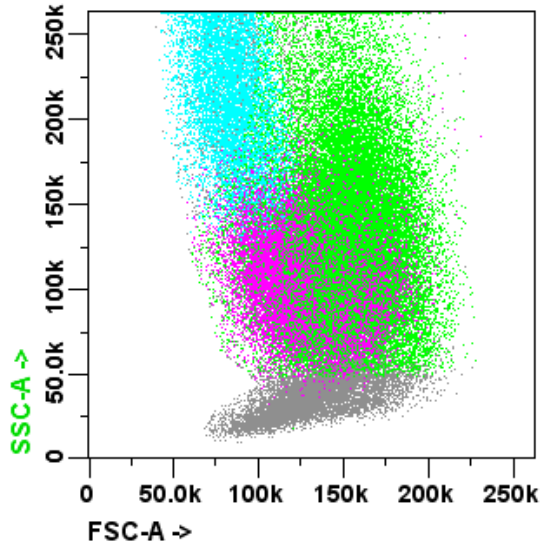
Red:

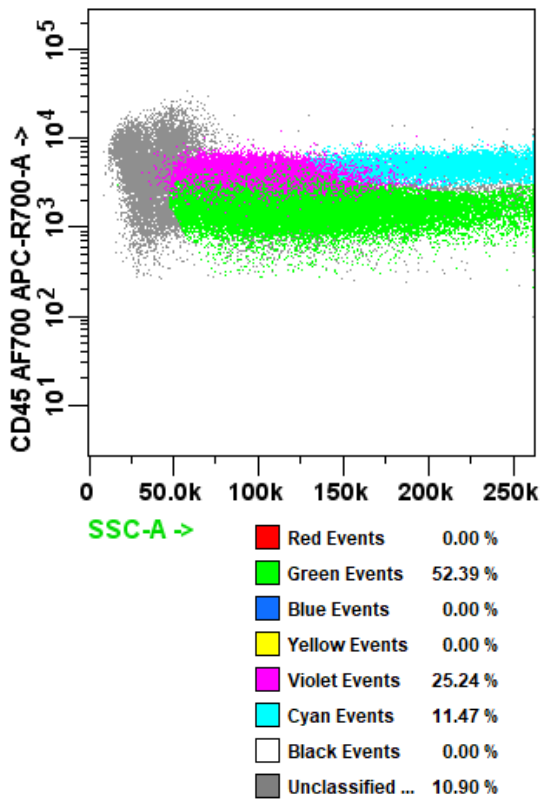
CD34(+) progenitors

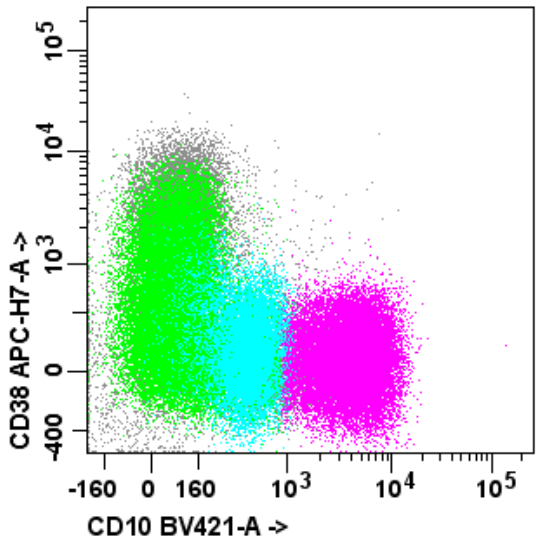
- These are negative for CD19 so they are not hematogones.
- The majority are likely peripheral blood progenitors or myeloblasts

- A very small subset is CD22(+).
- This subset lacks CD10 expression so is not a very early immature B-cell population (so called stage zero hematogones), which would be unusual to identify in the peripheral blood.
- This tiny subset may be a subset of plasmacytoid dendritic cell precursors

B-cell tube

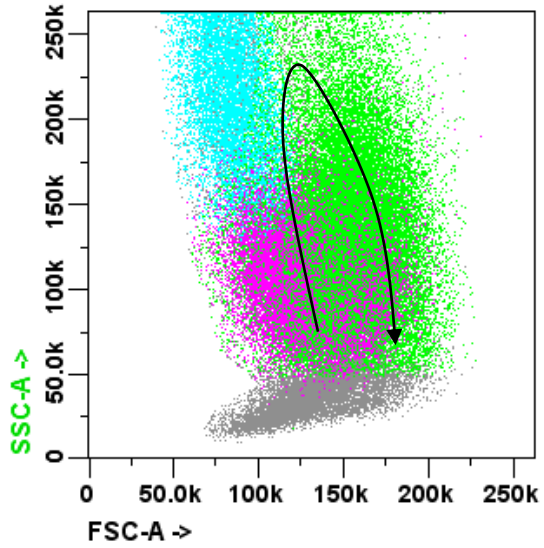


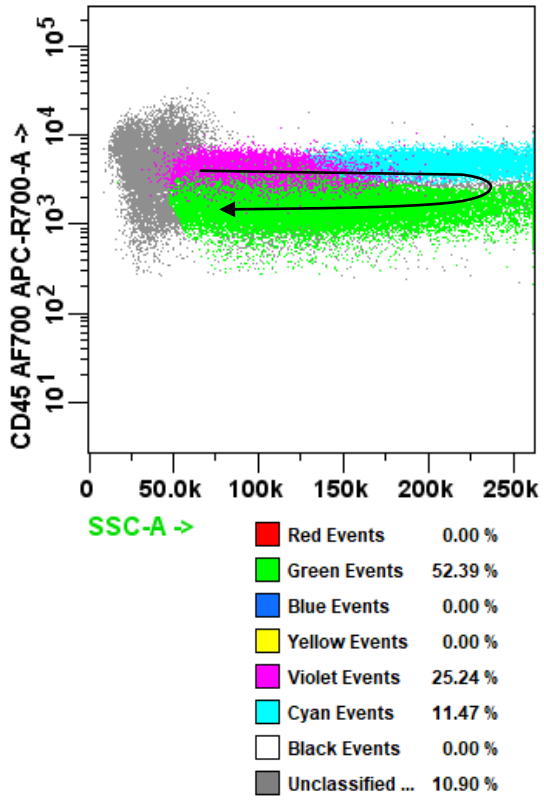


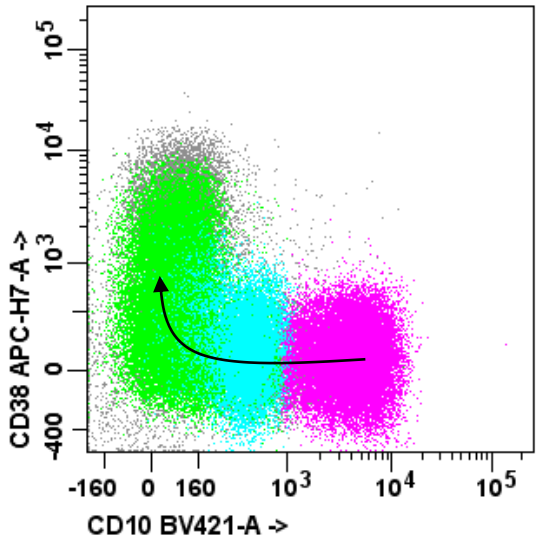


The colored populations are the non-basophil granulocytes, which all have moderate to high side scatter

B-cell tube







These are the non-basophil granulocytes, which all have moderate to high side scatter

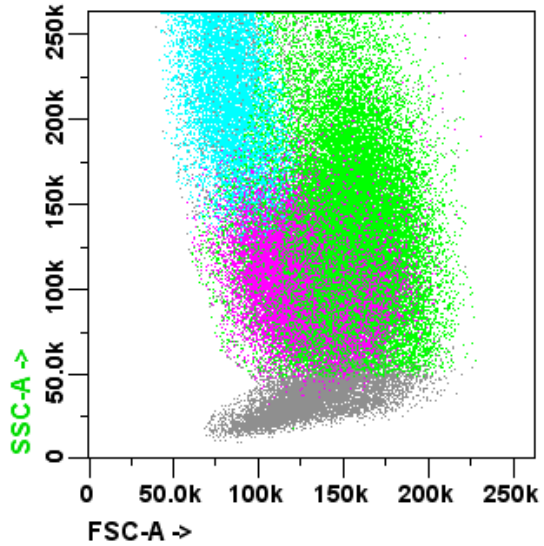
The neutrophilic granulocytes are clearly shifted toward immaturity (i.e., they show a left shift)

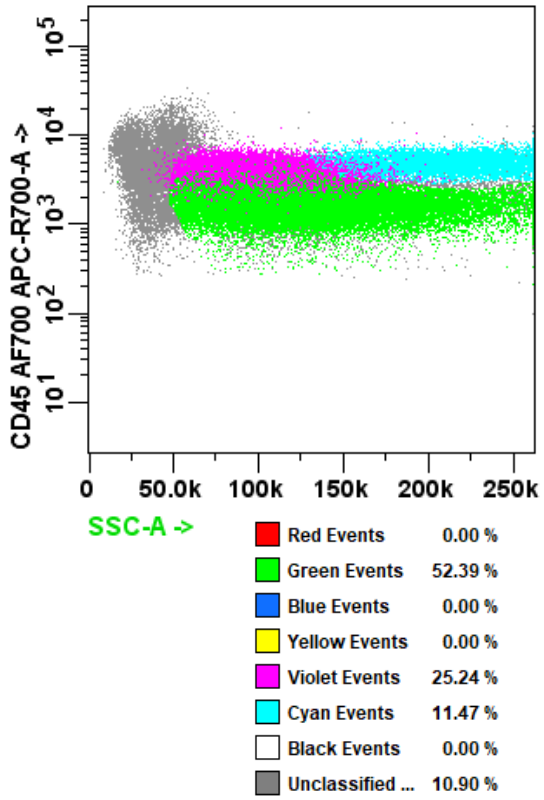
The purple are mature segmented neutrophils [CD10(+), CD45(brighter +), forward light scatter(relatively lower) and orthogonal light scatter(relatively lower)]

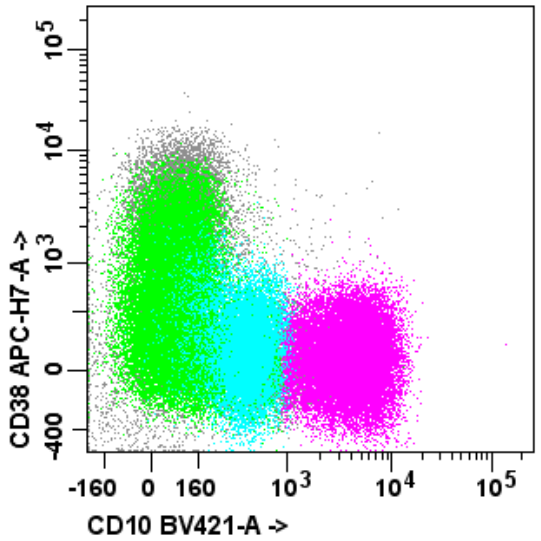
The green are left shifted granulocytes [CD10(-), CD45(dimmer +), forward light scatter(relatively higher) and orthogonal light scatter(low to high)]

Arrows are in the direction of the left shift (i.e., from most mature to least mature)

B-cell tube



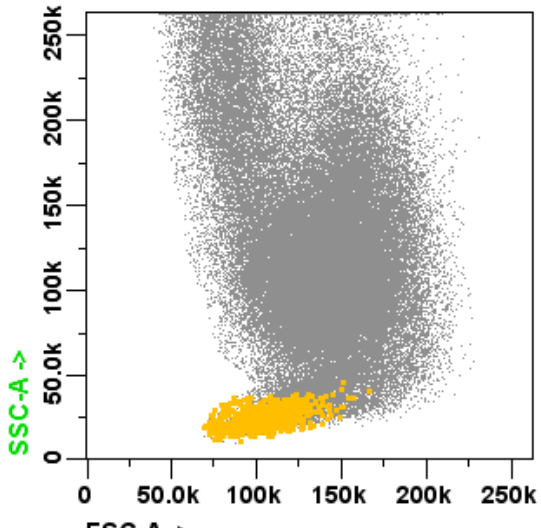


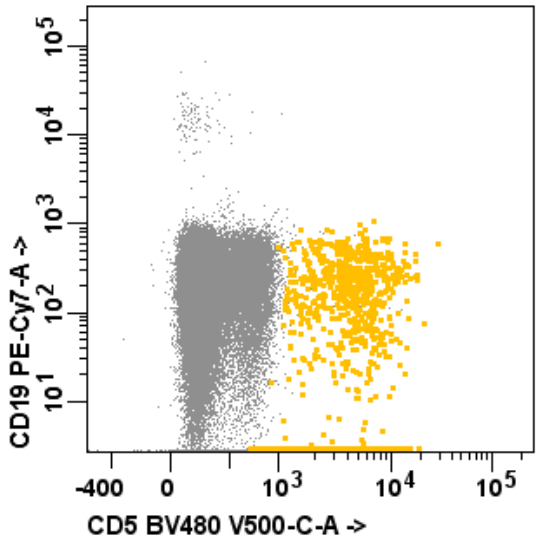


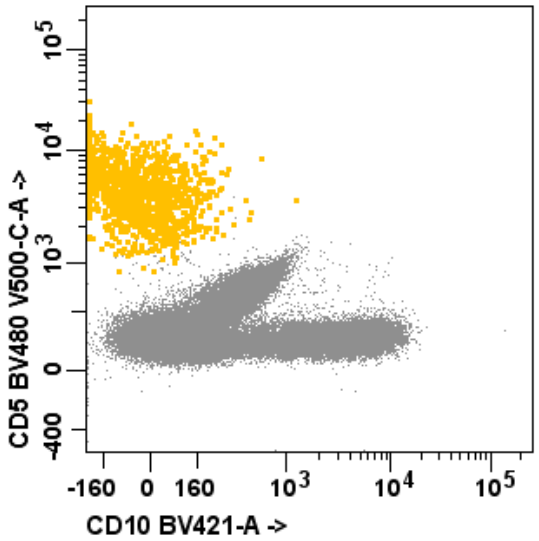
These are the non-basophil granulocytes, which all have moderate to high side scatter

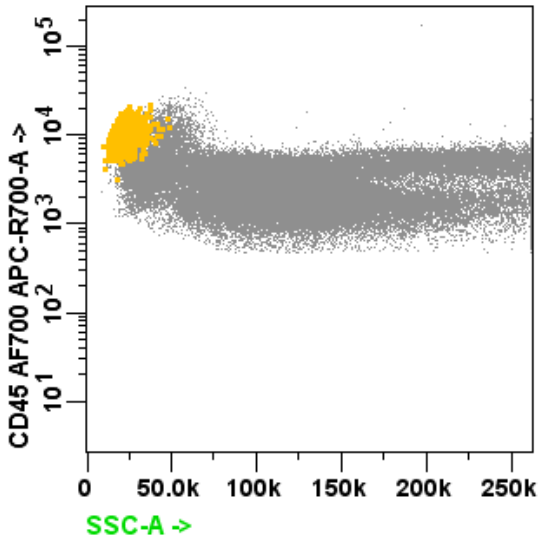
- Eosinophils are colored cyan/light blue
- When compared to neutrophils, eosinophils show lower forward light scatter, higher side light scatter and generally brighter intensity of expression for CD45
- Eosinophils are also CD10(-), the slight shift on the BV421 axis is due to artifact/autofluorescence.

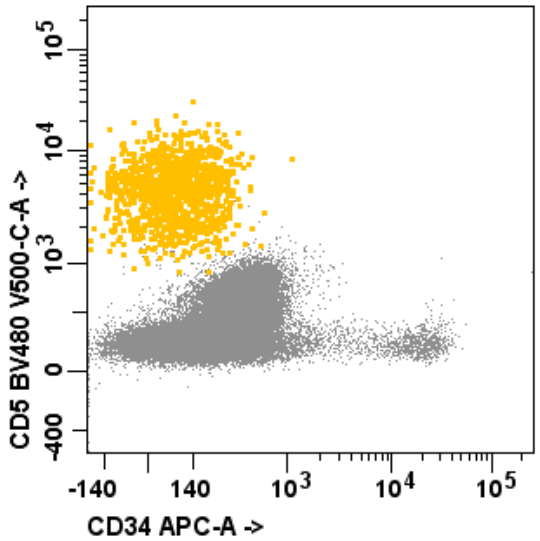
B-cell tube











Red Events	0.00 %
Green Events	0.00 %
Blue Events	0.00 %
Yellow Events	1.05 %
Violet Events	0.00 %
Cyan Events	0.00 %
Black Events	0.00 %
Unclassified ...	98.95 %

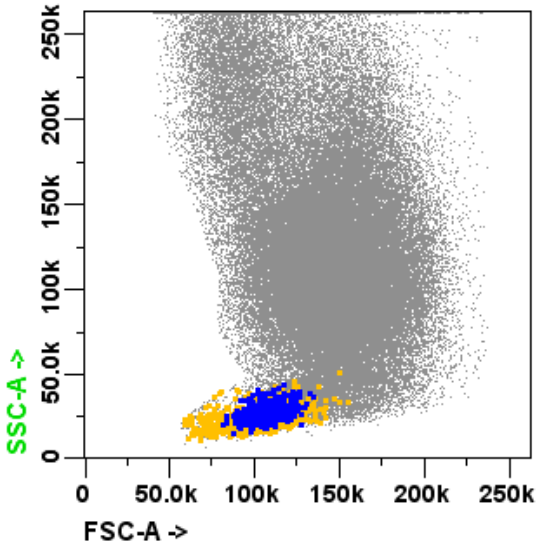
Yellow: T-cells appear unremarkable

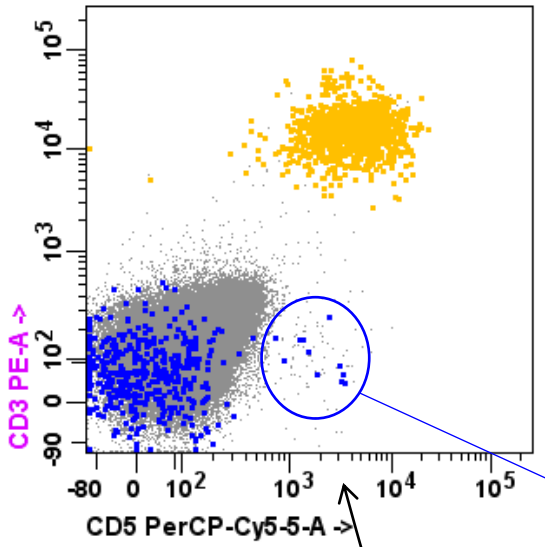
B-cell Tube Summary

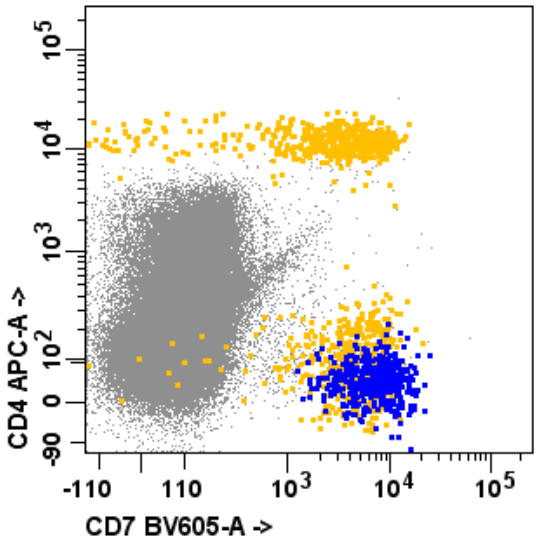
- B-cells look normal
- T-cells look unremarkable
- There is an increased proportion of non-B-lineage CD34(+) progenitor cells
- Neutrophils are left shifted
- There are increased proportions of eosinophils and likely basophils

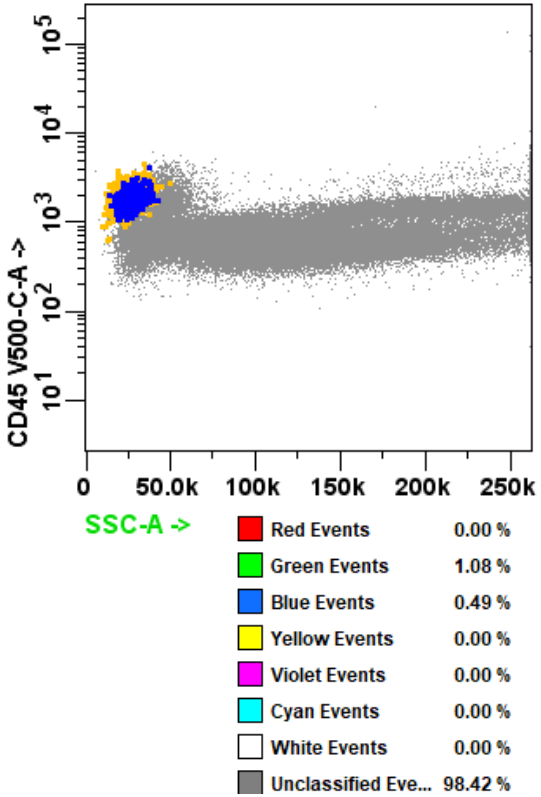
T-cell tube

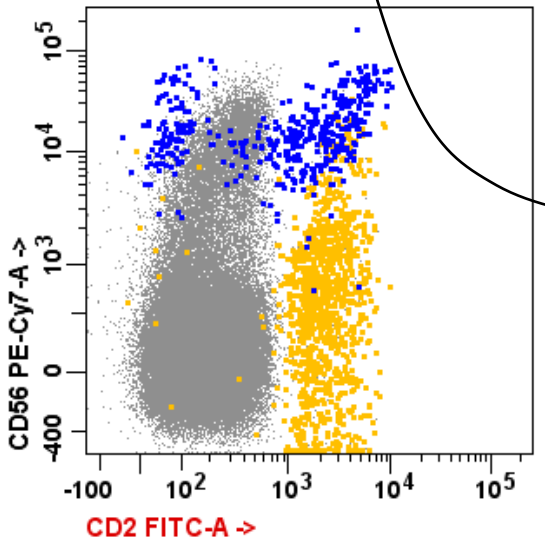
TUBE	FITC	PE	PerCp5.5	PE-Cy7	APC	APC-R700	APC-H7	BV421	V500c	BV605
PB T-cell Tube	CD2	CD3	CD5	CD56	CD4	CD64	CD8	CD14	CD45	CD7

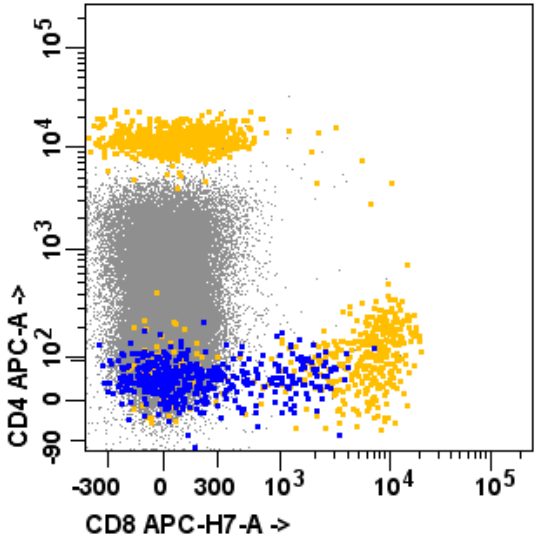












Green:

Mature T-cells appear unremarkable

Blue:

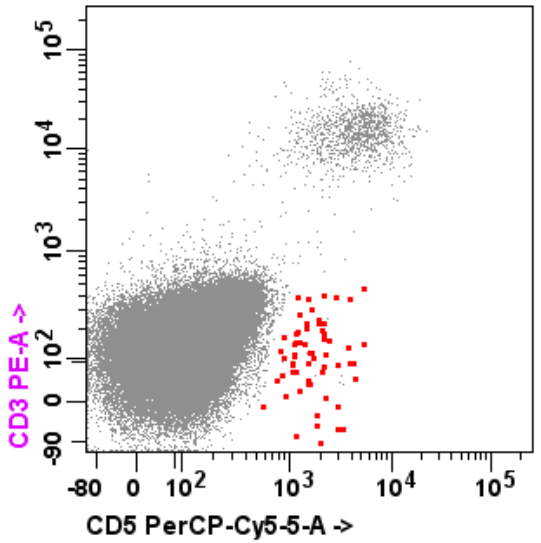
Mature NK-cells appear unremarkable

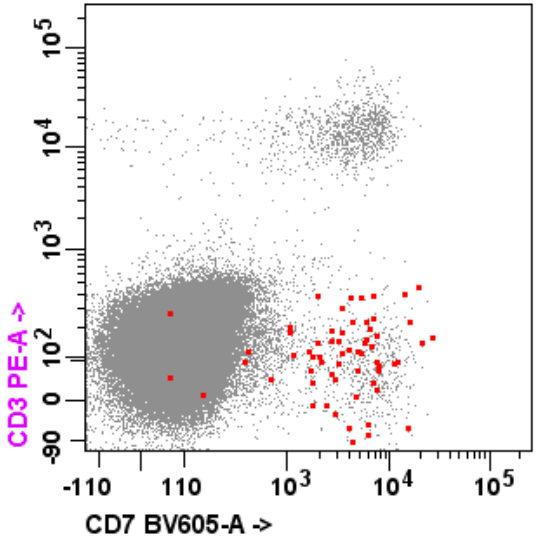
Note the minute subset of NK-cells that shows positive expression of CD5; this is a normal feature on small subsets of NK cells

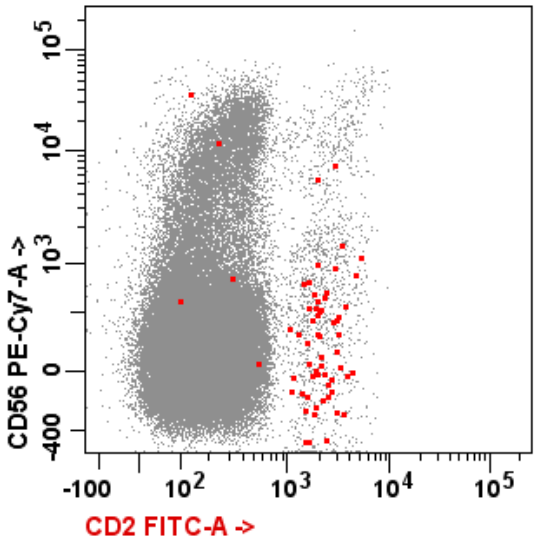
Since this is an ungated analysis on singlets/viable cells, we can see some features on uncolored/gated cells too

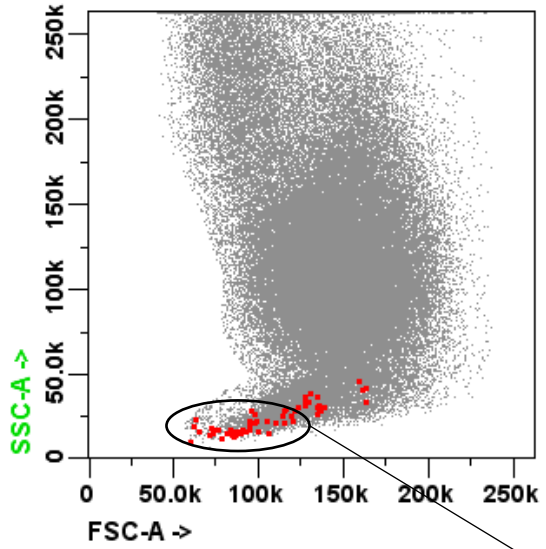
What are the remaining gray CD5(+)/CD3(-) dots?

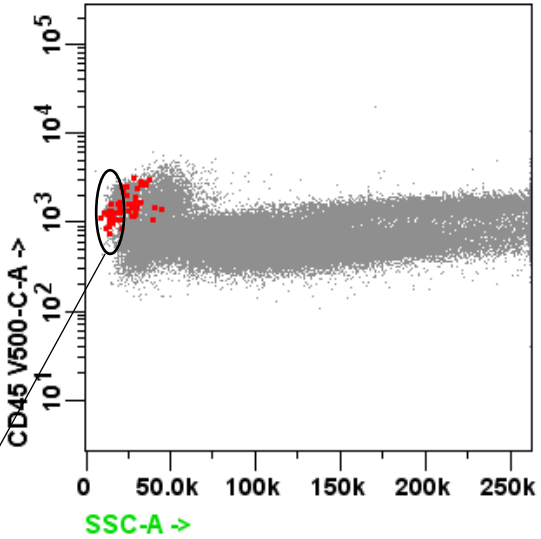
- What are the remaining CD5(+)/CD3(-) cells?
- These are now colored **RED** in the plots on this slide
- We know that subsets of plasmacytoid dendritic cells and other dendritic cells that can show expression of CD5, CD7 and CD2





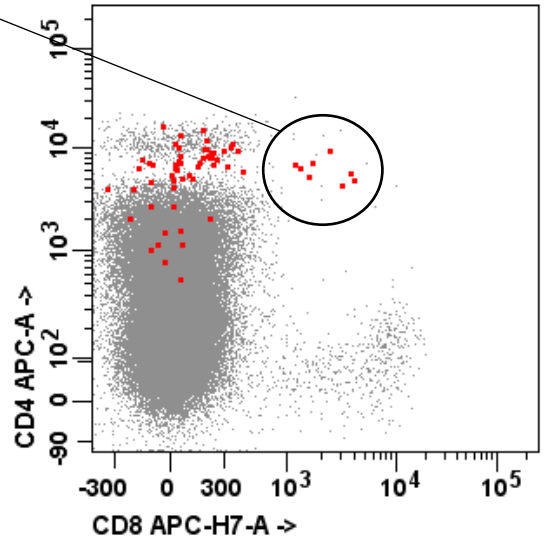






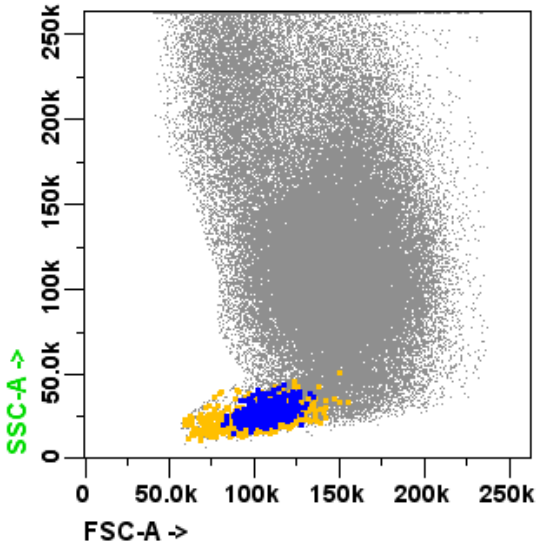
However, dendritic cells typically show higher forward and orthogonal light scatter than the cells encircled

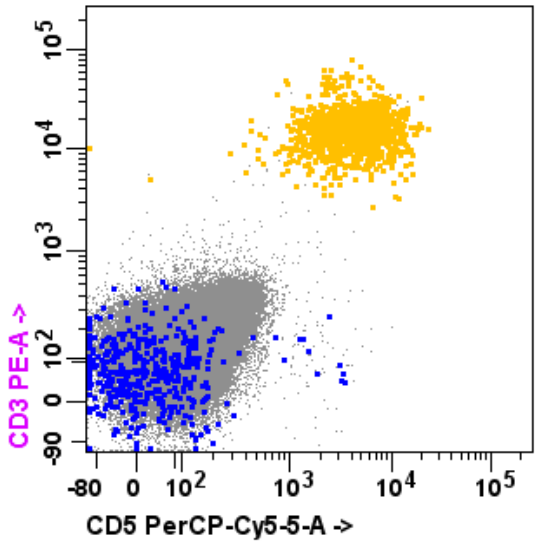
These smaller cells are also partial positive for CD8. In our experience it is not common to identify CD8(+) dendritic cells in peripheral blood

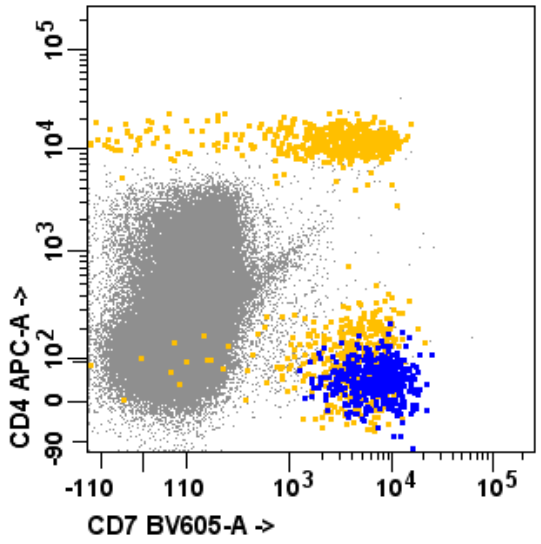


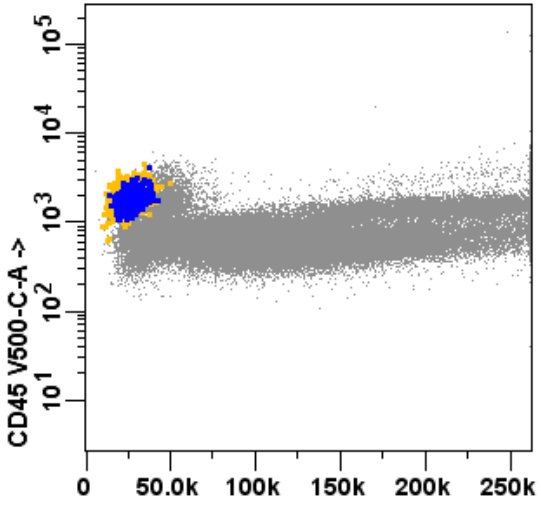
We will keep this population in the back of our minds as we continue analysis

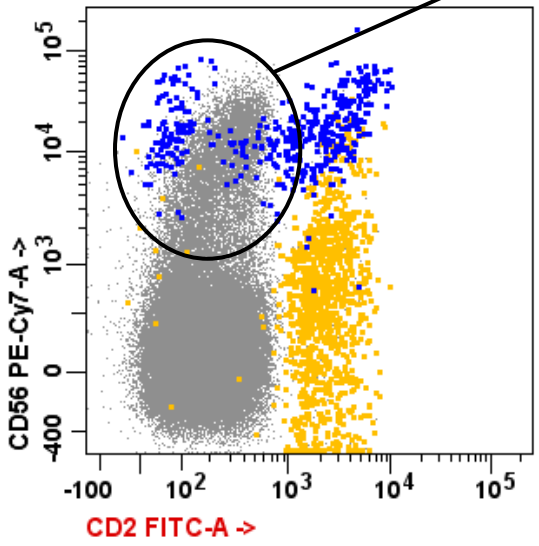
T-cell tube



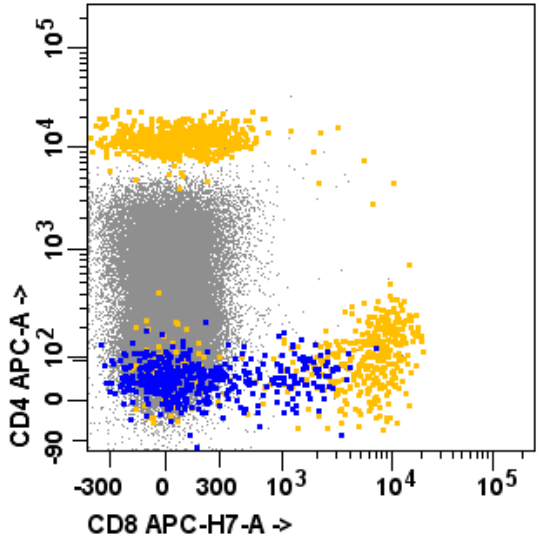






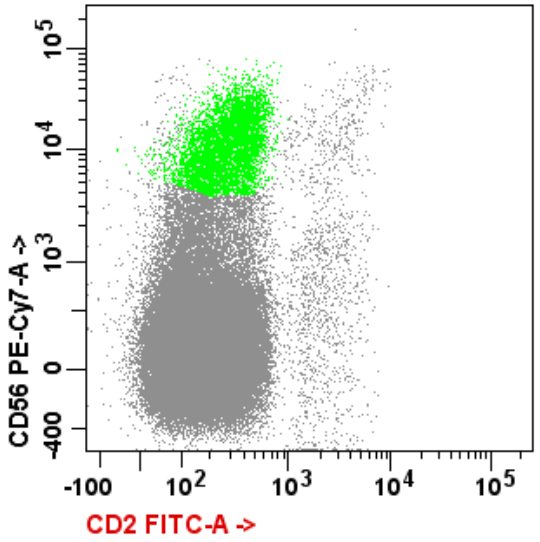


What are the remaining gray CD56(+)/CD2(-) dots?

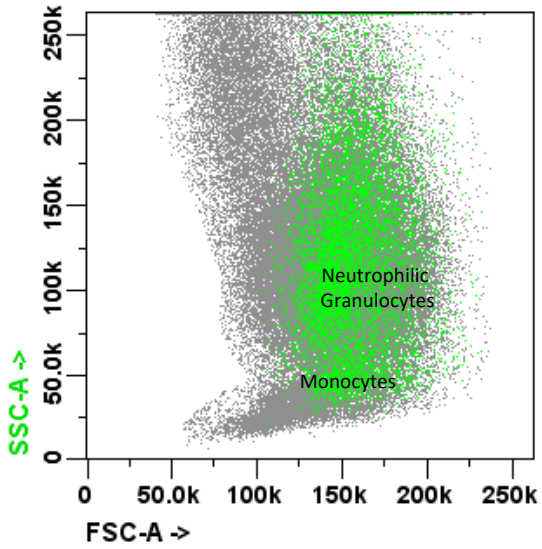


Red Events	0.00 %
Green Events	1.08 %
Blue Events	0.49 %
Yellow Events	0.00 %
Violet Events	0.00 %
Cyan Events	0.00 %
White Events	0.00 %
Unclassified Eve...	98.42 %

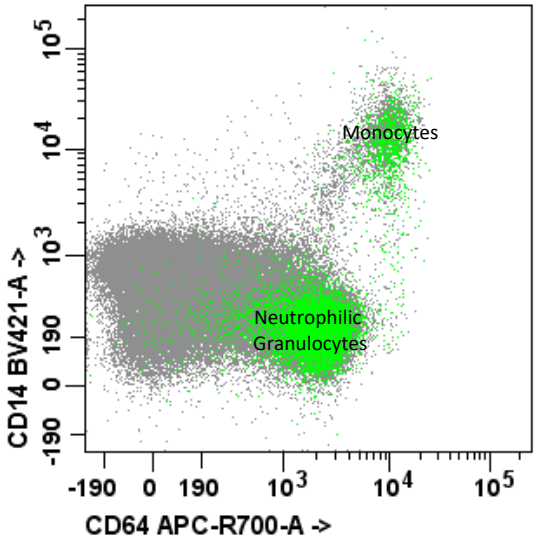
- What are the remaining gray CD56(+)/CD2(-) dots?
- We have colored these **GREEN** on this slide
- CD56(+) cells that are not T-cells or NK cells

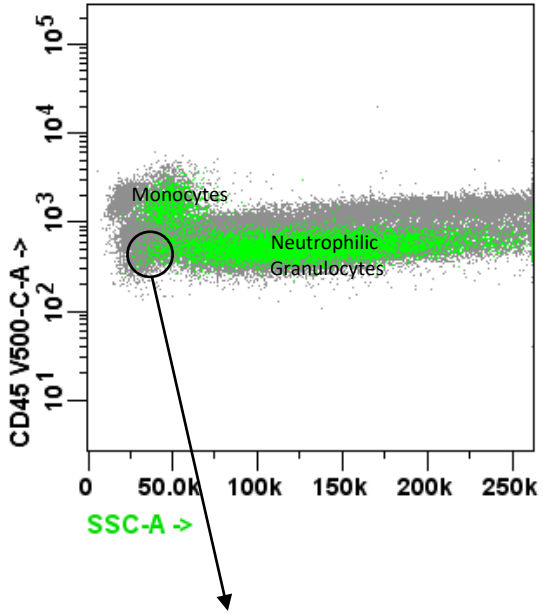


T-cell tube



Based on these 2 plots, the CD56 looks like it is being expressed by proportions of monocytes and granulocytes





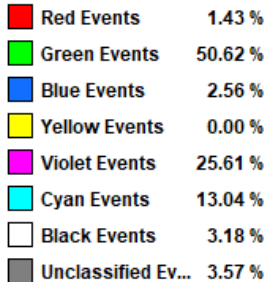
The green cells in this blast gate area may represent a small population of the CD34(+) blasts we identified in the B-cell tube

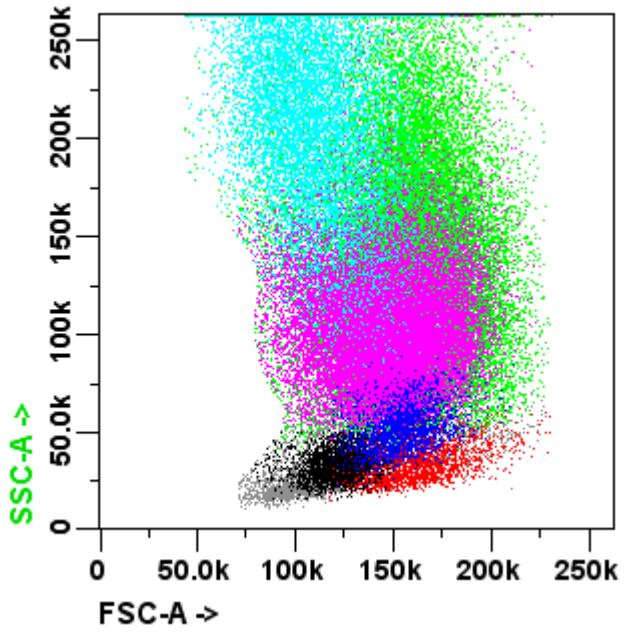
T cell tube summary

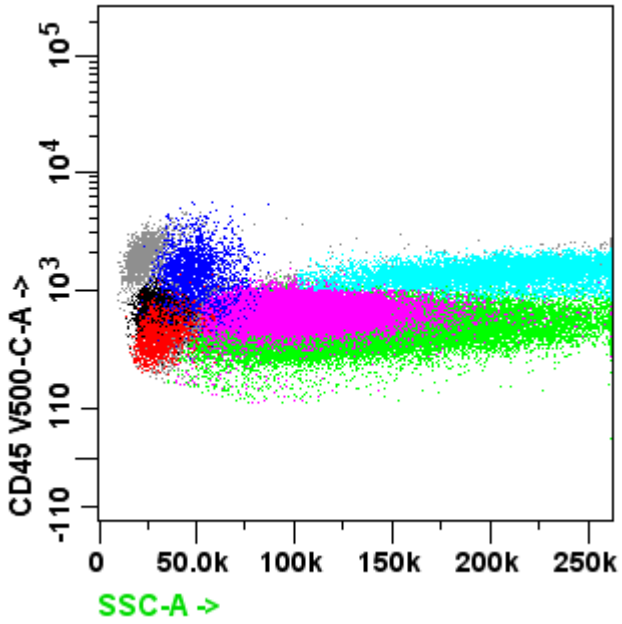
- Mature T-cells and NK cells look normal
- There is partial expression of CD56 on monocytes and granulocytes and a small subset of cells sitting in the CD45/SCC “blast gate”
- There is a minute population of CD5(+)/CD3(-) cells showing some unusual features that cannot be attributed to NK cells or dendritic cells with certainty

Additional Tube 1
Myeloid Tube

TUBE	FITC	PE	PerCp5.5	PE-Cy7	APC	APC-R700	APC-H7	BV421	V500c	BV605
Additional Tube 1	CD36	CD34	CD16	CD38	CD11b	CD64	CD7	CD56	CD45	CD13

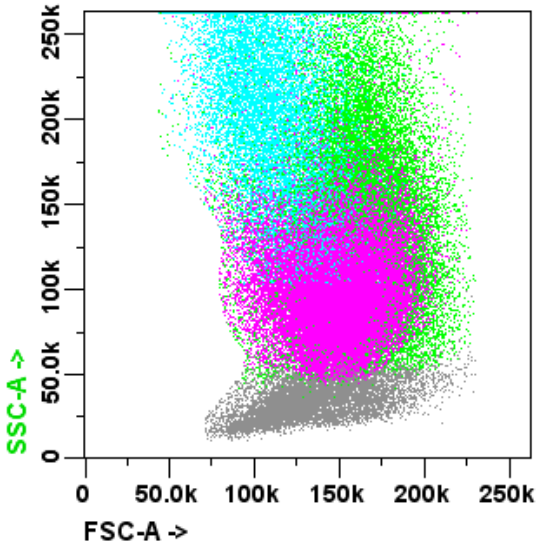


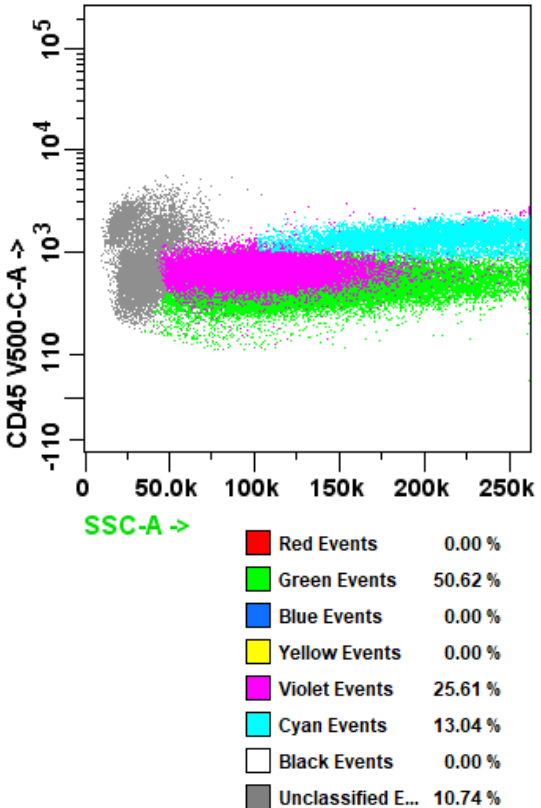


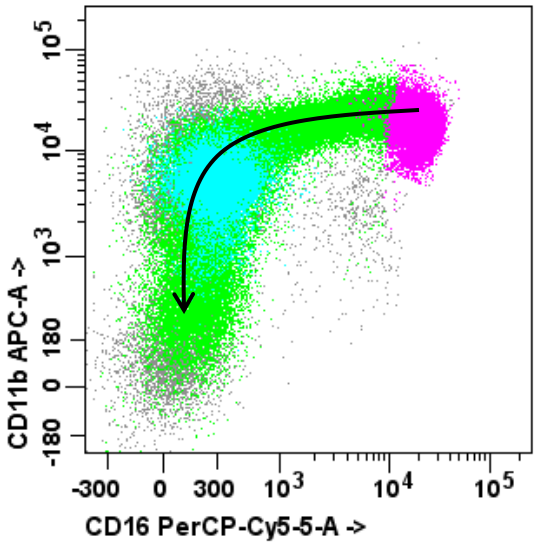


Additional Tube 1; Myeloid Tube

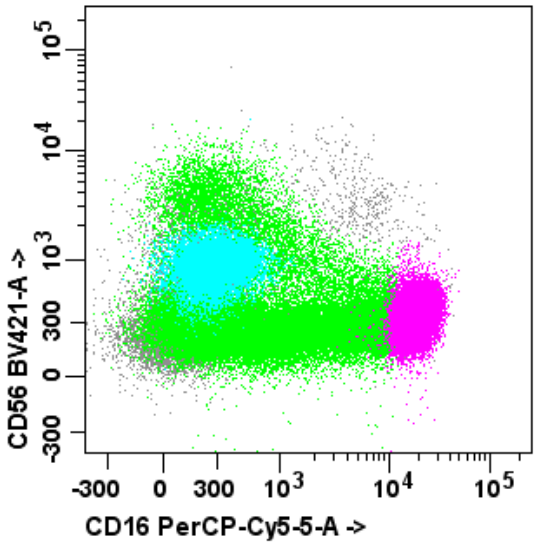
Purple: mature segmented neutrophils
Green: Left shifted neutrophils
Cyan/Light Blue: Eosinophils







- Granulocytes are shifted toward immaturity (see arrows – directed from most mature to least mature)
- Notice that if this was bone marrow the granulocytes would show normal maturation patterns (i.e., reverse the arrows)



- There is expression of CD56 on a proportion of the left shifted granulocytes
- CD56 expression can be seen on myeloid cells and is a non-specific finding that can be associated with myeloid neoplasms but can also be see in reactive/regenerative conditions
- Segmented neutrophils typically do not express CD56 even in such situations

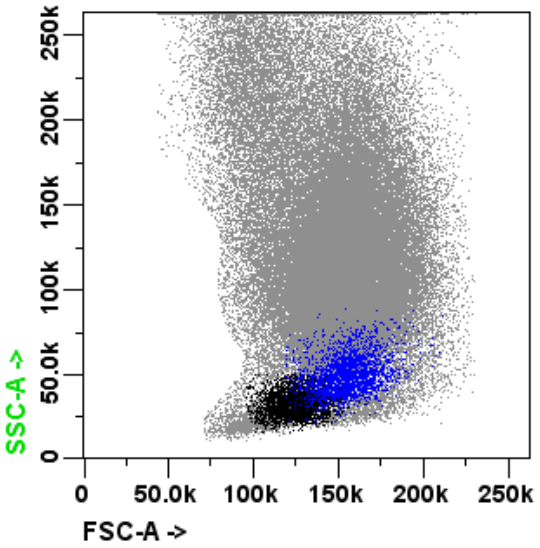
- While the eosinophils appear to show dim staining for CD56, this is artifact
- The eosinophils are CD56(-)
- As seen previously with CD10 in the B cell tube, eosinophils have high levels of autofluorescence in certain channels causing them to artificially appear dim positive

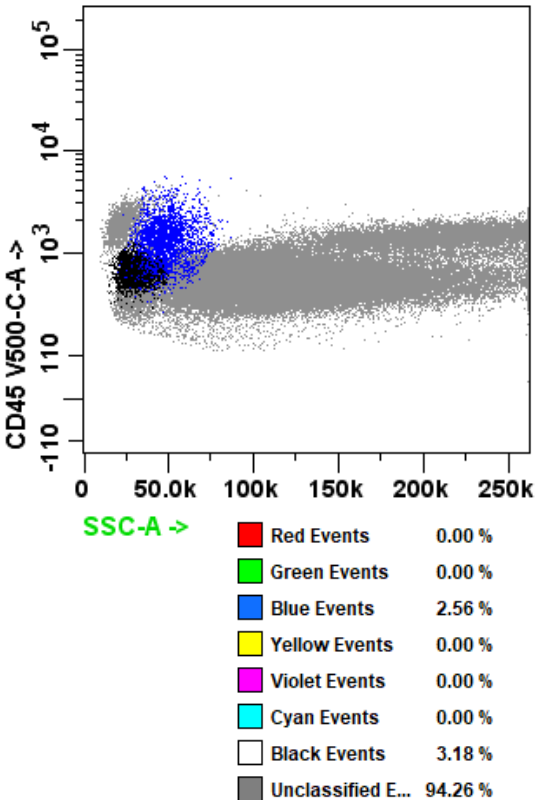
- Notably, eosinophils do not express CD16 and are slightly brighter for CD45 than neutrophils. These are characteristic and normal features.

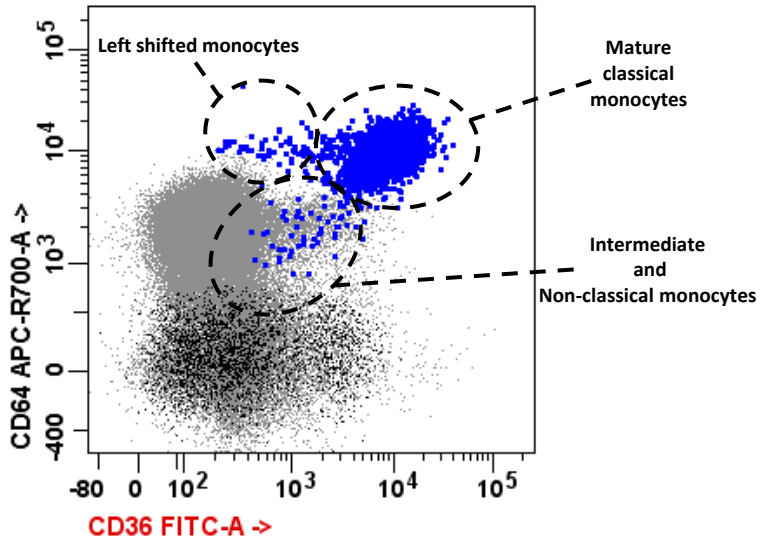
Additional Tube 1; Myeloid Tube

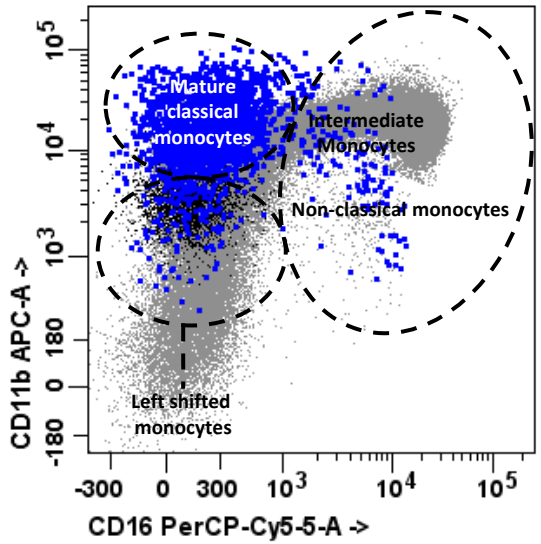
The monocytes (in blue) are highlighted here to visualize small subsets.

The basophils are in black.

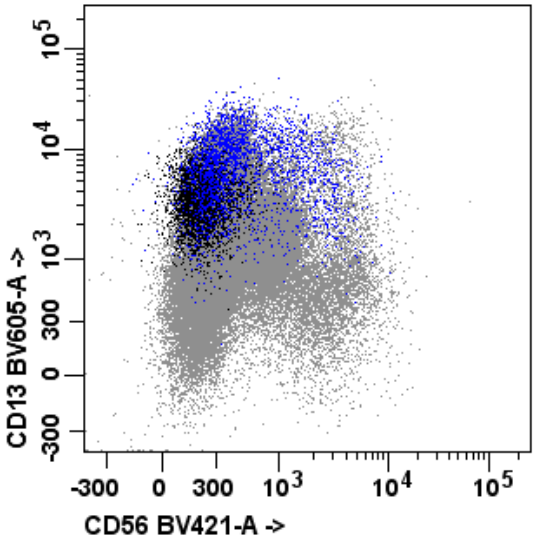






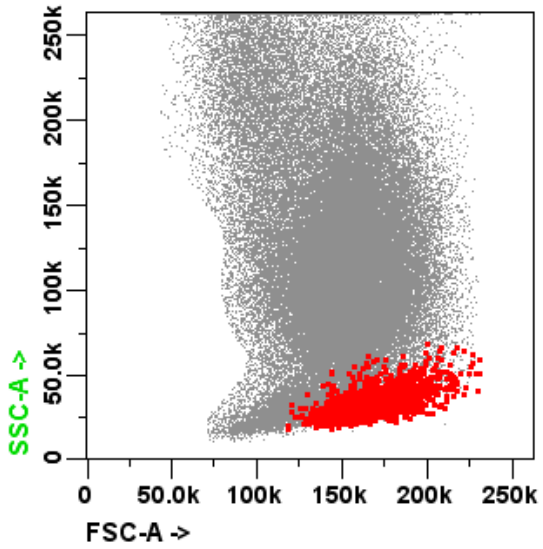


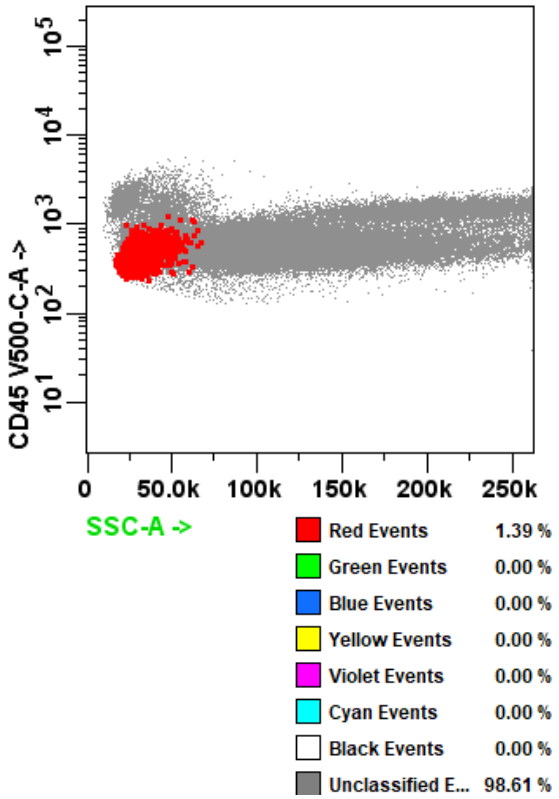
Overall, the monocytes and basophils show a relatively normal immunophenotype. As seen on the T-cell tube, a proportion of the monocytes show expression of CD56

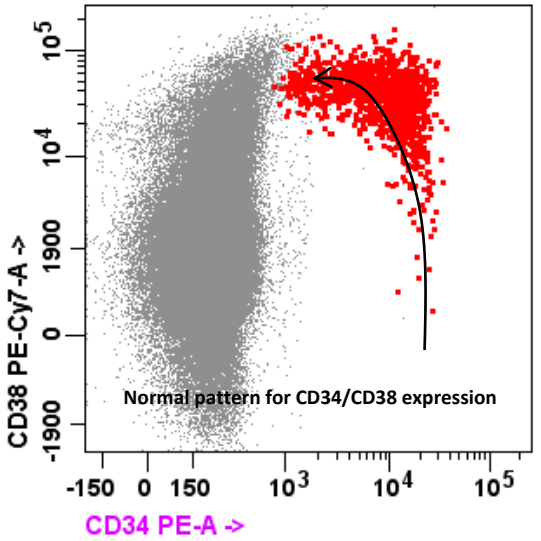


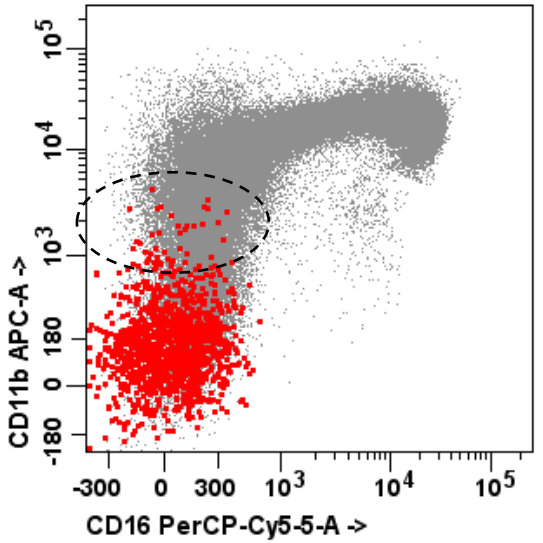
Additional Tube 1; Myeloid Tube

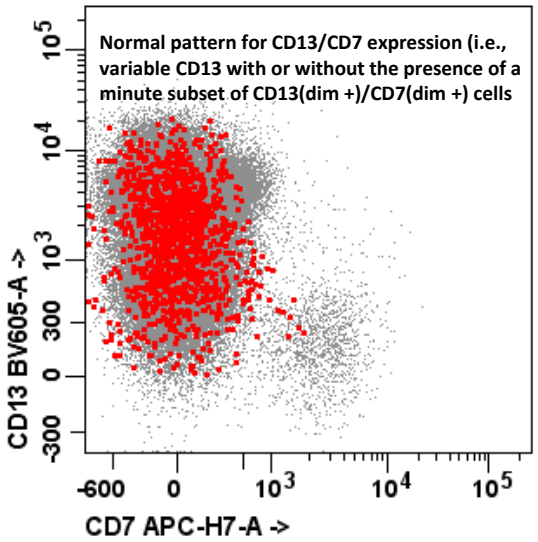
- The CD34(+) blasts show expression of CD13 suggesting that these are in fact myeloid in lineage.
- They are increased in proportion for the peripheral blood.
- This population shows a few immunophenotypic features worth noting.
- Similar to the monocytes and granulocytes, a proportion of blasts are CD56 positive.
- There is a subset of blasts that is CD36 positive. In the bone marrow CD36 is normally found on subsets of early progenitors of the myeloid lineage. In both peripheral blood and bone marrow, CD36 on myeloblasts can be seen in association with myeloid neoplasms[1].
- There is a small proportion of CD11b(dim +) blasts

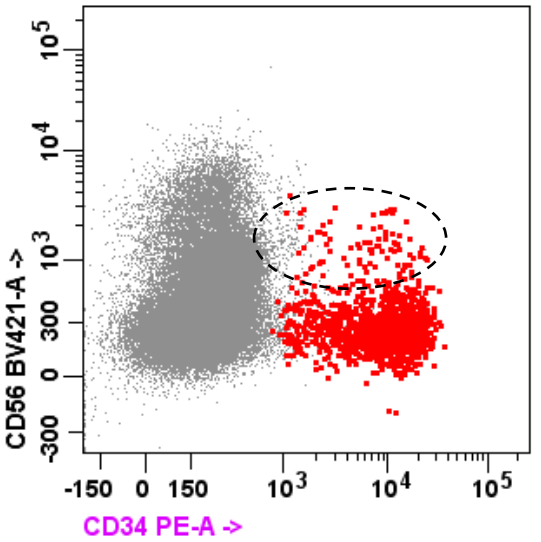


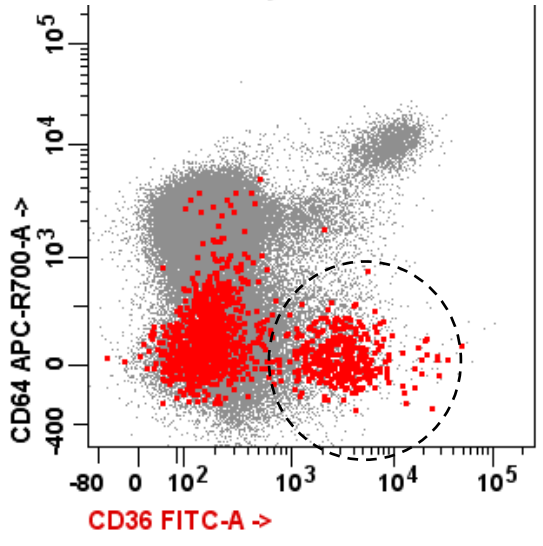










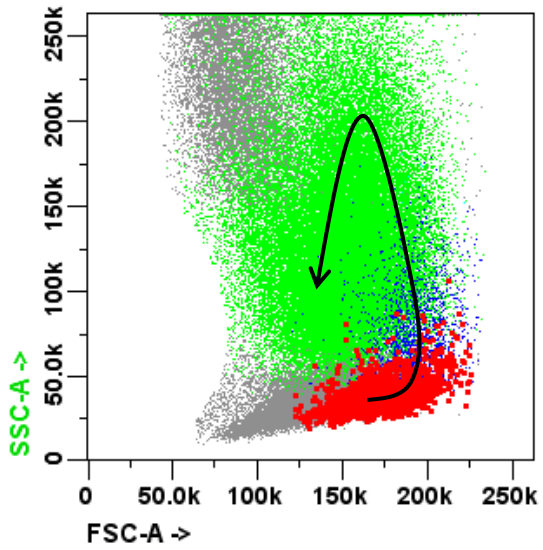


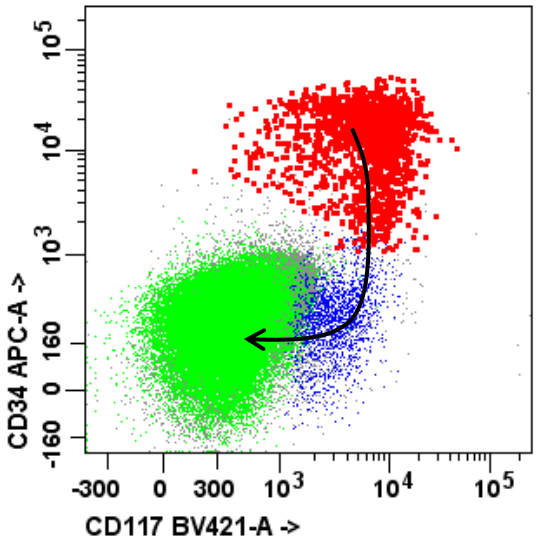
Additional Tube 1; Myeloid Tube Summary

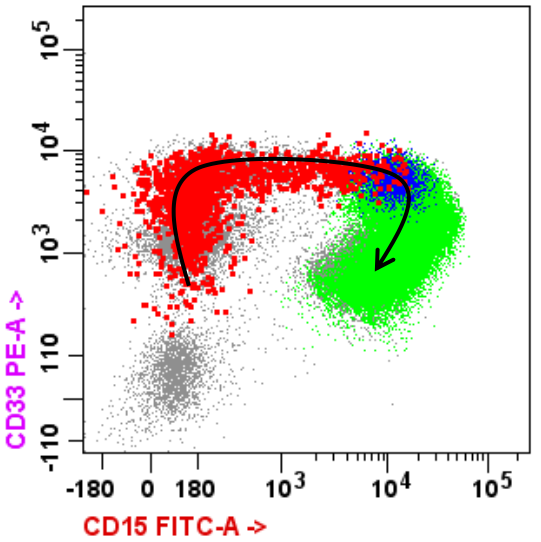
- There is an increased proportion of CD34(+) progenitor cells that show evidence of myeloid differentiation with mild aberrancy
  - i.e., subsets of blasts that are CD56 positive, CD36 positive and/or CD11b positive
- There is a left shift in granulocytes
- There is partial expression of CD56 on the monocytes and granulocytes

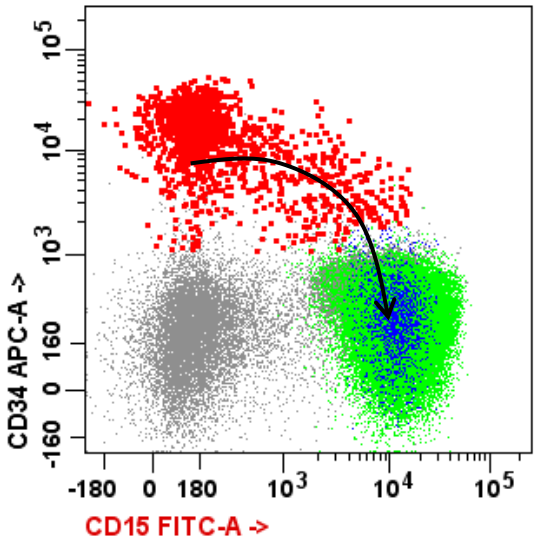
Additional Tube 2; Myeloid Tube

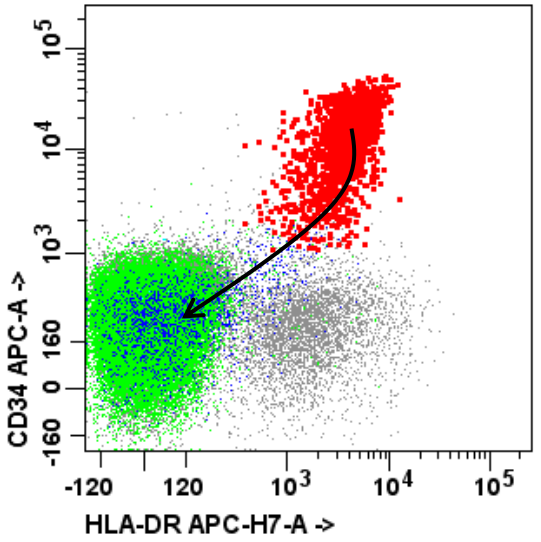
TUBE	FITC	PE	PerCp5.5	PE-Cy7	APC	APC-R700	APC-H7	BV421	V500c	BV605
Additional tube 2	CD15	CD33	---	---	CD34	---	HLA-DR	CD117	CD45	CD123



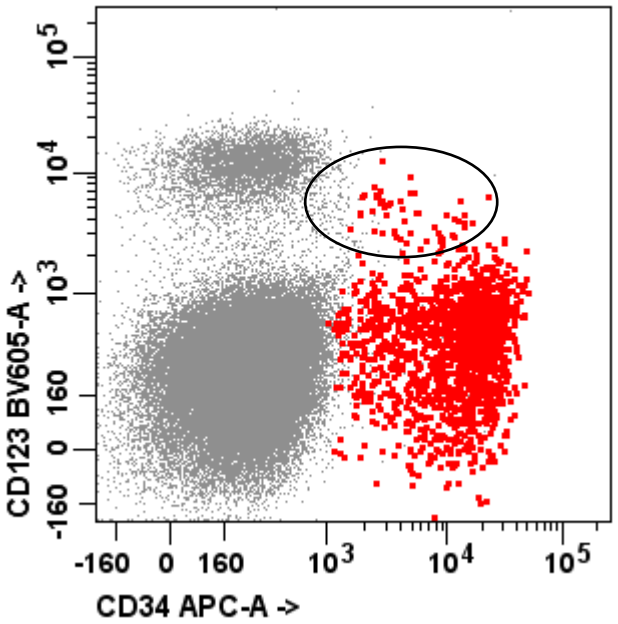






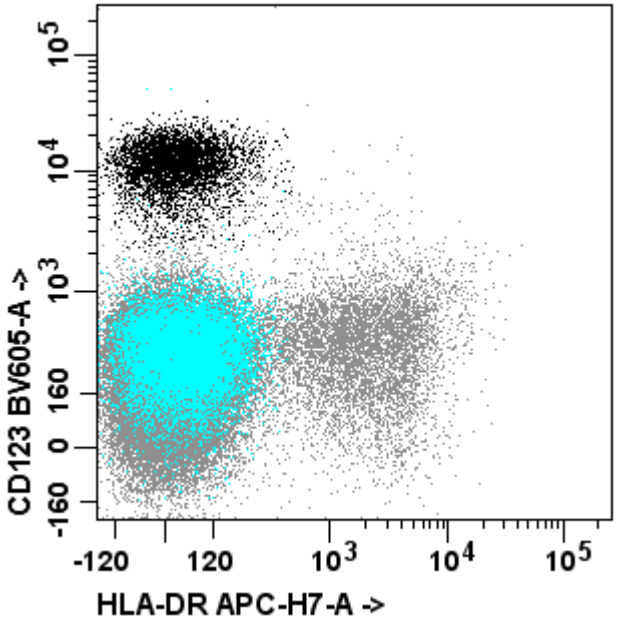


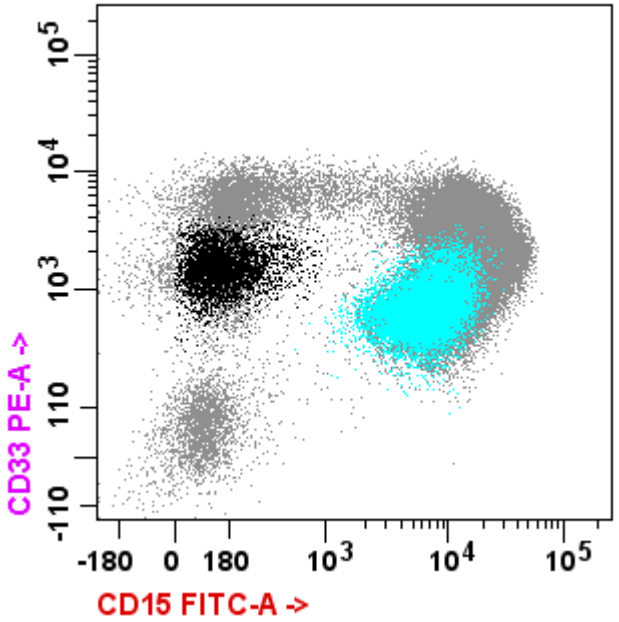
- In the second myeloid tube, the CD34(+) blasts (red) show further evidence of myeloid lineage differentiation (i.e., expression of CD33 and CD117 and partial expression of CD15).
- There is a progressive maturation spectrum (see arrows from least mature to most mature) from blasts (red) to promyelocytes (blue) to later stage granulocytes (green) in a manner that would be seen in bone marrow.
- No aberrancy is identified on the blasts or neutrophilic lineage in this tube.



- A minute subset of the **myeloblasts (RED)** shows expression of CD123.
- CD123 can be seen on both malignant myeloblasts as well as on normal subsets of normal myeloblasts[2].
- Plasmacytoid dendritic cell precursors also express CD34 and CD123[3]. The small subset of CD34(+)/CD123(+) blasts in this tube may correlate to the CD34(+)/CD22(+) blasts identified on the B-cell tube, although we did not run the markers in a combination to confirm this.

Additional Tube 2; Myeloid Tube





- Basophils **(BLACK)** and **eosinophils (CYAN/LIGHT BLUE)** are further characterized on this tube
- Basophils show bright intensity of CD123, moderate intensity for CD33, predominantly lack expression of CD15, and lack expression of HLA-DR
- Eosinophils generally show slightly dimmer expression of both CD15 and CD33 in relation to neutrophilic granulocytes and lack expression of CD123 and HLA-DR
- Neither the basophils or eosinophils show overt aberrancy in this tube

Additional Tube 2; Myeloid Tube Summary

- There is further immunophenotypic evidence of a left shift in the granulocytic lineage
- The myeloblasts, eosinophils and basophils are all increased in proportion
- No overt immunophenotypic aberrancy is seen on this tube
- Although not shown, the monocytes also lack aberrancy on this tube

Overall Summary of Results

28-year-old man with massive spleen and lymphadenopathy

+

- WBC
  - 165×10^9/L with absolute basophilia and eosinophilia
- Normochromic, normocytic anemia
- Peripheral Blood Smear
  - Neutrophilic left shift to blast stage, basophilia and eosinophilia

+

- Flow Cytometry
  - Small population of CD34(+) myeloblasts showing mild immunophenotypic aberrancy
  - Left shifted granulocytes
  - Partial expression of CD56 on granulocytes and monocytes
  - Increased proportions of basophils and eosinophils

=

Consistent with a myeloid neoplasm

Overall Summary of Results

28-year-old man with massive spleen and lymphadenopathy

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  - Left shifted granulocytes
  - Partial expression of CD56 on granulocytes and monocytes
  - Increased proportions of basophils and eosinophils

=

Consistent with a myeloid neoplasm

PLUS

Minute population of CD3(-)/CD2(+)/CD5(+)/CD7(+) small cells on the T-cell tube

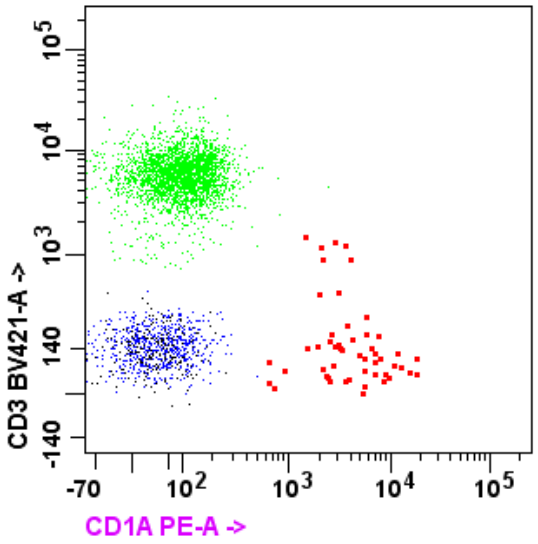
- We routinely reflex cytoplasmic tubes to establish a complete immunophenotype for myeloblasts in myeloid neoplasms, although it did not lend further significant information this case
- In addition, we decided to add an extra T-lineage tube at a higher sensitivity to further characterize the minute population of CD2(+)/CD3(-)/CD4(+)/CD5(+)/CD7(+)/CD8(partial +)/CD56(-) cells identified on the T-cell tube

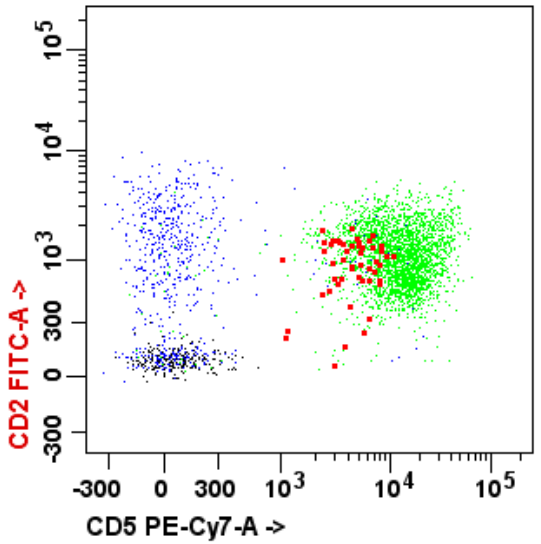
T-cell Reflex Tube

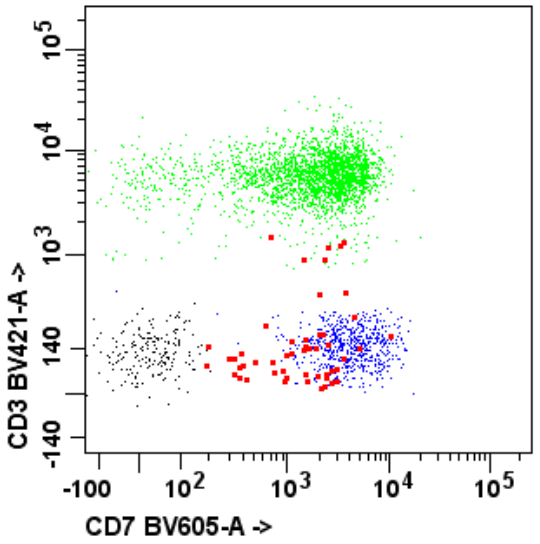
	FITC	PE	PerCp5.5	PE-Cy7	APC	APC-R700	APC-H7	BV421	V500c	BV605
Reflex T-ALL	CD2	CD1a	CD4	CD5	CD10	CD34	CD8	CD3	CD45	CD7

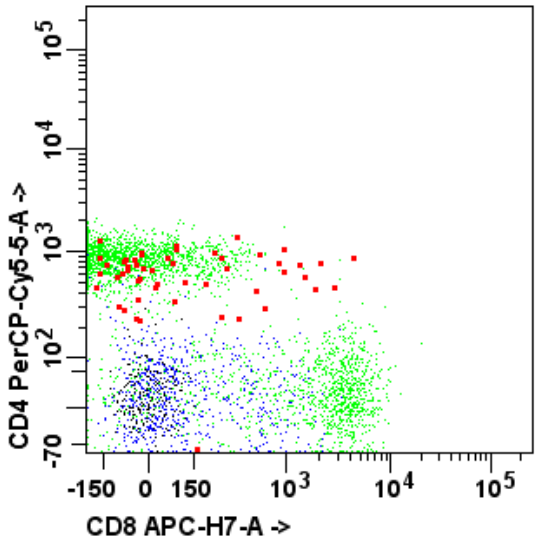
Gated on lymphocytes
Acquired 500,000 cells

- The cells colored in red are the cells of interest. They express the T-lineage associated markers CD4, CD5, CD7 and partially express CD2, CD3 and CD8.
- The cells are also CD1a positive, which is considered a marker of immaturity in the context of T-lineage cells.





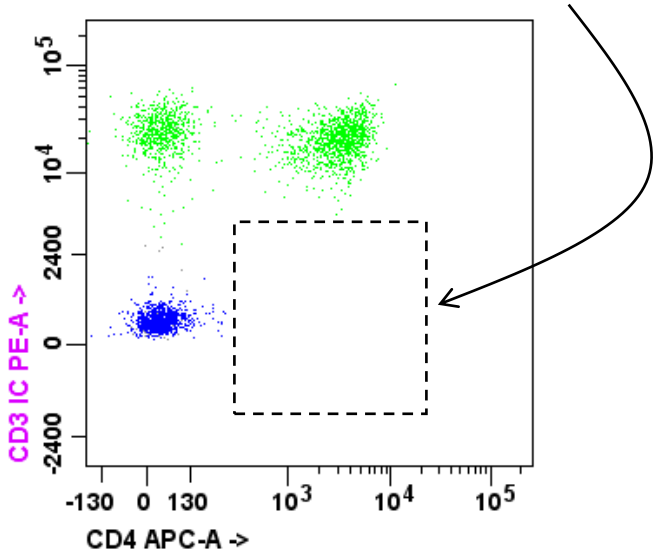




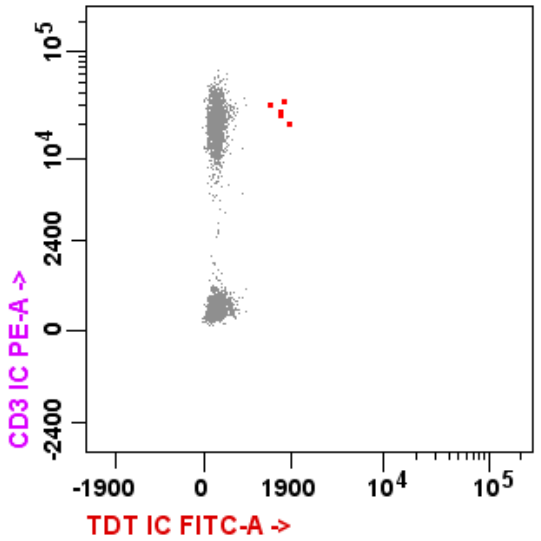
Red: ? Immature T-cells; 0.01% of total cells; 52 total events ?
Green: Mature T-cells
Blue: NK cells
Black: B-cells

Summary of reflex tubes

Remaining tubes were equivocal for further characterization of the minute population of suspected immature T-lineage cells. The cytoplasmic CD3 was suspected to be positive as there were no CD56(-)/CD5(+)/CD4(+)/cytoplasmic CD3(-) cells identified.



There was a very minute subset of cCD3(+) cells showing expression of TdT, which is generally considered to be a marker of immaturity



Overall summary of the peripheral blood flow cytometry:

- The findings were suggestive of a myeloid neoplasm with the presence of a minute population of immature T-lineage cells of unclear significance.
- Lymph node and bone marrow biopsies were recommended for further evaluation.

Bone Marrow Biopsy

- Flow cytometry showed similar findings to the peripheral blood.
- Morphologic examination was consistent with a myeloproliferative neoplasm with hypercellularity (95%), myeloid and megakaryocytic hyperplasia, left shifted granulopoiesis, mild myelofibrosis (MF1/2) and no increase in blasts.

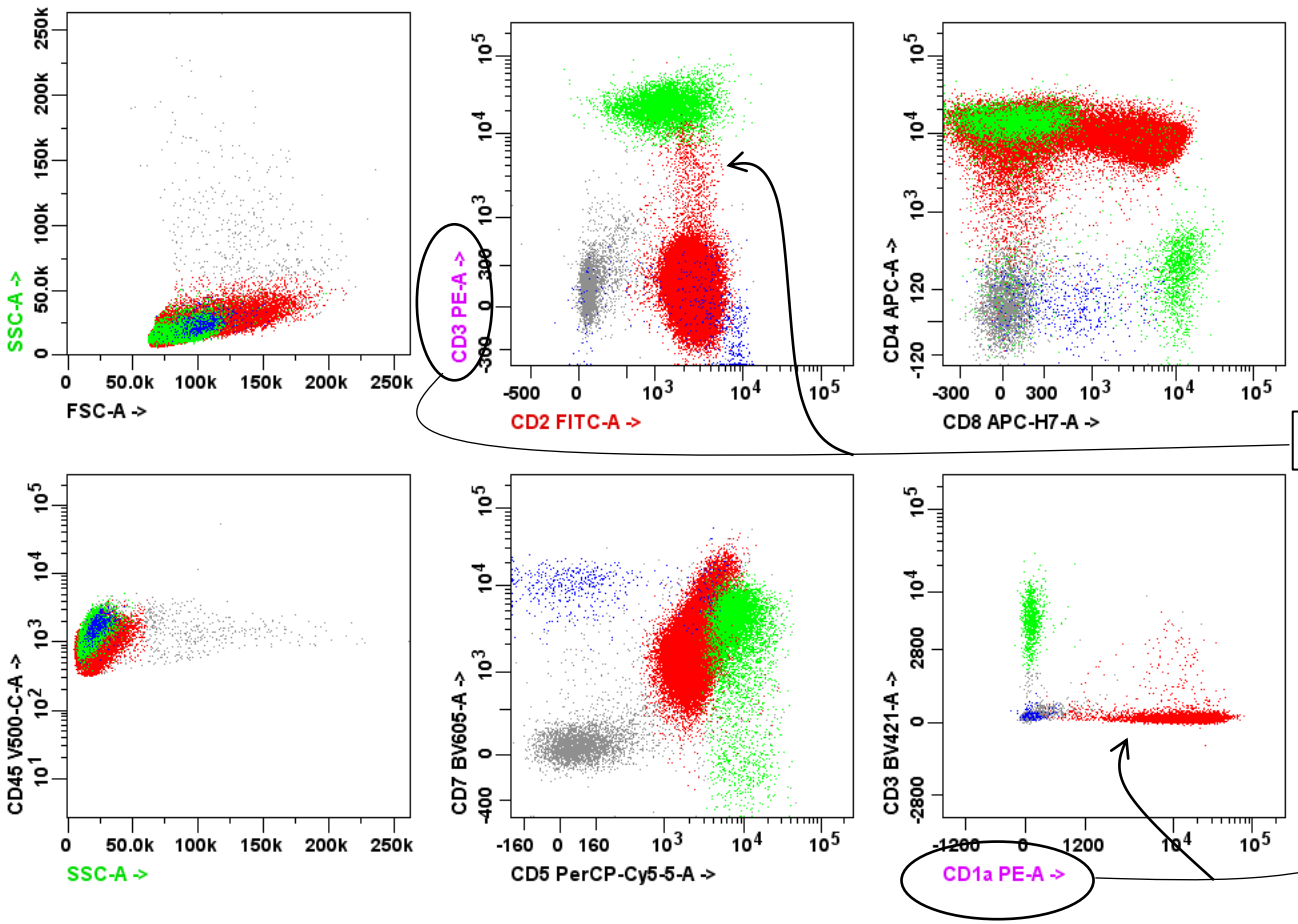
Lymph node biopsy

- An inguinal lymph node was biopsied and sent for flow cytometry and morphologic examination.
- Flow cytometry tubes included the following:

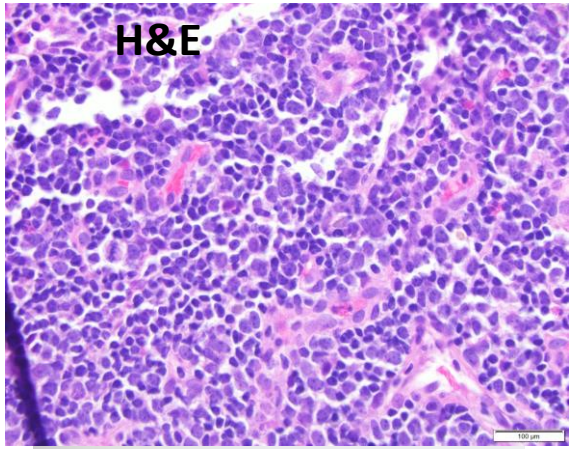
	FITC	PE	PerCp5.5	PE-Cy7	APC	APC-R700	APC-H7	BV421	V500c	BV605
Tissue T-cell Tube	CD2	CD3	CD5	CD56	CD4	CD64	CD8	CD30	CD45	CD7
Tissue B-cell Tube	mLambda	CD22	CD20	CD19	---	CD45 AF700	CD38	CD10	CD5 BV480	mKappa
Reflex T-ALL	CD2	CD1a	CD4	CD5	CD10	CD34	CD8	CD3	CD45	CD7

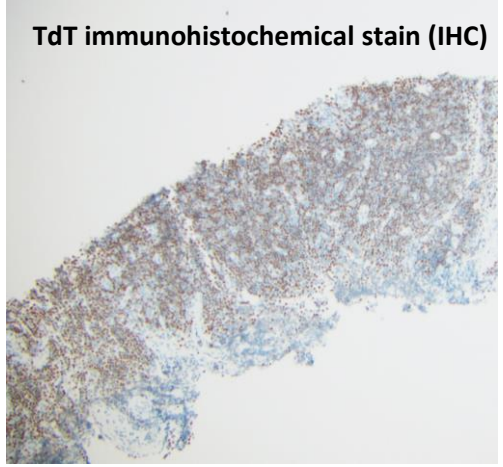
Lymph node biopsy

Flow cytometry revealed a large population of immunophenotypically aberrant, immature T-lineage cells with features similar to the minute population identified in the peripheral blood sample.



Lymph node biopsy showed architectural effacement by a diffuse infiltrate of lymphoid cells with dispersed chromatin and scant cytoplasm





Not shown: IHC for CD3 was positive

Overall lymph node diagnosis

- T-lymphoblastic leukemia/lymphoma

Summary

- Bone Marrow Morphology and Flow Cytometry
  - Myeloproliferative neoplasm and minimal involvement by T-lymphoblastic Leukemia/Lymphoma
- Lymph Node Morphology and Flow Cytometry
  - T-lymphoblastic Leukemia/Lymphoma

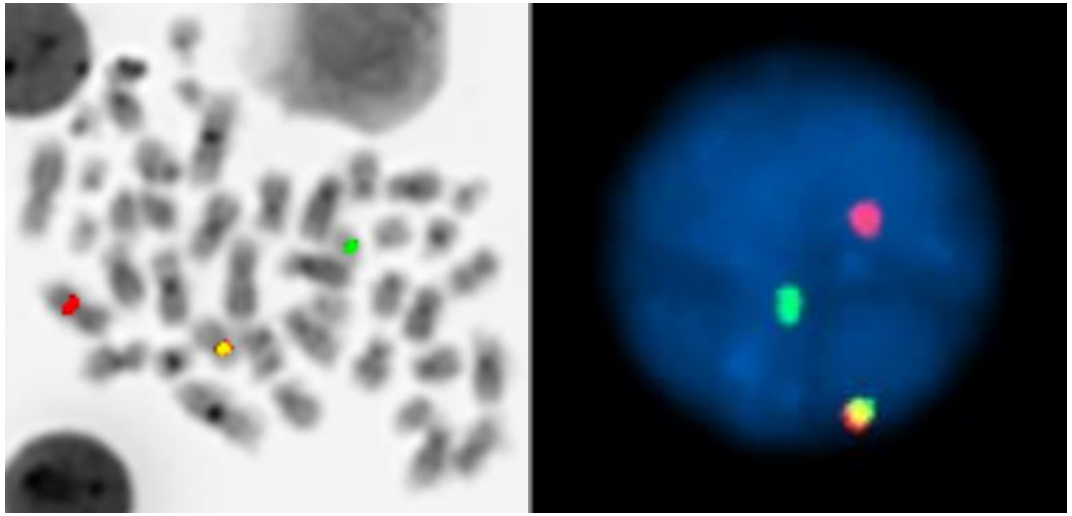
Overall

Myeloid neoplasm + T-ALL = Suspicious for a myeloid/lymphoid neoplasm with an FGFR1 rearrangement

Genetics

Fluorescence in situ hybridization (FISH) analysis

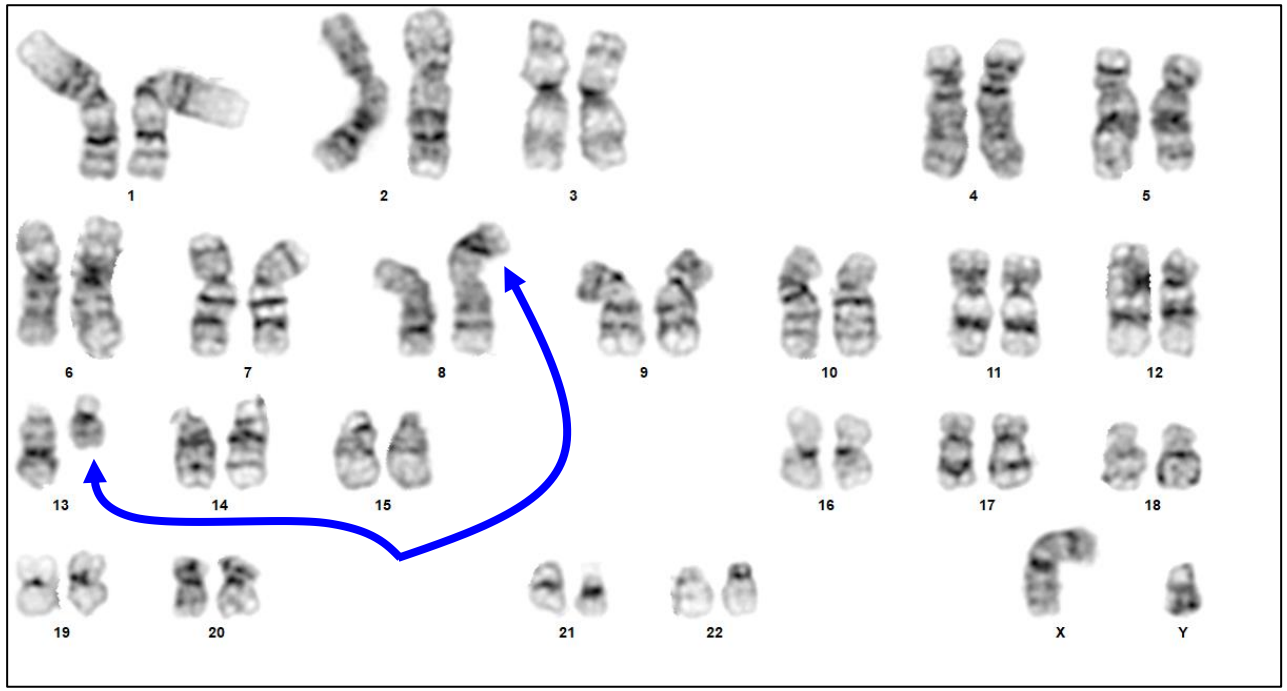
- Break apart FISH probe set with 3′FGFR1(red), 5′FGFR1(green), and yellow fusion signals
- Break apart probes are designed to flank either side of a gene, so that in the event of a translocation, the two colors will split
- In the panels below, the yellow signal (red and green combined) is normal
- The isolated green and red signals indicate that the FGFR1 gene is abnormal and is involved in a translocation event



An FGFR1 (8p11) gene rearrangement was detected in the lymph node (85% of cells) and bone marrow (95% of cells)

Cytogenetic Karyotype

- revealed t(8;13) in the bone marrow



FISH for BCR-ABL was negative

Myeloid/lymphoid neoplasm with an FGFR1 rearrangement

- Heterogeneous hematolymphoid neoplasms
- Derived from a pluripotent hematopoietic stem cell
- With different patients or at different stages of the disease, the neoplastic cells can present as either mature or immature or both
- Cases can present as myeloproliferative neoplasms (chronic phase or blast crises) or as acute leukemia (e.g., AML, B-ALL, T-ALL or MPAL)
- These neoplasms involve translocation of a receptor tyrosine kinase gene for fibroblast growth factors, i.e., the FGFR1 gene, located at chromosome 8p11.2 with variant gene partners
- Lymphoblastic leukemia is more common in cases with t(8;13) than with variant translocations
- Eosinophilia is common
- The myeloid neoplasms exhibit chemoresistance to first- and second-generation tyrosine kinase inhibitors (TKIs).
- Newer TKIs and small molecule FGFR1 inhibitors may be effective bridging therapies to stem cell transplant

References

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Authors

Buddy Fuda
Professor of Pathology
Division of Hematopathology
University of Texas
Southwestern Medical Center
Dallas, TX

Sharon Germans
Fellowship Trainee
Division of Hematopathology
University of Texas
Southwestern Medical Center
Dallas, TX

Mingyi Chen
Professor of Pathology
Division of Hematopathology
University of Texas
Southwestern Medical Center
Dallas, TX

Rolando Garcia
Assistant Instructor of Pathology
Division of Clinical Cytogenetics
University of Texas
Southwestern Medical Center
Dallas, TX