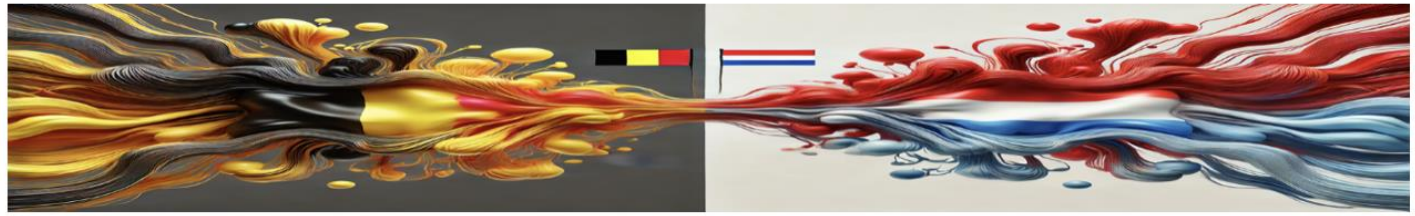


“Flow across borders”

Interactive case studies across the borders of gating, entities, diseases and classifications

PollEv.com/keesmeijer731

Jan Emmerechts, AZ Sint-Jan, Brugge

Kees Meijer, UMCG, Groningen

Activities

Visual settings

Edit

Which classification is used in your center

0

- WHO-HAEM4 (2016)
- WHO-HAEM5 (2022)
- ICC
- WHO-HAEM5 and ICC
- Another combination
- None of the above
- I don't know

Powered by Poll Everywhere

Case 1

- M 56 yr
- Persistent peripheral eosinophilia
- Increased IgE levels
- Skin abnormalities

- Is flow cytometry indicated?

Is flow cytometry indicated at this moment?

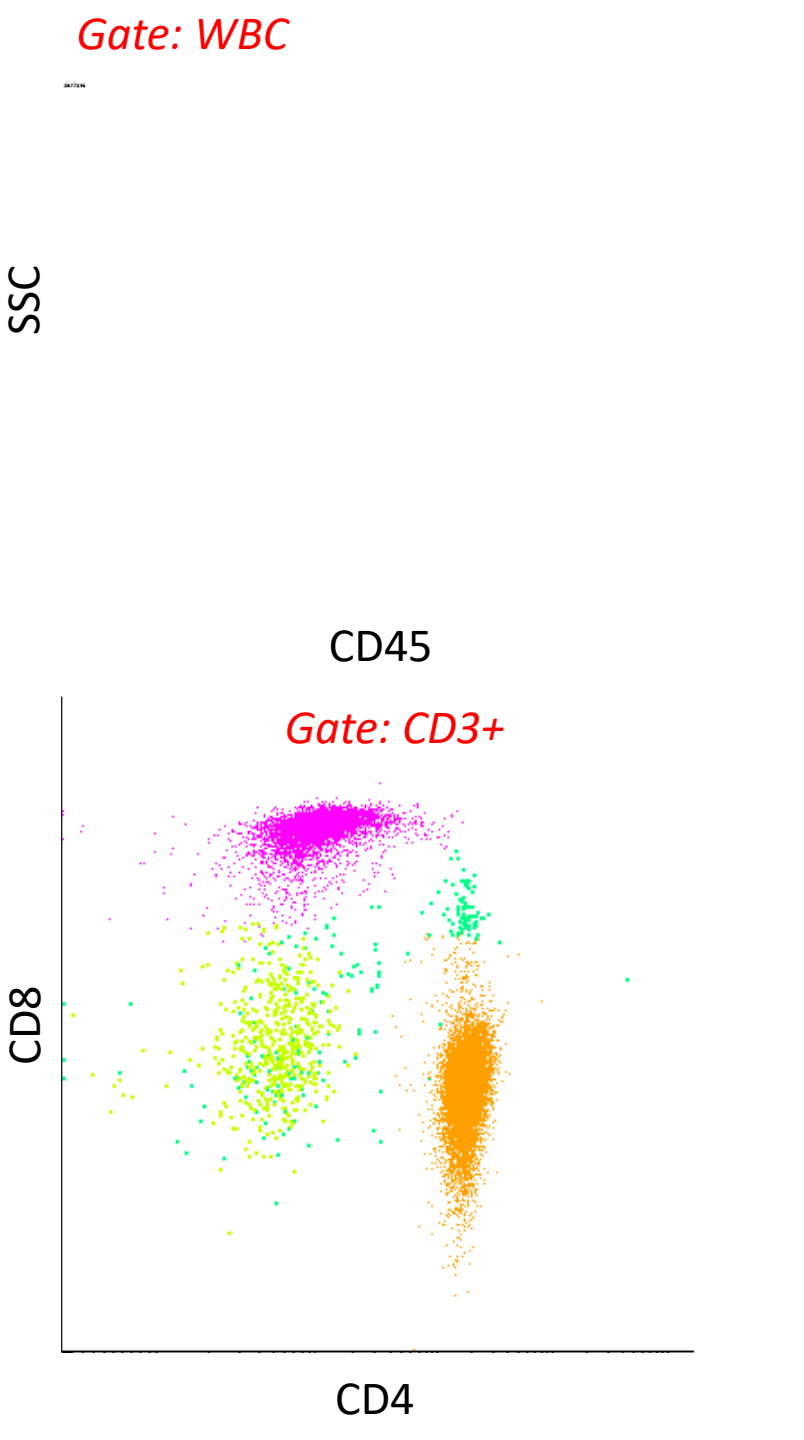
0

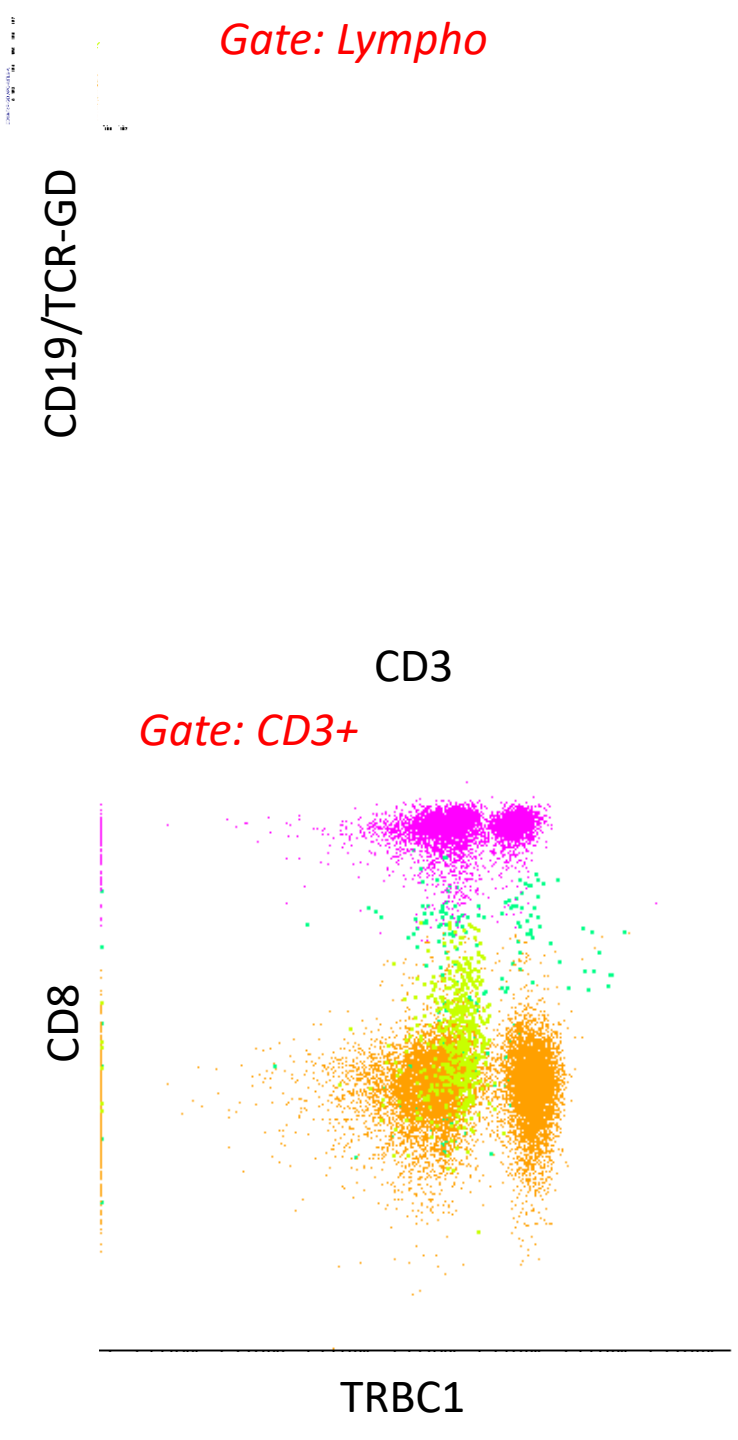
A) yes, panel for abnormal eosinophiles

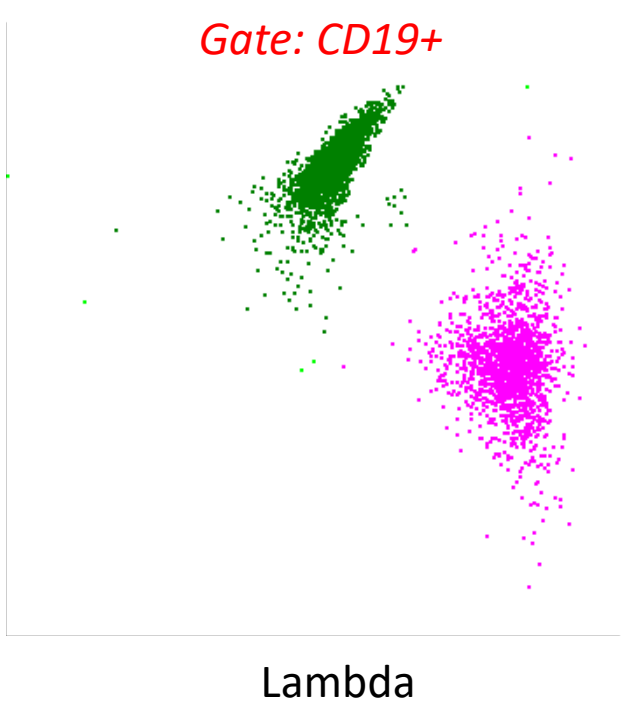
B) yes, mastocytosis panel on bone marrow

C) yes, lymphocytic screening panel on peripheral blood

D) Not indicated, molecular and parasitic testing first

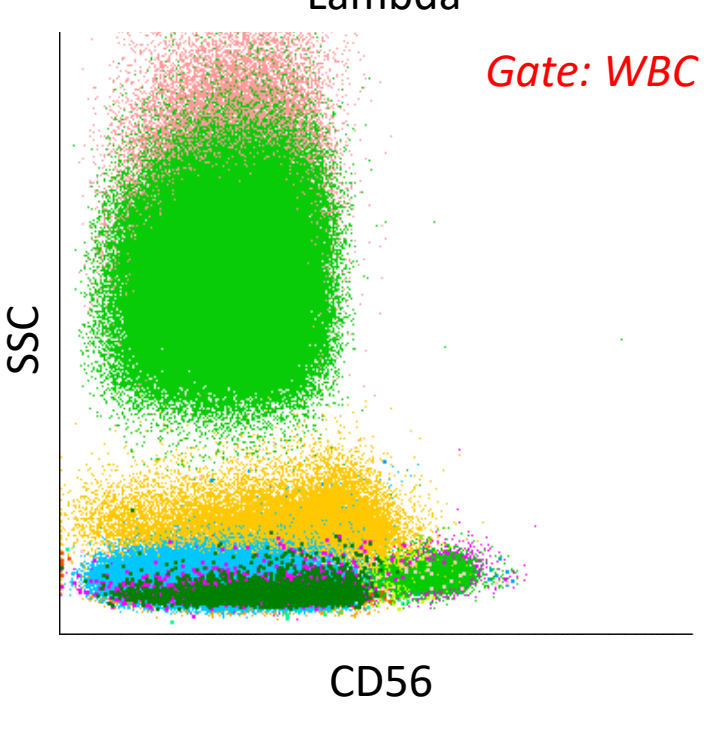

Gate: WBC
SSC
CD45


Gate: Lympho
CD19/TCR-GD
CD3


Gate: CD19+
CD19/TCR-GD
Lambda

Gate: CD3+
CD8
CD4

Gate: CD3+
CD8
TRBC1


Gate: WBC
SSC
CD56

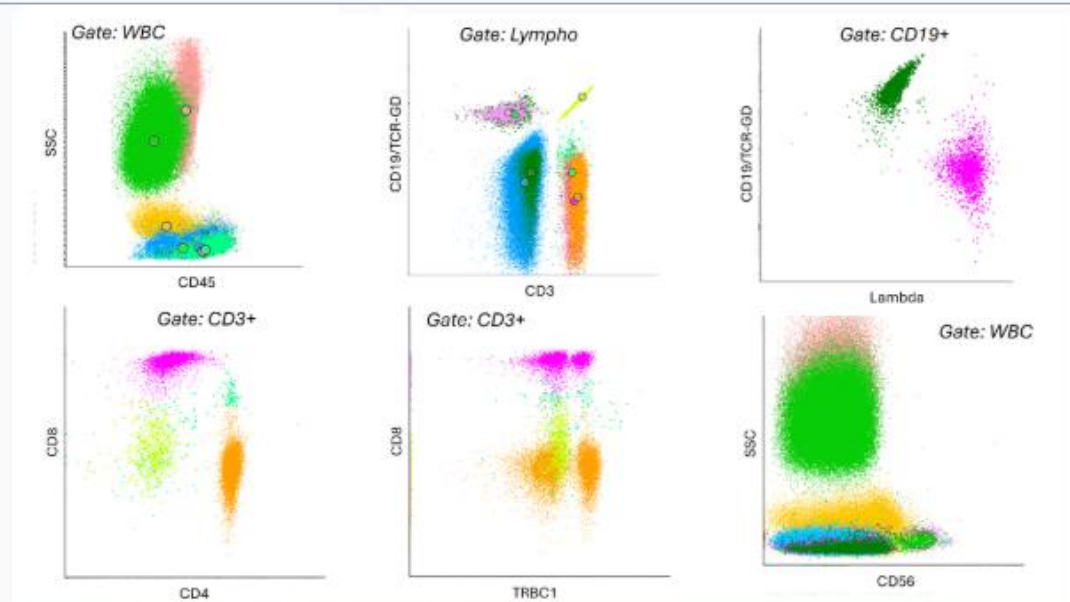
Activities

Visual settings

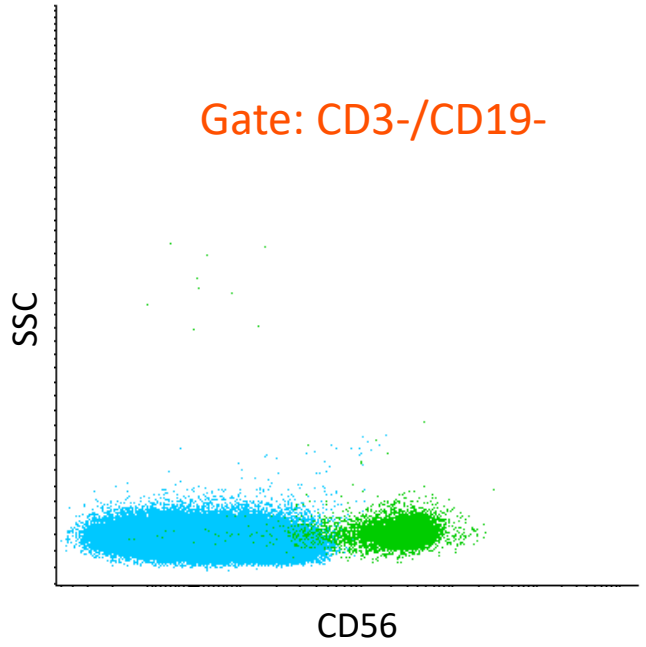
Edit

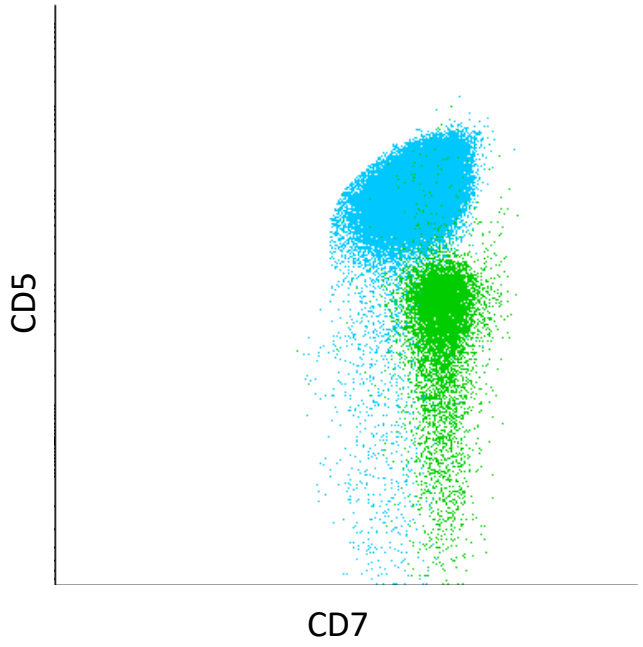
Where is the aberrant population?

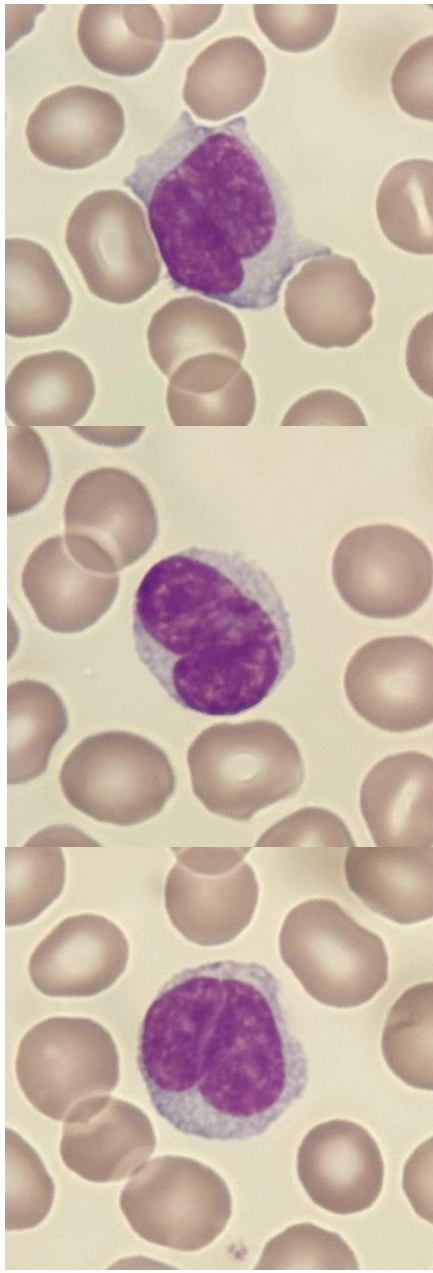
0

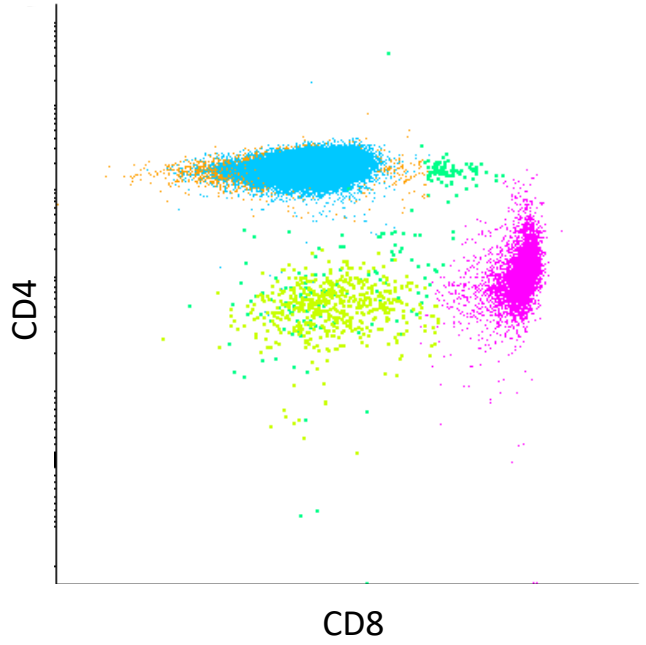


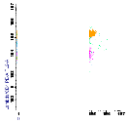
Powered by Poll Everywhere


Gate: CD3-/CD19-
SSC
CD56


CD5
CD7




CD4
CD8



CD4
TRBC1

Lymphocytic variant Hypereosinophilic syndrome (L-HES)

- Non-clonal eosinophilia secondary to abnormal IL-5 producing T-cells
  - Often T-cell clonality, but not required
  - Mostly **CD3-/CD4+**, but CD3+/CD4-/CD8- and CD3+CD4+CD7- is possible

- Indolent disease, but increased risk of progression to overt T-cell lymphoma

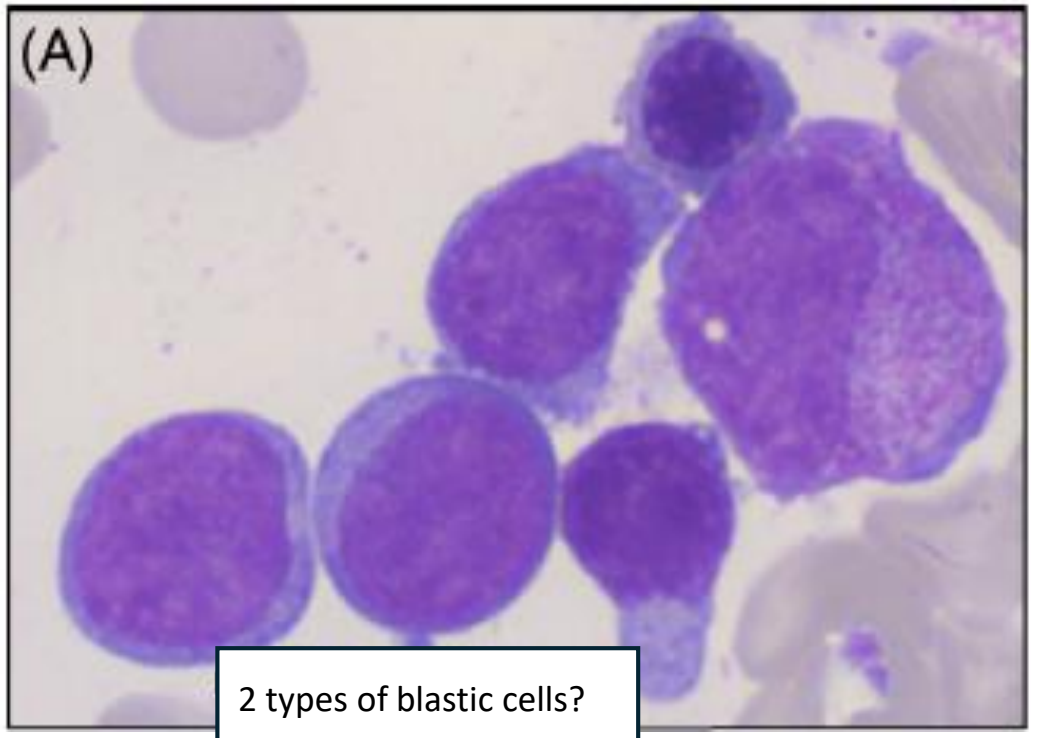
- CD3-/CD4+ T-cells also in TFH (AITL) and PTCL

Take home message:

- Unexplained eosinophila warrants screening for CD3-/CD4+ T-cells
- Be aware of loss of CD3 in mature T-cell malignancies
  - Don’t fully rely on fixed gates

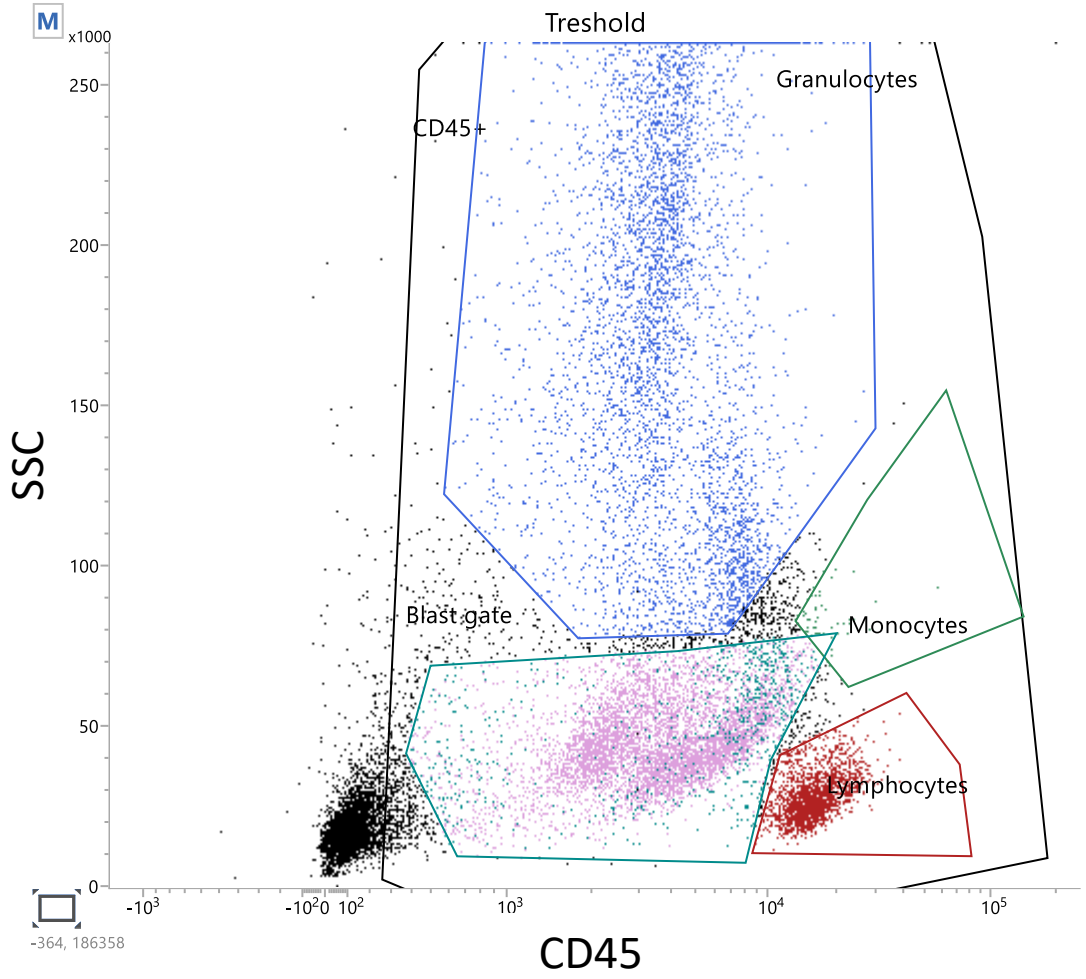
Case 2

- Man, 72y
- Medical history: known ET (JAK2+), treated
- Presentation: dizzyness, night sweats & loss of body weight (>10kg).
- PB: Pancytopenia
  10% blasts
- BM: 41% blastic cells

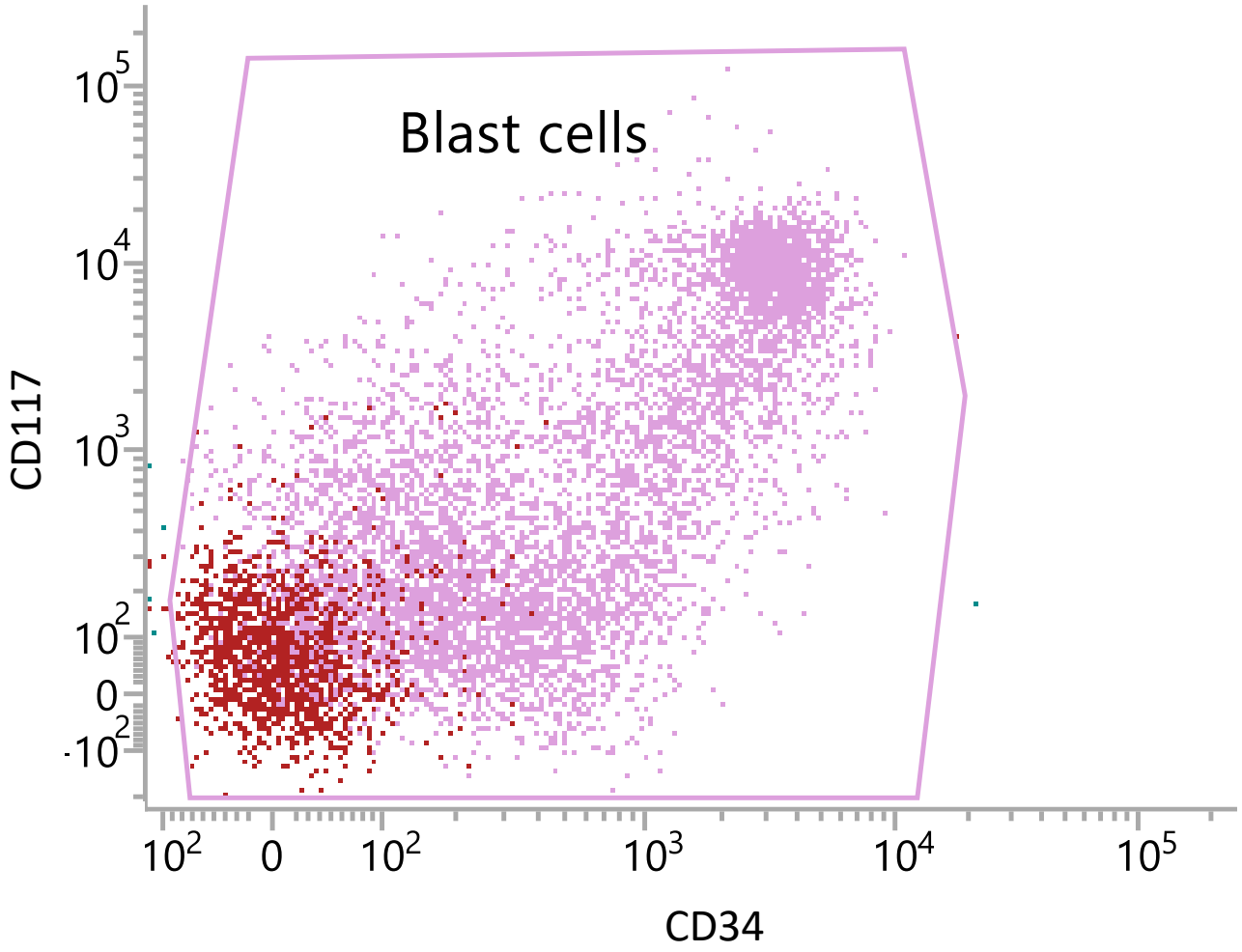


2 types of blastic cells?

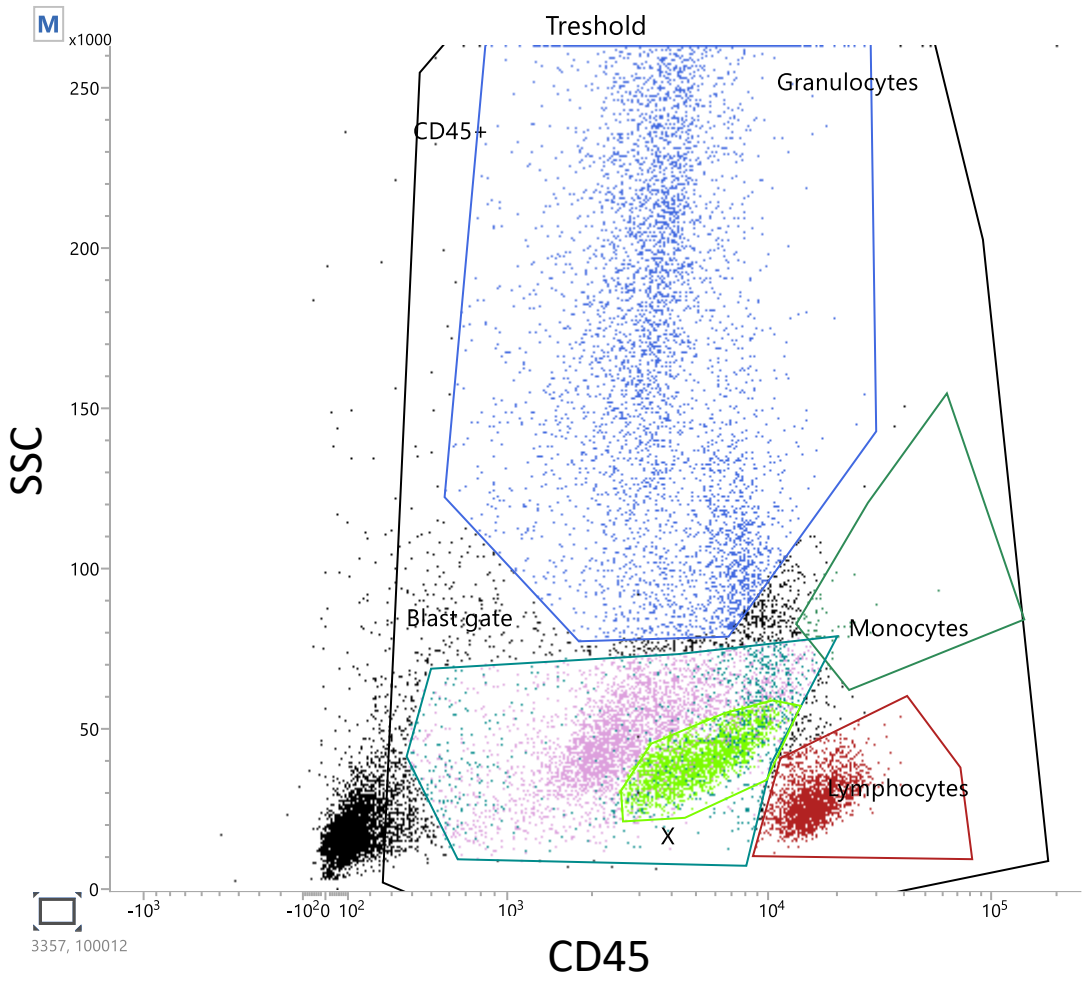
Case 2

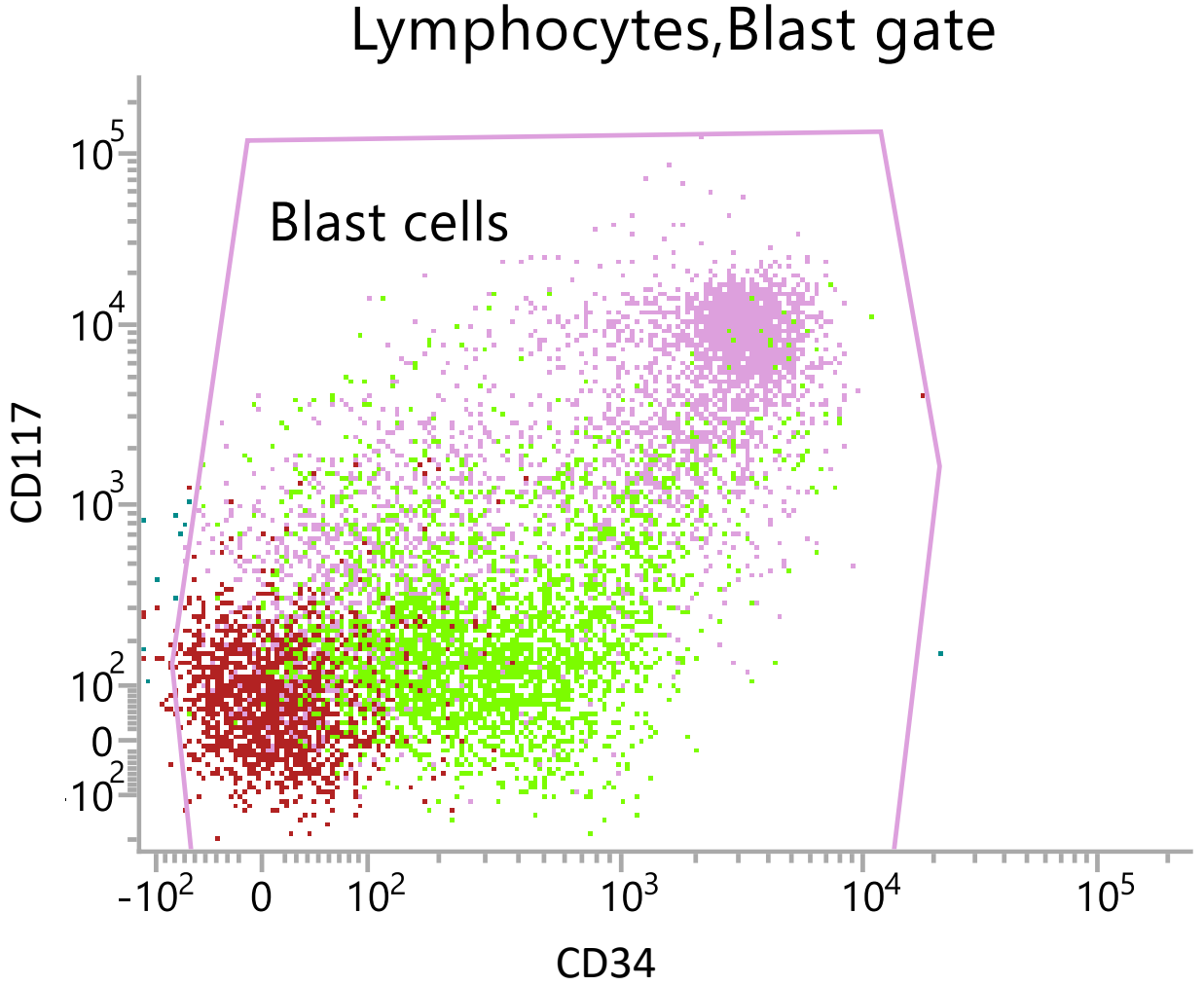


Lymphocytes,Blast gate

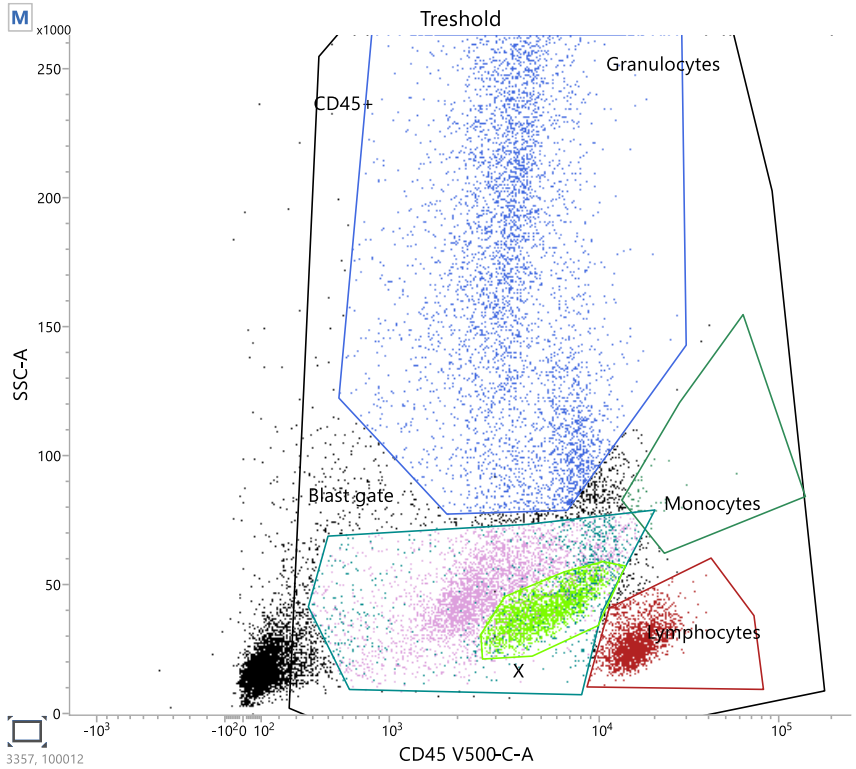


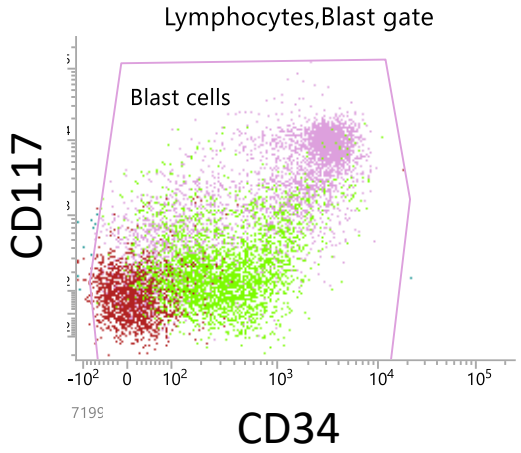
Case 2

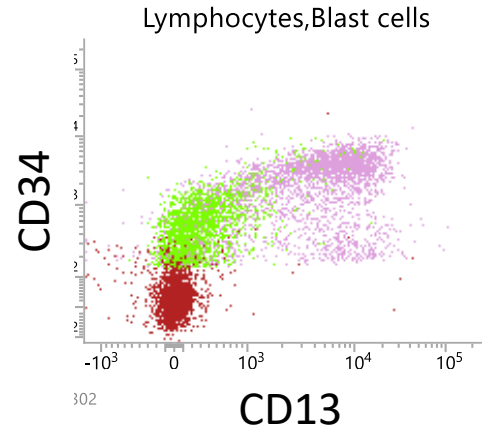

Treshold
Granulocytes
CD45+
Blast gate
Monocytes
Lymphocytes
X
SSC
CD45
x1000
250
200
150
100
50
0
-10^3
-10^2 0 10^2
10^3
10^4
10^5
3357, 100012

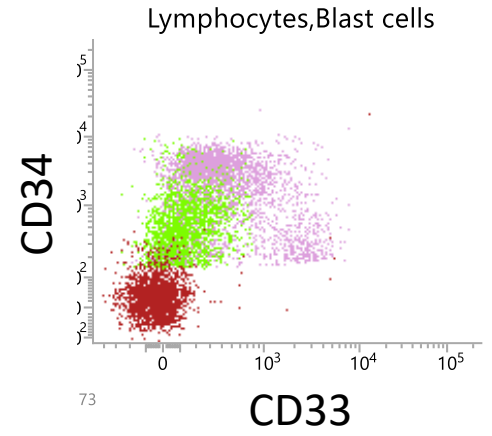

Lymphocytes,Blast gate
Blast cells
CD117
CD34
10^5
10^4
10^3
10^2
0
-10^2
-10^2
0
10^2
10^3
10^4
10^5

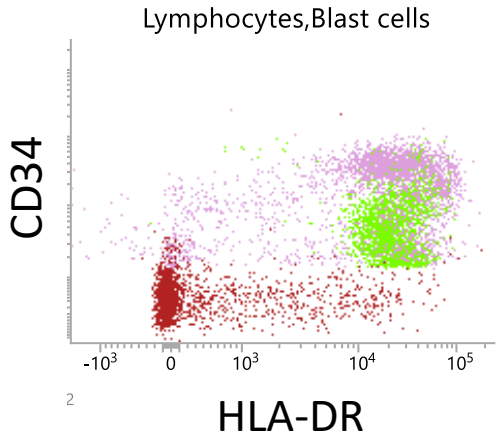
Case 2











Lineage defining markers:

Pink: cyCD3-/sCD3-/CD13+/CD19-/CD22-/CD33-(+weak?) /CD41CD61-/CD79a-/CD117+/MPO-

Green: cyCD3-/sCD3-/CD13-/CD19-/CD22-/CD33- /CD41CD61-/CD79a-/CD117-/MPO-

How would you define the **pink** population?

- Undifferentiated
- Myeloid
- B-lymphoid
- T-lymphoid
- One can't tell

Activities

Visual settings

Edit

How would you define the pink population?

0

A) Undifferentiated

B) Myeloid

C) B-lymphoid

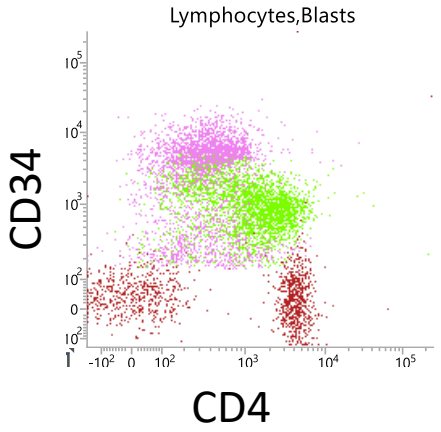
D) T-lymphoid

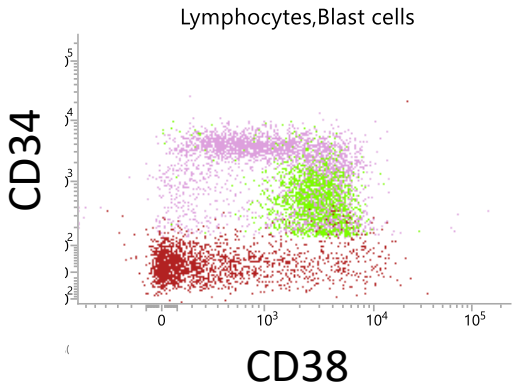
E) One can’t tell

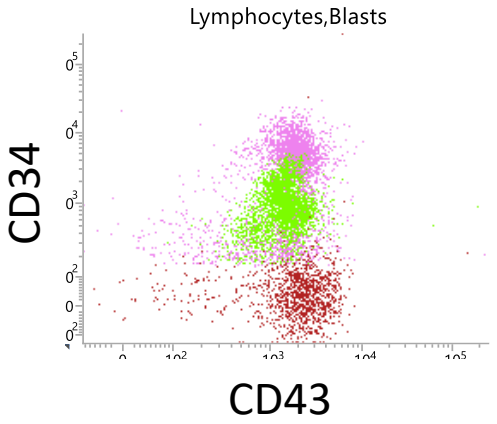
Powered by Poll Everywhere

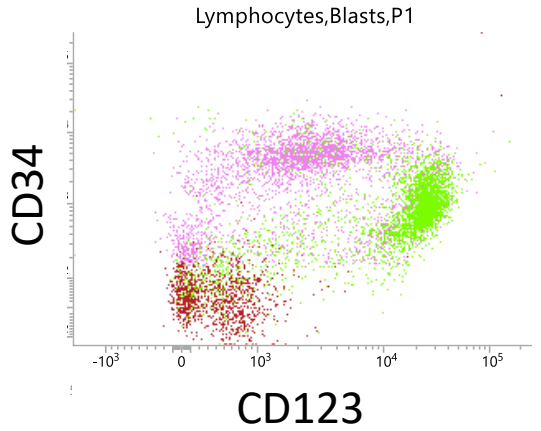
Case 2

Green: CD4+/CD38+/CD43+/CD123+





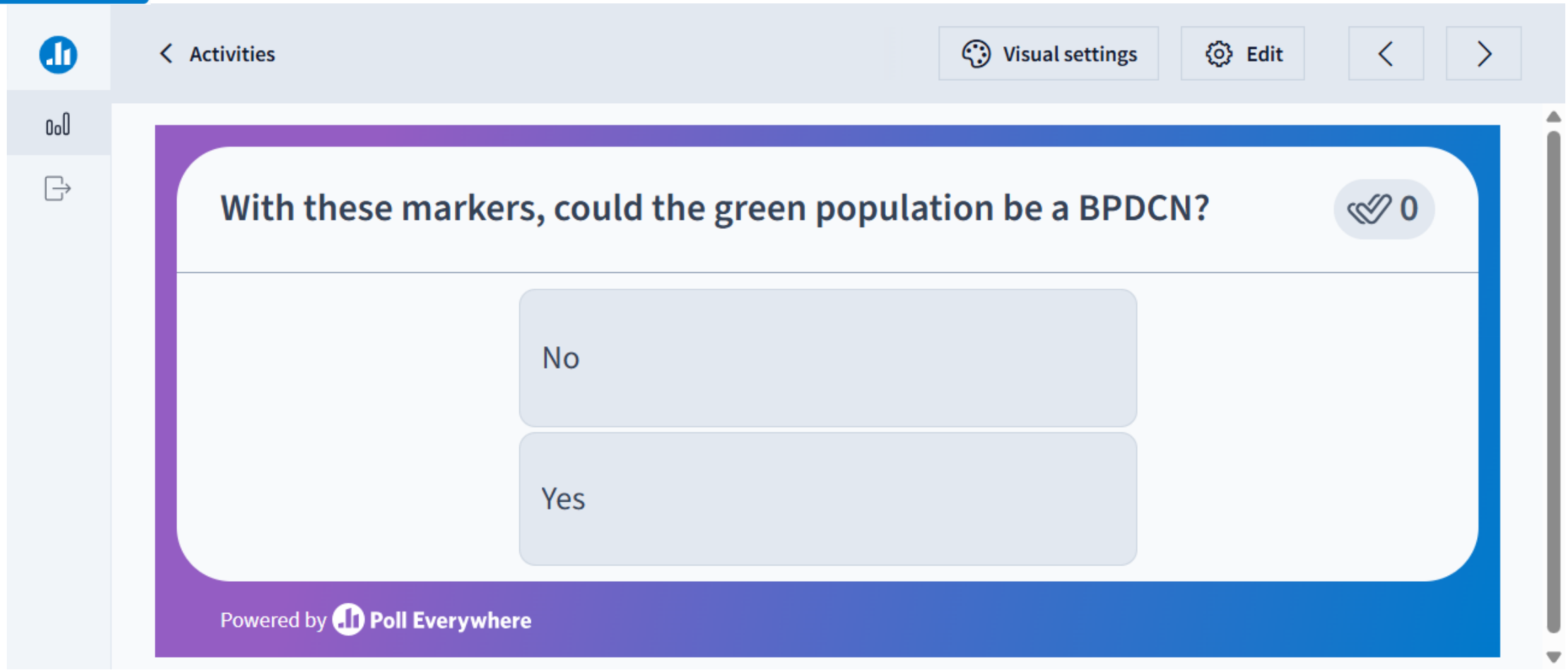




With these markers, could the green population be a BPDCN?

- Yes
- No

CD34dim/CD4+/CD38+/CD43+/CD123+



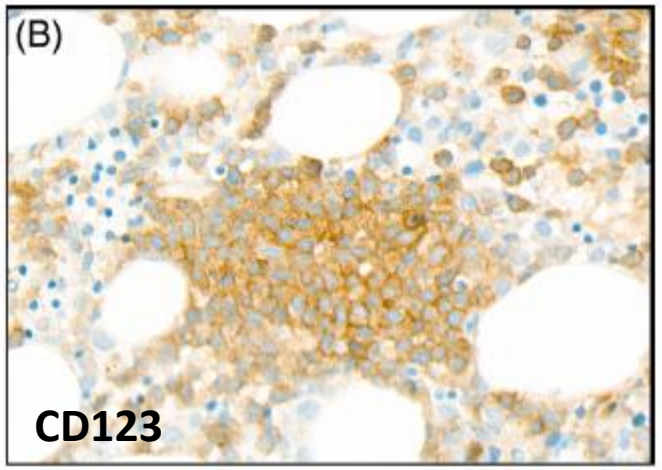
Case 2

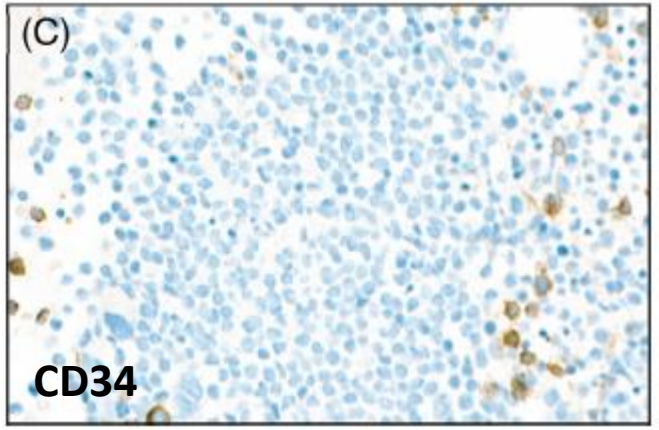
Green:

IF: all other tested markers negative, including CD56-/cyTCL1-, so BPDCN is unlikely

Molecular biology: RUNX1 mutation

IH:





pDC-AML

Case 2: pDC-AML

- pDC-AML is no separate entity in either WHO or ICC

- **<u>WHO-HAEM5:</u>**
- Part of MPDCP
- pDC: >2% on NEC
- pDC: CD123+/CD303+/CD304+. Possible aberrant loss (CD303, TCL1) or gain (CD34, CD56, TdT) of markers
- pDC-CMML: mature pDC
- pDC-AML: spectrum of pDC maturity: from (CD34+/303-) to (CD34-/CD303+)
- up to 5% of AML cases

Mature plasmacytoid dendritic cell proliferation associated with myeloid neoplasm

Definition
Mature plasmacytoid dendritic cell proliferation (MPDCP) associated with myeloid neoplasm is a clonal proliferation of plasmacytoid dendritic cells (pDCs) with low-grade morphology identified in the context of a defined myeloid neoplasm.

ICD-O coding
9727/1 Mature plasmacytoid dendritic cell proliferation associated with myeloid neoplasm

ICD-11 coding
2B31.Y Other specified histiocytic or dendritic cell neoplasms

Related terminology
Acceptable: mature plasmacytoid dendritic cell proliferation.

Subtype(s)
None

Localization

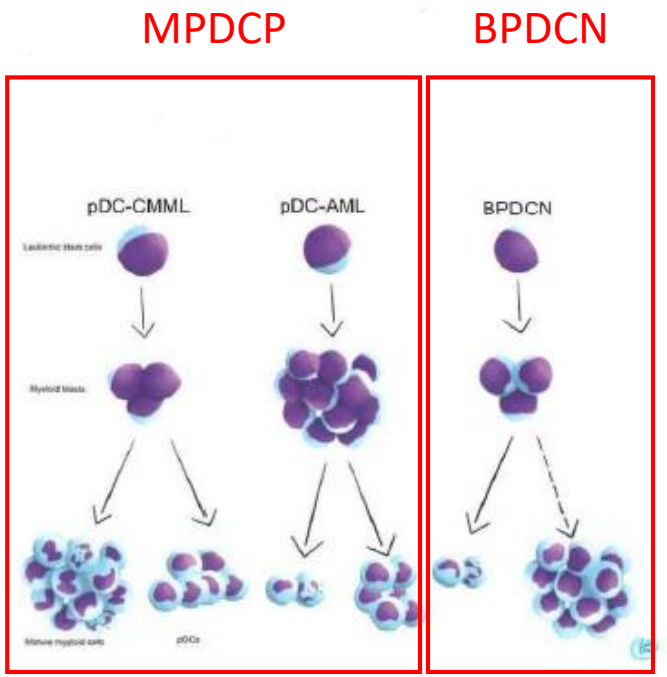


Fig. 3.04 Clonal plasmacytoid dendritic cell proliferations. Proposed scheme outlining the relationships between mature plasmacytoid dendritic cell proliferations, associated with myeloid neoplasms, and blastic plasmacytoid dendritic cell neoplasm [1864].

Case 2: pDC-AML

- pDC-AML is no separate entity in either WHO or ICC
- **ICC:**
  - Only small note under BPDCN:

The distinction of BPDCN from pDCs hyperplasia or clonal proliferations of mature pDCs associated with CMML is mainly based on morphology (blastoid vs mature cells) and immunophenotype: in contrast to mature pDCs, BPDCN cells strongly express BCL2, CD56, and SOX4 (Table 38.1).[3,10] BPDCN must also be distinguished from AML associated with increased pDCs (AML-pDCs) in the bone marrow and peripheral blood, where pDCs can express CD34 and are typically CD56 and TCL1 negative. Notably, 75% of cases of AML-pDCs are characterized by *RUNX1* mutations, which are rarely detected in BPDCN[11]

Case 2: pDC-AML

	Benign pDC	BPDCN	pDC-CMML	pDC-AML	
				WHO	ICC
Populations		1	2	2	2
CD4	+	+			
CD34	-	-	-	+(-)	+
CD56	-	+	-(+)	-(+)	-
CD123	+	+	+	+	
CD303/304	+	+	+(-)	+(-)	
TCL1	+	+	+(-)	+(-)	-
TdT	-	+-		-(+)	

WHO: min 3
ICC: min 2

WHO: BPDCN

Box 3.01 Immunophenotypic diagnostic criteria for blastic plasmacytoid dendritic cell (pDC) neoplasm

Expected positive markers

pDC markers:

- CD123
- TCF4
- TCL1
- CD303
- CD304

Other markers:

- CD4
- CD56

Expected negative markers

- CD3
- CD14
- CD19
- CD34
- Lysozyme
- Myeloperoxidase

Immunophenotypic diagnostic criteria

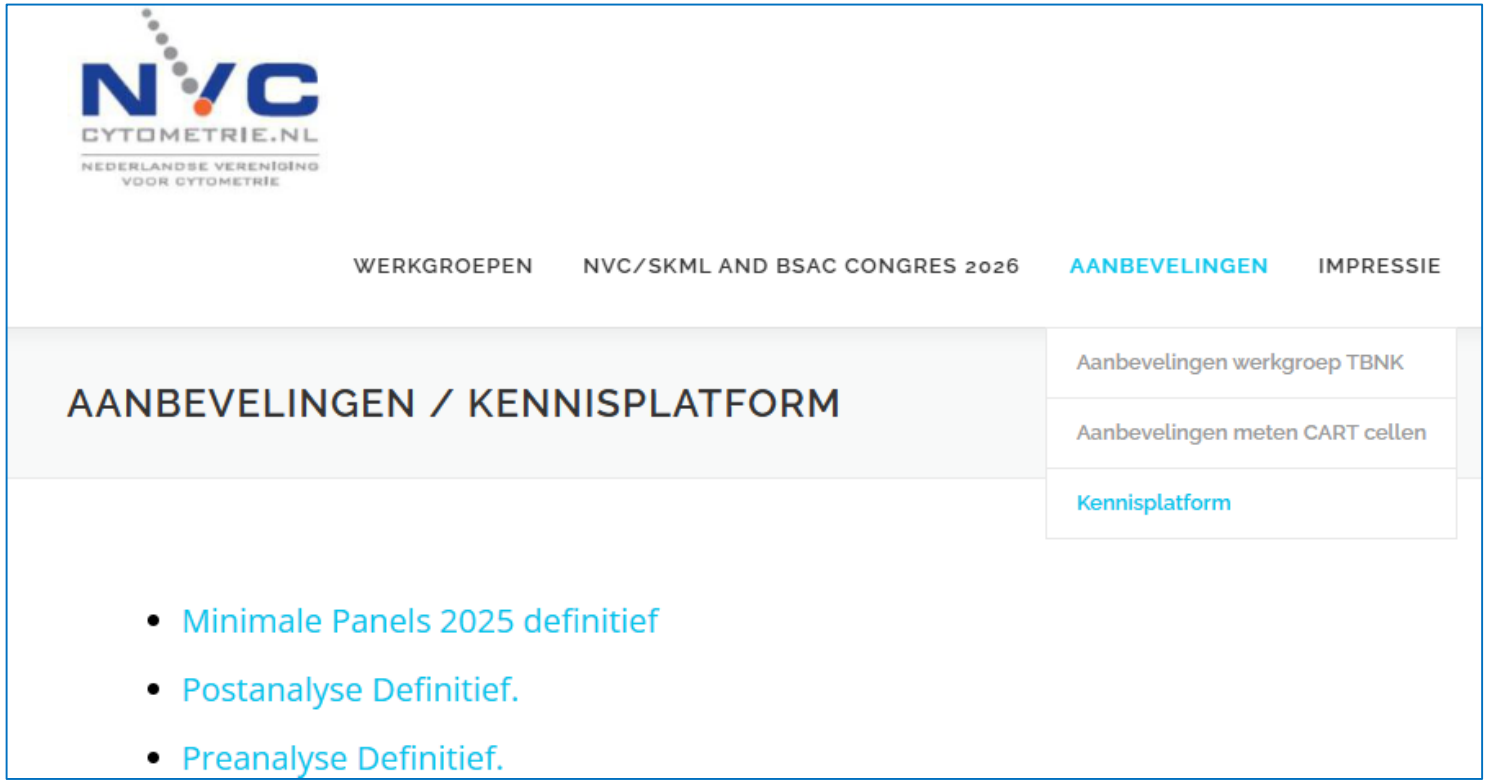
Expression of CD123 and one other pDC marker in addition to CD4 and/or CD56

OR

Expression of any three pDC markers and absent expression of all expected negative markers

Case 2: pDC-AML

- www.cytometrie.nl
- Aanbevelingen/Kennisplatform/Minimale panels 2025 definitief



Case 2: pDC-AML

BLASTAIR PLASMACYTOID DENDRITISCHE CEL NEOPLASMA (BPDCN)

Tabel 6. Minimale panel BPDCN.

	Minimale panel 2024	Verwachte expressie
CD45	Verplicht	+
CD4	Verplicht	+
CD34	Verplicht	-
CD56	Verplicht	+
CD123	Verplicht	+
CD303	Sterk aanbevolen	+
CD304	Sterk aanbevolen	+
cTCL1	Sterk aanbevolen	+

Diagnostische criteria WHO 2022:

- Expressie van CD123 en eventueel 1 andere plasmacytoïde dendritische marker* (cTCL1, CD303, CD304), naast CD4 en/of CD56

  of

- Expressie van drie plasmacytoïde dendritische markers* (CD123, cTCL1, CD303, CD304) en afwezige expressie van alle verwachte negatieve markers (CD3, CD14, CD19, CD34, cMPO).

Voetnoten:

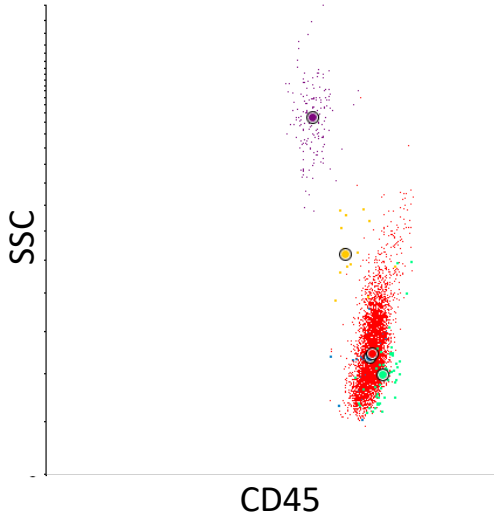
- Positiviteit van CD34 maakt dat voorkeur moet worden gegeven aan AML met pDC-uitrijping boven BPDCN (zie hiervoor het WHO-hoofdstuk over Mature plasmacytoid dendritic cell proliferation associated with myeloid neoplasm MPDCP)
- In de differentiaaldiagnose van BPDCN staan onder andere rijpe pDC afwijkingen, deze worden voorlopig buiten beschouwing gelaten (zie MPDCP).
- TCF4 wordt door WHO 2022 als pDC-marker genoemd, omdat de beschikbaarheid zeer beperkt en het gebruik door experts onduidelijk is, wordt deze marker voorlopig buiten beschouwing gelaten.

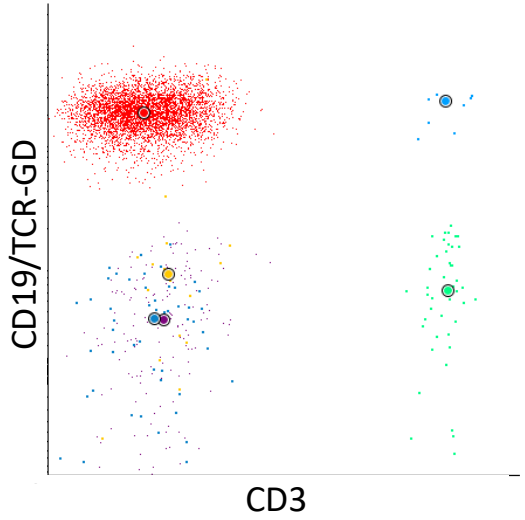
Case 2

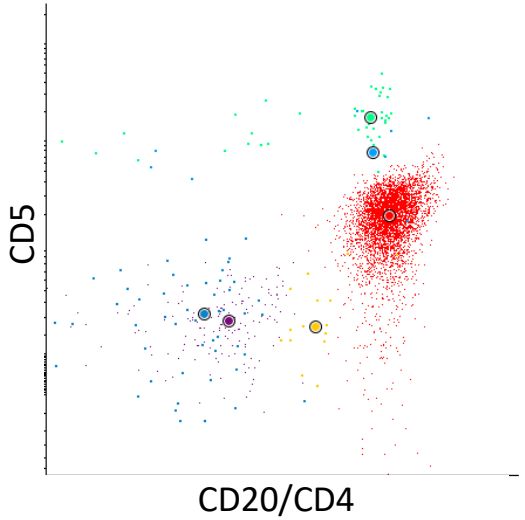
Take home message:

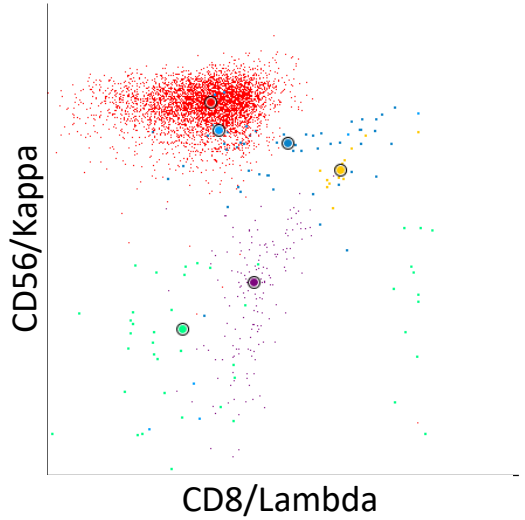
- MPDCP exist, but they are rare
- Distinction with BPDCN: 2 populations, CD34+-/CD56-/TCL1-

Case 3

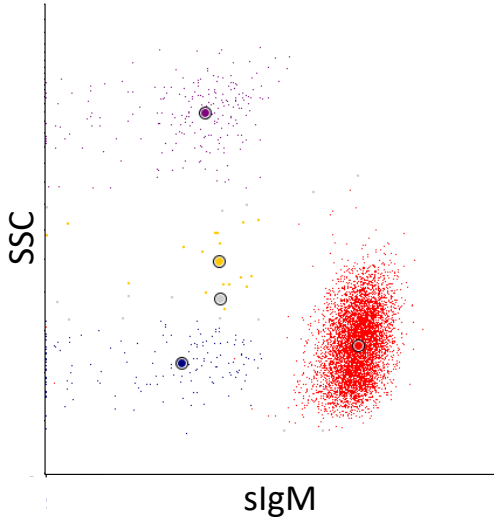


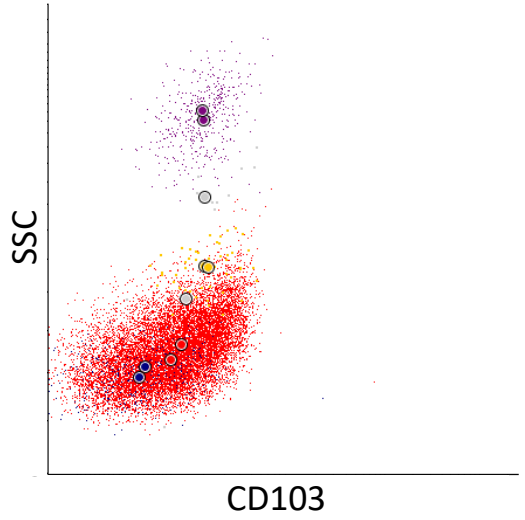


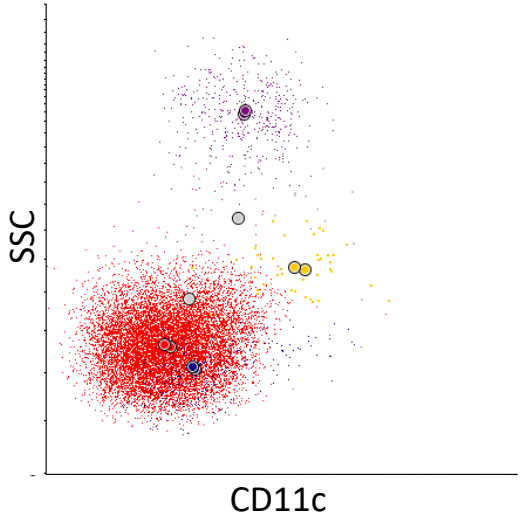




CD79b+
CD22+
IgD+
CD10-
CD23-
CD43-
CD200-



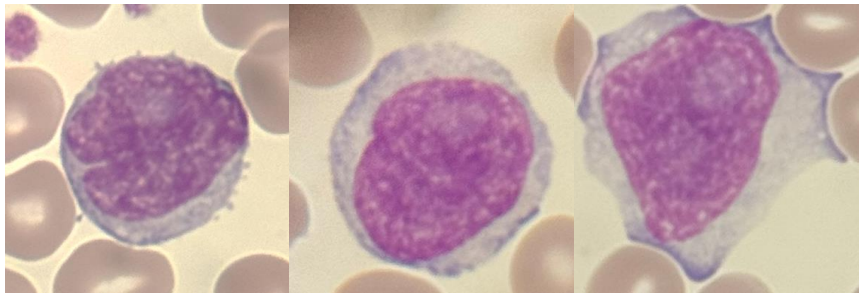




Male 60y
Accidentally discovered leukocytosis
L: 88 x10^9/L
Hb: 9,4 mmol/L
T: 197 x10^9/L

No lymphadenopathy
No splenomegaly

How would you classify this disease?



Activities

Visual settings

Edit

How would you classify this disease?

Nobody has responded yet.

Loading...

Hang tight! Responses are coming in.

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Additional diagnostic information

Cytogenetics:

46,XY,i(8)(q10),-17,+r[4]/46,XY[22] → gain of MYC (on 8q) and loss of one TP53 copy (on 17p)

NGS:

Gene	Transcript	cNomen	pNomen	VAF (%)	ID
TP53	NM_000546	c.524G>A	p.(Arg175His)	42	COSM10648

Pathology:

No alterations in cyclin D1/D2/D3 or SOX11 – MCL unlikely

What's your preferred diagnosis?

Activities

Visual settings

Edit

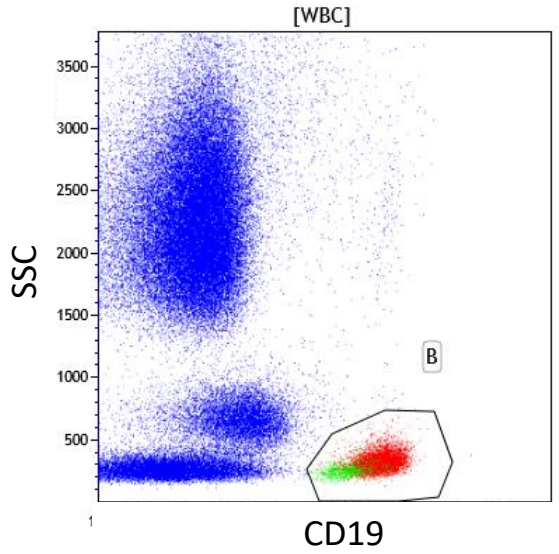
What's your preferred diagnosis? You can vote on the disgnoses submitted.

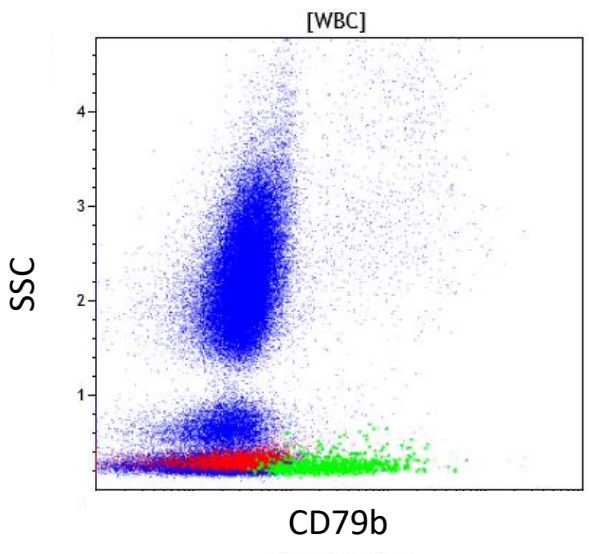
0

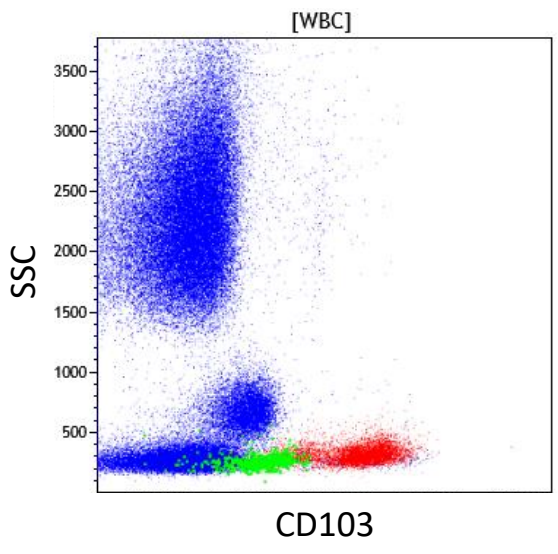
Nobody has responded yet.

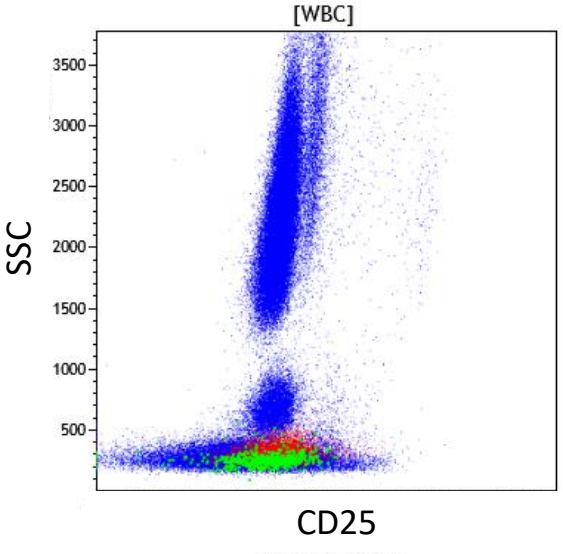
Hang tight! Responses are coming in.

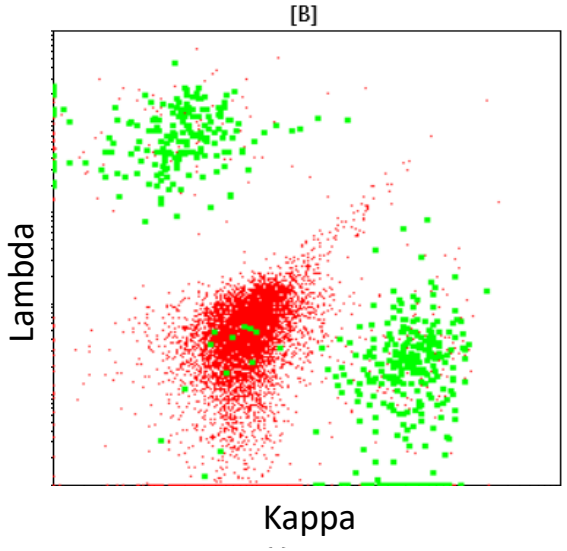
Powered by Poll Everywhere





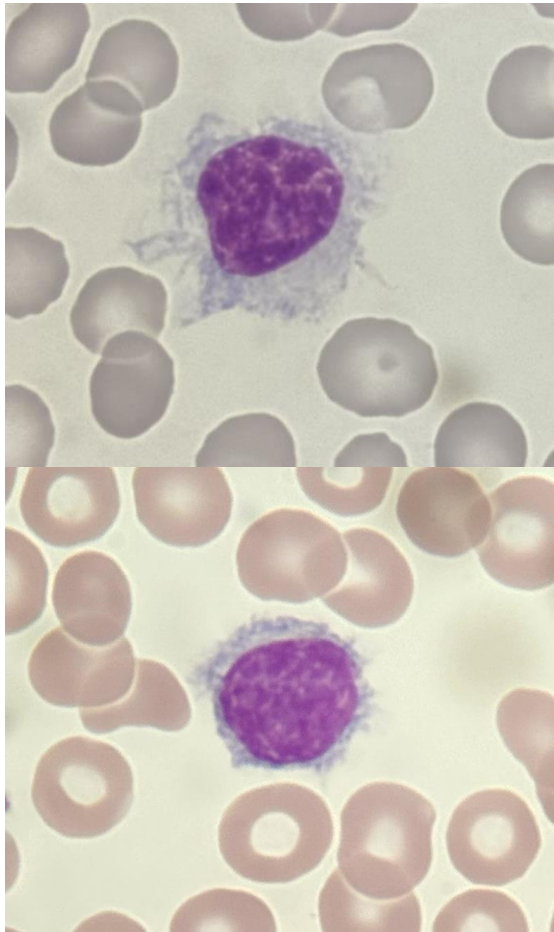






CD5-
CD11c+
CD22++
CD20++
CD200 wk
CD43-

BRAF negative



What's your preferred diagnosis?

Activities

Visual settings

Edit

And how would you classify this disease?

Nobody has responded yet.

Loading...

Hang tight! Responses are coming in.

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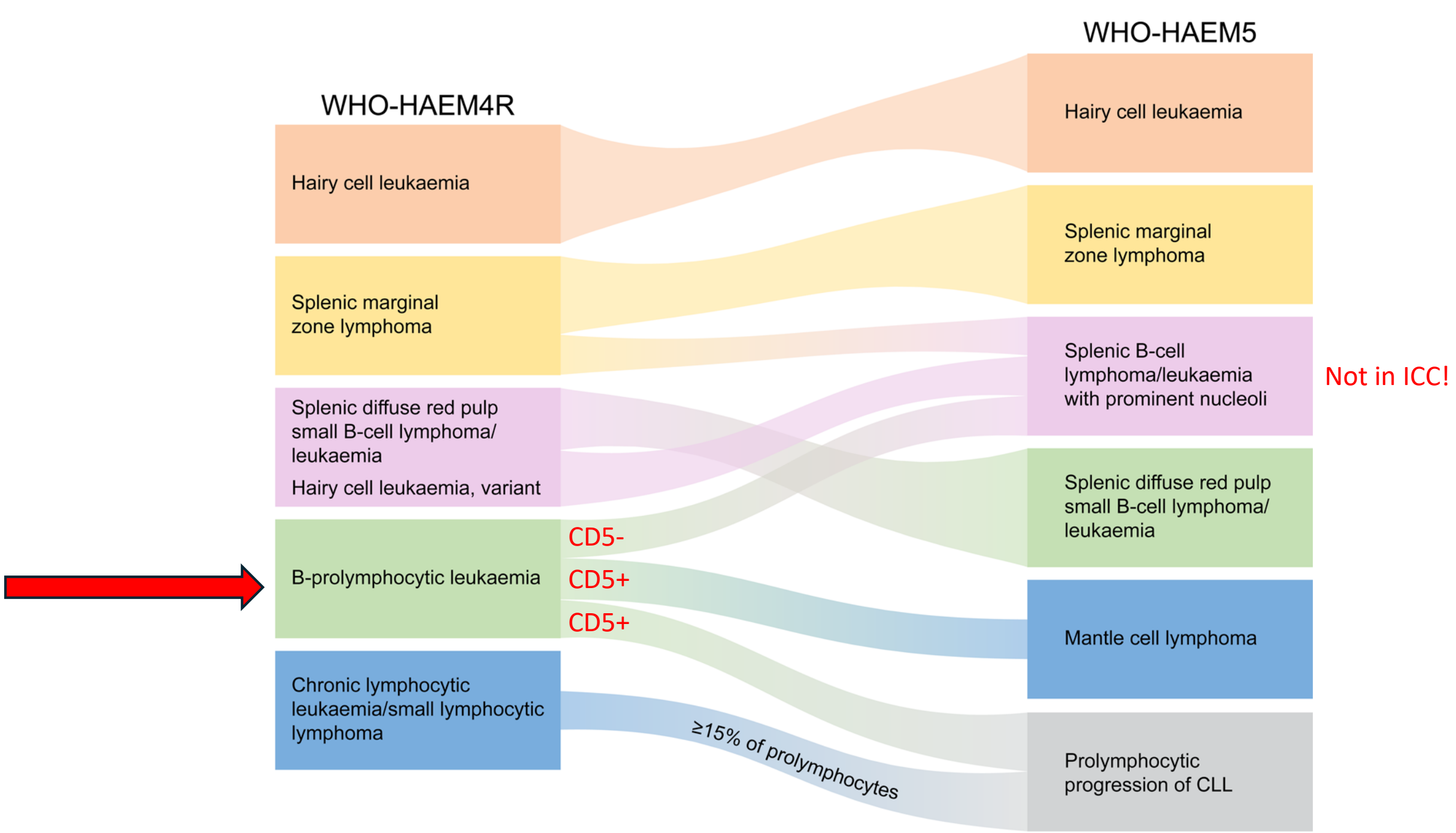
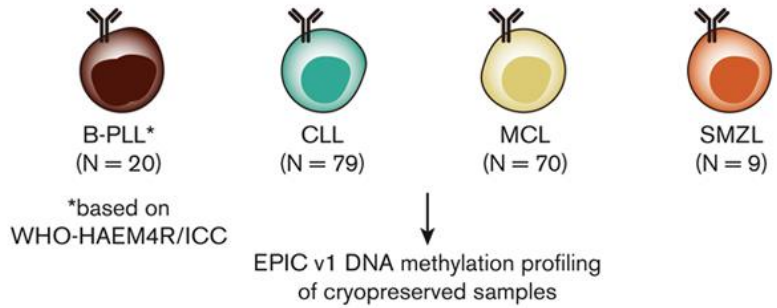
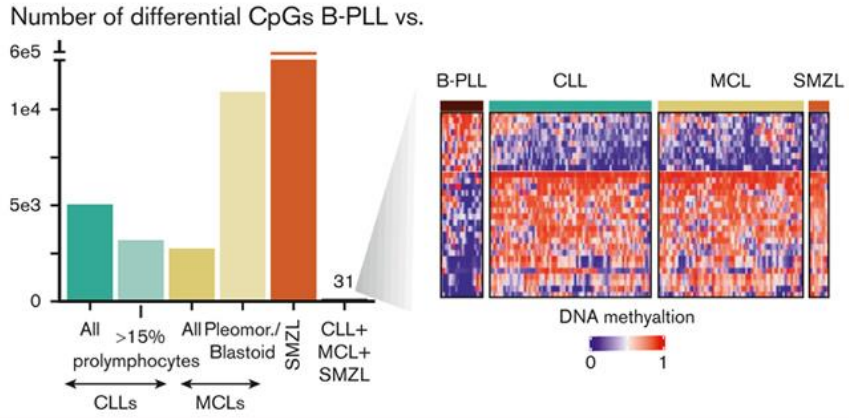
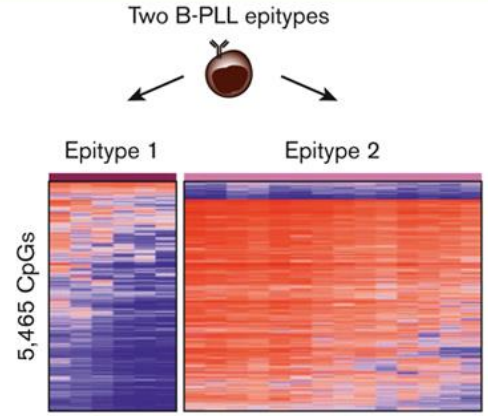


Leukemia **volume 36**, pages1720–1748 (2022)

Epigenetic features support the diagnosis of B-cell prolymphocytic leukemia and identify two clinico-biological subtypes







Differential features	Epitype 1	Epitype 2
GC* epigenetic imprint:	↑	↑↑
% IGHV SHM*:	↑	↑↑
Genetic alterations:	*MYC, SF3B1* mutations Gain 1q	
Clinical outcome:	✗✗	✗

*GC, germinal center
*SHM, somatic hypermutation

Conclusions: 1) B-PLL diagnosed with WHO-HAEM4R guidelines shows a different DNA methylome from CLL, MCL and SMZL. 2) Two B-PLL epitypes with different biological and clinical features can be identified.

*Charalampopoulou et al. DOI: 10.1182/**blooda**.2024013327*

blood advances Visual Abstract

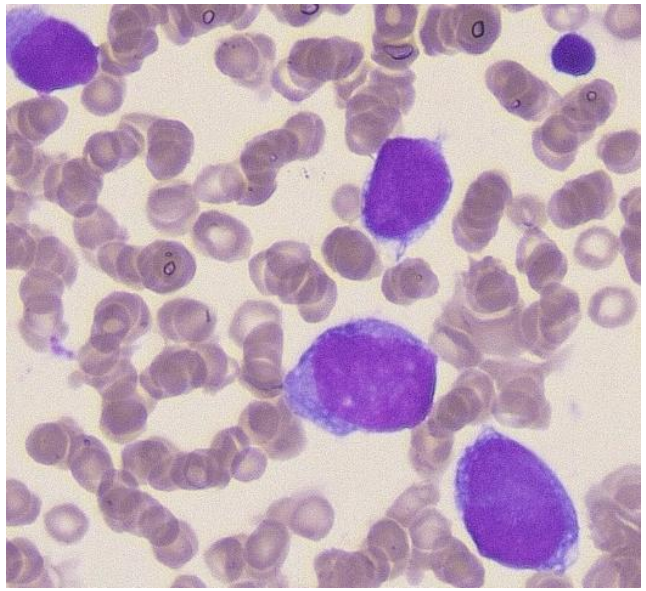
- Be carefull with assigning B-PLL to another entity (CLL, MCL, etc):
  - Interdisciplinary diagnosis
- CD5- B-PLL ≠ HCL-variant?
  - WHO criteria not clear
- Prolymphocytic progression of CLL is continuous with indolent CLL, but distinct from B-PLL
- More research needed: probably a spectrum of B-cell disorders, not as black and white as our classification systems

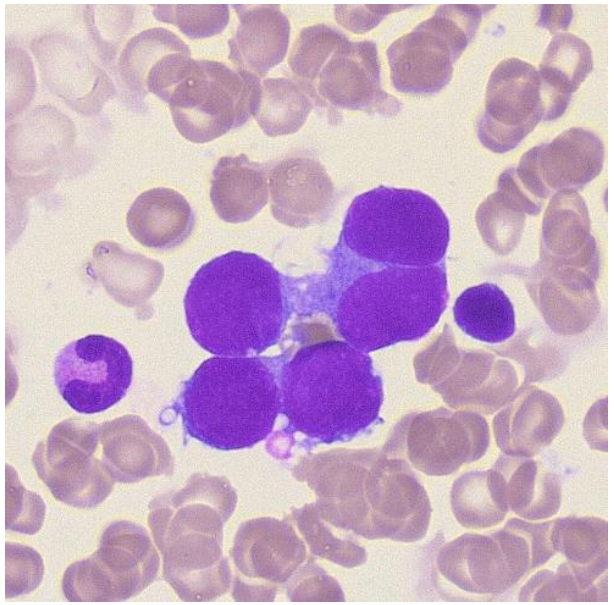
Take home message:

- Cases formerly known as B-PLL need careful, interdisciplinary classification
- Probably partly overlapping with CLL, MCL and HCL-v (SBLPN), but also distinct features

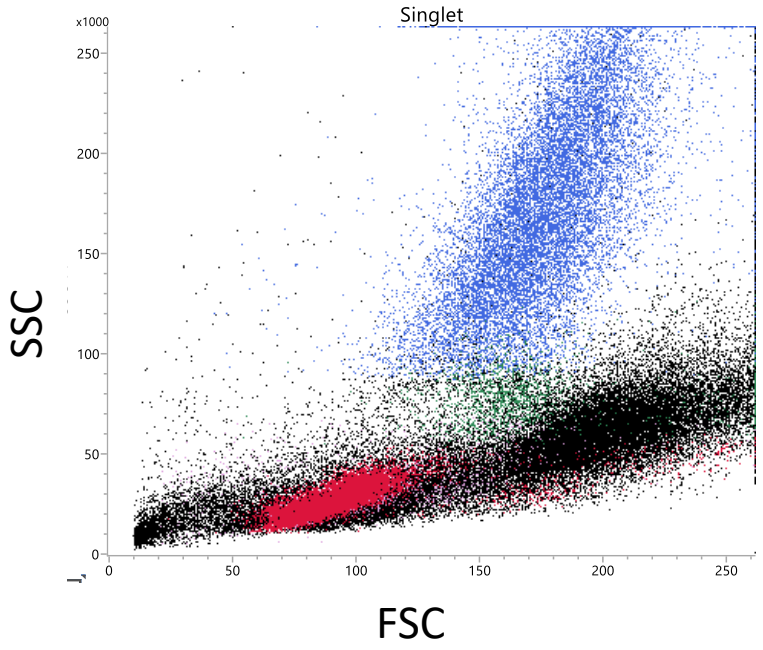
Case 4

- Man, 19y
- Medical history: /
- Presentation: nasopharyngeal swelling, blurred vision + exophtalmia, no B-symptoms
- CT-scan: mass in nasal cavity, slight hepatomegaly
- PB: anemia, thrombocytopenia, normal leukocytosis & differentiation.
- BM: 83% blastic cells:





Case 4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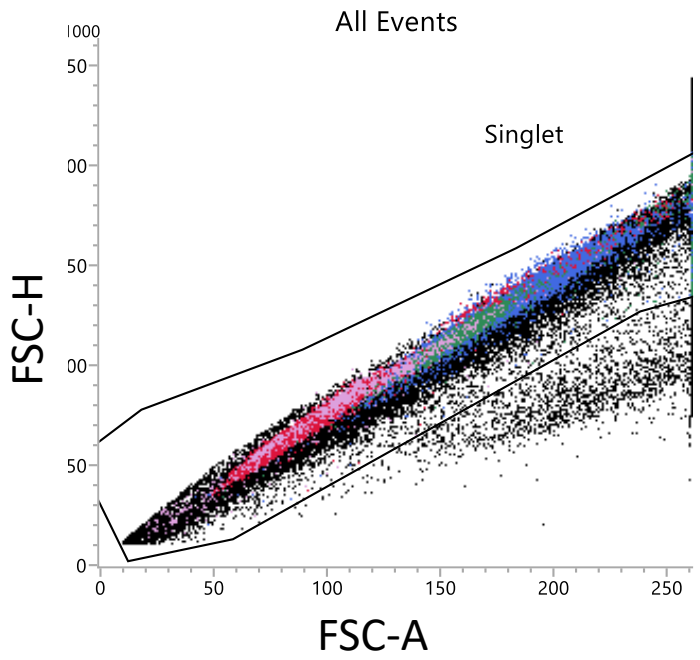
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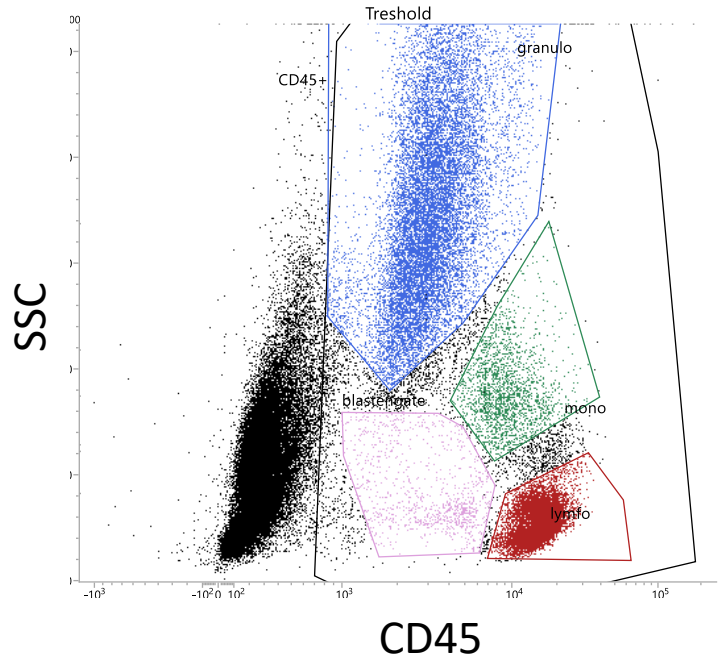
Treshold
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CD45+
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CD45

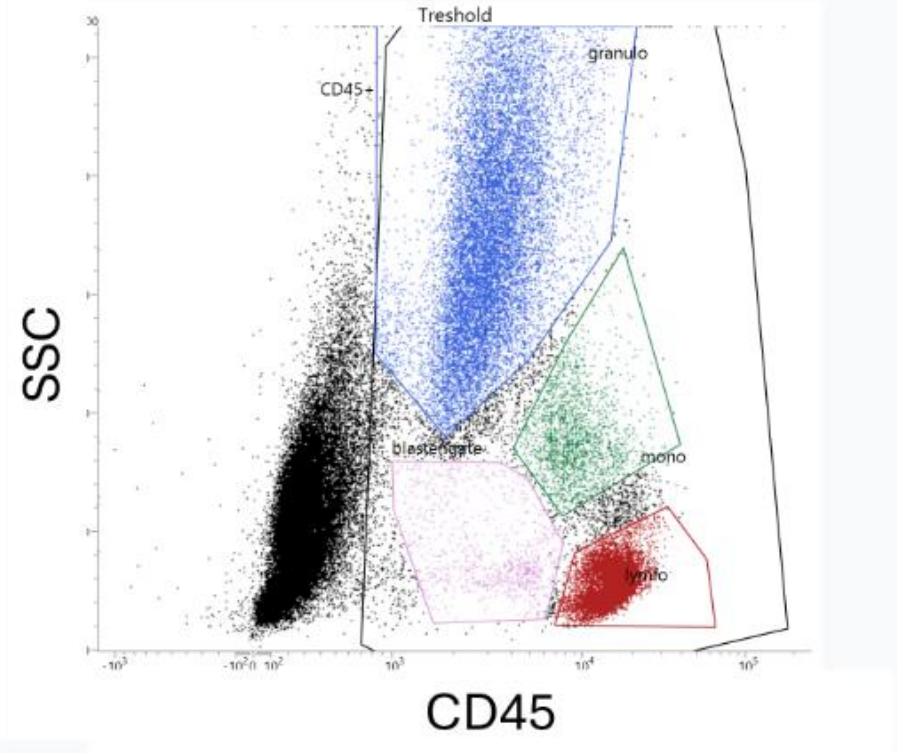
Activities

Visual settings

Edit

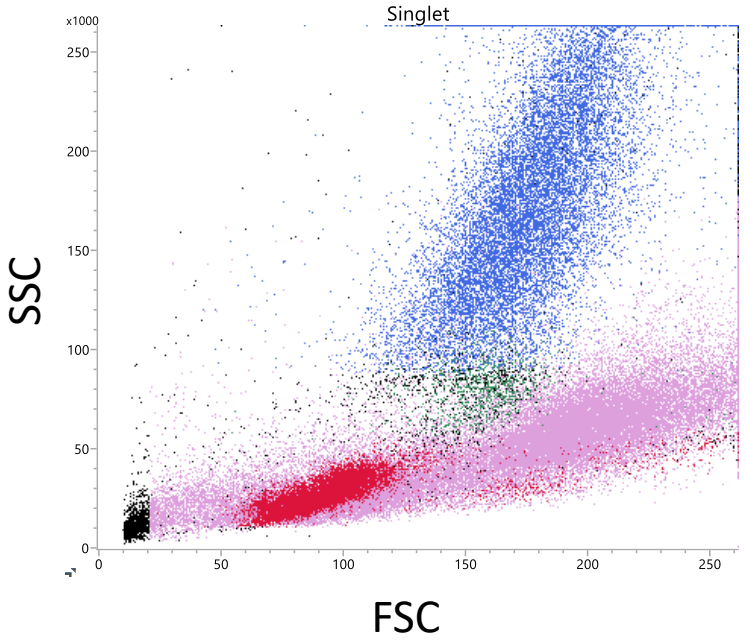
Click on the abnormal population

0



Powered by Poll Everywhere

Case 4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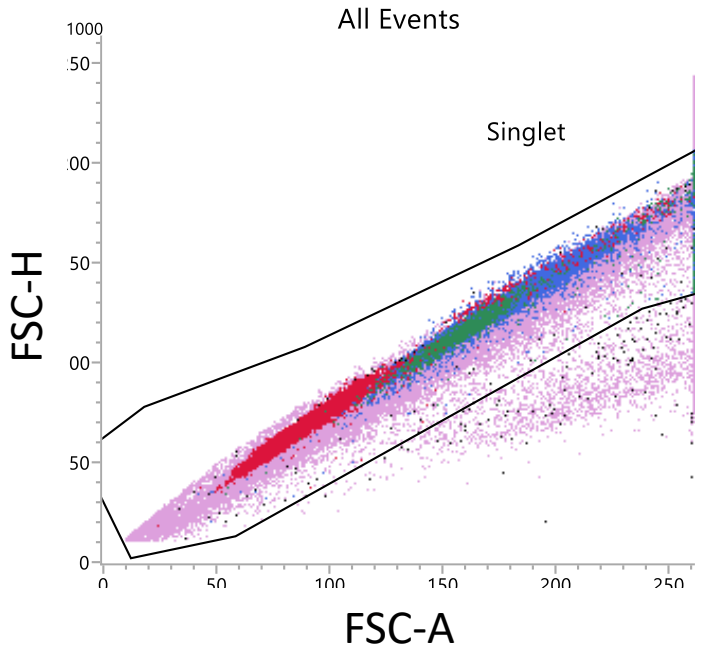
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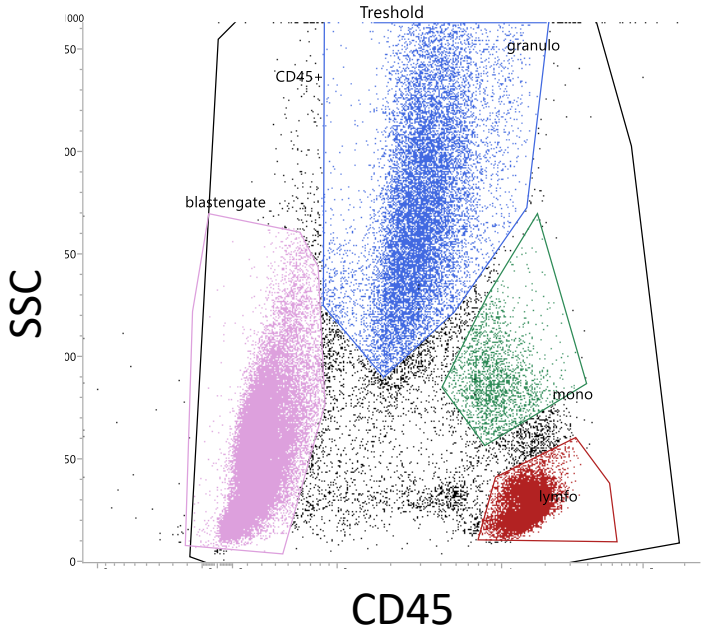
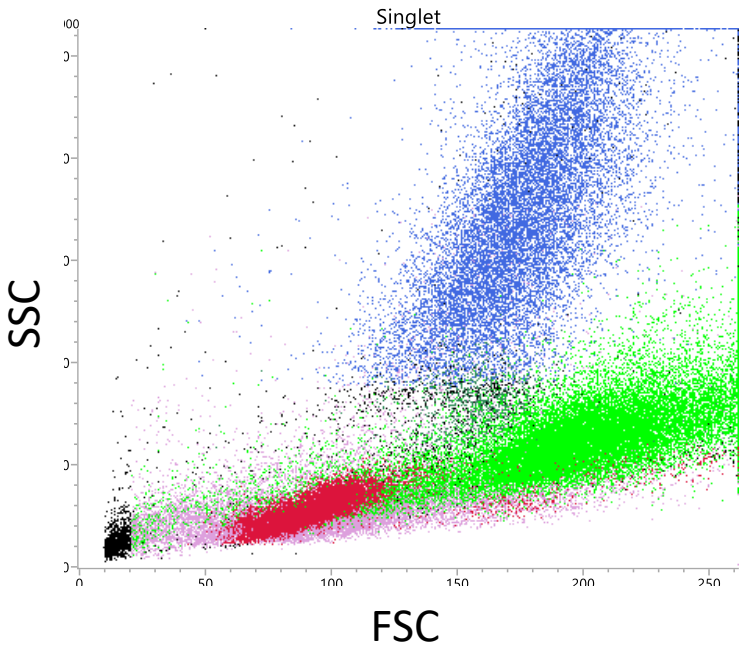
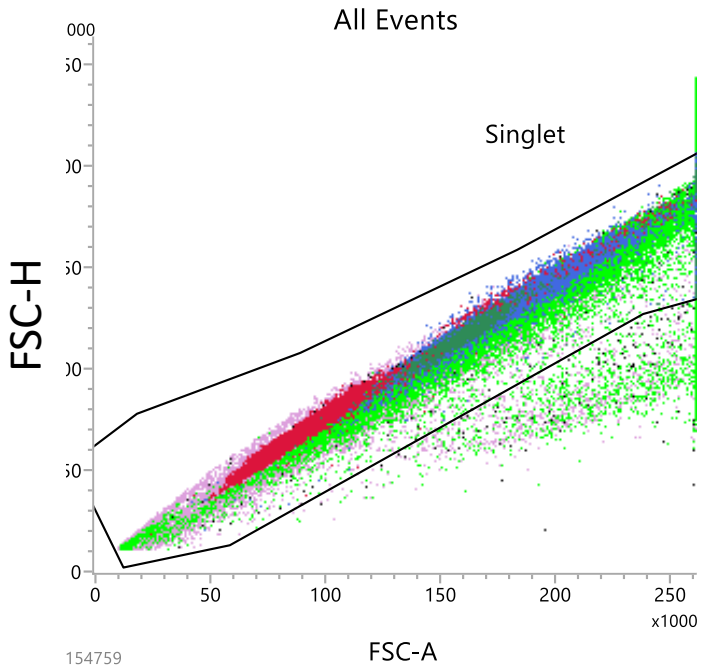
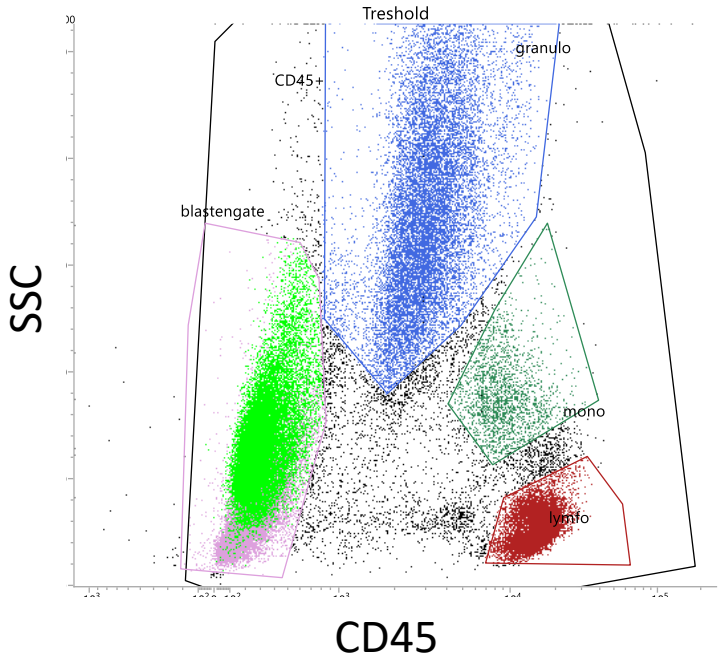

Treshold
granulo
CD45+
blastengate
mono
lymfo
SSC
CD45

Case 4







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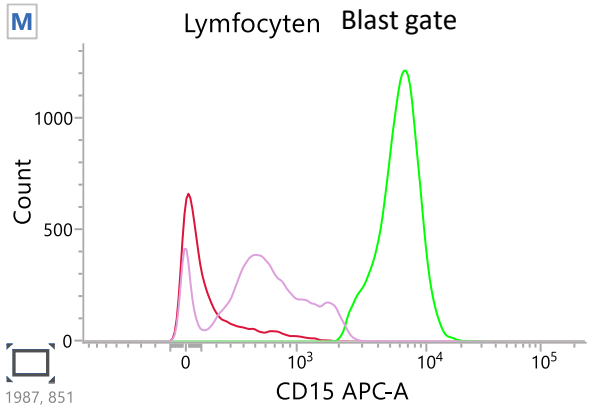
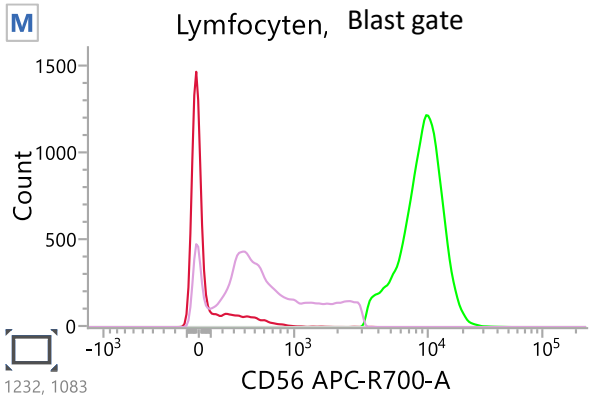
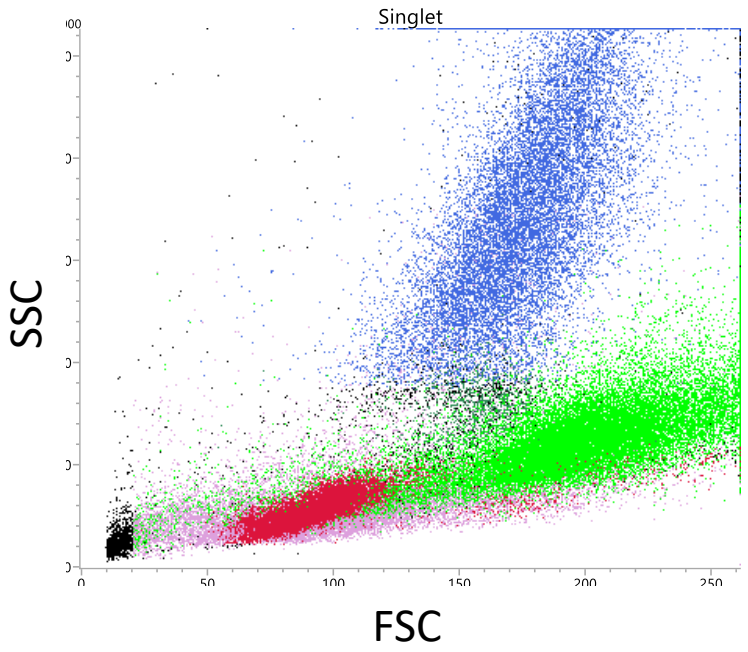
cyCD3/sCD3/CD4/CD10/CD13/CD14/CD15/CD16/CD20/CD22
/CD30/CD34/CD35/CD41CD61/CD64/CD43/CD45/
CD56/CD57/CD117/CD123/HLA-DR/TdT/cyTCL1/cyMPO

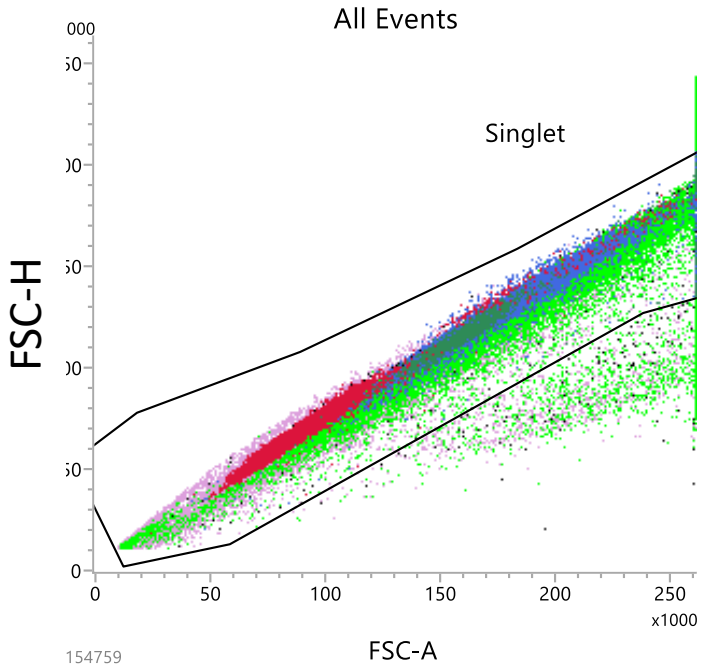
- Pink: all markers negative
- Green: all markers negative, except CD15 & CD56

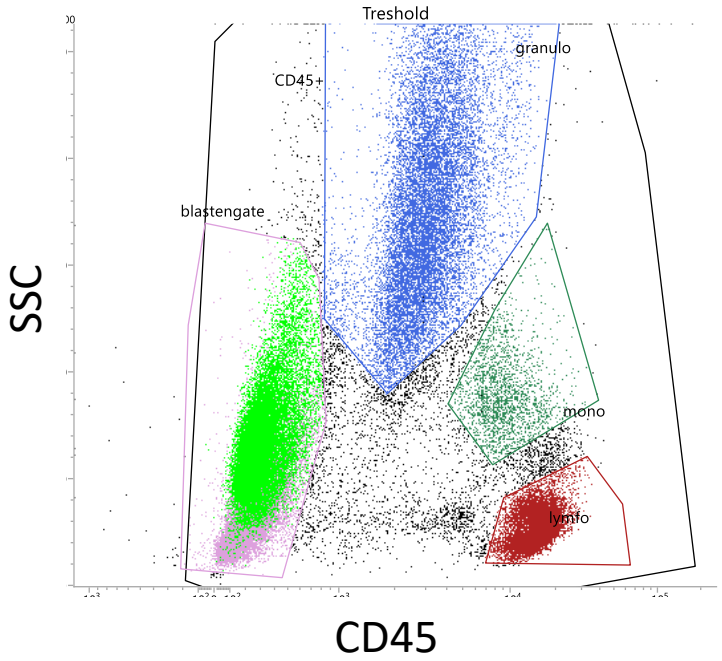




Case 4







Markers evaluated:
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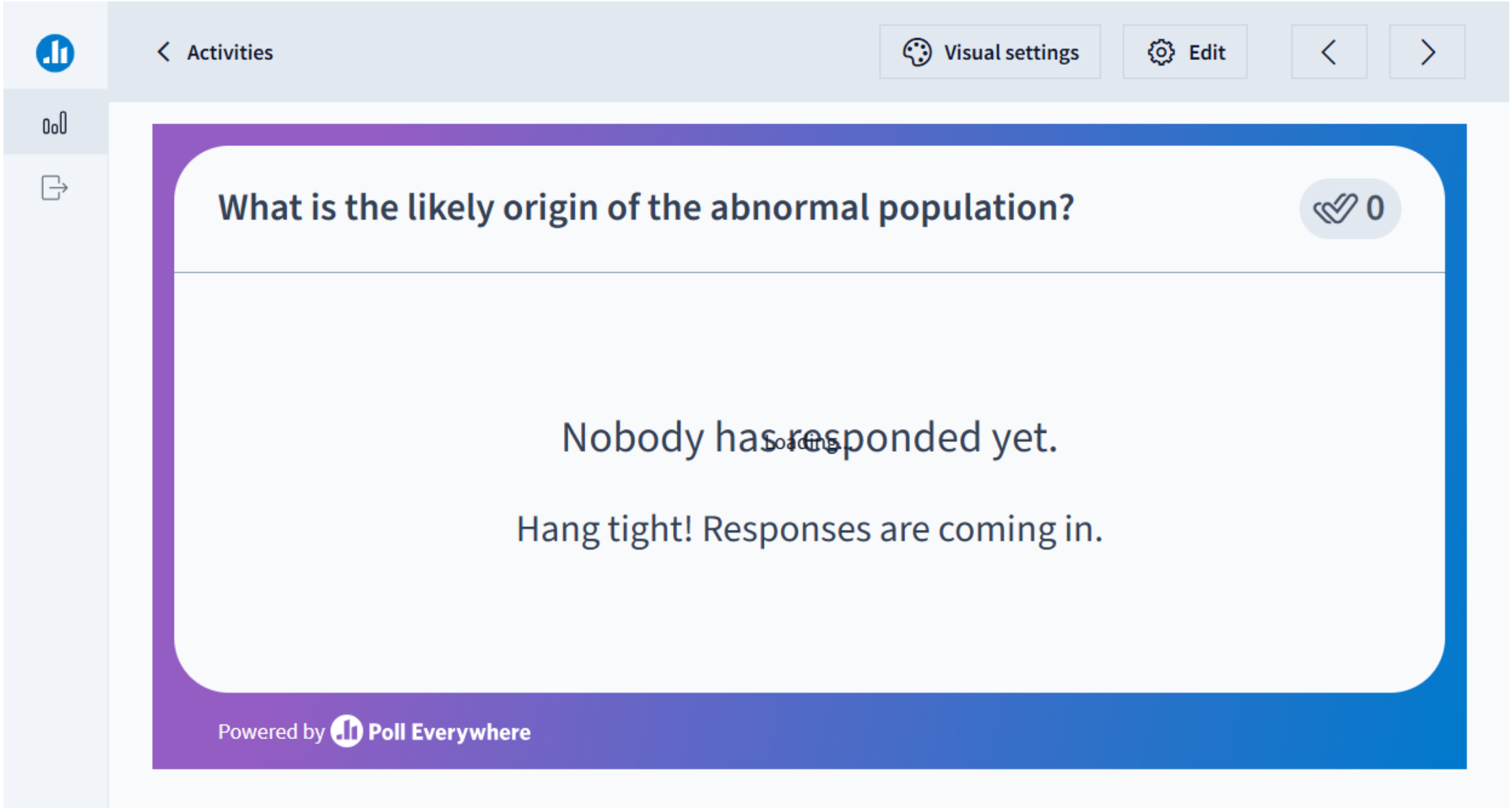
/CD30/CD34/CD35/CD41CD61/CD64/CD43/CD45/
CD56/CD57/CD117/CD123/HLA-DR/TdT/cyTCL1/cyMPO

What is the most likely origin of the green population?

- Pink: all markers negative
- Green: all markers negative, except CD15 & CD56

CD15 & CD56 positive



Case 4

- IH: CD56+/vimentin+/desmin+/muscle-specific actin/myogenin
- FISH: FOXO1 rearrangement

Non-hematological tumor: rhabdomyosarcoma

- A rare malignant soft tissue sarcoma made up of cells that normally develop into skeletal muscle tissue.
- Occurs most frequently in adolescents, but can affect all age groups.
- Most often presents in the head and neck region.
- Bone marrow involvement is found in 15% of patients.
- Difficult to distinguish from acute leukemia owing to the presence of cytopenia and BM infiltration by blast-like cells with a loosely arranged distribution.

Other rare non-hematological malignancies that have been reported to display an AL-like morphology in BM (and often CD56+):

- Neuroblastoma
- Medulloblastoma
- Anaplastic oligodendroglioma
- Small cell carcinoma
- Ewing's sarcoma
- Neuroendocrine tumors

Case 4

ChatGPT 5.2

Is CD15 expressed on non-hematological cells?

Yes — **CD15 is primarily expressed on hematological cells, but it can also be found on certain non-hematological cells.**

🔬 What is CD15?

CD15 (Lewis X antigen, SSEA-1) is a carbohydrate antigen mainly known for expression on:

- Neutrophils (strong expression)
- Other granulocytes
- Some monocytes
- Reed–Sternberg cells in classical Hodgkin lymphoma

🧬 Expression on non-hematological cells

1️⃣ Normal tissues

CD15 can be expressed on certain **non-hematopoietic cells**, including:

- Some **epithelial cells** (e.g., gastrointestinal epithelium)
- Certain **endothelial cells**
- **Embryonic cells** (as SSEA-1)

Expression in normal non-hematologic tissues is usually **weaker and context-dependent** compared to granulocytes.

2️⃣ Non-hematological tumors

CD15 may be expressed in several **epithelial malignancies**, such as:

- Lung carcinomas

Is CD15 expressed on rhabdomyosarcoma?

Short answer: **No — CD15 is not typically expressed in rhabdomyosarcoma.**

🔬 Immunophenotype of Rhabdomyosarcoma (RMS)

Rhabdomyosarcoma is a skeletal muscle–lineage tumor. The characteristic markers are:

- **Desmin**
- **Myogenin**
- **MyoD1**
- Often **vimentin**

CD15 is **not part of the standard immunophenotypic profile** of RMS.

🧪 Can CD15 ever be positive?

- Rare cases may show **focal or weak staining**
- Staining may reflect **background granulocytes**
- Occasional nonspecific reactivity can occur

However:

- CD15 expression in RMS is **not consistent**
- It has **no diagnostic utility** for RMS
- Strong CD15 positivity should prompt consideration of other entities

---

✅ Bottom line

CD15 is **not a typical marker for rhabdomyosarcoma**.

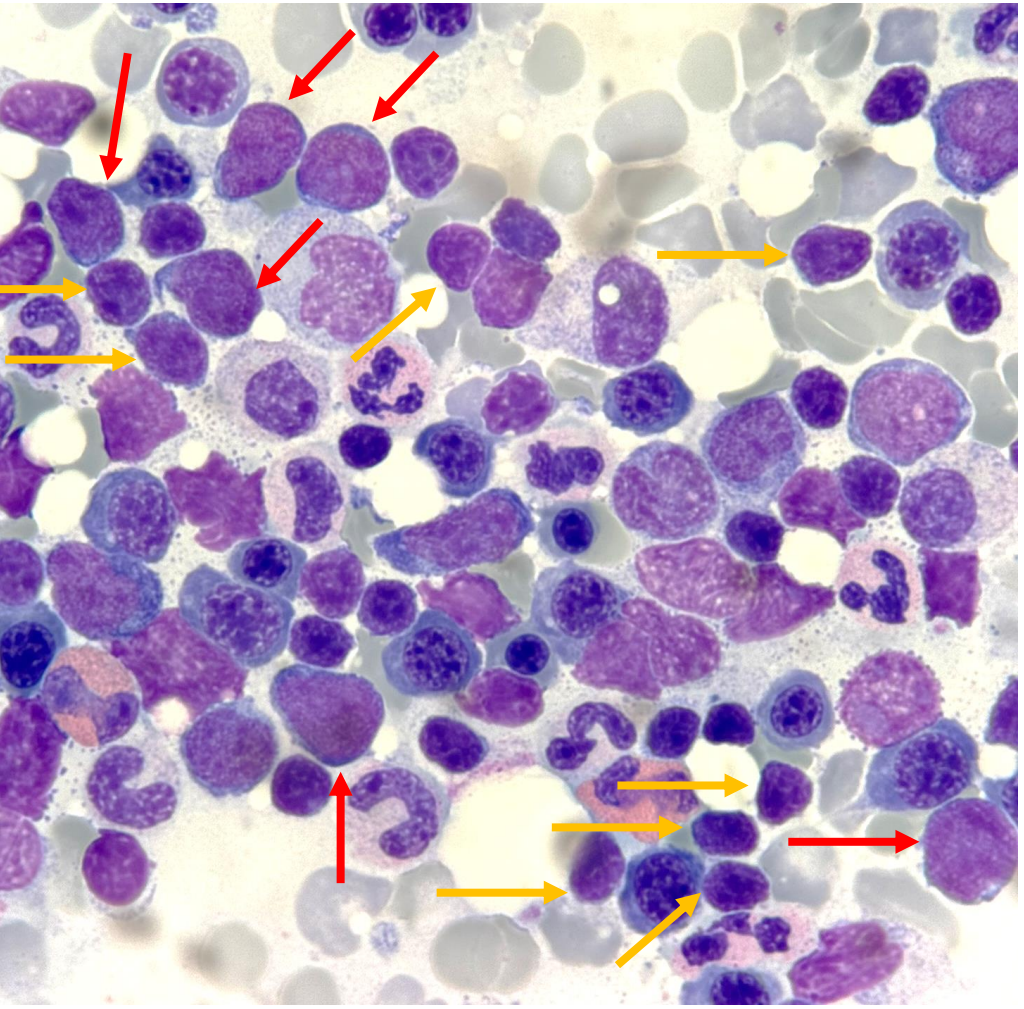
If a tumor suspected to be RMS shows strong CD15 expression, alternative diagnoses (e.g., Hodgkin

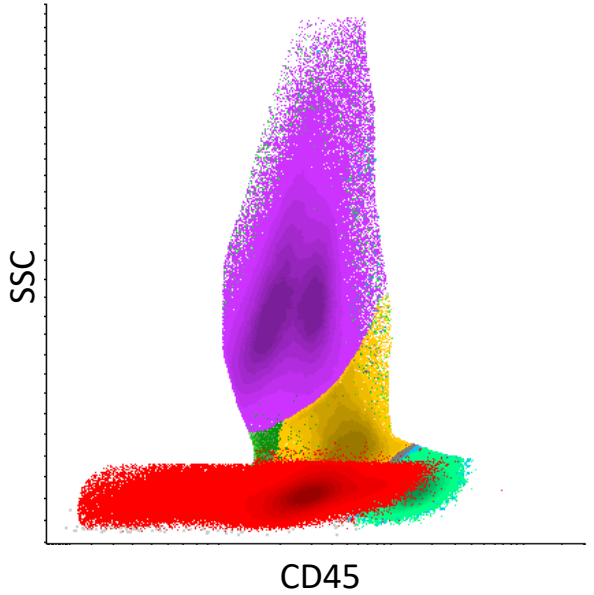
Case 4

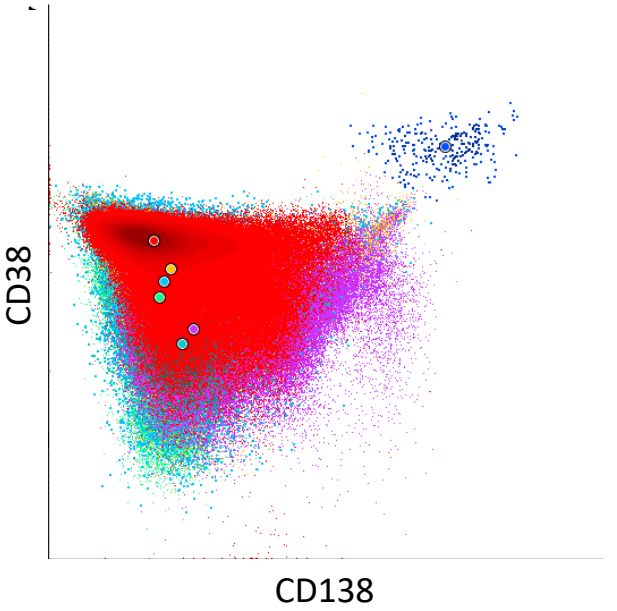
Take home message:

- Exclude doublets
- Not all blastic cells are blasts!
- Not all hematological cells are CD45+

Case 5







- Follow-up Multiple Myeloma
- Autologues stem cell transplantation
- Maintenance lenalidomide
- No MRD MM

What flow panel would you like to see next?

Activities

Visual settings

Edit

What additional flow panel would you like to see next

0

AML

No additional flow needed

Lymphatic screening

Plasma cell

B-ALL

Acute Leukemia Screening

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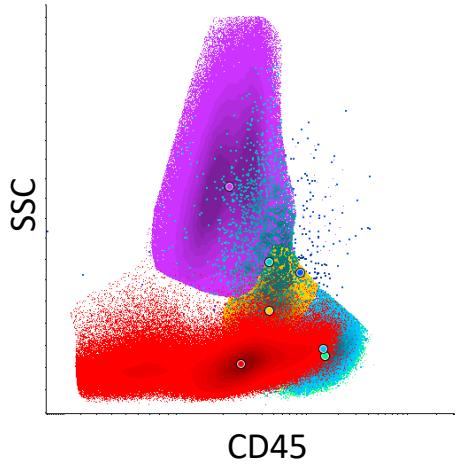
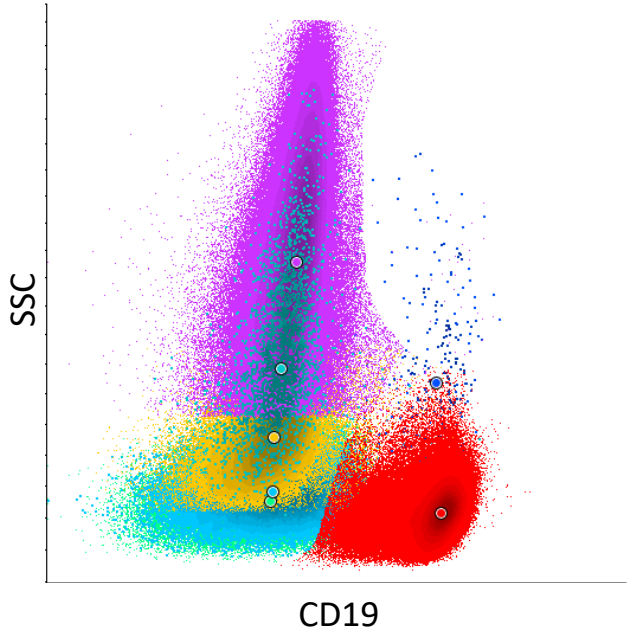
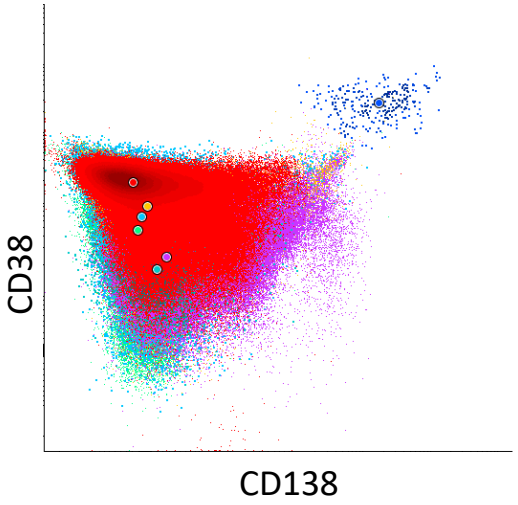
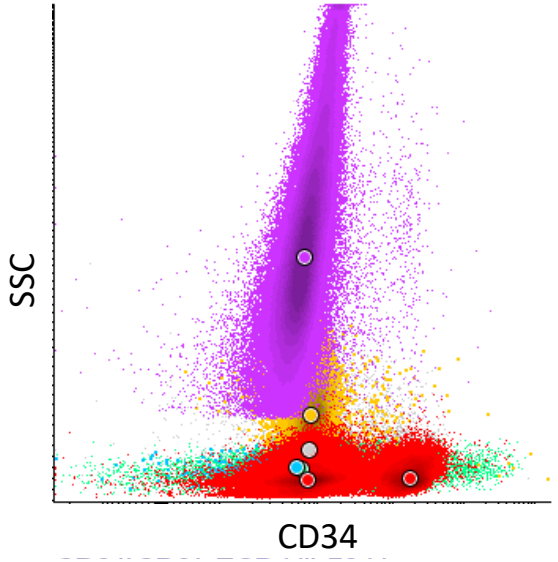


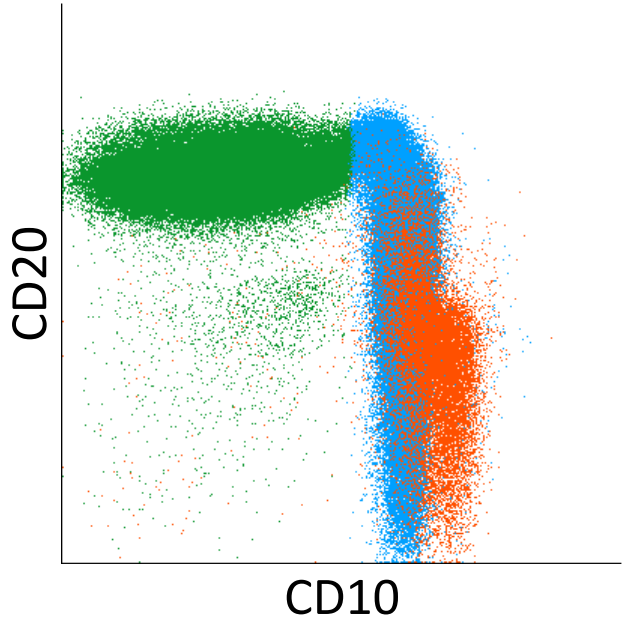


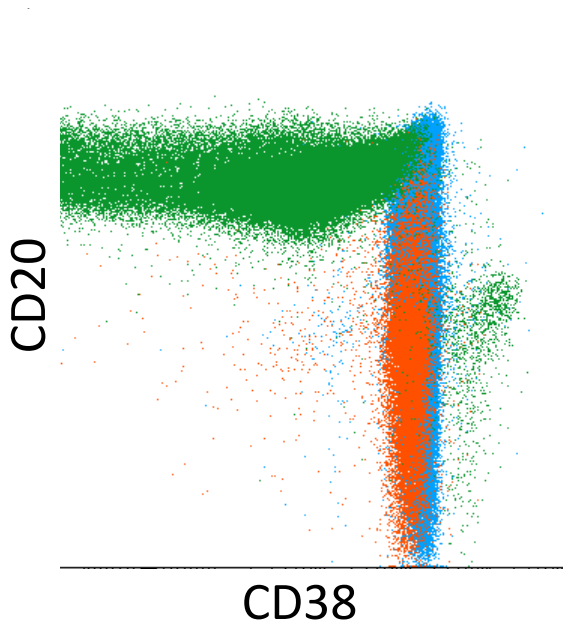


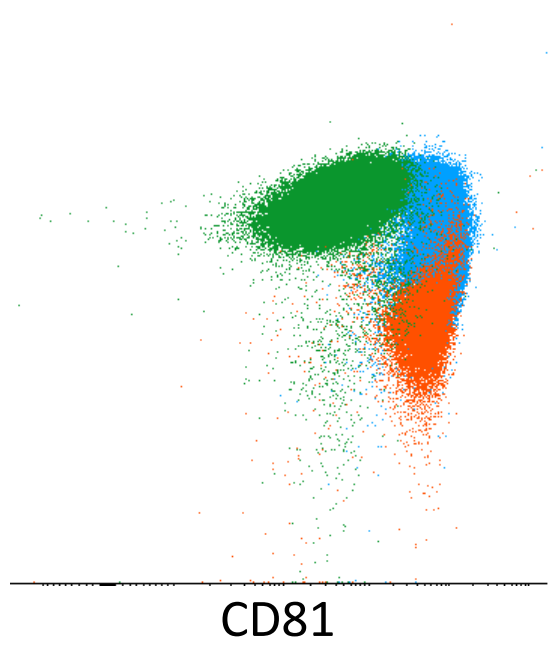


Red population: 50%

Normal BM





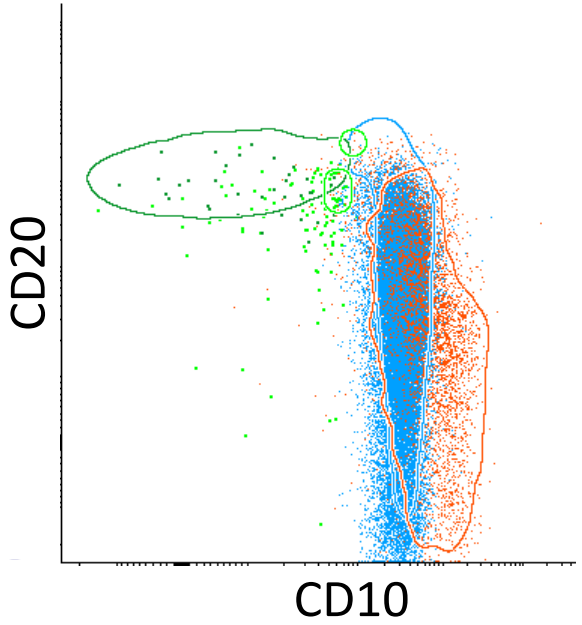


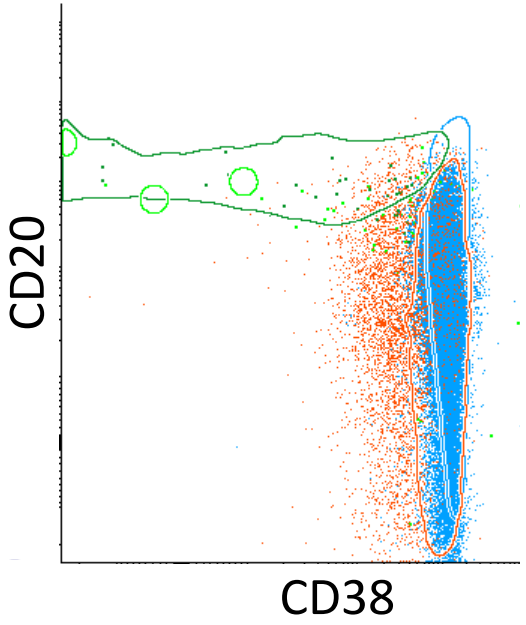
CD34+/CD10+/CD20-

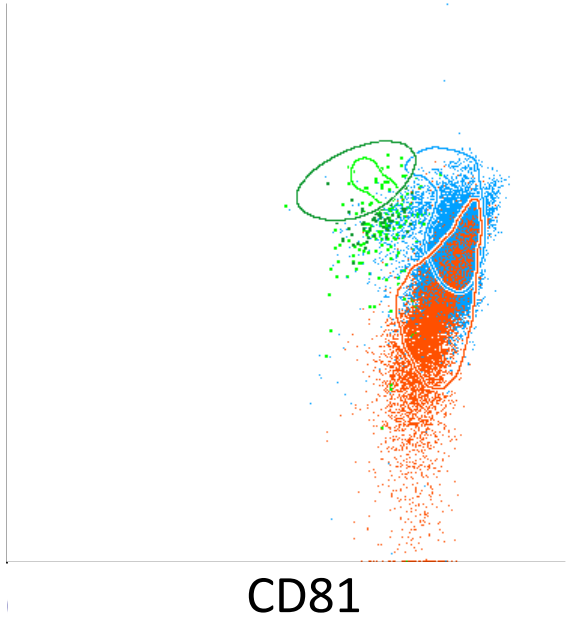
CD34-/CD10+/CD20-

CD34-/CD10-/CD20+

Patient BM







What's your final verdict?

Activities

Visual settings

Edit

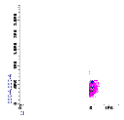
It's B-ALL!?

0

True

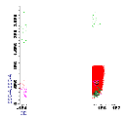
False

Powered by Poll Everywhere



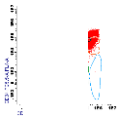
SSC

CD45



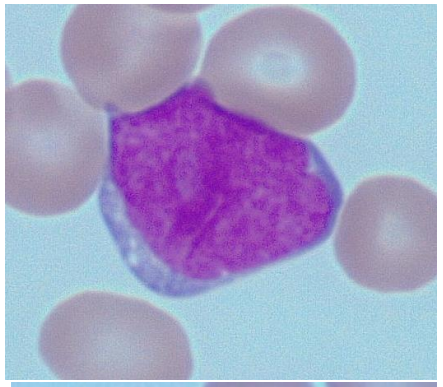
SSC

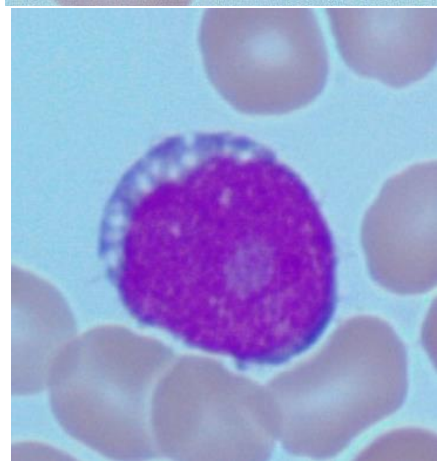
CD19

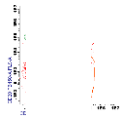


SSC

CD81

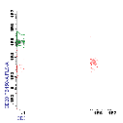






CD20

CD10



CD20

CD38

Case 5B

- Follow-up Multiple Myeloma
- Autologues stem cell transplantation
- Maintenance lenalidomide
- No MRD MM

- TP53 mutated B-ALL

So case 5A, is no B-ALL, 5B is

5A: Transient increased number of normal hematogones after lenalidomide – typically with maturation block

Be aware of increased blasts in Multiple Myeloma

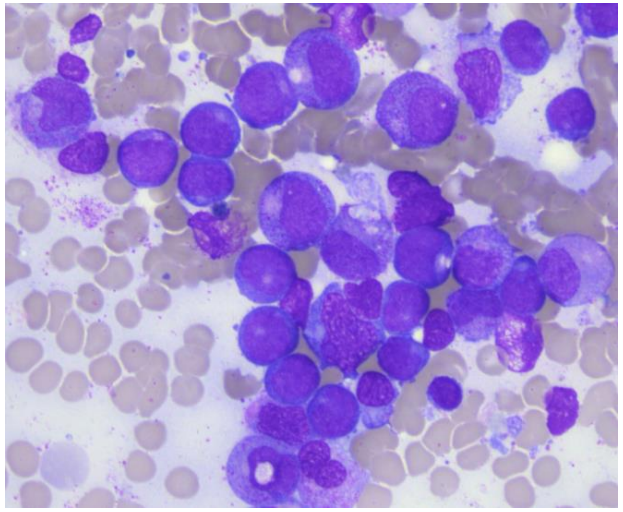
- Secondary MDS/AML can be seen in MM-patients
- Lenalidomide can induce B-ALL, many TP53, IKZF1 or IDH2 mutated (hypothetical due to pressure on B-cell genesis in the presence of CHIP-markers

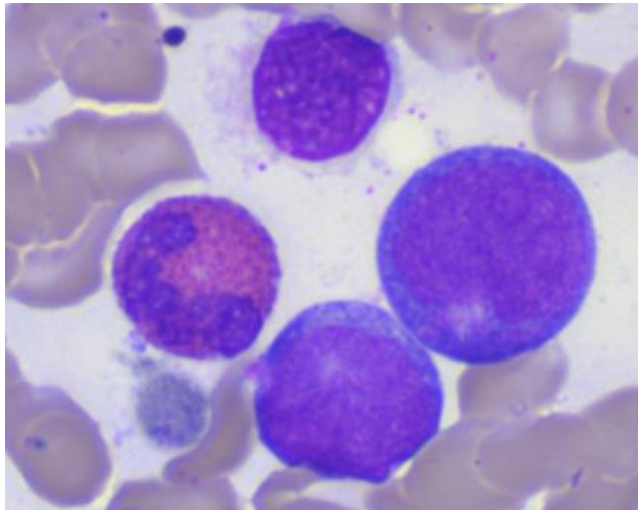
Take home message:

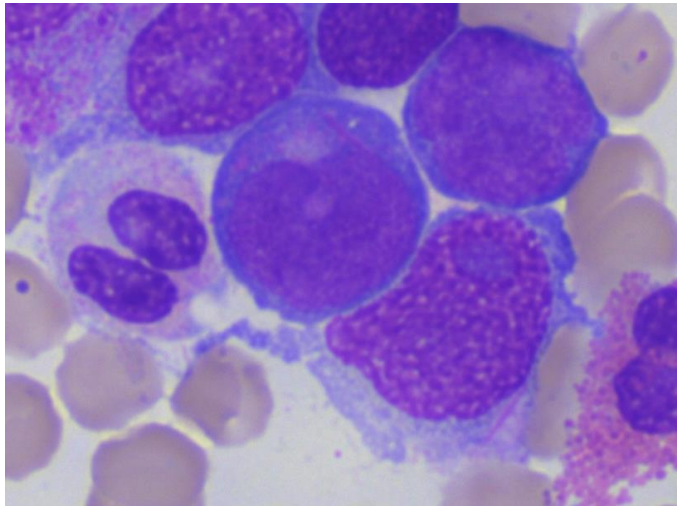
- Vast increase of normal B-cell precursors can be observed with lenalidomide (even up to 50%)
- Knowledge of normal B-cell maturation is essential in these cases

Case 6

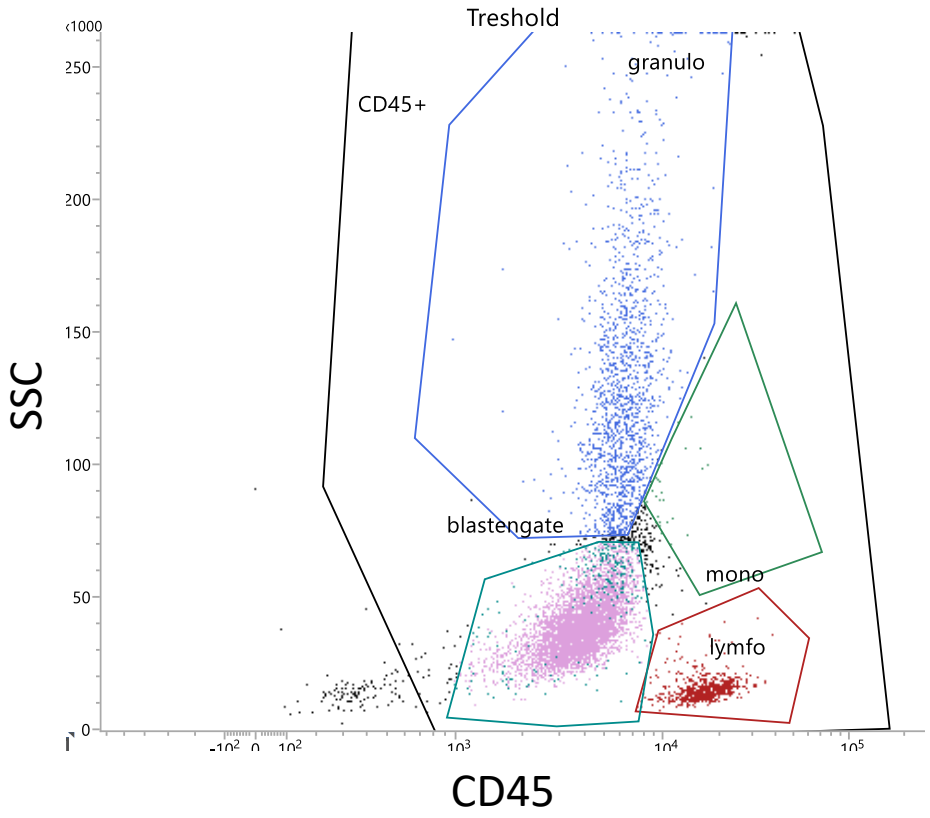
- Man, 46y
- Clinic: Fever, left flank pain: urologic inflammation?
- PB: anemia, thrombocytopenia, normal leukocytosis but 45% blasts
- BM: 74% blasts

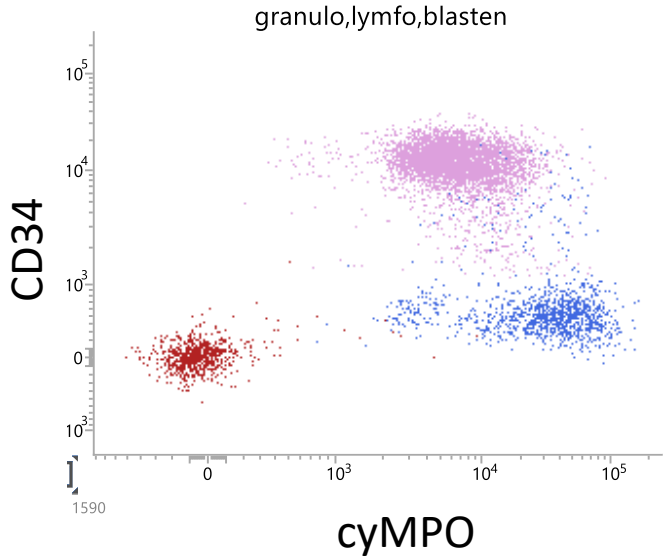


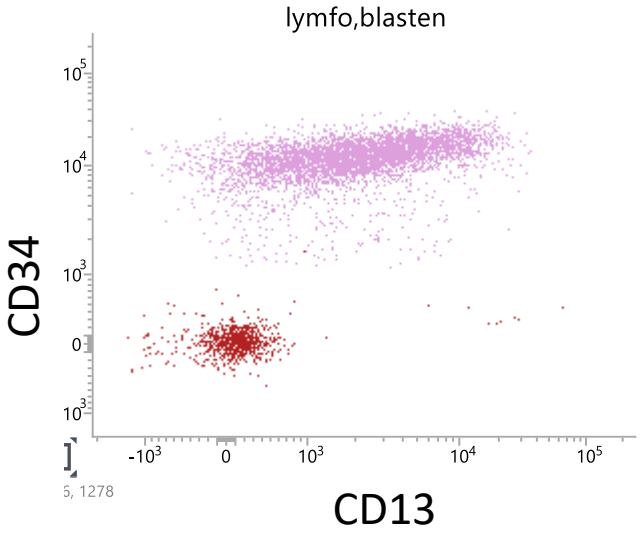


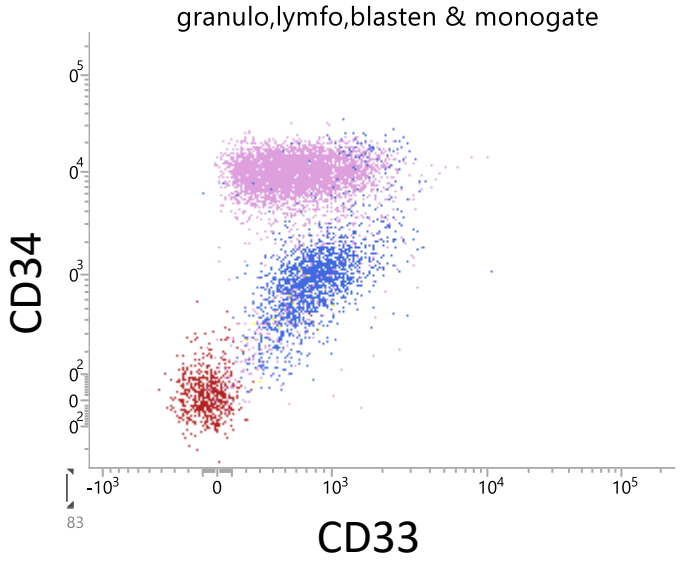


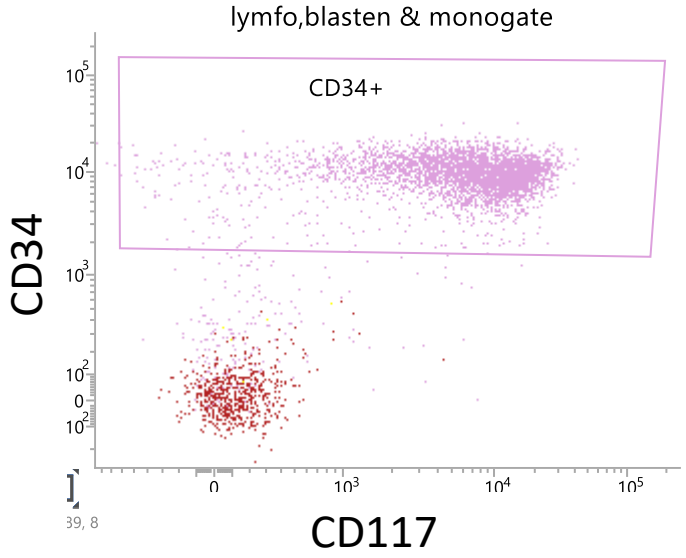
Case 6


Treshold
granulo
CD45+
blastengate
mono
lymfo
SSC
CD45

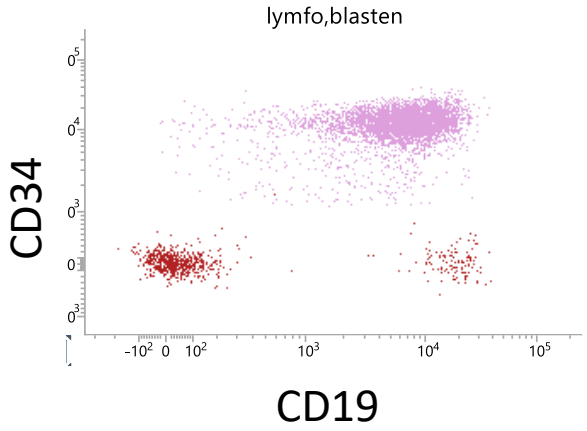

granulo,lymfo,blasten
CD34
cyMPO

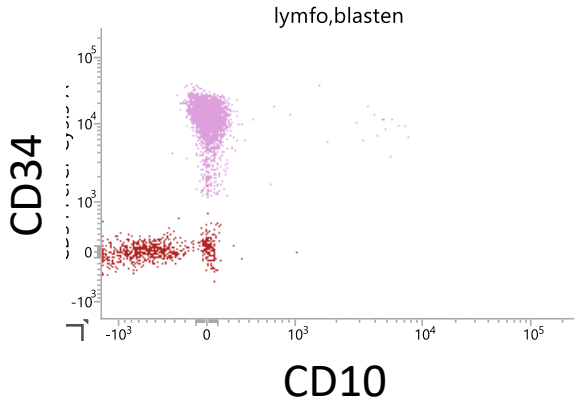

lymfo,blasten
CD34
CD13

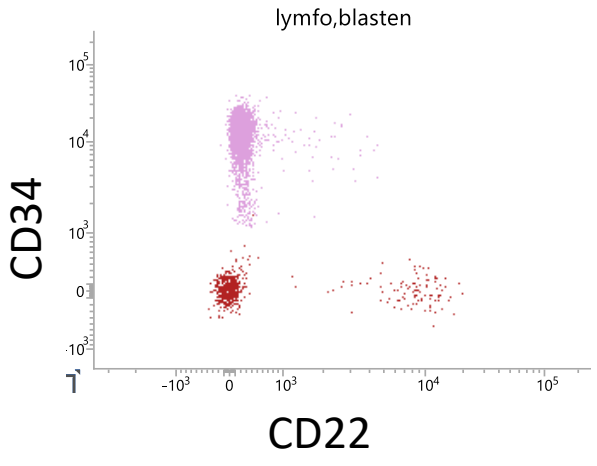

granulo,lymfo,blasten & monogate
CD34
CD33


lymfo,blasten & monogate
CD34+
CD34
CD117

Case 6







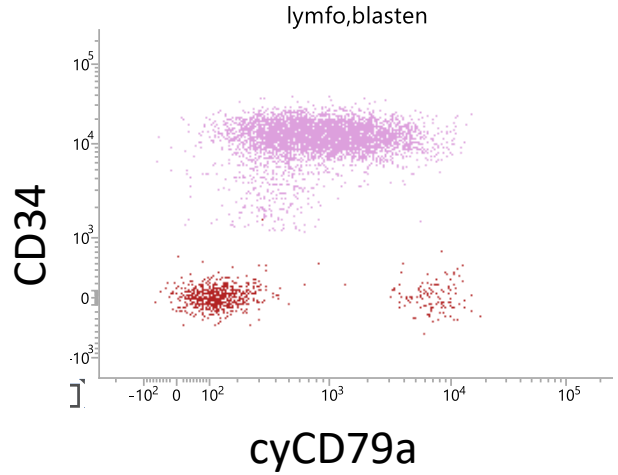


Table 2.25 Lineage assignment criteria for mixed-phenotype acute leukaemia

Lineage	Criterion
B lineage	
CD19 strong[a]	One or more of the following also strongly expressed: CD10, CD22, CD79a[c]
OR	
CD19 weak[b]	Two or more of the following also strongly expressed: CD10, CD22, CD79a[c]
T lineage	
CD3 (cytoplasmic or surface)[d]	Intensity in part exceeds 50% of that of mature T cells by flow cytometry **OR** Immunocytochemistry positive with non–ζ-chain reagent
Myeloid lineage	
Myeloperoxidase	Intensity in part exceeds 50% of mature neutrophil level
OR	
Monocytic differentiation	Two or more of the following expressed: nonspecific esterase, CD11c, CD14, CD64, lysozyme

[a]CD19 intensity in part exceeds 50% of that of normal B-cell progenitor by flow cytometry. [b]CD19 intensity does not exceed 50% of that of normal B-cell progenitor by flow cytometry. [c]Provided T lineage is not under consideration; if it is, CD79a cannot be used. [d]Using CD3 epsilon-chain antibody.

Which type of acute leukemia?

- Myeloid
- B-lymphoid
- MPAL
- To hell with flow cytometry, let's perform molecular biology!

Activities

Visual settings

Edit

How would you classify this leukemia?

0

A) Myeloid

B) B-lymphoid

C) MPAL

D) To hell with flow cytometry,
let's perform molecular biology!

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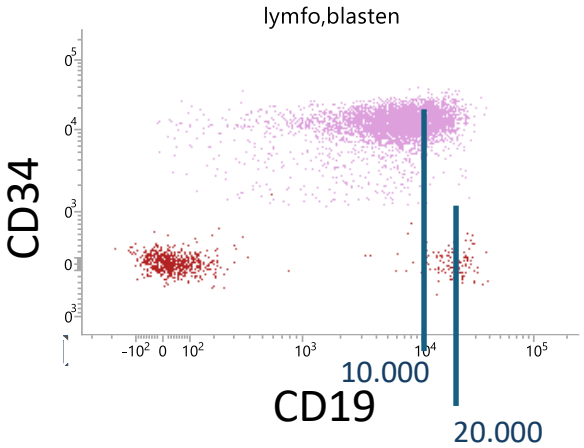
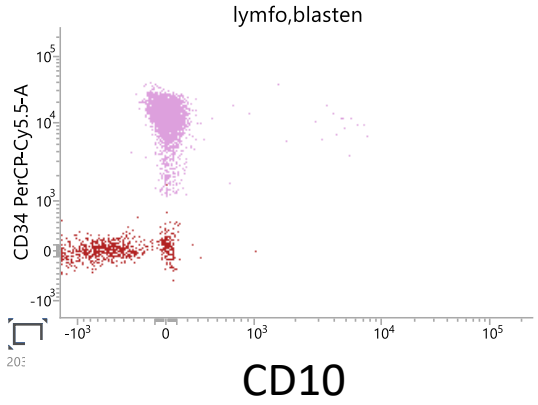
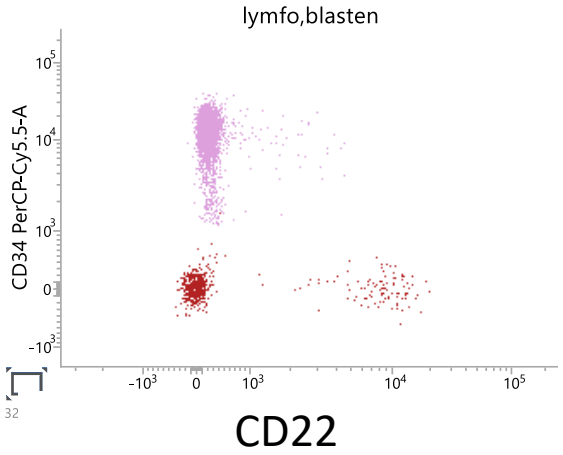
Case 6



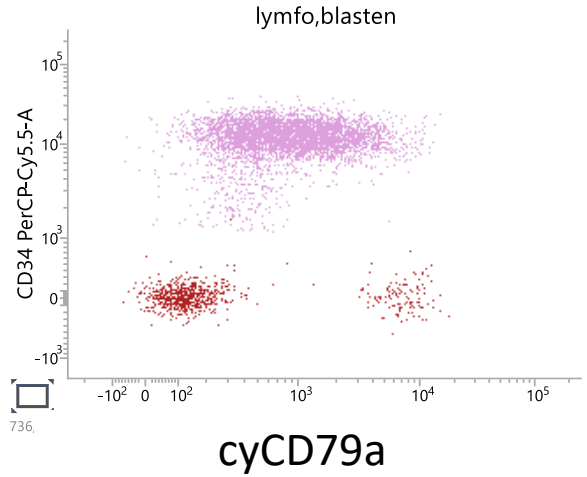
CD19 strong



CD10 neg



CD22 neg



cyCD79a (weak?)

CD19 ‘strong’:

- **WHO**: *“CD19 intensity in part exceeds 50% of that of normal B-cell progenitors”*
- **ICC**: *“CD19 intensity as seen in stage 1 B-cell precursors or mature B cells”*

No definition of ‘strong’ for CD10, CD22 or cyCD79a in either WHO or ICC

Case 6

WHO:

- Table:

Table 2.25 Lineage assignment criteria for mixed-phenotype acute leukaemia

Lineage	Criterion
B lineage	
CD19 strong[a]	One or more of the following also strongly expressed: CD10, CD22, CD79a[c]
OR	
CD19 weak[b]	Two or more of the following also strongly expressed: CD10, CD22, CD79a[c]
T lineage	
CD3 (cytoplasmic or surface)[d]	Intensity in part exceeds 50% of that of mature T cells by flow cytometry **OR** Immunocytochemistry positive with non–ζ-chain reagent
Myeloid lineage	
Myeloperoxidase	Intensity in part exceeds 50% of mature neutrophil level
OR	
Monocytic differentiation	Two or more of the following expressed: nonspecific esterase, CD11c, CD14, CD64, lysozyme

[a]CD19 intensity in part exceeds 50% of that of normal B-cell progenitor by flow cytometry. [b]CD19 intensity does not exceed 50% of that of normal B-cell progenitor by flow cytometry. [c]Provided T lineage is not under consideration; if it is, CD79a cannot be used. [d]Using CD3 epsilon-chain antibody.

- Text:

B lineage

No single antigen is entirely specific for B lineage, so it is the expression of multiple antigens in combination that provides specificity. Expression of CD19 approaching the level seen on normal B progenitors (exceeding 50% of the level seen on normal B progenitors on at least a portion of the leukaemia) has considerable specificity for B lineage, but it still should occur with expression of one or more other B-lineage antigens (CD10, CD22, or CD79a) to define B lineage. Lower CD19 expression is less specific for B lineage, and demonstration of at least two of the other B antigens listed above is required. When T lineage is

Case 6

ICC:

- Table:

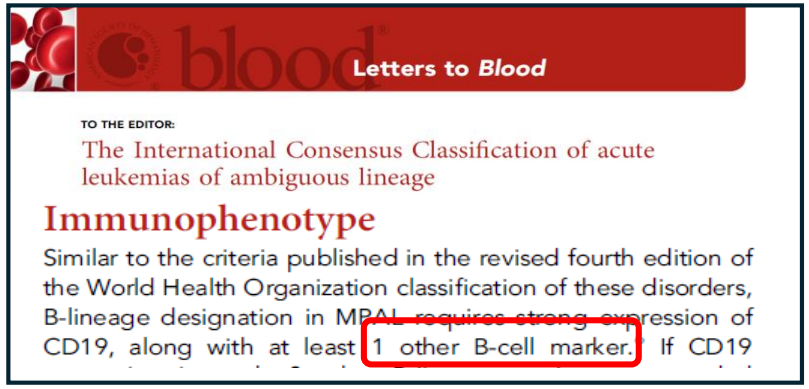
Table 39.2 Criteria for diagnosis of mixed phenotype acute leukemia with a biphenotypic pattern

B lineage		
	Strong CD19 *and*	1 or more marker expression of CD10, CD22, CD20, or CD79a
	Weak CD19 *and*	2 more strongly expressed: CD10, CD22, CD20, or CD79a
	Immunohistochemistry for B-lineage-associated markers	PAX5
T lineage		
	CD3 cytoplasmic or surface	Other T-lineage markers possible but not required
Myeloid lineage		
	MPO	
	Monocytic differentiation	NSE, CD64, CD11c, CD14, or lysozyme

MPO, myeloperoxidase; NSE, non-specific esterase.

- Text:

B-cell component of MPAL: Following previously published criteria,[1,2] B-lineage designation in MPAL requires CD19 expression, but CD19 alone is not a sufficient criterion for assigning B-cell lineage. In cases with strong expression of CD19 (as seen in stage I B-cell precursors or mature B cells), at least one other B-cell-associated marker should be strongly expressed (cyt.CD79a, CD10, or cyt./sCD22). If CD19 expression is weak, two other B-lineage markers are needed. Of these, cyt.CD79a is probably the most sensitive, although it may occur in T-ALL and cannot be used alone in the diagnosis of B/T leukemia.



blood Letters to *Blood*

TO THE EDITOR:

The International Consensus Classification of acute leukemias of ambiguous lineage

Immunophenotype

Similar to the criteria published in the revised fourth edition of the World Health Organization classification of these disorders, B-lineage designation in MPAL requires strong expression of CD19, along with at least 1 other B-cell marker.[1] If CD19

Case 6

	WHO	ICC book	ICC article
Text	+	+strong	+
Table	+strong	+	+

According to the WHO-HEM5/ICC [1–3], leukemias of mixed phenotype that can be assigned to other clinically or genetically defined categories should not be diagnosed as MPAL despite meeting the lineage criteria, as described below (immunophenotypic section). These include acute myeloid leukemia (AML) with defining genetic abnormalities (such as *RUNX1*::*RUNX1T1* and *CBFB*::*MYH11* fusion), chronic myeloid leukemia (CML) in blast crisis, myeloid/lymphoid neoplasms with eosinophilia and kinase gene fusions (such as *FGFR1*-rearranged myeloid/lymphoid neoplasms), therapy-related AML, and AML with myelodysplasia-related changes (AML-MR). Such cases should be classified by

AML with t(8;21) (RUNX1::RUNX1T1)

AML with t(8;21) (RUNX1::RUNX1T1)

- Large blasts with abundant basophilic cytoplasm, often containing numerous azurophilic granules and perinuclear clearing. Variable degree of dysplasia. Eosinophil precursors often increased.
- Often aberrant expression of CD19 and cyCD79a
- CD33 usually weak or negative

Case 6

Take home message:

- Consider only strong expression of lineage defining markers for MPAL
- Always wait for molecular/cytogenetic results for definitive diagnosis

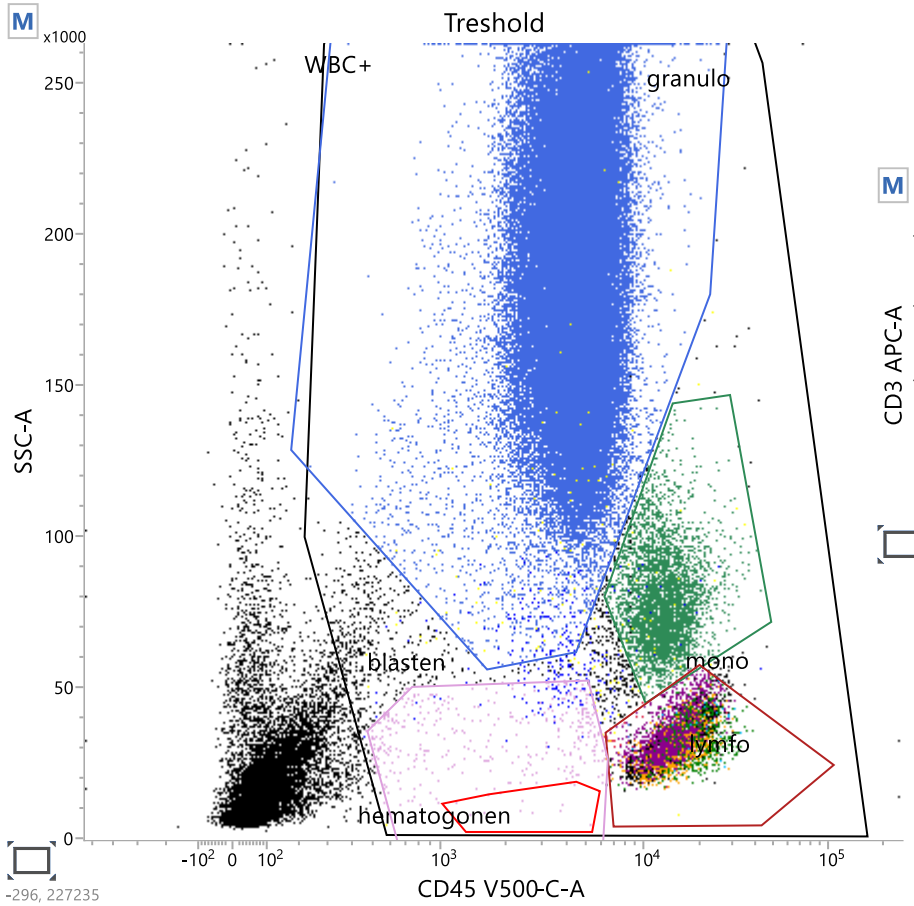
Case 7

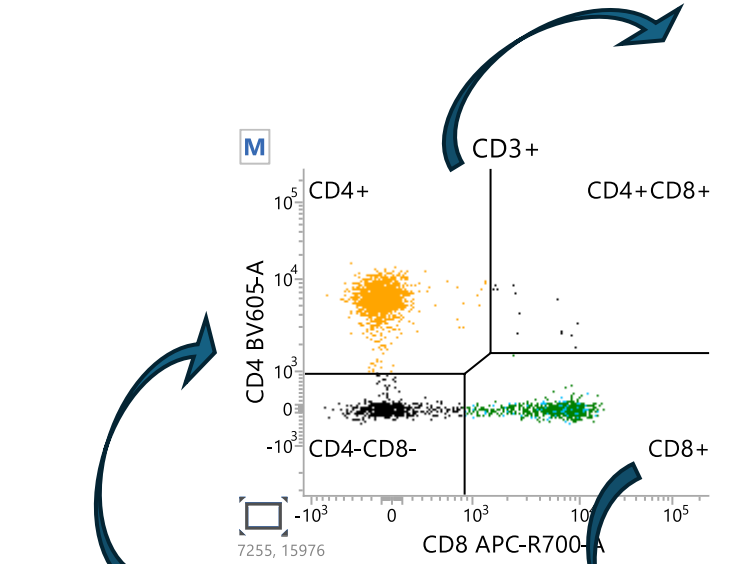
- Man, 56y
- Clinic: diffuse inflammatory syndrome, fever, synovitis, arthritis, oedema of lower limbs, multiple adenopathies but no hepatosplenomegaly.
- PET: hypercaptation of BM: inflammation? Malignancy?

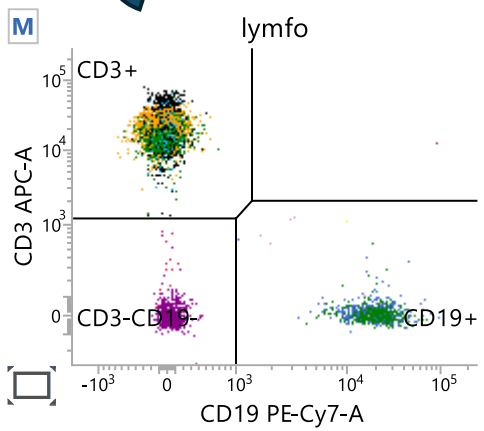
- PB: anemia, thrombocytosis
- BM: no specific morphological findings, no vacuolisation (><VEXAS)
  flow: LST

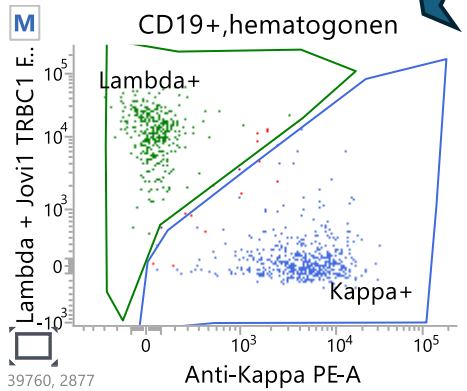
Case 7

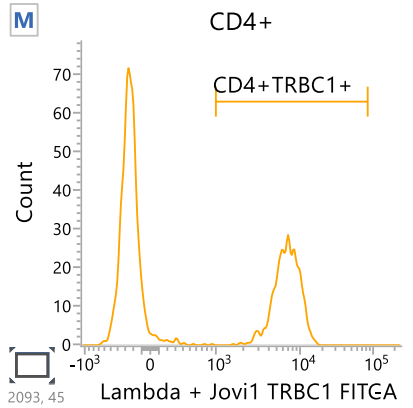
- Flow:

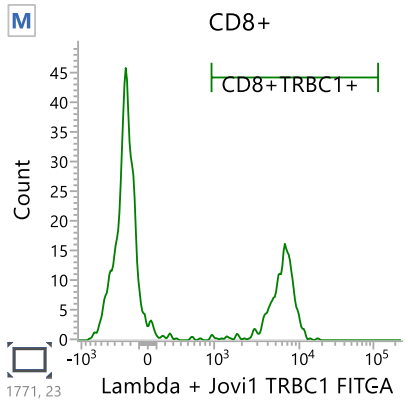


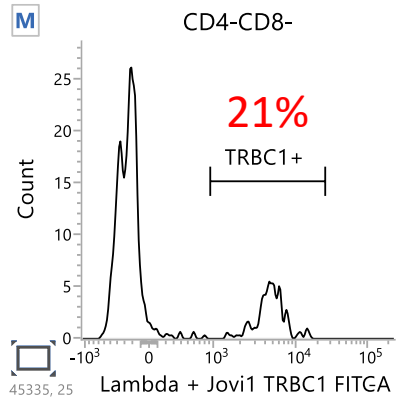












The CD4-/CD8- population in healthy individuals is:

- TRBC1<15%
- TRBC1 15-85%
- TRBC1 >85%

Activities

Visual settings

Edit

The TRBC1 percentage within CD4-/CD8- population in healthy individuals is:

0

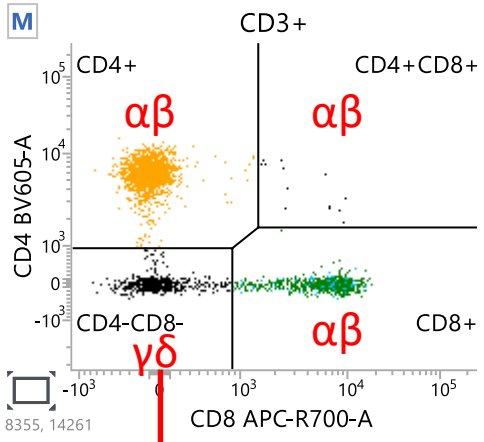
A) <15%

B) 15-85%

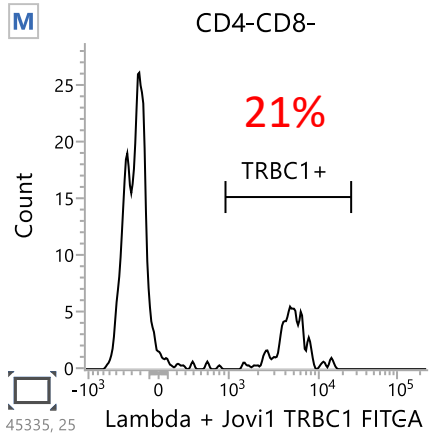
C) >85%

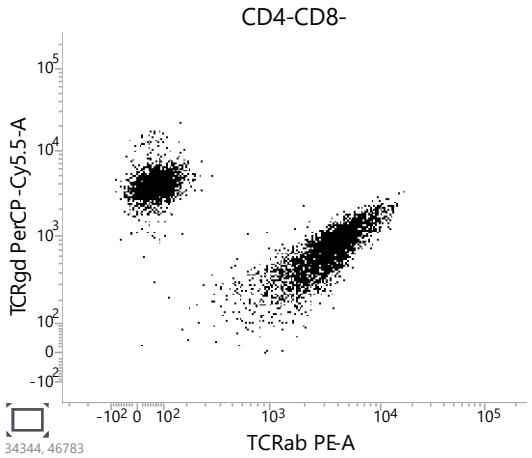
Powered by Poll Everywhere

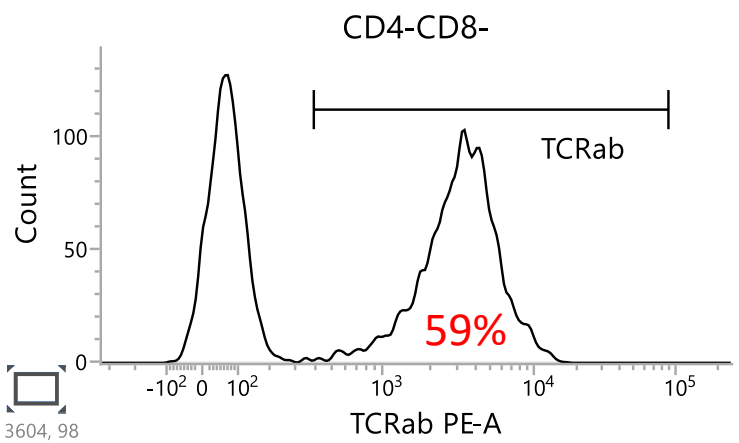
Case 7



No β -chain, so no TRBC1 (nor TRBC2)







9% of all lympho's
12% of all T-lympho's

<u>Auto-immune lymphoproliferative syndrome (ALPS)?</u>

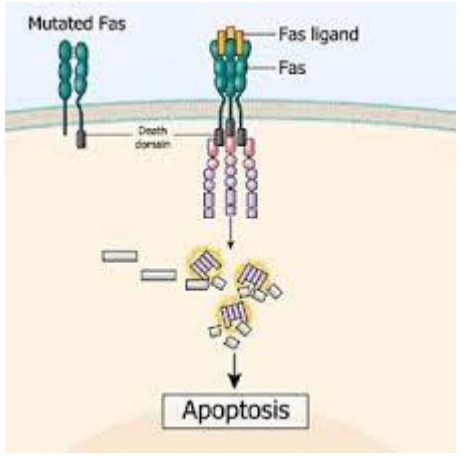
- Genetic (autosomal dominant), non-malignant auto-immune disorder
- Inborn error of immunity (IEI)
- Defect in apoptosis of lymphocytes
- Lymphadenopathy, splenomegaly, cytopenia, auto-immune manifestations
- Increase in CD3+/CD4-/CD8-/TCRab+:
  - >1,5% of all lymphocytes
  - >2,5% of T-lymphocytes

Case 7

Additional testing for ALPS:

- **Flowcytometry:**

Investigation of FAS(CD95)-mediated apoptosis *(CHU Brugmann, Brussels)*: neg



- **Molecular biology:**

Evaluation of mutations in FAS, FASL, CASP10: neg

Immunophénotype

Cellules T

CD3 absolus	↘ 336.0	/µL	605.0-2460.0
CD4 absolus	↘ 181.0	/µL	410.0-1590.0
CD8 absolus	↘ 102.0	/µL	190.0-1140.0
CD3/CD45	65.1	%	55.0-83.0
CD4/CD45	35.2	%	28.0-57.0
CD8/CD45	19.7	%	10.0-39.0
rapport CD4/CD8	1.8		0.6-2.8
CD3+CD4-CD8- (calcul)	↗ 16	%	0-5
CD3+CD4+CD95+	57.12 *100% apres stimulation*	%	
CD3+CD8+CD95+	40.40 *100% apres stimulation*	%	
C. apopototiques (contrôle négatif)	20.61	%	
C. apopototiques (anti-Fas inducteur)	88.8	%	

Cellules B

CD19/CD45	11.0	%	8.0-24.0
CD19 absolus	↘ 57.0	/µL	100.0-500.0

Cellules NK

CD16/CD56 (NK)	23.7	%	7.0-31.0
NK absolus	122.0	/µL	90.0-600.0

Conclusion

~ Augmentation des lymphocytes T CD4-CD8-. Pas de deficit d'apoptose Fas-induite par un anticorps inducteur.
L'expression du CD95 (Fas) est normale et bien upregulee apres la stimulation.
En cas de suspicion d'ALPS, completer par l'evaluation des TCRab/gd et par le sFAS.

Case 7

- DD with VEXAS: clinic (absence of adenopathy&splenomegaly), morphology (vacuoles) & genetics (UBA1), no flowcytometric aberrencies
- DD with CD4-/CD8- lymphoma:

	ALPS	Hepatosplenic TCL	Gamma delta T-LGL
Hepato-splenomegaly	+	+	-
B-symptoms	+	+	-
CD4	-	-	-
CD8			
CD5			-
CD7			-
CD16		+-	
CD56		+-	-
CD57		-	+
TCR	ab	gd	gd

No ALPS, no VEXAS, no CLPD, diagnosis unclear

Case 7

Take home message:

- Don’t forget CD4-/CD8-
- Most increases in CD4-/CD8- are inflammatory
- Most CD4-/CD8- cells express TCRgd . Do not consider TRBC1 & 2 on these cells!
- ALPS is an IEI with increased polyclonal CD4-/CD8- that express TCRab