Mass General Brigham

Having a Blast? Acute Leukemia and Beyond

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February 6, 2023

Disclosures

Scopio:

- Consultant

Sysmex:

- Speaker

Objectives

Recognize blood and/or bone marrow smears with increased blasts and identify important morphologic clues.

Appropriately apply and interpret pertinent ancillary methods to cases presenting with increased blasts.

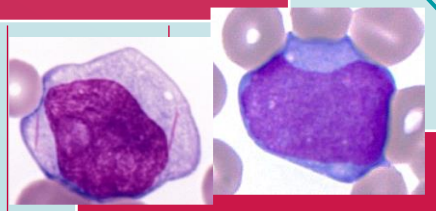
Understand the diagnostic and prognostic significance of ancillary testing.

Blasts do NOT read pathology books!

Bone marrow

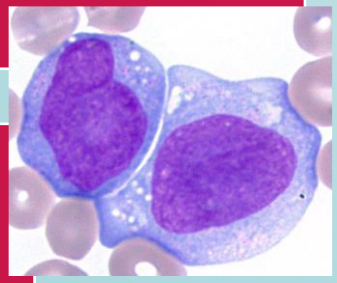
Myeloid SC

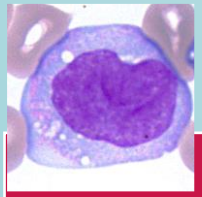
Lymphoid SC



Myeloblast

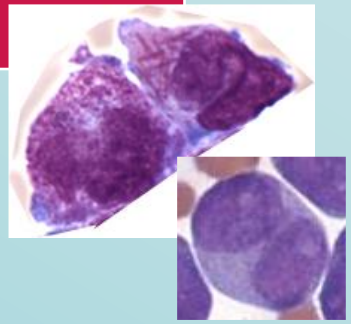
Monoblasts





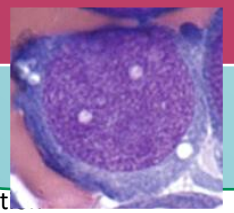
Promonocyte

Promyelocyte

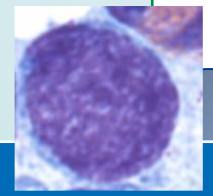


Megakaryoblast

Erythroblast

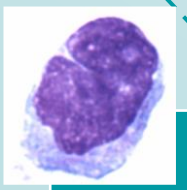


Thymus



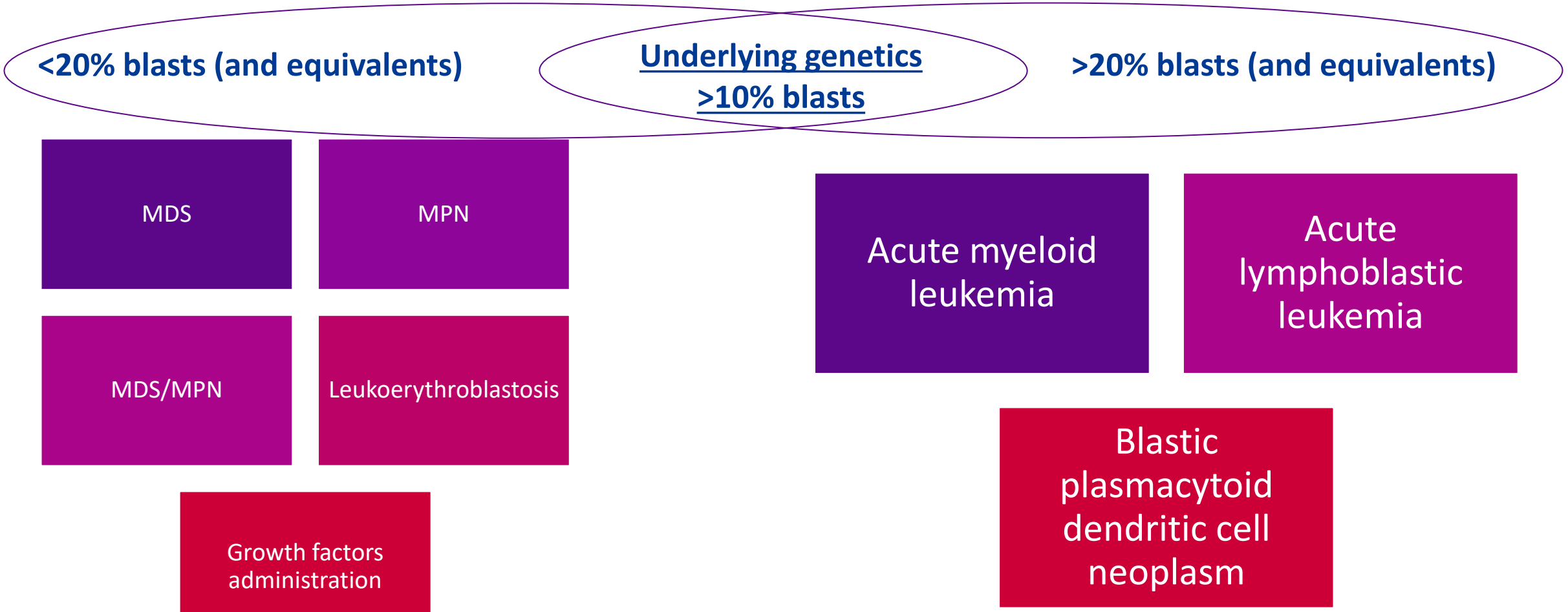
Lymphoblast

Tissues

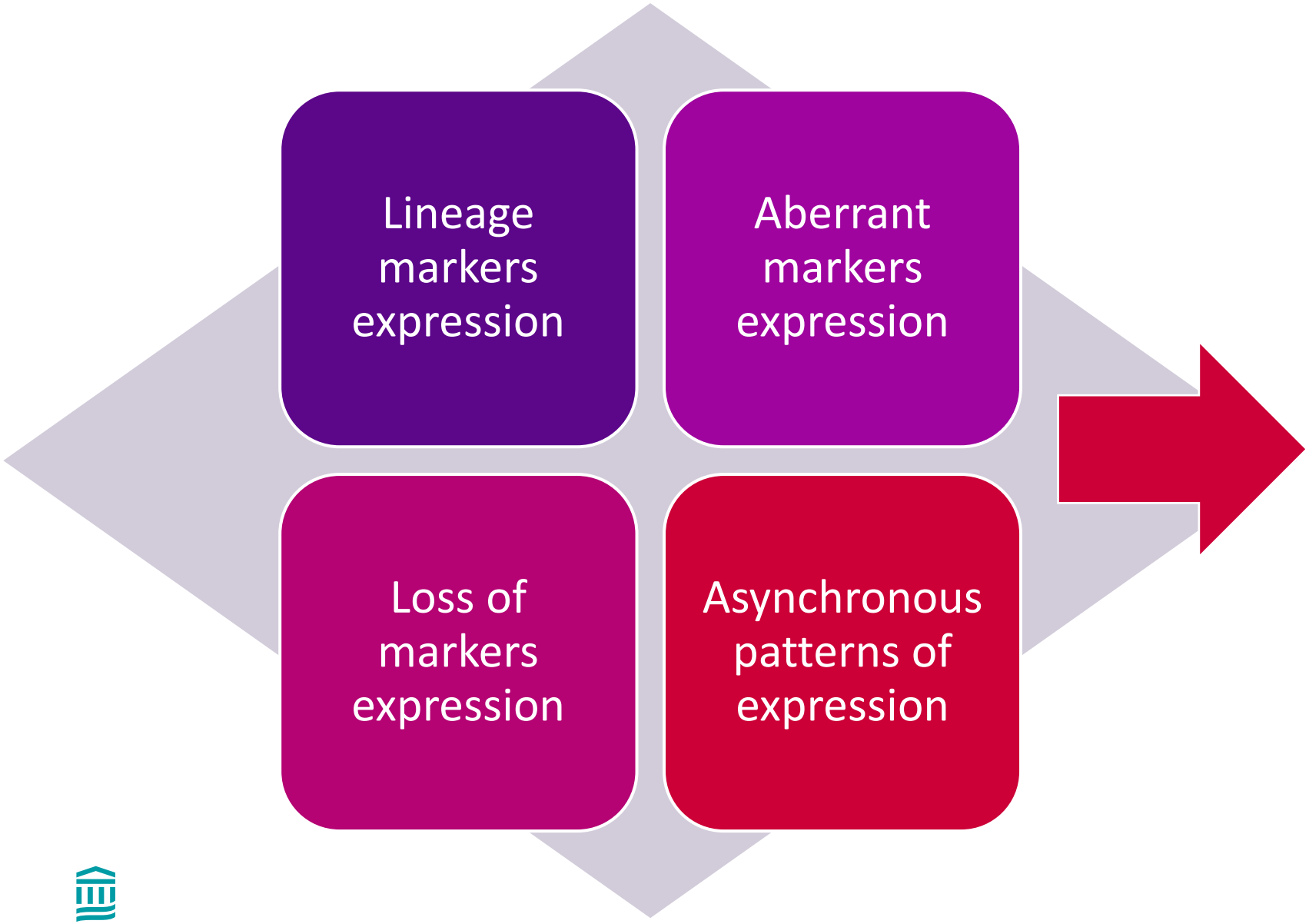


Plasmacytoid DC

Differential diagnosis for blood or marrow specimens with increased blasts



Immunophenotype is essential !!!



Blast population:

- Cell lineage (quantity)
- Underlying genetic abnormalities
- Antigen expression for targeted therapies
- Make (preliminary) diagnosis

Case 1. I spy with my little eye …

62-year-old patient with unremarkable medical history is presenting with atypical lymphocytosis

Complaints:

Fatigue, feeling “run down”

PMH:

Unremarkable

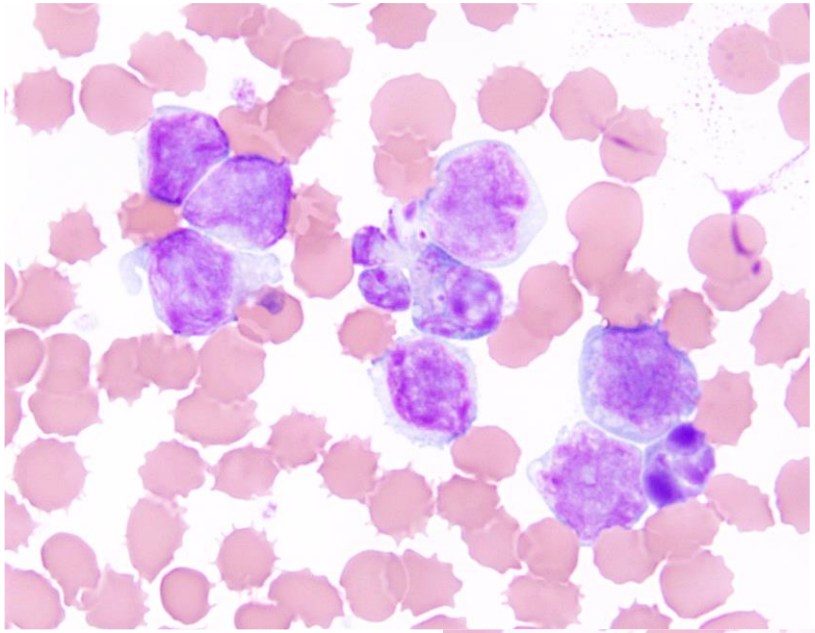
ROS:

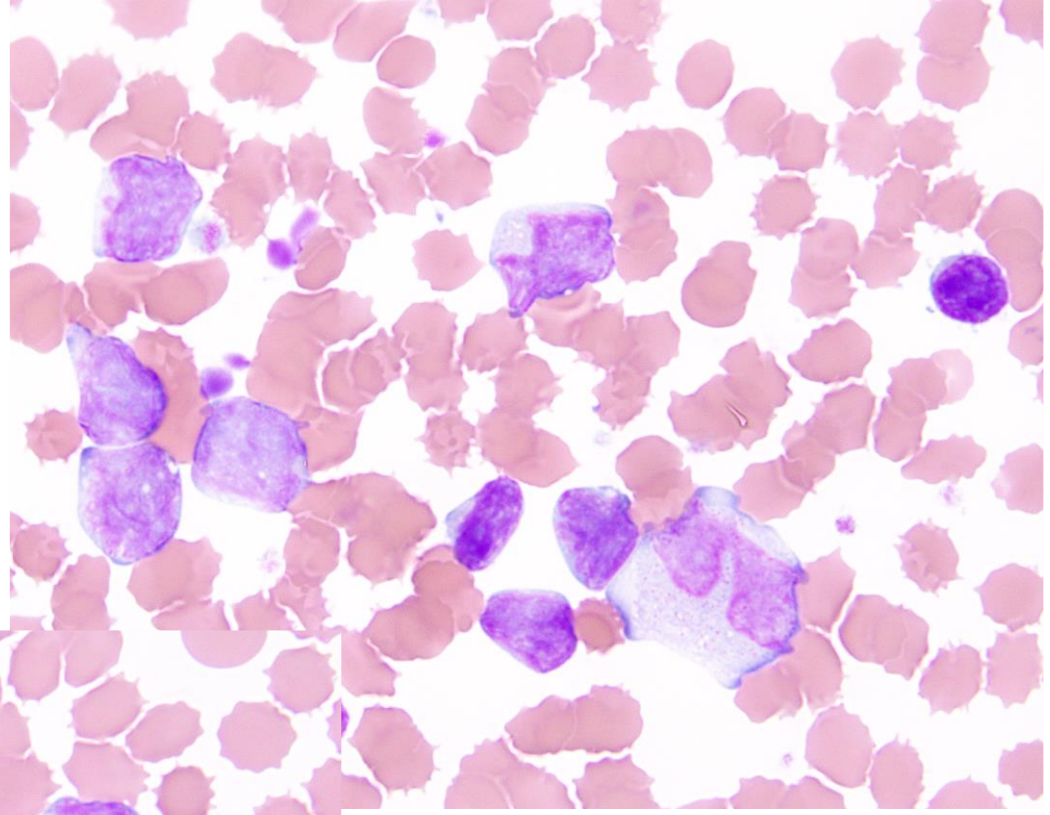
Unremarkable

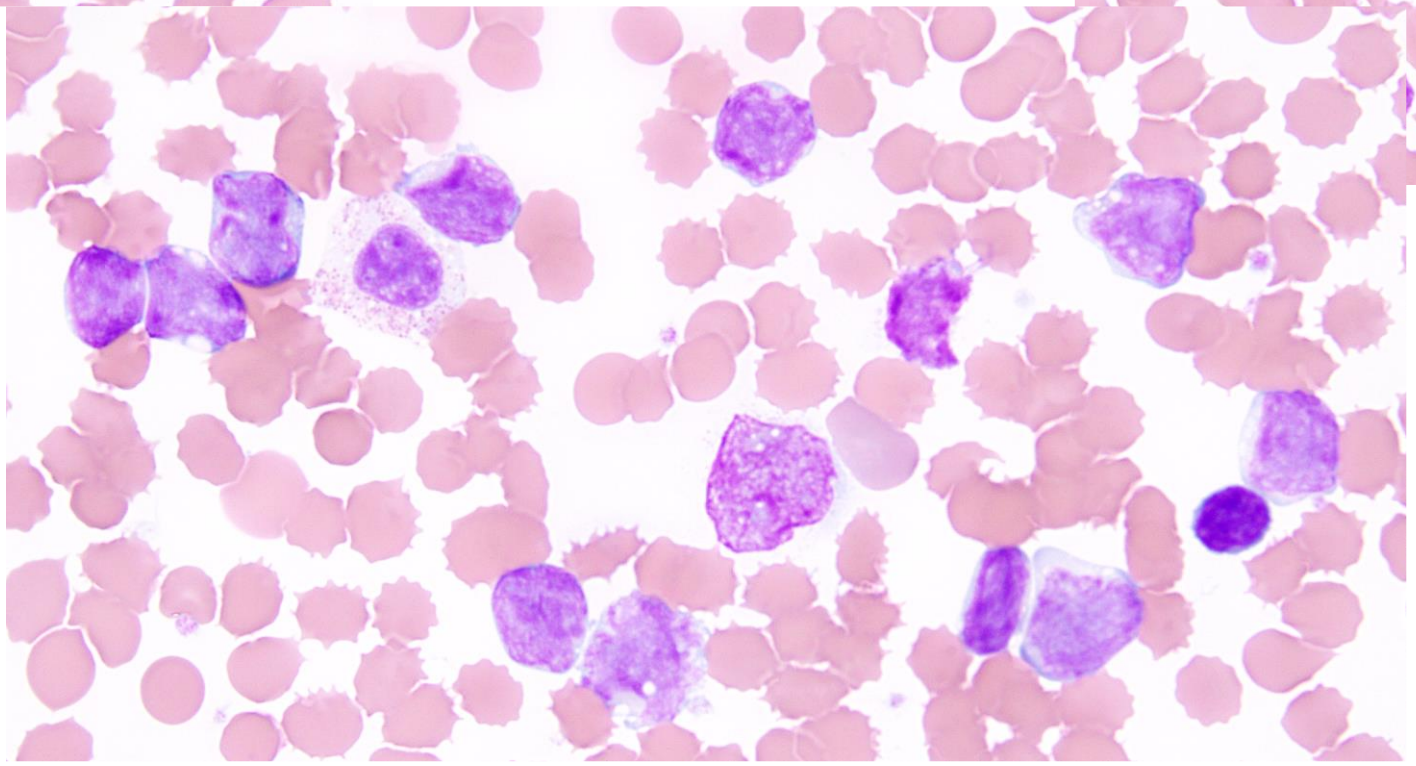
The abnormal population = 77%

Parameters	Result	Reference range
WBC	222.22 (HH)	4.00 - 10.90 K/µL
- Neutrophils	8.89 (H)	1.92 - 7.60 K/µL
- Lymphocytes	171.11 (HH)	0.72 - 4.10 K/µL
- Monocytes	6.67 (H)	0.16 - 1.10 K/µL
Hgb	12.9	11.5 - 16.4 g/dL
HCT	40.3	36.0 - 48.0 %
MCV	93.1	80.0 - 100.0 fL
PLT	176	150 - 450 K/K/µL

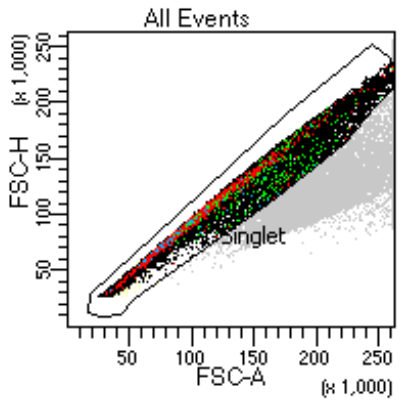
Blood smear at presentation

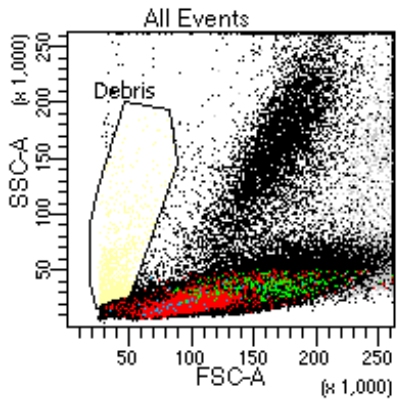


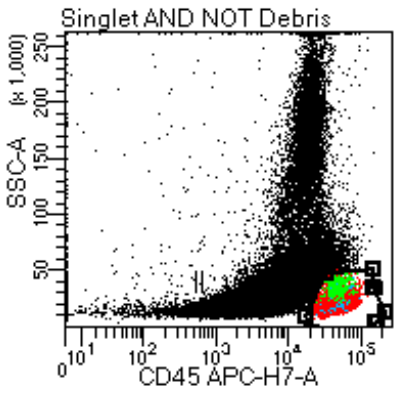




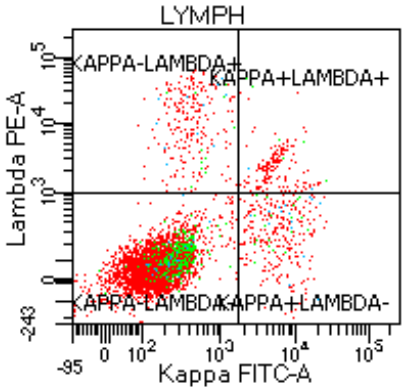
Lymphoma panel
“typical” lymphocyte gate ($CD45^{br}$ + SSC^{low})

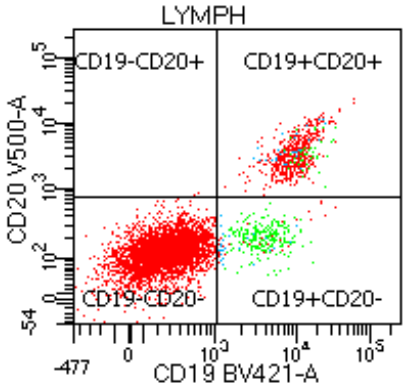


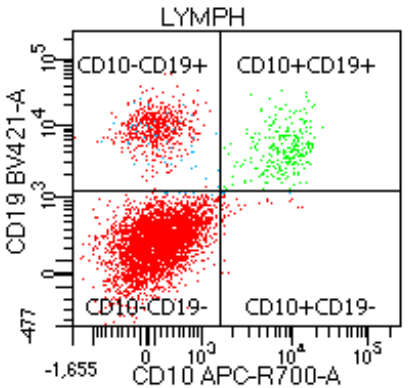




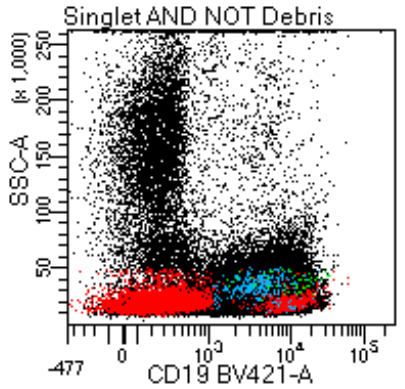
3% of all events

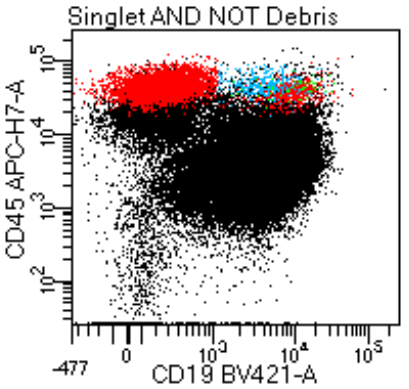




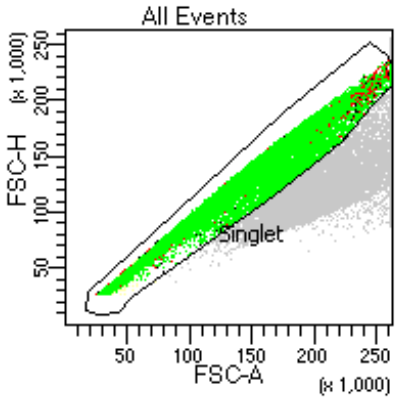


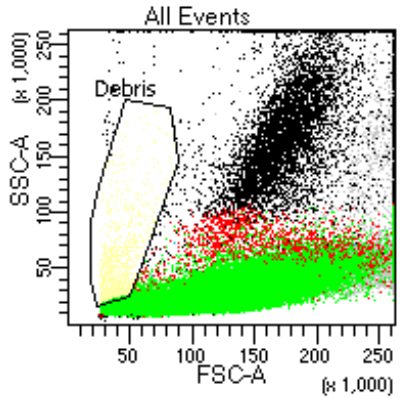
Without gating

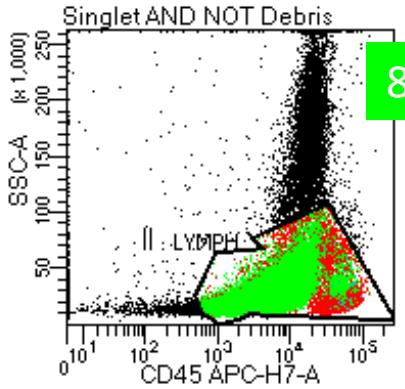




Lymphoma panel – expand the lymphocyte gate CD45 (dim + bright)

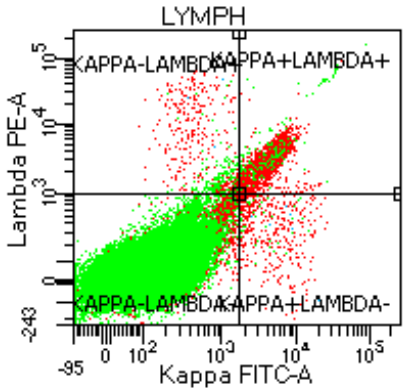


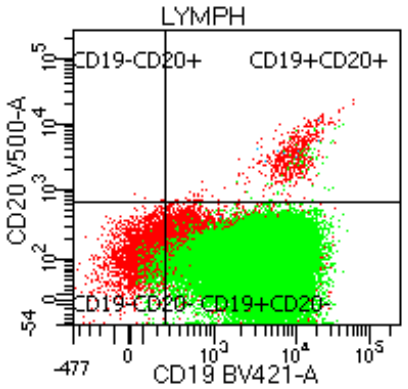


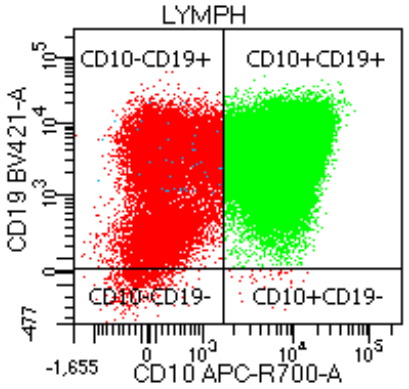


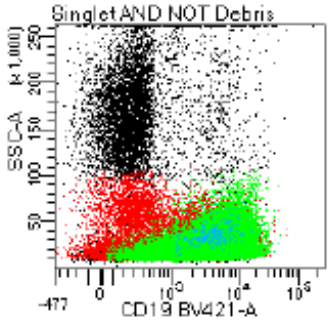
84% of all events

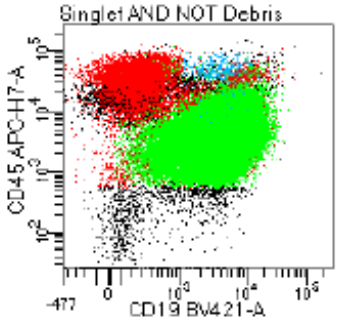
(+): CD45^{dim}, CD19, CD10
(-): CD20, (s)kappa/lambda
T-cell markers



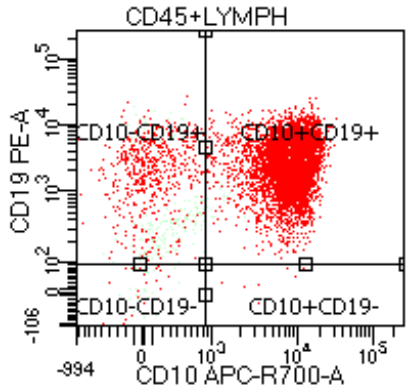


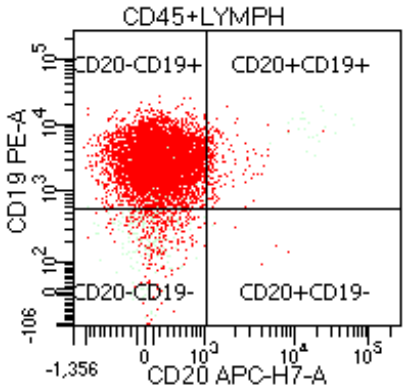


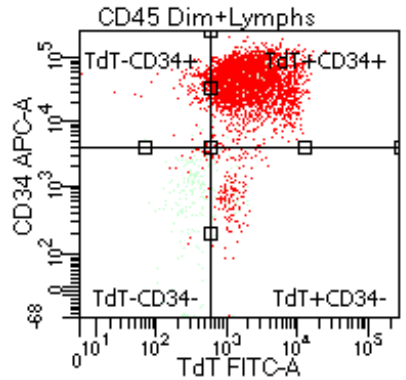


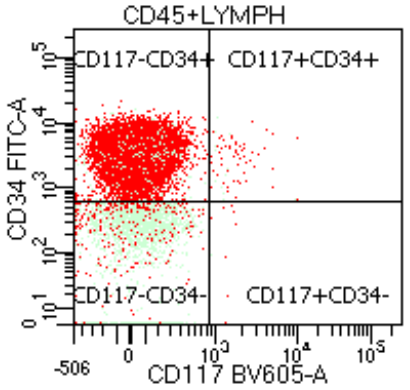


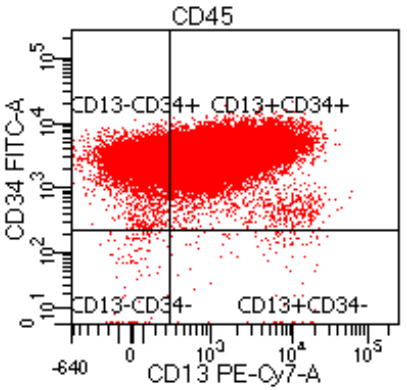
Leukemia panel

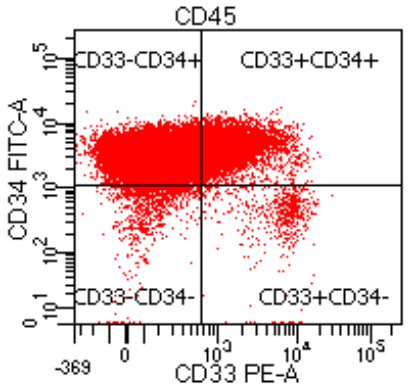








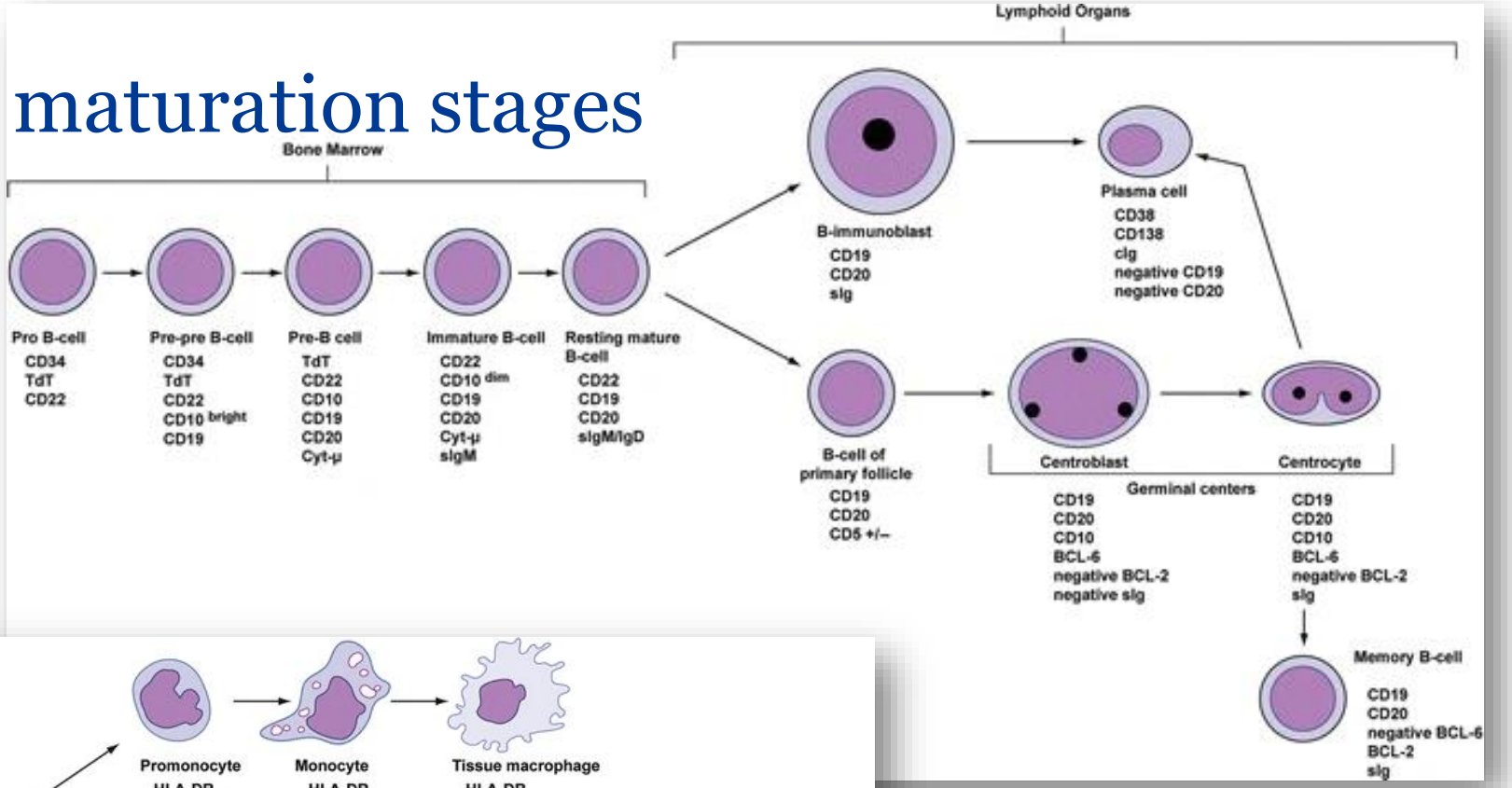


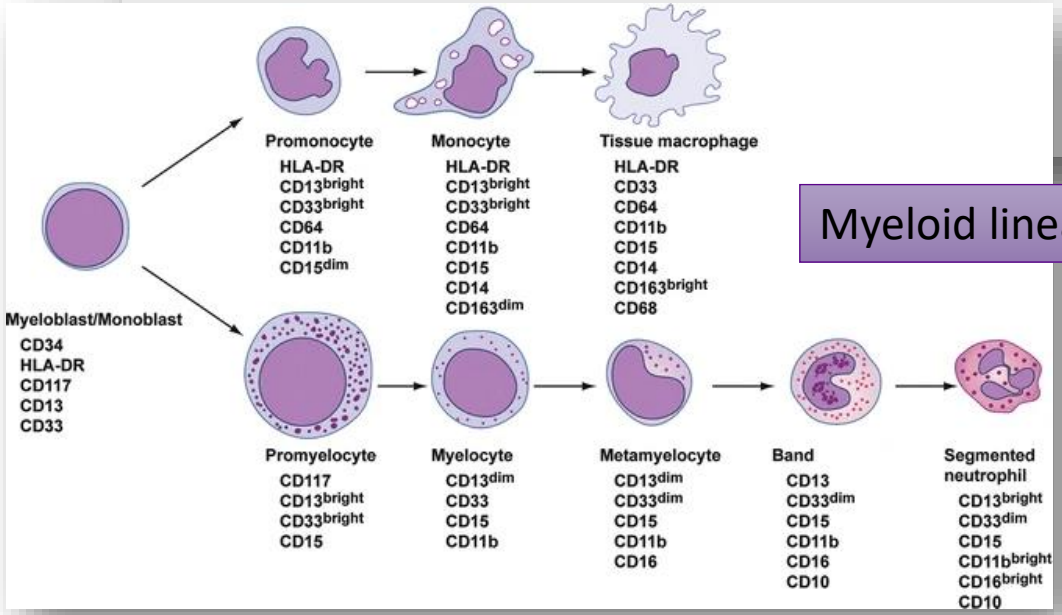


(+): CD45^{dim}, CD19, CD10, <u>CD34, TdT, CD13, CD33^{dim}</u>

(-): CD20, (s)kappa/lambda
T-cell markers

B-cell maturation stages





Myeloid lineage markers

(+): CD45^{dim}, CD19, CD10, CD22
<u>CD34, TdT, CD13, CD33^{dim}</u>

(-): CD20, (s)kappa/lambda
T-cell markers

Czader, M. (2013). Clinical Flow Cytometry in Molecular Genetic Pathology. In: Cheng, L., Zhang, D., Eble, J. (eds) Molecular Genetic Pathology. Springer, New York, NY.
https://doi-org.ezp-prod1.hul.harvard.edu/10.1007/978-1-4614-4800-6_10

Other results

Cytogenetics

- 46,XX,t(9;22)(q34;q11.2)[20]

Molecular

- *BCR::ABL1* (p190, e1a2) >50% *BCR-ABL1/ABL1* (above limit of quantification)

- *BCR::ABL1* (p210): NOT Detected (adequate control gene)
- 1 copy deletion IKZF1 (on 7p) (exons 5-7)

Diagnosis

- B-ALL with t(9;22)/*BCR::ABL1*

Typical B-lymphoblast phenotype: Positive: CD19, CD10, CD22, CD20+/-, CD34, TdT Negative: sIg, myeloid markers

B-ALL with	Frequency	Prognosis	Phenotype
t(9;22)(q34.1;q11.2)/*BCR::ABL1*	25% adults, 2-4% AYA	Poor	myeloid markers, CD25
t(v;11q23.3)/*KMT2A* rearranged	Most common <1 yo	Poor	CD10-, CD15+
t(12;21)(p13.2;q22.1)/*ETV6::RUNX1*	25% AYA	Good	myeloid markers, CD9-, CD20-
t(1;19)(q23.3;p13.3)/*TCF3::PBX1*	6% AYA	Variable	(c)mu+, CD9+, CD34-
BCR::*ABL1*–like	10-25% adults	Variable	myeloid markers, CRLF2+
MYC rearrangement	2-5%	Poor	CD34-, sIg+/-
DUX4 rearrangement	5-10%	Favorable	CD371+, CD2+
MEF2D rearrangement	3-5%	Poor	CD10dim/-, (c)mu+
ZNF384(362) rearrangement	5-10%	Variable	CD10dim/-, myeloid markers
NUTM1 rearrangement	<2%, mostly in infants lacking *KMT2A* rearrangement	Good	CD10dim/-, myeloid markers

Arber DA, et al. Blood. 2022 Sep 15;140(11):1200-1228. PMID: 35767897.

BCR::ABL1-like B-ALL

Gene expression profiling identifies a subset of B-ALL cases with similar gene expression profile to *BCR::ABL1*+ B-ALL

- Share *IKZF* deletion with *BCR::ABL1+* B-ALL

10% of pediatric, up to 25% of adult B-ALL

Altered *ABL/JAK* pathway signaling

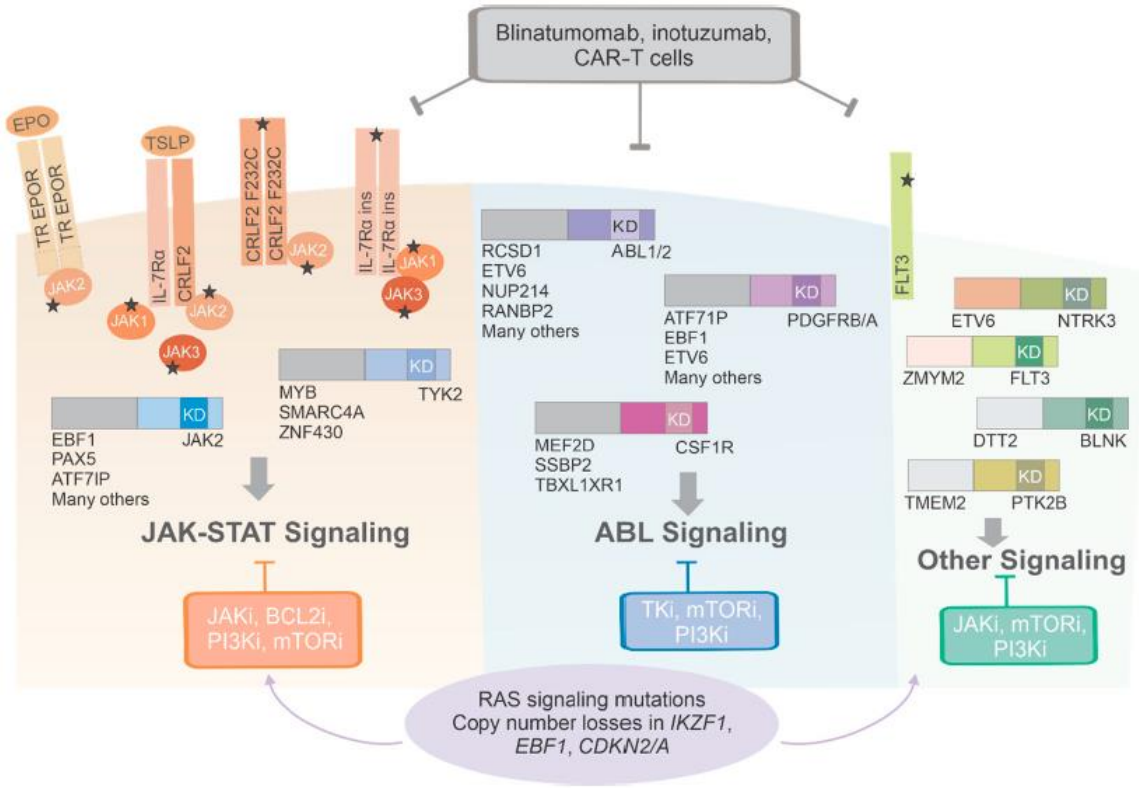
- Often have rearrangements of tyrosine kinase or cytokine genes
- Can respond to appropriate targeted therapies

Poorer prognosis than other *BCR::ABL1* negative B-ALLs

Owattanapanich W et al. Clin Lymph Myel Leuk 2020

Roberts KG et al. NEJM 2014;371:1005. Loh et al. Blood 2003;121:485

Schematic representation of main genomic alterations in Ph-like B-ALL

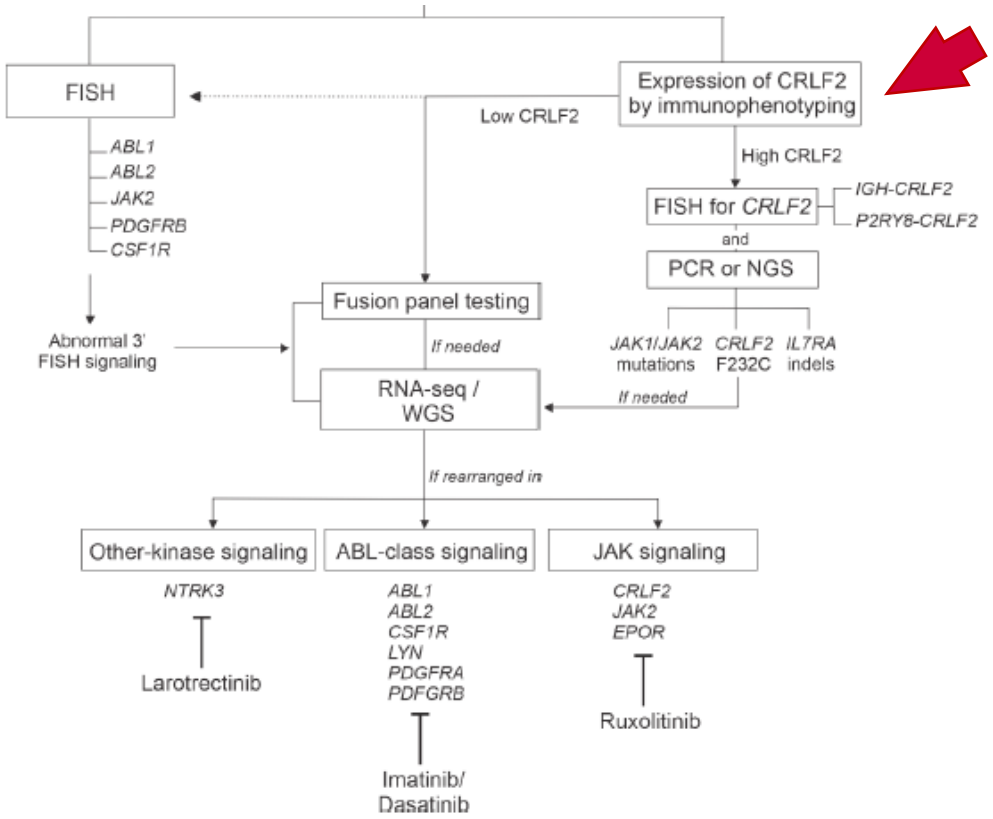


>60 genetic alterations (many cryptic)
Three main types:
1. *JAK/STAT* alterations including mutations activating cytokine receptors (e.g., *CRLF2* and *IL7R*); gene fusions hijacking cytokine receptor expression (e.g., *IGH-CRLF2* and *P2RY8–CRLF2*); gene fusions and/or mutations activating kinases (e.g., *JAK1, JAK2, JAK3, TYK2*); rearrangements hijacking and truncating cytokine receptor expression (e.g., cryptic *EPOR* rearrangements)
2. Fusions involving *ABL*-class genes (*ABL1, ABL2, CSF1R, LYN, PDGFRA, PDGFRB*)
3. Less common fusions (*FLT3, FGFR1, NTRK3, PTK2B*); number is growing with increasing sequencing studies of different cohorts

Iacobucci I and Roberts KG Genes 2021;12:687

Diagnostic approach to detect Ph-like B-ALL

BCR::ABL1 or other translocations are not detected

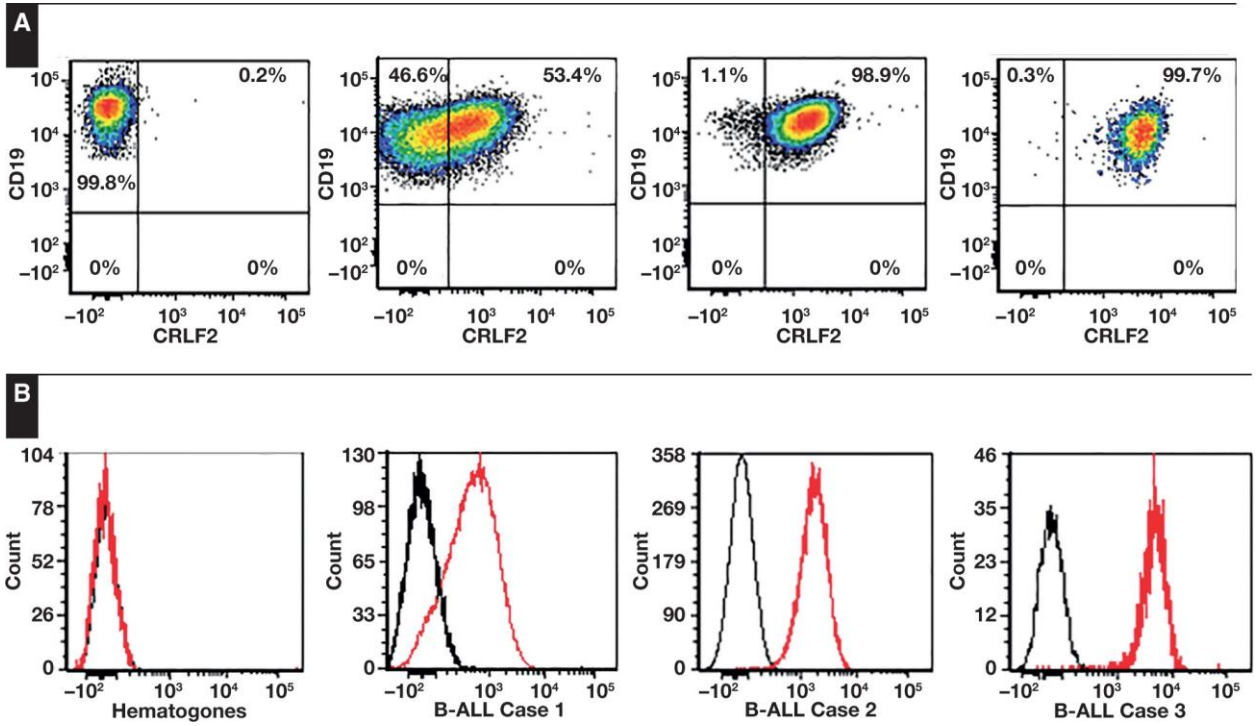


Starts with flow cytometry for CRLF2 overexpression
Cytokine receptor like factor 2
Cost-effective and predictive of CRLF2 rearrangements (~50% of cases) -
FISH
Fusion panels
Clinical RNA-seq is the “gold standard” but the slowest, most expensive and intensive with regards to analyses

Poor prognosis
Targeted TKI therapies

Iacobucci I and Roberts KG Genes 2021;12:687

CRLF2 flow cytometry detects B-ALL cases with *CRLF2* rearrangements



- Absent in normal precursor B cells (hematogones)
- Levels of CRLF2 expression B-ALL can vary from dim to moderate to bright

Am J Clin Pathol, Volume 147, Issue 4, April 2017, Pages 357–363, https://doi.org/10.1093/ajcp/aqx005

Favorable prognostic factors for B-ALL

Age (<50 years)

White Blood Cell count (<30,000)

Genetics

- Hyperdiploidy
- t(9;22); *BCR::ABL1* – targeted therapy with TKI

Response to therapy

- Negative MRD after induction therapy

Measurable (Minimal) Residual Disease (MRD) Concept

MRD is the presence of aberrant cells below the limit of morphologic detection (< 5%)

MRD detection methods:

- Flow Cytometry (aberrant immunophenotype)
- RT-PCR (specific fusions)
- Next Generation Sequencing

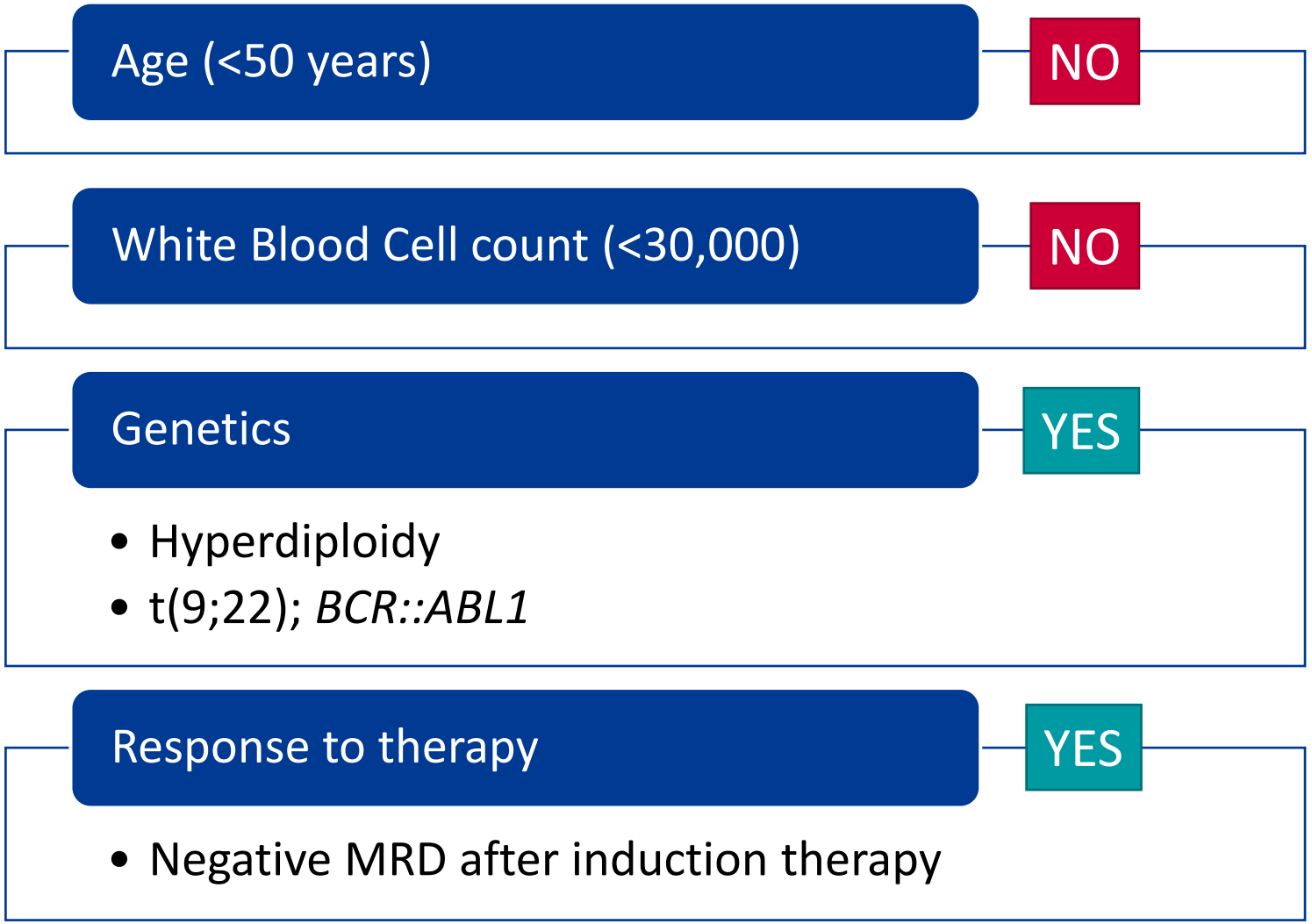
MRD sensitivity:

- Flow cytometry: 1×10^{-4}
- RT-PCR: 1×10^{-4}
- Next Generation Sequencing: 1×10^{-6}

MRD use:

- Prognosis
- Treatment stratification

Favorable prognostic factors for B-ALL: What about our patient?



Our patient falls into the intermediate prognostic group and is undergoing alloSCT

Summary of Case 1:

1

Flow cytometry is essential for assigning cell lineage

- Need to know what populations to gate on!
  - Morphology and ungated events

2

Aberrant marker expression can predict underlying genetic alterations

- Myeloid markers in B-ALL often signify the presence of Ph+ chromosome

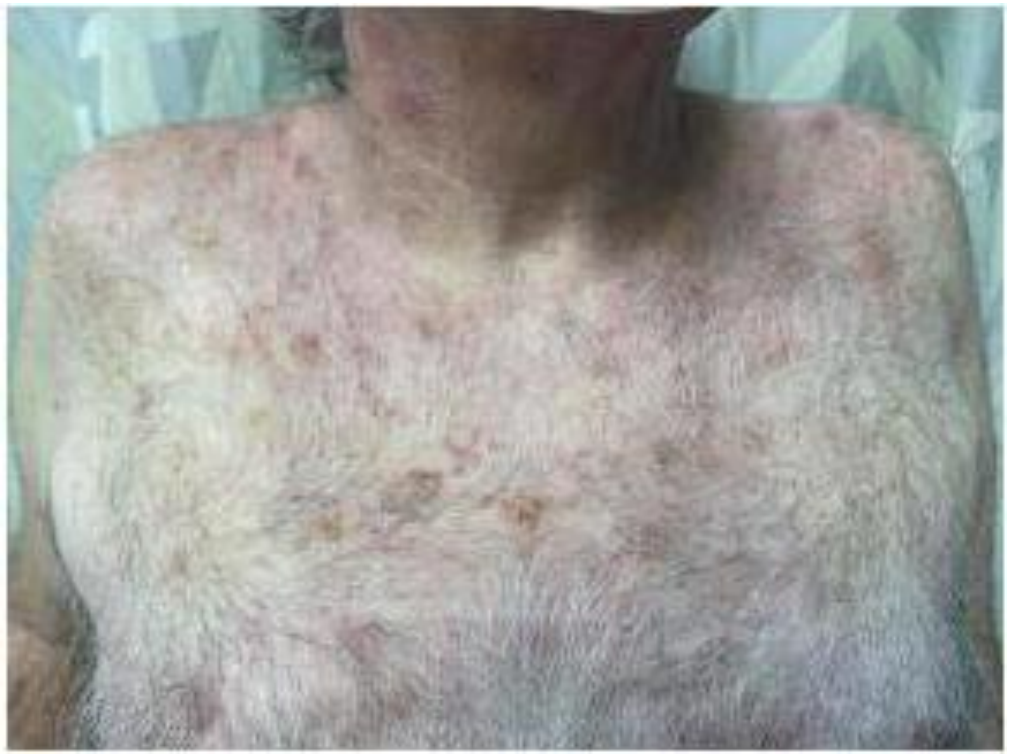
3

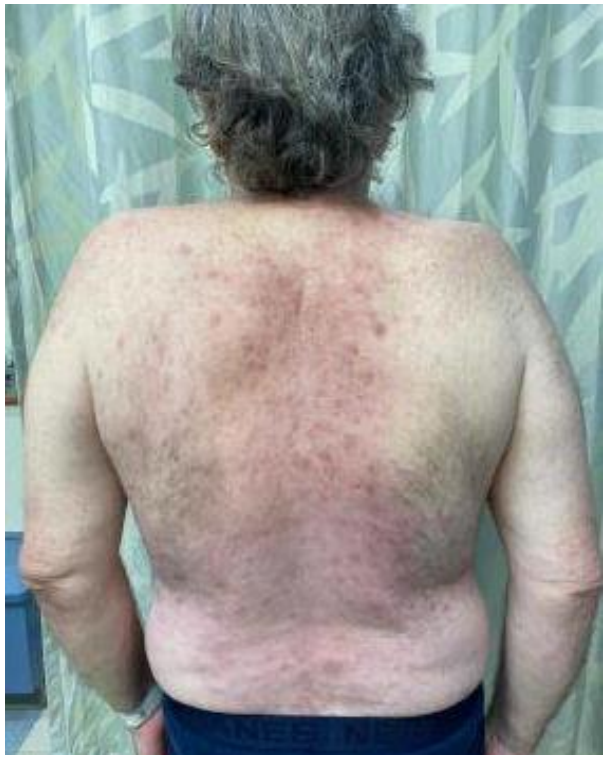
Flow cytometry can be used for identification of products of genetic alterations

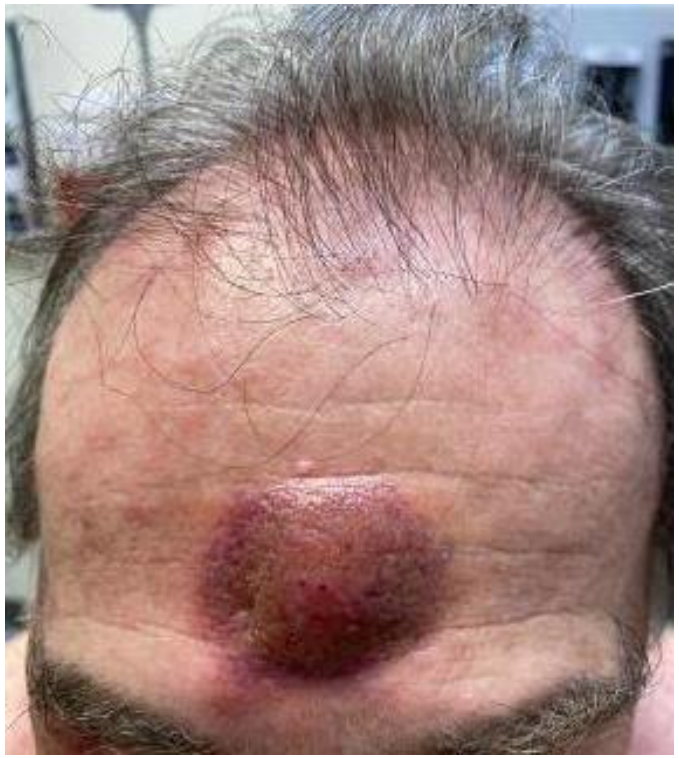
- *CRLF2* is overexpressed in 50% of B-ALL and can guide further testing

Case 2. Hit me with your best shot …

65-year-old patient presenting with widespread rapidly progressing skin lesions

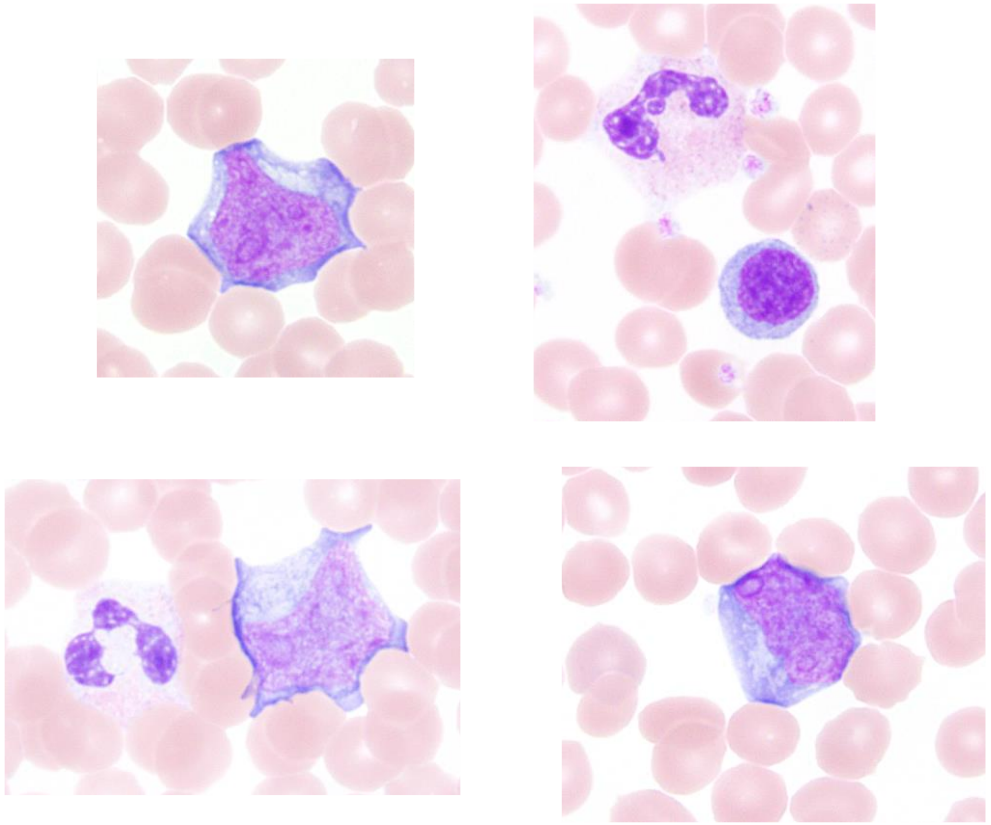






Skin biopsy at OSH – High-grade lymphoid neoplasm

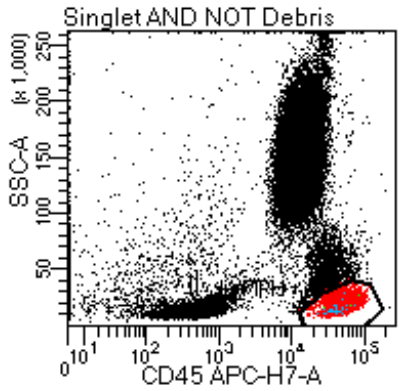
CBC at presentation:

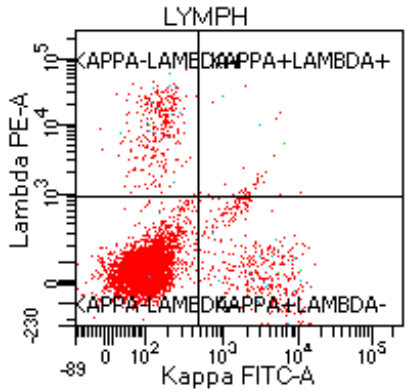


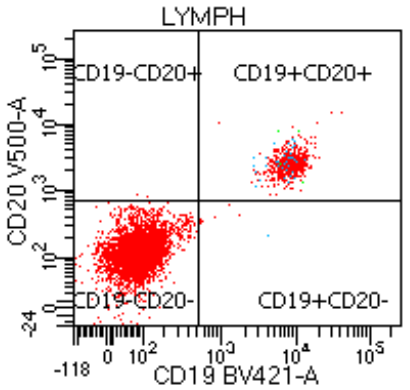
6% OTHERS

Parameters	Result	Reference range
WBC	8.92	3.81 – 8.94 K/µL
- Neutrophils	6.23 (H)	2.23 – 6.11 K/µL
- Lymphocytes	1.49	0.21 – 2.74 K/µL
- Monocytes	0.83	0.20 – 0.87 K/µL
Hgb	14.2	12.5 - 16.3 g/dL
HCT	41.4	37.1 – 49.5 %
MCV	83.2	79.0 - 97.0 fL
PLT	125 (L)	152 - 440 K/K/µL

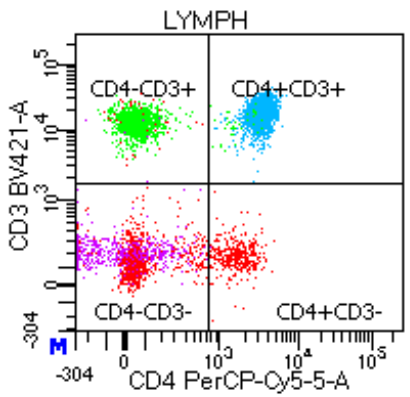
Lymphoma panel: Lymphocyte gate (small)

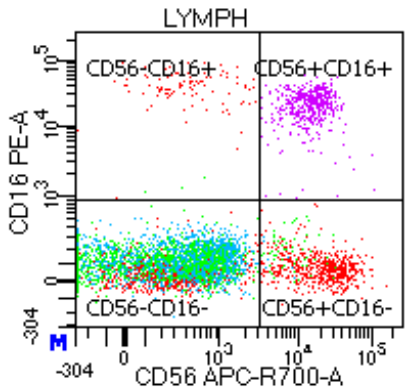


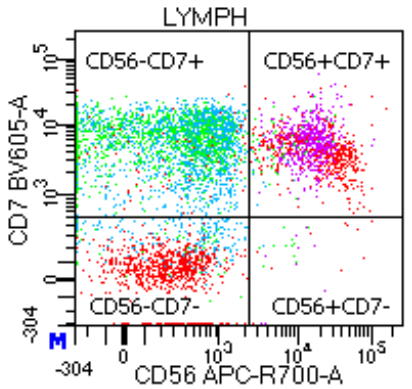




B cells

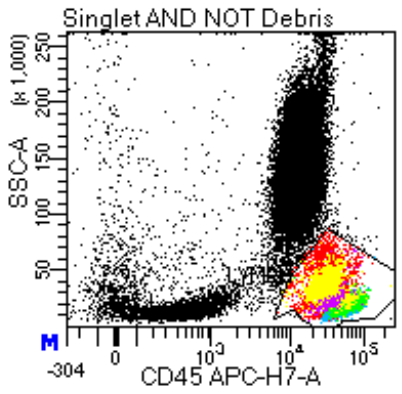


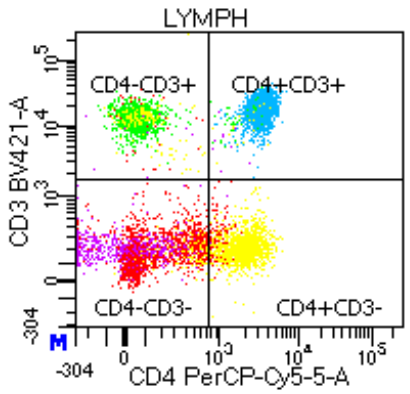


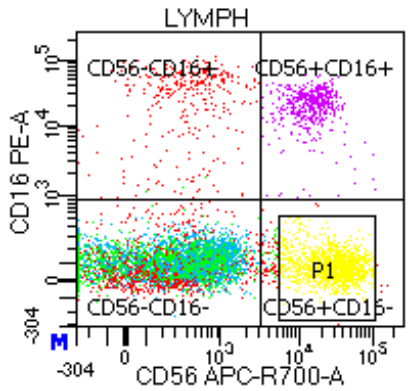


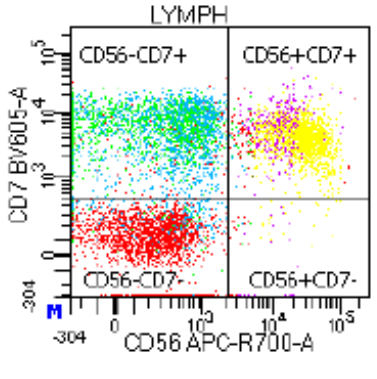
T cells (green+blue)
NK cells (purple)
What are the red cells?

Lymphoma panel: Lymphocyte gate (7% large cells)





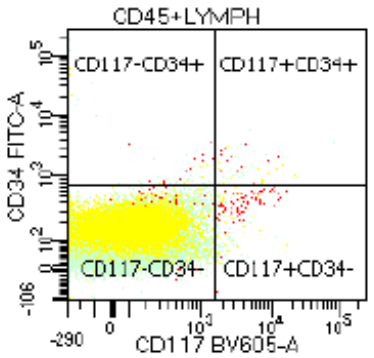


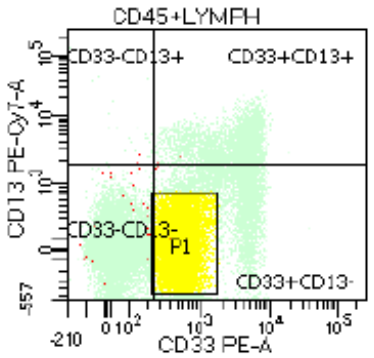


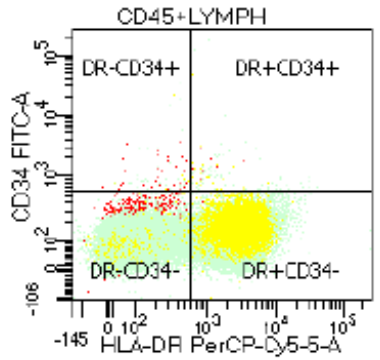
(+): CD45, CD4, CD56^{br}, CD123^{br} CD7, CD33^{dim}

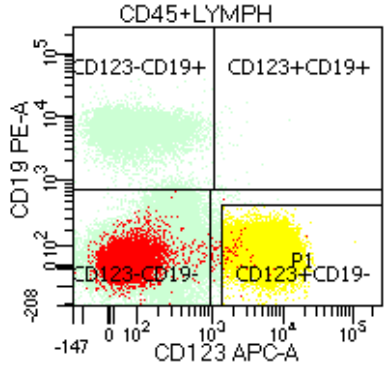
(-): CD34, other myeloid, B-cell, T-cell markers

Leukemia panel:









(+): CD45, CD4, CD56^{br}, CD123^{br}
CD7, CD33^{dim}

How to interpret immunophenotypic markers?

Lineage specific (LS)

Myeloid lineage
- Myeloperoxidase

Monocytic lineage
- Non-specific esterase
- Lysozyme

B-cell lineage
- CD19 (strong) + 1 LA antigen
- CD19 (weak) + 2 LA antigens

T-cell lineage
- CD3 (surface or cytoplasmic)

NK-cell lineage

Lineage associated (LA)

Myeloid lineage
- CD13, CD15, CD33, CD117, CD123

Monocytic lineage
- CD11b, CD14, CD15, CD16, CD33, CD64

B-cell lineage
- CD10, CD20, CD22, CD27, PAX5, CD79a
- Light and heavy chains

T-cell lineage
- CD2, CD4, CD5, CD7, CD8

NK-cell lineage
- CD8 (dim), CD16, CD27, CD28, CD56, CD94, KIR

No lineage specific markers!

Additional information:

Karyotype:

46,XY[20].nuc ish(D6Z1,MYBx2)[100],(MYCx2)(5''MYC sep 3''MYCx1)[36/100]

Molecular:

ASXL1	p.E635Rfs*15 7.5% VAF
NRAS	p.G13D 7.7% VAF
PPM1D	p.E475Kfs*8 1.5% VAF
TET2	p.Q644* 10.3% VAF

Summary of diagnostic findings

Multiorgan involvement:

- ✓ Skin, bone marrow, peripheral blood, CSF
- ✓ Likely lymph nodes (FDG-avid lymph node above and below diaphragm) and spleen (marked splenomegaly of 17 cm)

Blastoid morphology:

- ✓ Intermediate to large neoplastic cells with round to slightly irregular nuclei, prominent small nucleoli, scant to moderate amounts of agranular cytoplasm

Immunophenotype - no lineage specific markers:

- ✓ CD45(dim)+, CD123+, CD4+, CD56+, CD7+, CD33+, HLA-DR+

Normal karyotype but MYC rearrangement by FISH

Myeloid-associated mutations

- ✓ *TET2, ASXL1, NRAS, PPM1D*

Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN)

Clonal hematopoietic neoplasm:

- ✓ Cell of origin – plasmacytoid dendritic cell
- ✓ Distinct WHO entity

Epidemiology:

- ✓ 0.04 cases per 100,000 people
- ✓ Median age: 53-68 years
- ✓ Male to female ratio: 2 to 3.3:1

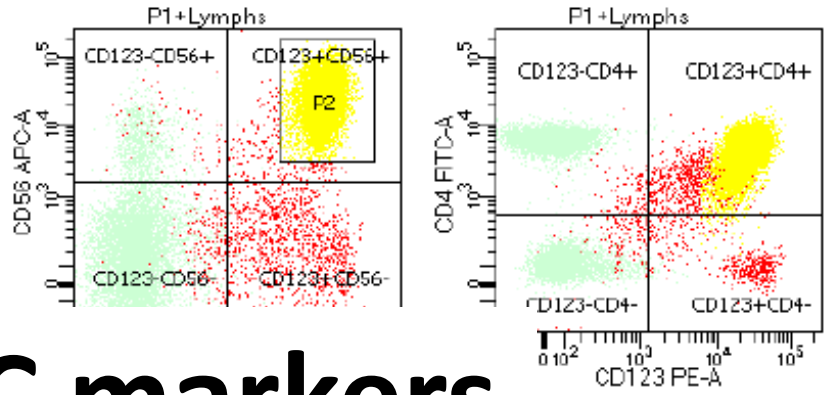
Molecular pathogenesis:

- ✓ Genomic losses: 5q, 6q, 12p, 13q, 17p, 15q, -9
- ✓ Commonly deleted regions: 9p21.3 (CDKN2A/CDKN2B), 13q13.1-q14.3 (RB1), 12p13.2-p13.1 (CDKN1B), 13q11-q12 (LATS2), and 7p12.2 (IKZF1)
- ✓ Myeloid mutations: *TET2, ASXL1, NRAS, ATM, MET, KRAS, IDH2, KIT*

Prognosis:

- ✓ OS <2 years (17-34 months)

BPDCN Diagnosis:



Inv
ski
BM
LN - 30%
CSF – 30%
Liver and spleen – 20%

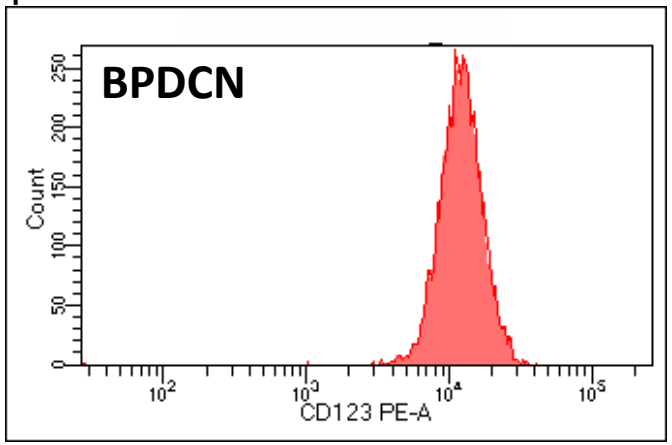
123-4-56 and one other PDC markers

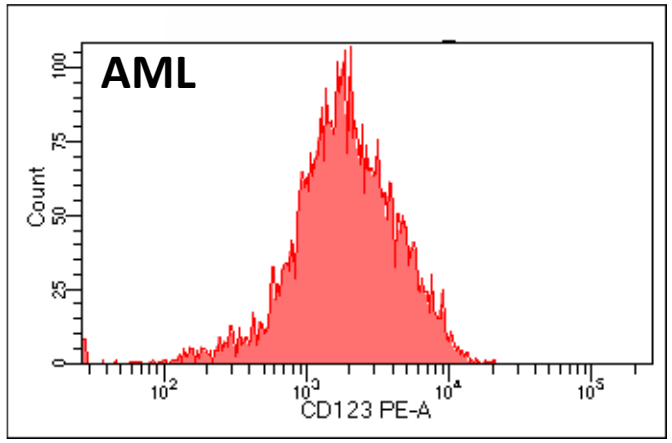
Immunophenotype is the key!

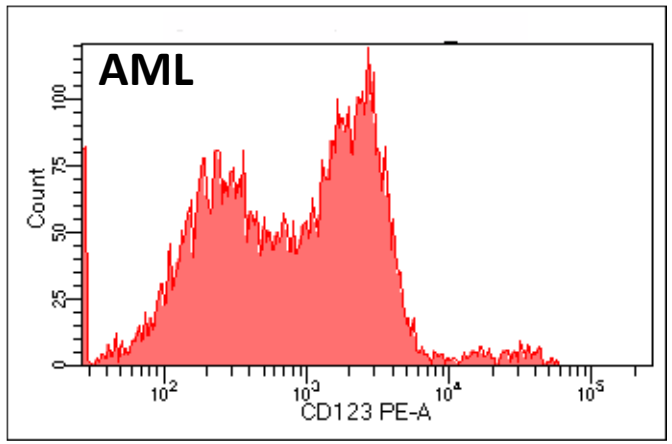
1. Positive for pDC markers:
   CD123/IL-3Ra, CD2AP, TCL1, CD303/BDCA-2, BCL11A, SPIB
2. CD4 and CD56
3. Absence of lineage specific markers

	Myeloid			Monocytic			NK/T		PDC			Immature	
	CD13	CD33	MPO	CD4	NSE	Lysozyme	CD7	CD56	CD123	TCL1	CD303	TdT	CD34
BPDCN													
AML													
AMoL													

How to treat?







No standard care

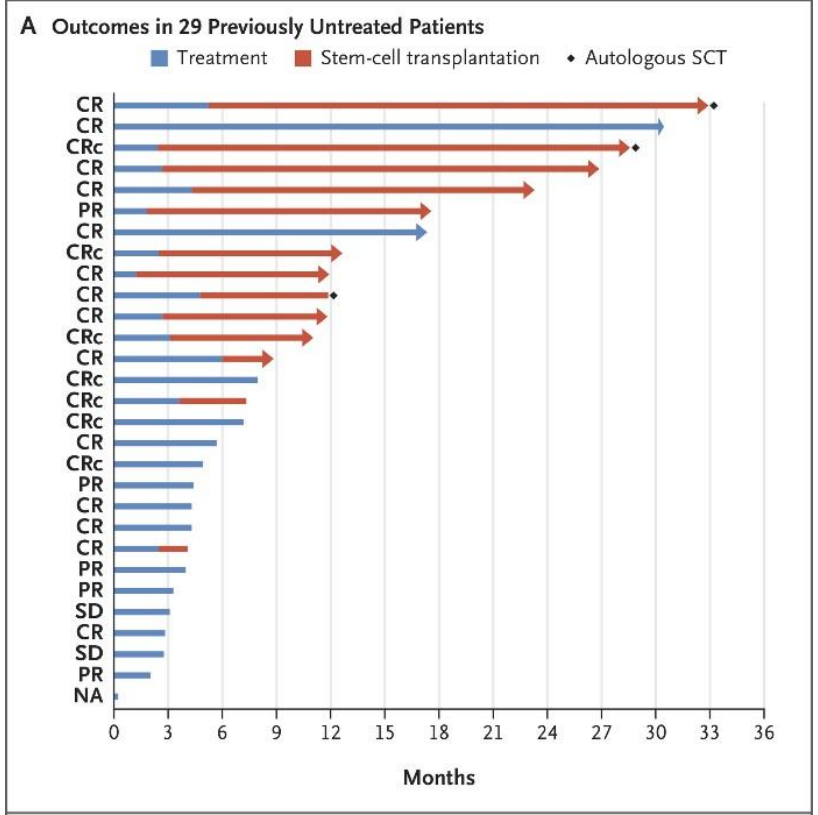
ALL-like regimens followed by SCT

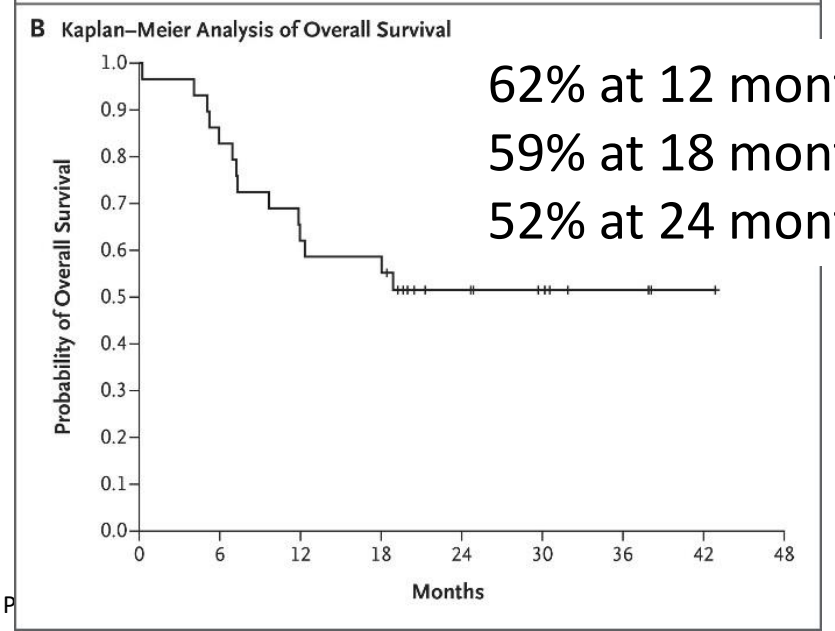
TABLE 4 | Clinical trials of anti-CD123 mAbs in myeloid neoplasms.

Disease type and inclusion criteria	Drug or drug combination, other therapies	Outcome measures	(Estimated) enrollment	Clinical trials identifier	Trial status
AML in first remission, high risk of relapse, patients not eligible for post-remission chemotherapy or aHSCT	CSL362 (anti-CD123)	Primary: AE and DLT Secondary: PK and ADA	30	NCT01632852	Completed (mid 2015)
R/R AML R/R MDS Patients not eligible for curative therapy	KHK2823 (anti-CD123)	Primary: AE Secondary: PK, ORR, OS, EFS, RFS, DFS, and ADA	60	NCT02181699	Active, not recruiting (completed mid 2017)
R/R or de novo AML Patients not eligible for curative therapy	HMA vs. HMA + JNJ-56022473 (talacotuzumab, CSL362, anti-CD123)	Primary: CRR, OS Secondary: ORR, DOR, CR, EFS, RFS, AE, ADA, QOL, and PK	326	NCT02472145	Active, not recruiting (estimated completion mid 2018)
AML and MDS after HMA failure	JNJ-56022473 (talacotuzumab, CSL362, anti-CD123)	Primary: ORR Secondary: AE, OS, PFS, HI, QOL, and DOR	43	NCT02992860	Suspended (estimated completion late 2018)
R/R AML	SGN-CD123A (anti-CD123, PBD ADC)	Primary: AE, LA, and DLT Secondary: PK, ADA, ORR, DFS, and OS	102	NCT02848248	Recruiting (estimated completion mid 2019)
R/R AML R/R BPDCN High-risk MDS R/R CMML, R/R bc CML, R/R MPN Patients not eligible for curative therapy	IMGN632 (anti-CD123, DGN549 ADC)	Primary: MTD Secondary: AE, ORR, PK, and ADA	155	NCT03386513	Recruiting (estimated completion early 2021)

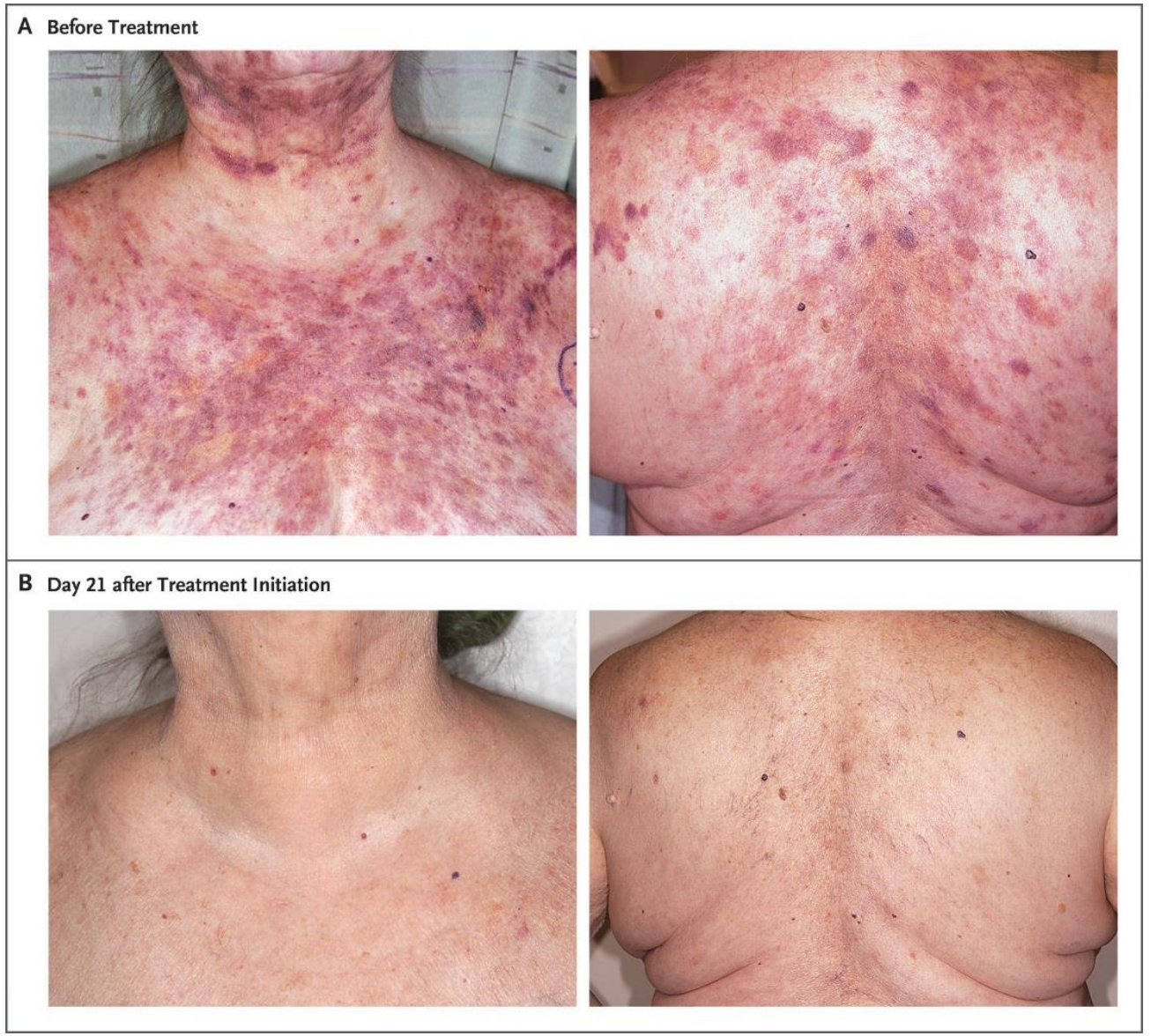
Schürch CM. Front Oncol. 2018 May 18;8:152.

Outcomes of 29 previously untreated BPDCN patients who received first-line treatment with Tagraxofusp





62% at 12 months
59% at 18 months
52% at 24 months



Pemmaraju N and Lane AA et al. N Engl J Med. 2019 Apr 25;380(17):1628-1637

Back to our patient: after one cycle of CD123-targeted ADC the patient is in complete remission

- Skin - clear
- CSF - clear
- BM – negative, including flow cytometry MRD
- Imaging - Most of the prior intensely FDG-avid lymph nodes, FDG uptake in the spleen, skin and cutaneous/subcutaneous tissues has resolved
- CBC – mild leukocytosis and anemia

Targeted therapies

Small molecules

- ❑Tyrosine kinase inhibitors
  - Gleevec in Ph+ B-ALL
- ❑Epigenetic regulatory proteins
  - Ivosidenib (*IDH1*) in AML
- ❑DNA damage repair enzymes
- ❑Proteasome inhibitors
  - Bortezomib in MM

Macromolecules

- ❑Monoclonal antibodies
  - Blinatumomab (CD19/CD3) in B-ALL
- ❑Polypeptides
  - Romiplostim (TPO) for ITP
- ❑Antibody-drug conjugates
  - Tagtaxofusp (anti-CD123) in BPDCN
- ❑CAR-T cells
  - Yescarta for DLBCL

Zhong, L., Li, Y., Xiong, L. *et al.* Small molecules in targeted cancer therapy: advances, challenges, and future perspectives. *Sig Transduct Target Ther* **6**, 201 (2021). https://doi.org/10.1038/s41392-021-00572-w

Summary of Case 2:

1

Immunophenotyping is essential for assigning cell lineage

- Need to know what populations to gate on!
  - Morphology and ungated events

2

123-4-56

- BPDCN diagnosis could be tricky
- Remember this markers combination

3

Antigen expression and intensity is vital for targeted therapies

- CD123 is a target for BPDCN, as well as AMLs

Case 3. Blue's Clues …

46-year-old man is presenting with lymphadenopathy

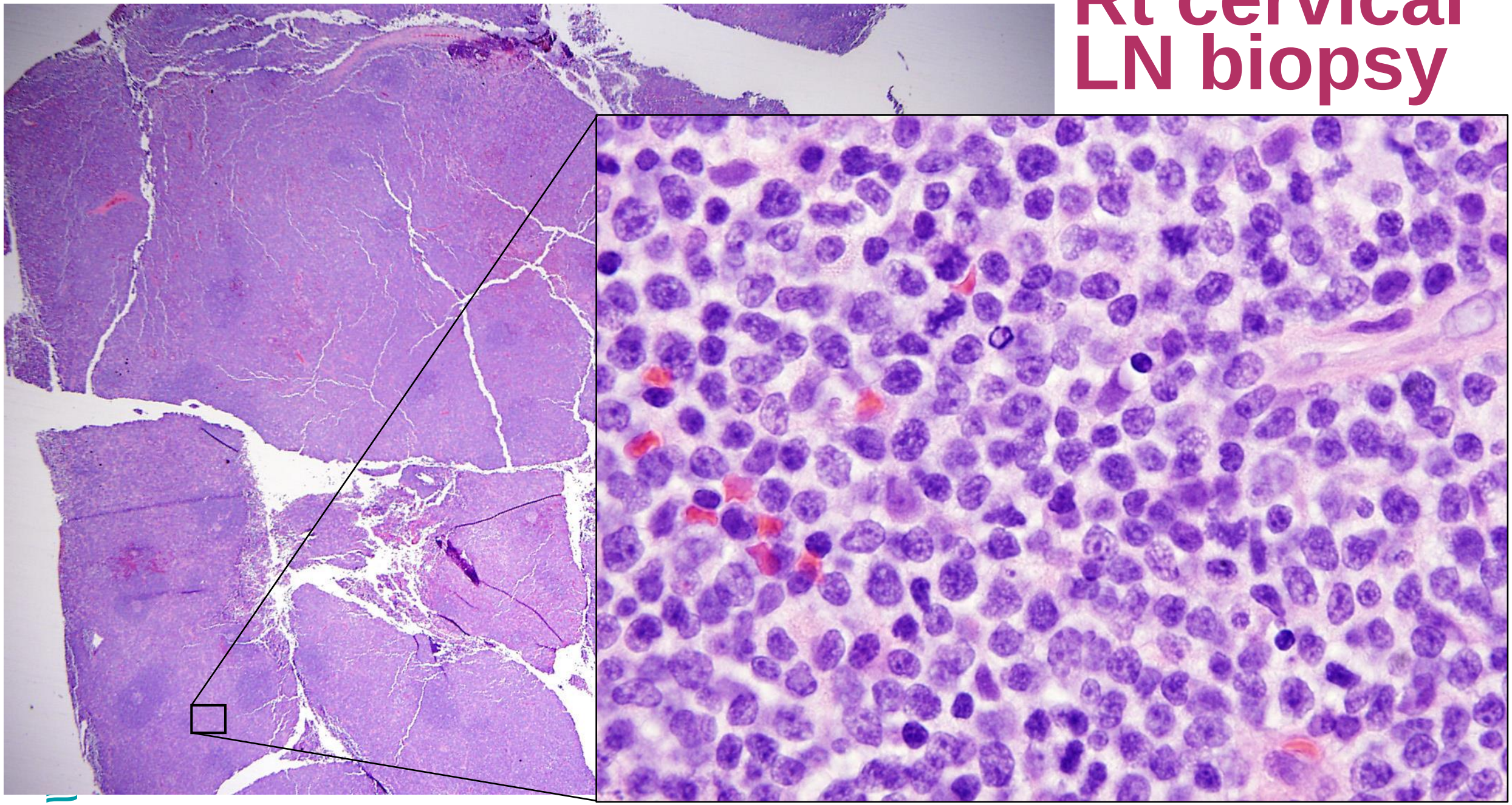
Diffuse lymphadenopathy:

- CT: left axillary lymphadenopathy with lymph nodes measuring up to 3.3 x 2.3 cm, bilateral supraclavicular adenopathy, left periaortic retroperitoneal adenopathy along the iliac chain bilaterally measuring up to 5 cm. There is no splenomegaly.

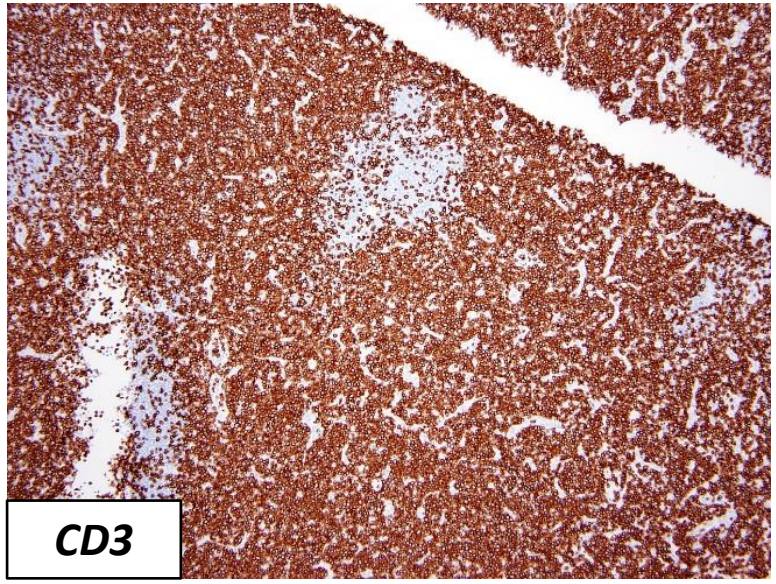
CBC: WBC 10.8, Hgb 13.6 and PLT 215

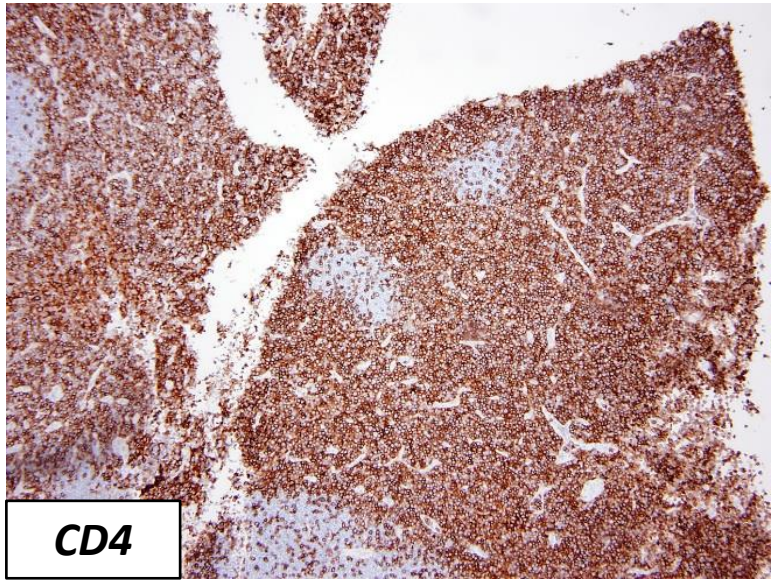
- No WBC differential is provided

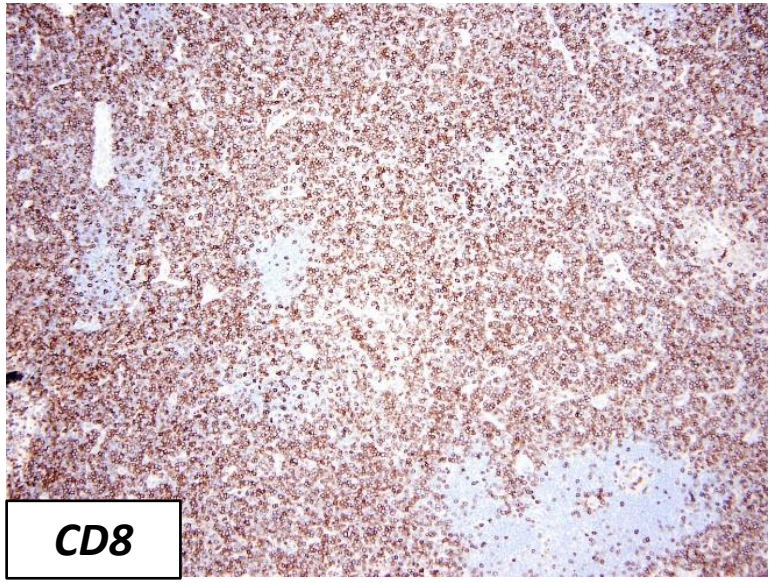
Rt cervical LN biopsy

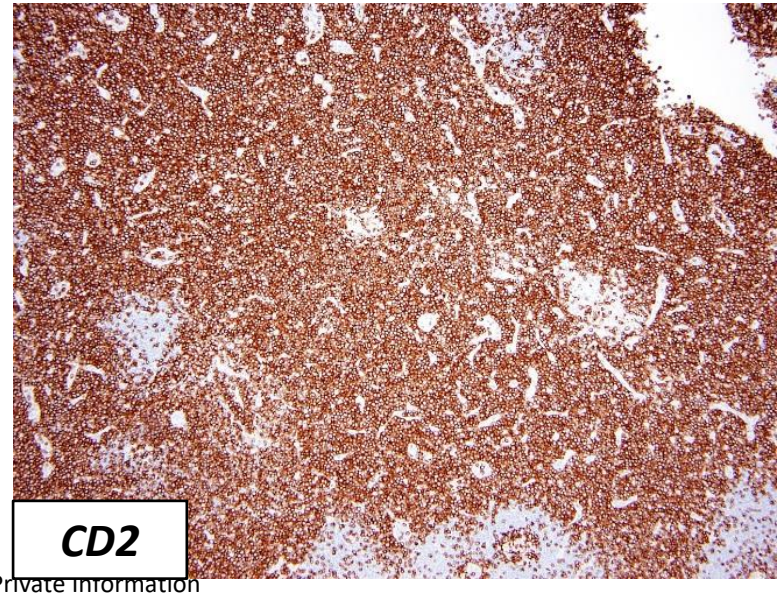


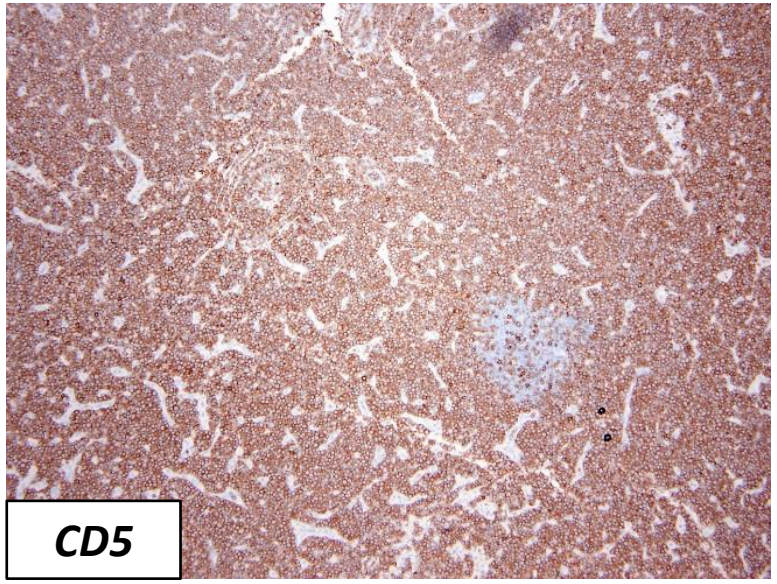
Positive for many T-cell markers

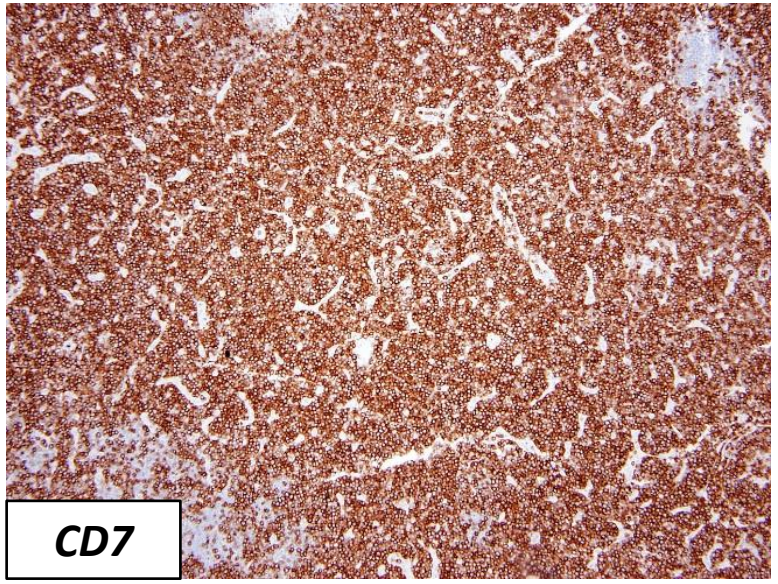




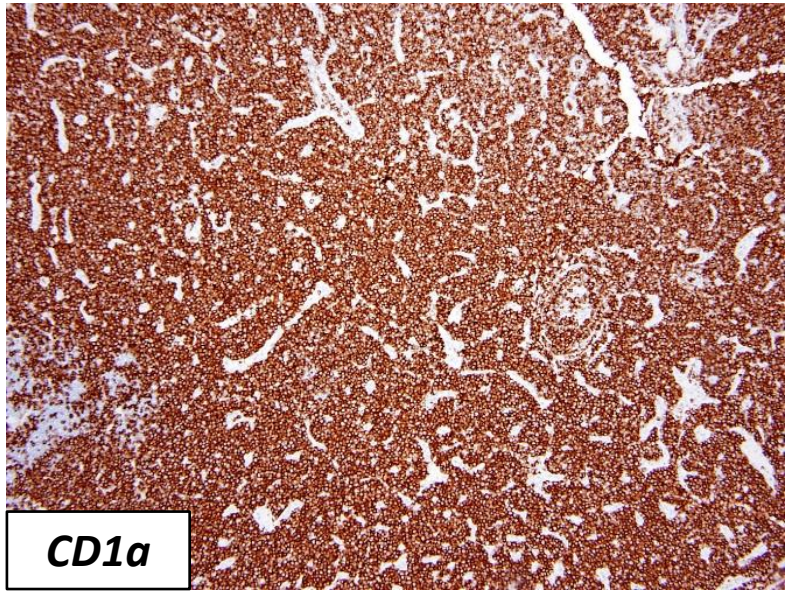




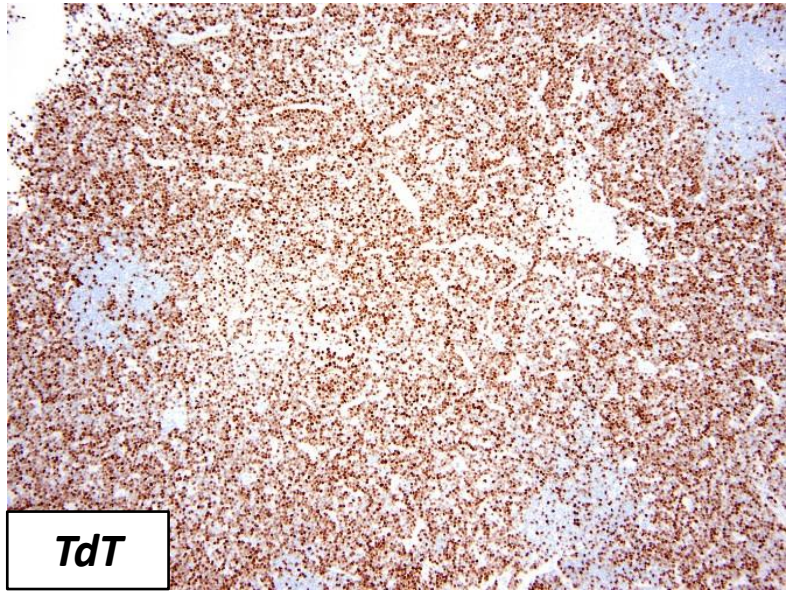




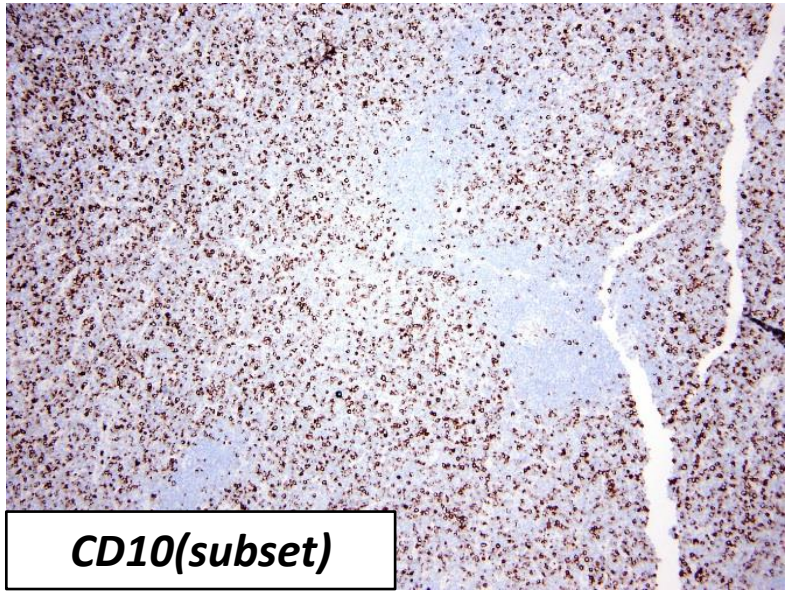
Positive for markers of immaturity



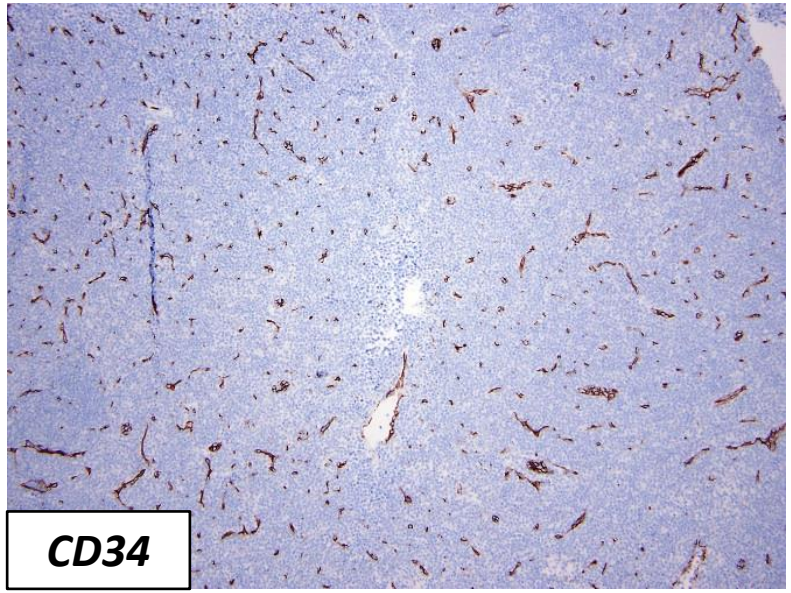
CD1a



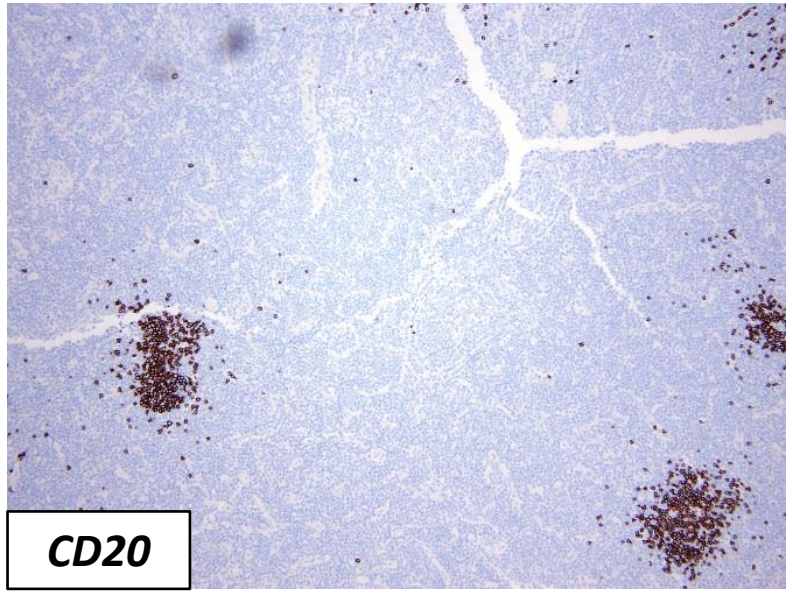
TdT



CD10(subset)



CD34



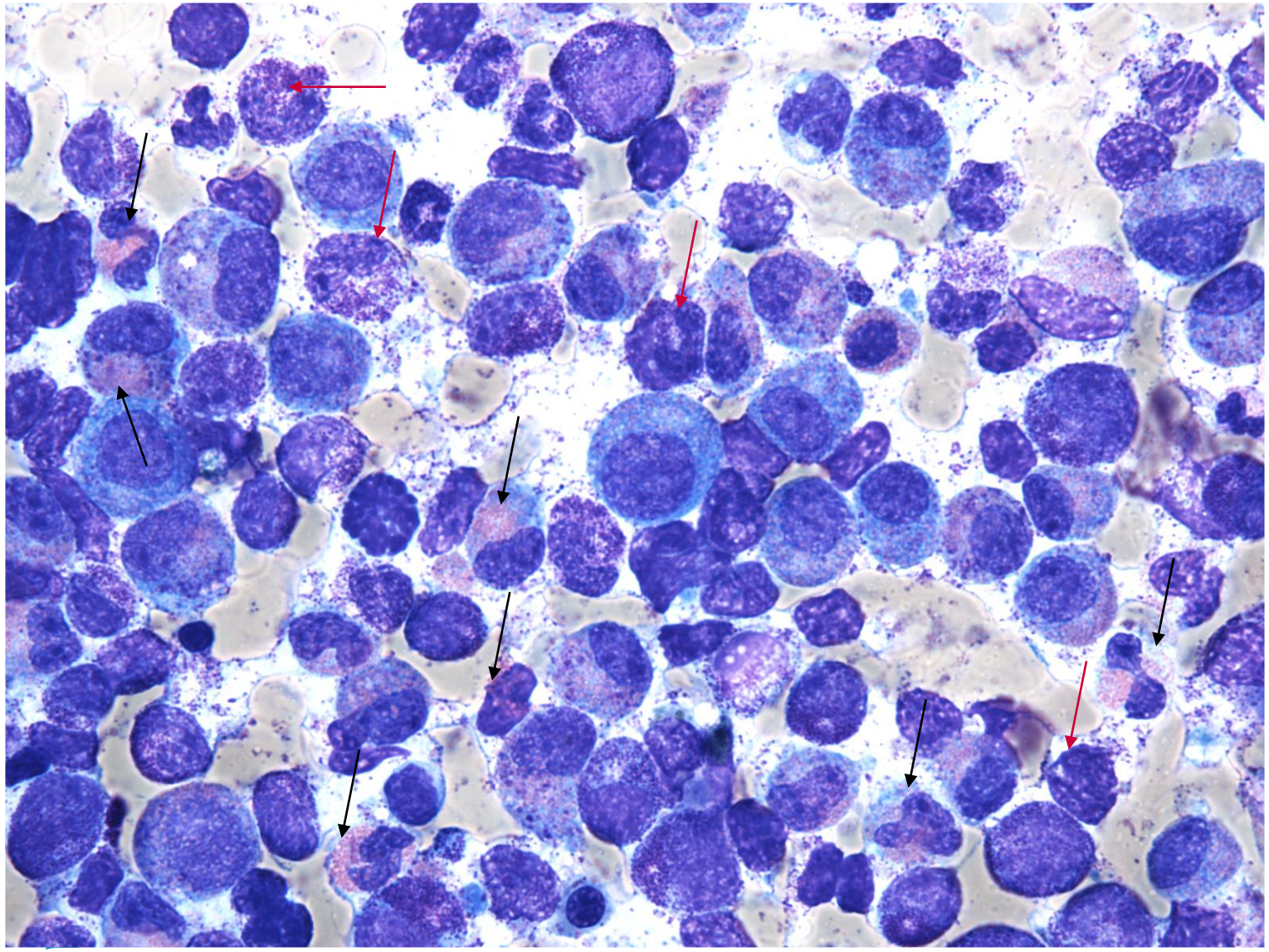
CD20

T lymphoblastic lymphoma

(+) CD3, CD4, CD8, CD2, CD5, CD7, CD1a, TdT, CD10(subset), PD1

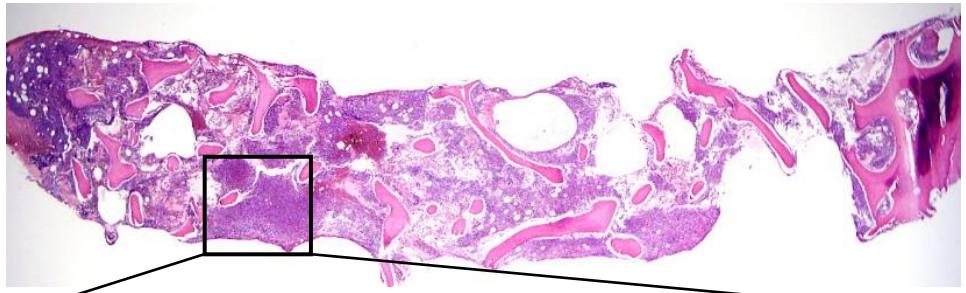
(-) ALK1, CD34, TCL1, FoxP3, CD25, CD30, CD20, PAX5

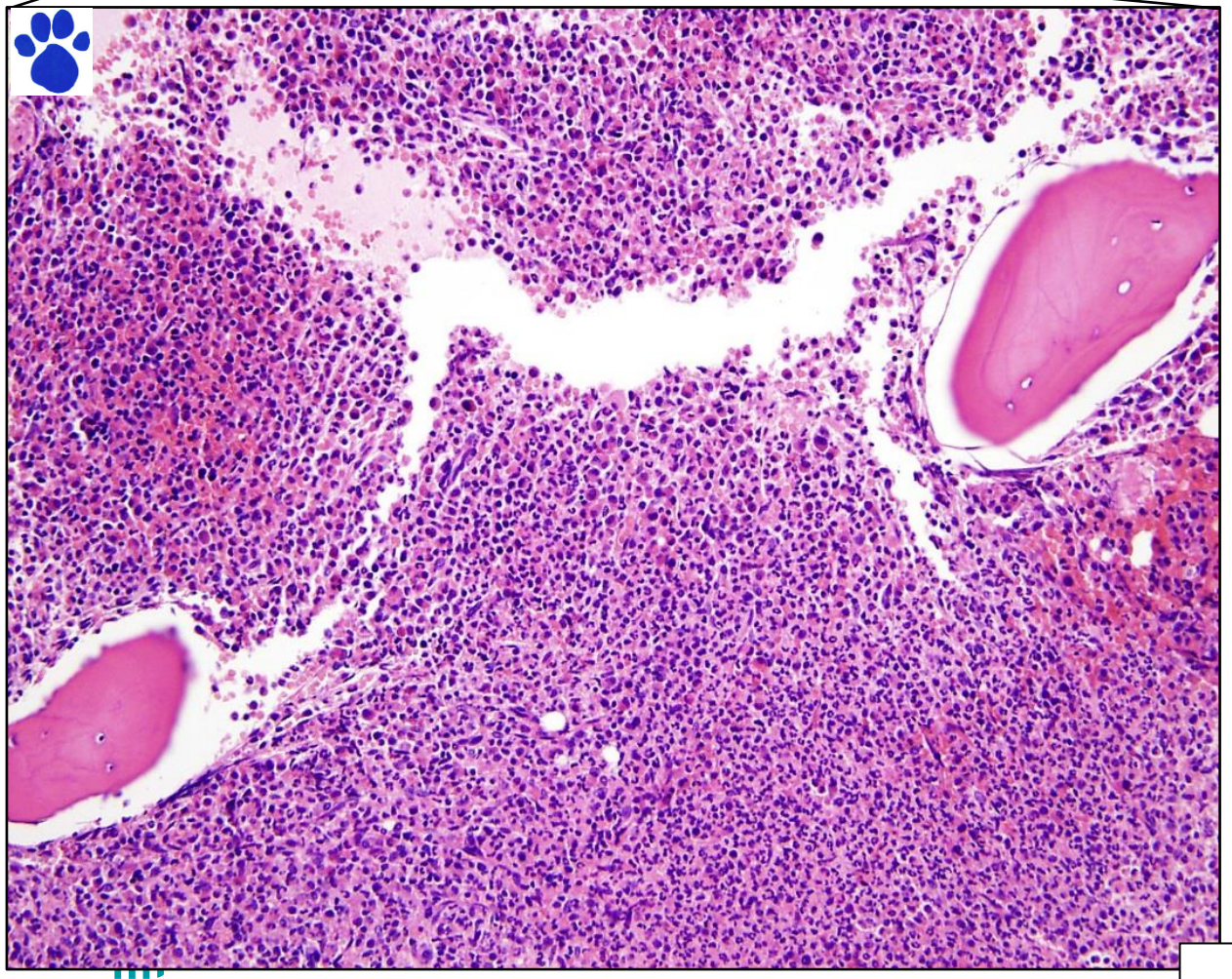
Bone marrow aspirate



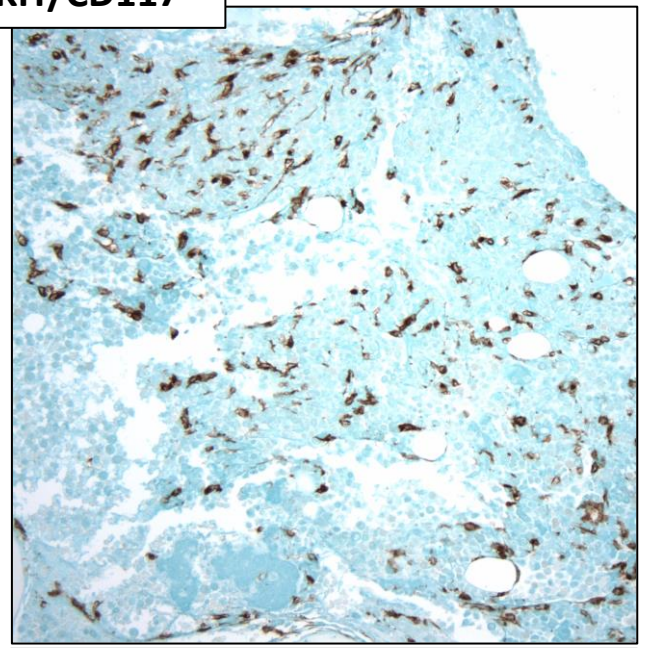
Differential count:

Cellularity : Increased
Megakaryocytes : Present
Blasts : -
Promyelocytes : 4%
Myeloid : 78%; markedly increased eos (including immature forms) and some eo-baso forms, neutrophils with prominent granules
Erythroid : 10%
Lymphocytes : 8%
Plasma cells : -
Others : -; ?Mast cells, partially degranulated
M:E ratio : 7.8:1

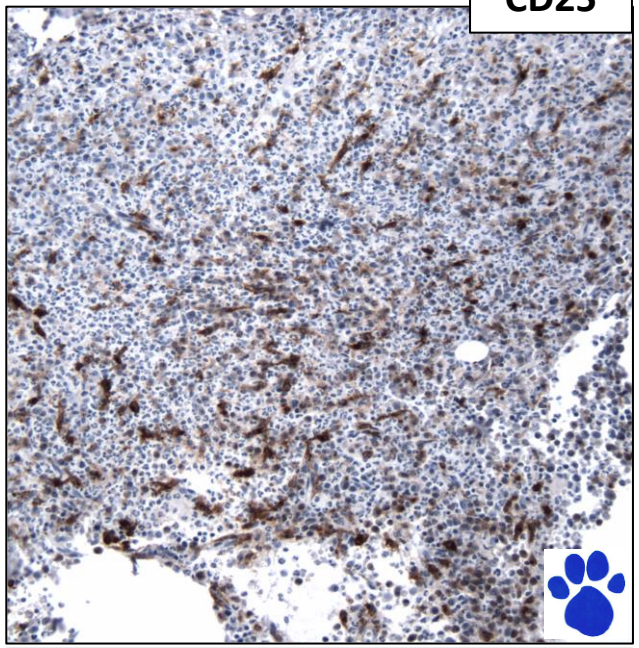


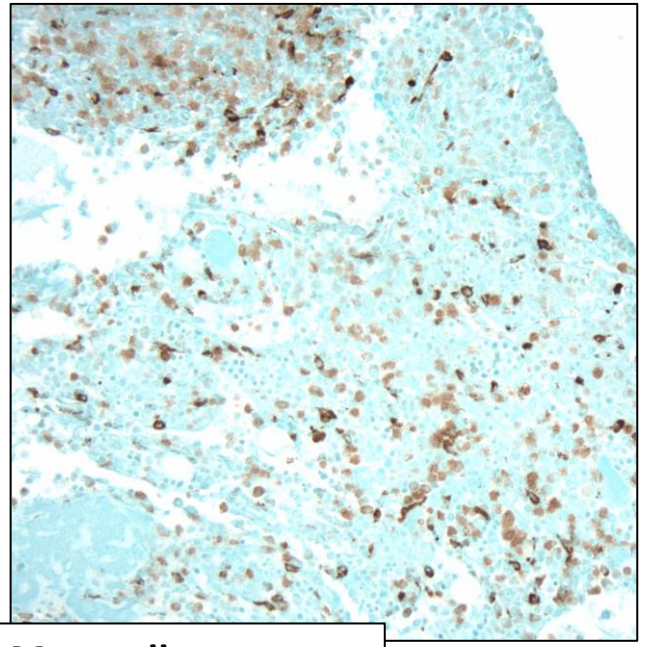


KIT/CD117

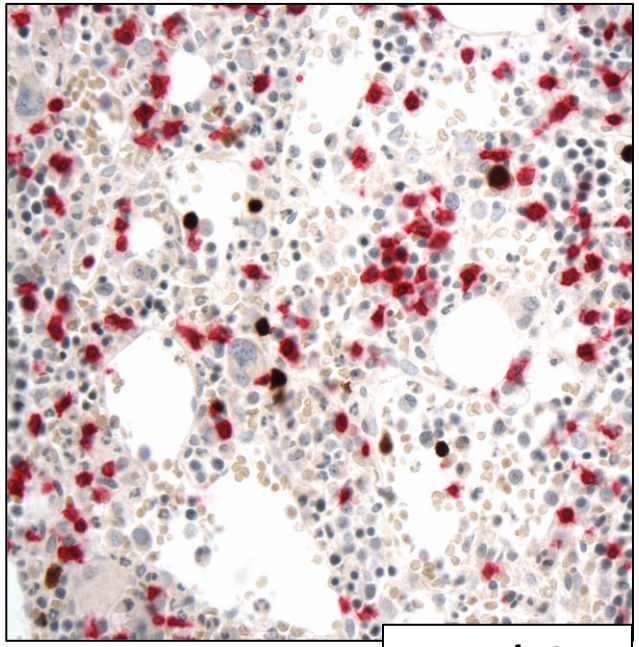


CD25



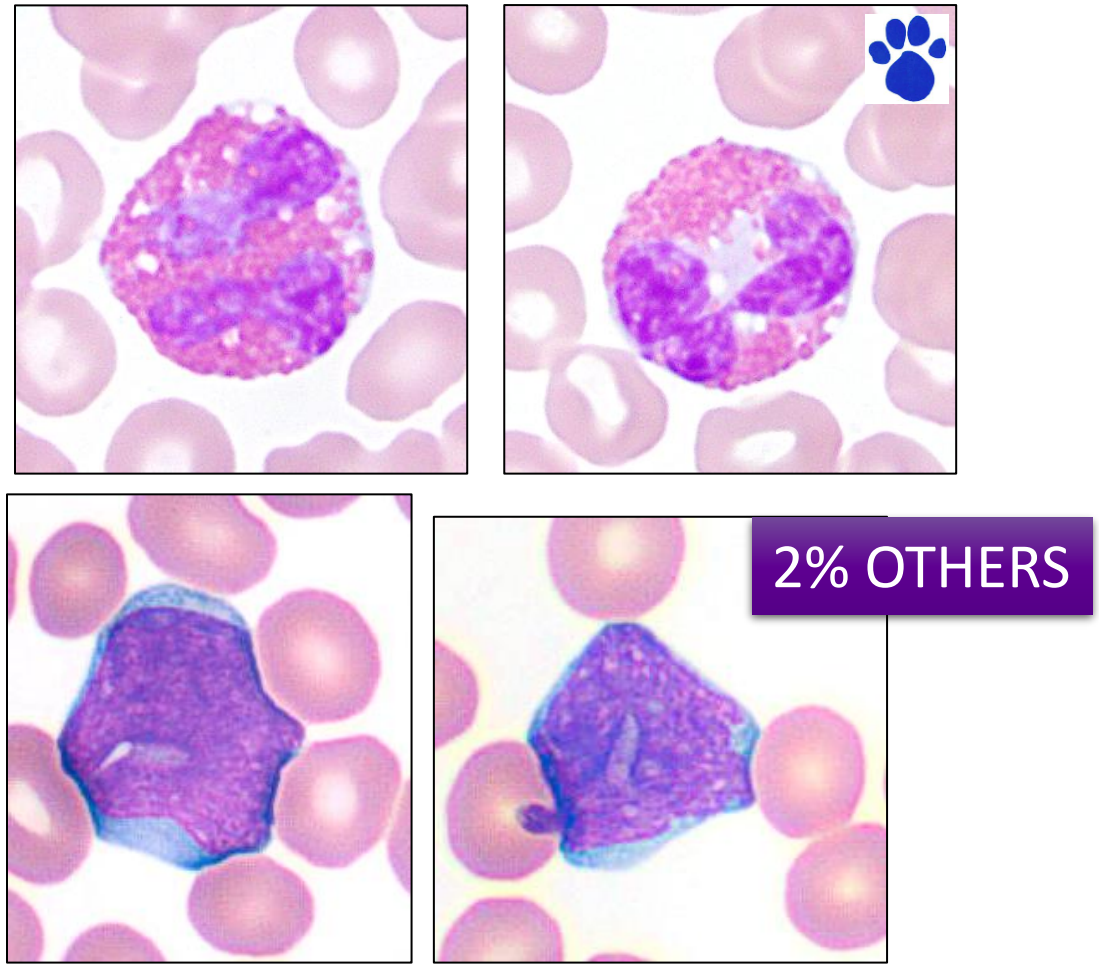


Mast cell tryptase



CD3/TdT

CBC at the time of BM biopsy:



Parameters	Result	Reference range
WBC	12.32 (H)	3.81 – 8.94 K/µL
- Neutrophils	9.11 (H)	2.23 – 6.11 K/µL
- Lymphocytes	1.11	0.21 – 2.74 K/µL
- Monocytes	0.49	0.20 – 0.87 K/µL
- Eosinophils	0.49	0 – 0.52 K/µL
- Basophils	0.25 (h)	0 – 0.11 K/µL
Hgb	8.7 (L)	12.5 - 16.3 g/dL
HCT	27.8 (L)	37.1 – 49.5 %
MCV	86.3	79.0 - 97.0 fL
PLT	374	152 - 440 K/K/µL

Markedly elevated tryptase at 82.1 ng/mL

Genetic analysis

Karyotype:

46,XY[20]

NGS molecular analysis:

Negative for Pathogenic Single Nucleotide Variants and Small Insertions/Deletions

Summary of diagnostic findings (so far …)

CBC

- Mild leukocytosis with 2% blasts, 4% eosinophils with abnormal morphology
- Anemia but no thrombocytopenia

Lab tests:

- Markedly elevated tryptase at 82.1 ng/mL

Multiorgan involvement:

- LN – T-LBL
- BM – ?reactive or ?some kind of MPN - ?CEL ?SM ?SM-AHN:
- Markedly hypercellular
- Marked myeloid hyperplasia (hypergranular)
- Marked eosinophilia (partially granulated, cytoplasmic vacuoles, abnormal nuclear segmentation)
- Increased mast cells (hypogranular, mostly round, clusters <15 cells, aberrant CD25)

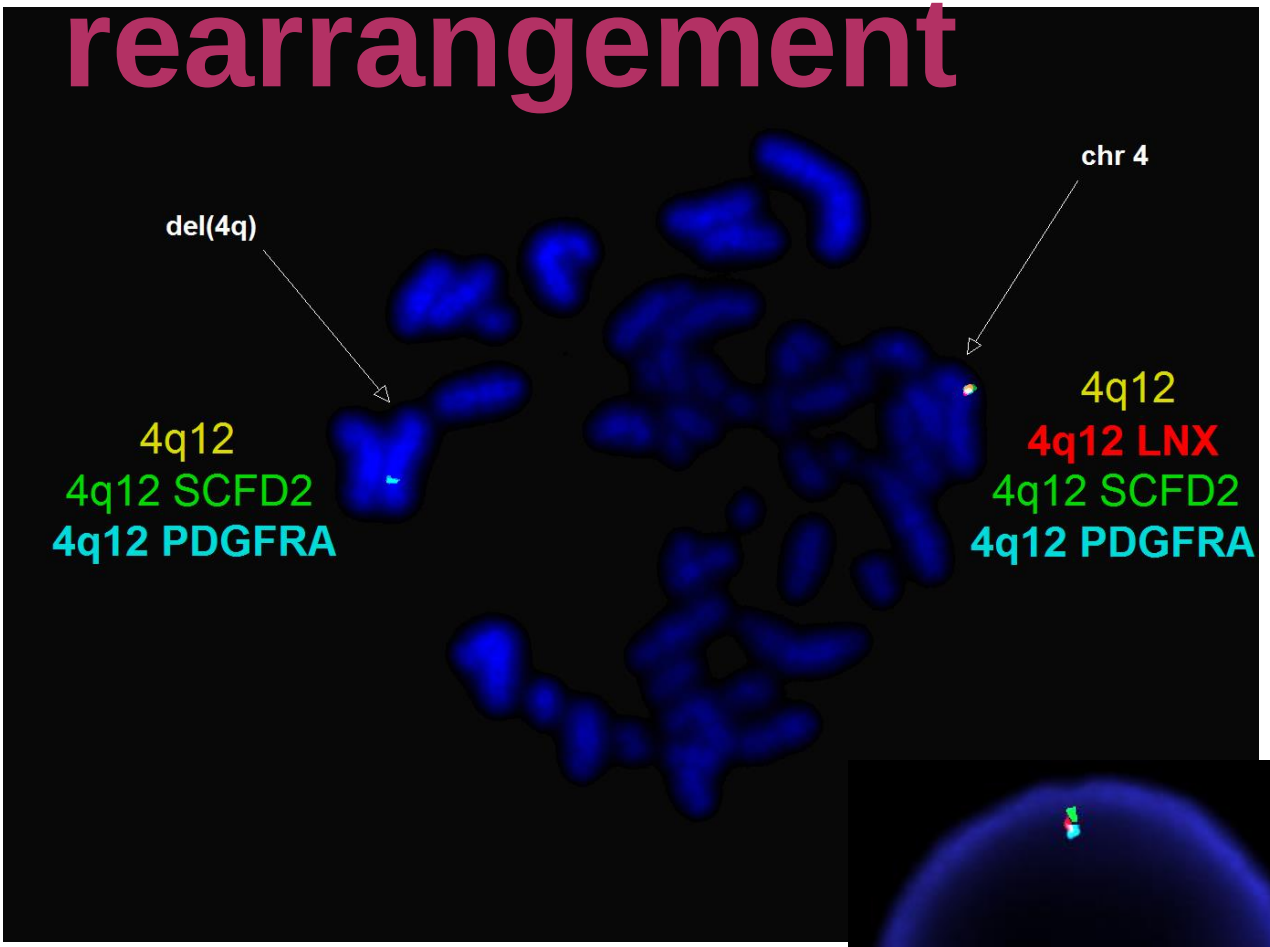
Genetics:

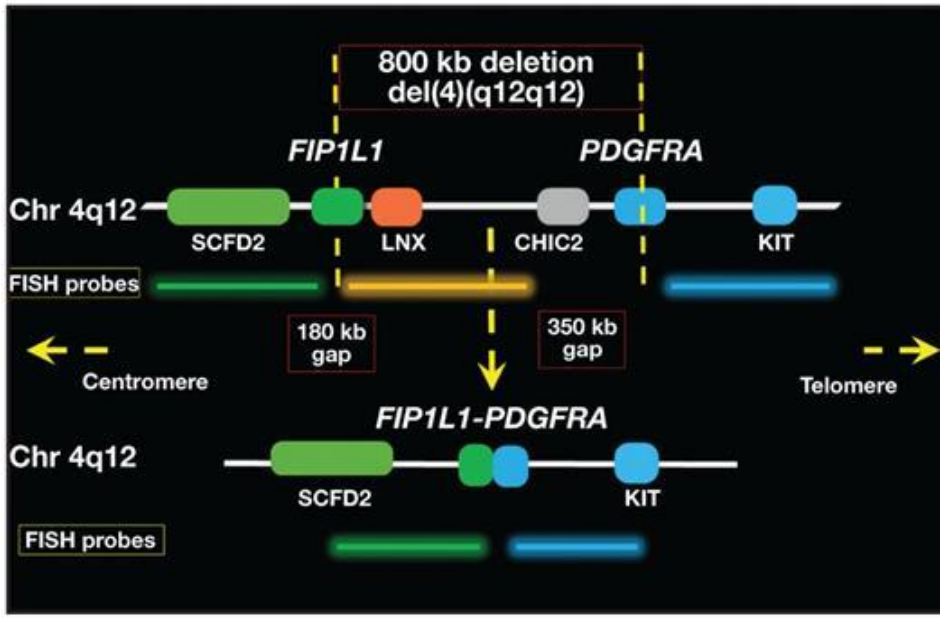
- Normal

We have options!

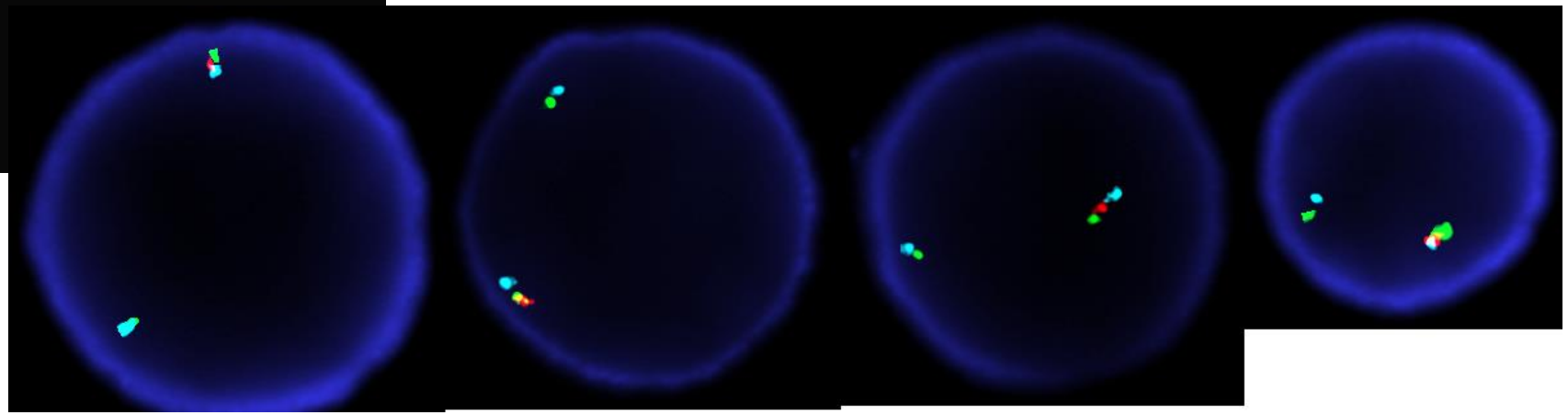
1. T lymphoblastic lymphoma (extramedullary)
   - Unequivocal
2. Systemic mastocytosis
   - Pro – increased mast cells in BM, forming clusters, spindled morphology, aberrant CD25, increased tryptase
   - Con – absence of *KIT* mutation
3. Systemic mastocytosis with an associated hematological neoplasm
   - Pro – see #2 + hypercellularity with marked neutrophilia and eosinophilia with abnormal morphology
   - Con – see #2 + absence of myeloid-associated pathogenic variants
4. #1 + #2 + #3
   - Pro – unifying diagnosis, such as MLN-eo (some are cryptic), male gender, targeted therapy
   - Con – absence of PB eosinophilia

FISH for *PDGFRA* rearrangement





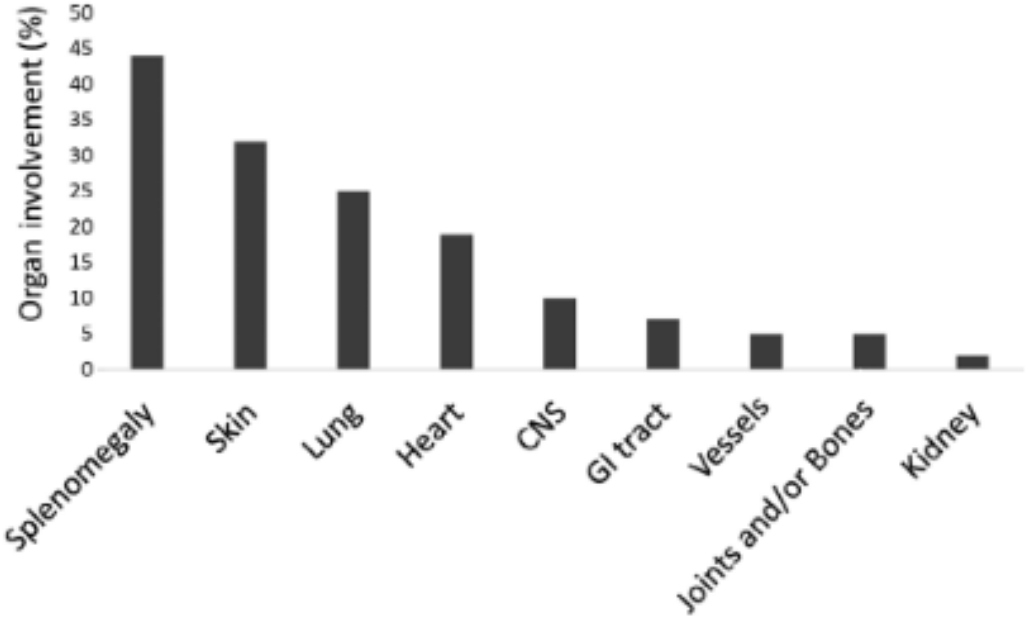
Positive in 69%



Patients	N = 151
Male	143 (96)
Age at diagnosis	49 +/– 12
Number of organs involved	
Asymptomatic	26 (17)
1	41 (28)
2	36 (24)
3 or more	31 (21)
CBC	
Eosinophils (/mm3)	10 309 +/– 5960
Hemoglobin (g/dl)	13 +/– 2
Platelets (/mm3)	195 700 +/– 63 600
Neutrophils (/mm3)	6850 +/– 5330
Lymphocytes (/mm3)	2650 +/– 1120
Basophils (/mm3)	240 +/– 270
Monocytes (/mm3)	640 +/– 415
F/P transcript screening	
PCR	140/140 (100)
FISH	87/87 (100)
Other	
High B12 levels	74/79 (94)
Median (IQR) serum B12 levels (pmol/l)	1741 (1170-2080)
High tryptase levels	45/57 (79)
Median (IQR) serum tryptase levels (ng/mL)	23 (14-43)
High CRP levels	34/118 (29)
Median (IQR) serum CRP levels (mg/L)	19 (9-30)
High total IgE levels	12/86 (14)
Median (IQR) serum IgE levels	20 (8-168)

Exclusively sensitive to imatinib!
OS: 1-year 99%, 5-year 95% and 10-year 84%
None developed accelerated phase

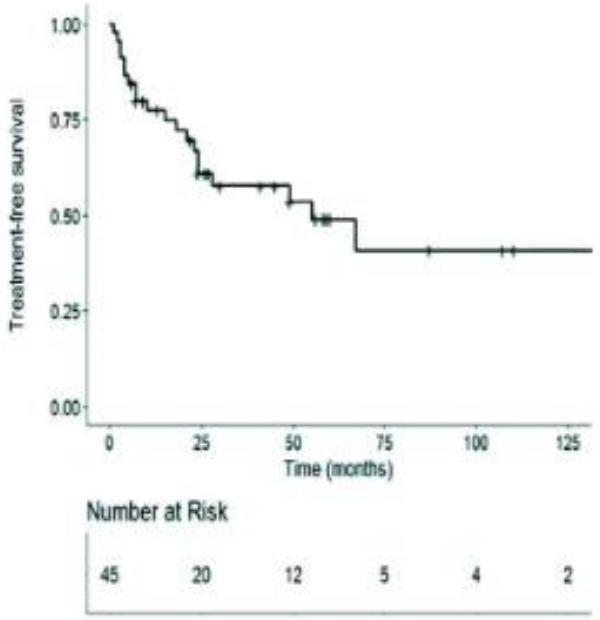
Frequency of organ involvement



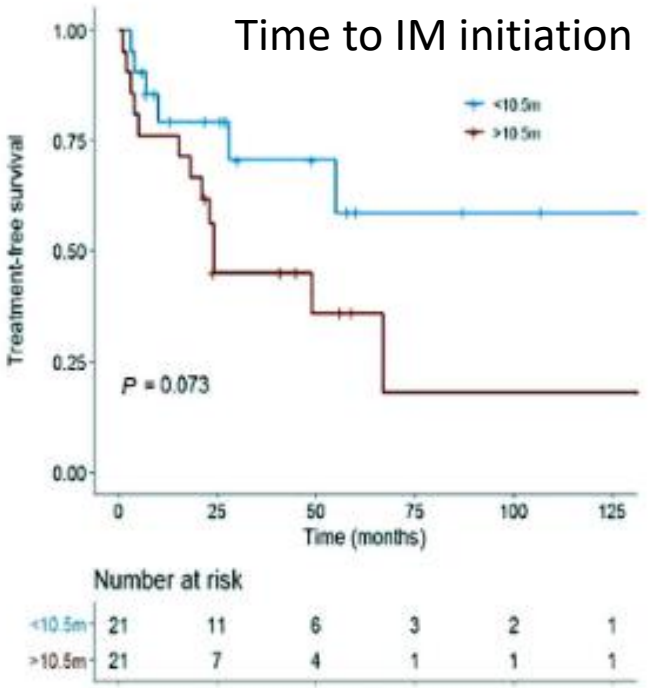
Rohmer J et al. Am J Hematol. 2020 Nov;95(11):1314-1323PMID: 32720700.

Predictors of relapse after imatinib (IM) treatment withdrawal (n=46 pts)

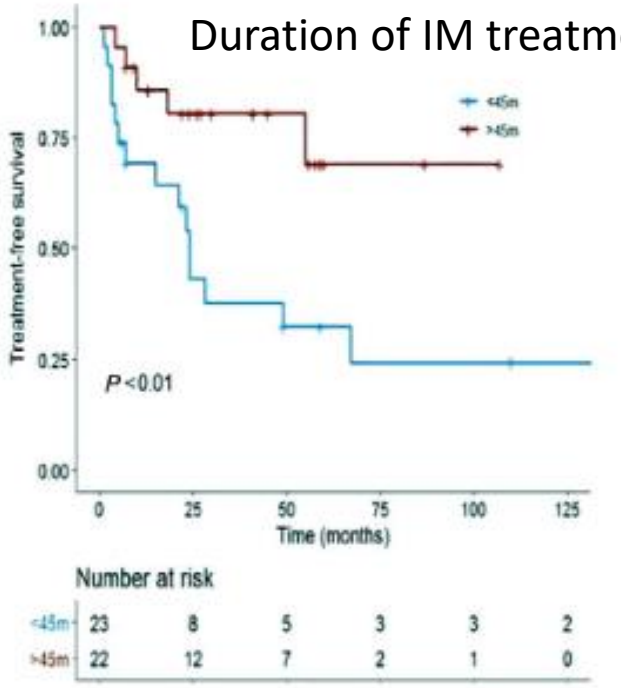
All pts



Time to IM initiation



Duration of IM treatment



	Univariate analysis			Multivariate analysis		
	HR	CI95%	*P*-value	HR	CI95%	*P*-value
Treatment with IM						
Time to IM initiation (months)	**1.01**	**[1.00–1.03]**	**.01**	**1.01**	**[0,99–1.03]**	**.05**
Sudden withdrawal	1.16	[0.48-2.85]	.74			
Total IM treatment duration	**0.97**	**[0.95–0.99]**	**.002**	**0.97**	**[0.95–0.99]**	**.001**
IM starting dose	1	[0.99–1.00]	.77			
Last IM dose before withdrawal	1	[0.99-1.01]	.95			

Rohmer J et al. Am J Hematol. 2020 Nov;95(11):1314-1323PMID: 32720700.

Myeloid/lymphoid neoplasms with *PDGFRA* rearrangement

- >90% of *PDGFRA* rearrangements are cytogenetically **cryptic**
  - *FIP1L1::PDGFRA* – cryptic 4q12 deletion
  - Other partners (7): *BCR* (22q11), *ETV6* (12p13), *KIF5B* (10p11), *CDK5RAP2* (9q33), *STRN* (2p22), *TNKS2* (10q23), *FOXP1* (3p13)
  - *PDGFRA* mutation
- **Diverse** morphologic spectrum usually with hypereosinophilia
  - CEL, AML, ALL and SM
  - abnormal eosinophil morphology (uneven granulation, hypo- or hypersegmentation)
- ~20% of cases present **without** PB eosinophilia
  - Some have variant *PDGFRA* rearrangements other than *FIP1L1*; may show abnormal karyotype
  - Some show myeloid/eosinophilic proliferations in biopsies that can be a clue
- **Extramedullary** presentation is common ~50% of cases and it can be the primary site of *PDFGRA*-rearranged neoplasm
  - LN is the most common site of involvement
  - MPN with eosinophilia is the most common pattern (but can vary)
- ~40% of cases show aberrant mast cell proliferations in the absence of a *KIT* mutation (**helpful feature!**)
- If *PDGFRA* FISH is negative and suspicion is high use **other** molecular methods
  - RNAseq, SNP-CN microarray
- Consider a **trial** of TKI in cases with hypereosinophilia not responsive to conventional therapy and perform comprehensive retrospective testing in responders

Pozdnyakova O et al. Am J Clin Pathol. 2021 Feb 4;155(2):160-178 PMID: 33367495.

Clinical, diagnostic pathologic features and treatment of MLN-eo and gene rearrangements

Fusion	Marrow Myeloid Presentation	Extramedullary and/or lymphoid Presentation	Progression	Diagnosis	Treatment
FIP1L1 – *PDGFRA* Other partners: *KIF5B, CDK5RAP2, STRN, ETV6, BCR, TNKS2, FOXP1* 25-55 y (median 40s) M>>>F 17:1	CEL AML SM, other MPN	AML/MS T-LBL B-LBL	AML T-LBL B-LBL	Cryptic by KT *SCFD2/LNX/PDGFRA* FISH RT-PCR RNA (or DNA) Fusion NGS	Imatinib
ETV6 – *PDGFRB* Numerous other partners (n = 30) 8-72 y (median 40s) M>F 2:1	CMML CEL aCML SM, other MPN AML	AML/MS T-LBL	AML T-LBL Excludes Ph-like B-LBL	May be cryptic by KT Complex rearrangements *PDGFRB* breakapart RT-PCR confirmation or monitoring of *ETV6-PDGFRB* RNA (or DNA) Fusion NGS	Imatinib
FGFR1 Several other partners (n=14) 3-84 y (median 32) M>F 1.5:1	CEL SM, other MPN AML	AML/MS T-LBL	AML T- or B-LBL MPAL	KT RNA (or DNA) fusion NGS Common additional somatic variants (especially *RUNX1*)	In development
PCM1 – *JAK2* Other partners: *ETV6, BCR* 12-75 y (median 47) M>>F 5.4:1	CEL MDS/MPN +fibrosis	AML/MS T- or B-LBL	AML T- or B-LBL Excludes Ph-like B-LBL	*KT* *JAK2* breakapart confirmation RNA (or DNA) Fusion NGS	Ruxolitinib (transient)

Kim A and Pozdnyakova O. Clinical Lymphoma, Myeloma and Leukemia. In Press

What happens when we excluded MLN-eo with gene rearrangements and other MPNs?

Chronic eosinophilic leukemia, NOS

- Clonality and abnormal BM morphology (i.e. dysplasia)
- 2-19% PB or 5-19%% BM blasts

Idiopathic HES

- Exclude above + reactive + LV HE
- BM morphology is normal and shows only increased eosinophils

HE of unknown significance

- Persistent HE and absence of tissue damage
- BM morphology is normal and shows only increased eosinophils

Arber DA et al. Blood. 2022 Sep 15;140(11):1200-1228.PMID: 35767897; PMCID: PMC9479031.
Khoury JD et al. Leukemia. 2022 Jul;36(7):1703-1719. PMID: 35732831; PMCID: PMC9252913.

Summary of Case 3:

1

Cases with *PDGFRA* rearrangement may present without eosinophilia

- Look for clues, such as tissue eosinophilia, abnormal eosinophil morphology, increased mast cells with aberrant CD25 in BM

2

PDGFRA rearrangement is cryptic

- Be vigilant!
- If suspicion is high, insist on using other molecular methods

3

Correct diagnosis is essential for patient's treatment and prognosis

- TKI have revolutionized treatment
- SeruTime to treatment initiation affects prognosis

What we have covered:

Recognize blood and/or bone marrow smears with increased blasts and identify important morphologic clues:

- Recognition of blasts is essential
- Some blasts have unique morphologies
- Pay attention to other cells (numbers and abnormal morphologies)

Appropriately apply and interpret pertinent ancillary methods to cases presenting with increased blasts:

- Always perform immunophenotyping to confirm the presence of blasts and their lineage – morphology is deceptive!
- There are immunophenotypic patterns that are associated with certain underlying genetic alterations
- Targeted therapies have been developed against certain antigens

Understand the diagnostic and prognostic significance of ancillary testing:

- Current classification of acute leukemias is based on genetic findings
- Absence of genetic findings could be a clue! Know potentially cryptic rearrangements
- Many genetic findings guide treatment choices


Mass General Brigham