Molecular aspects of precursor lymphoma/leukemia in the bone marrow

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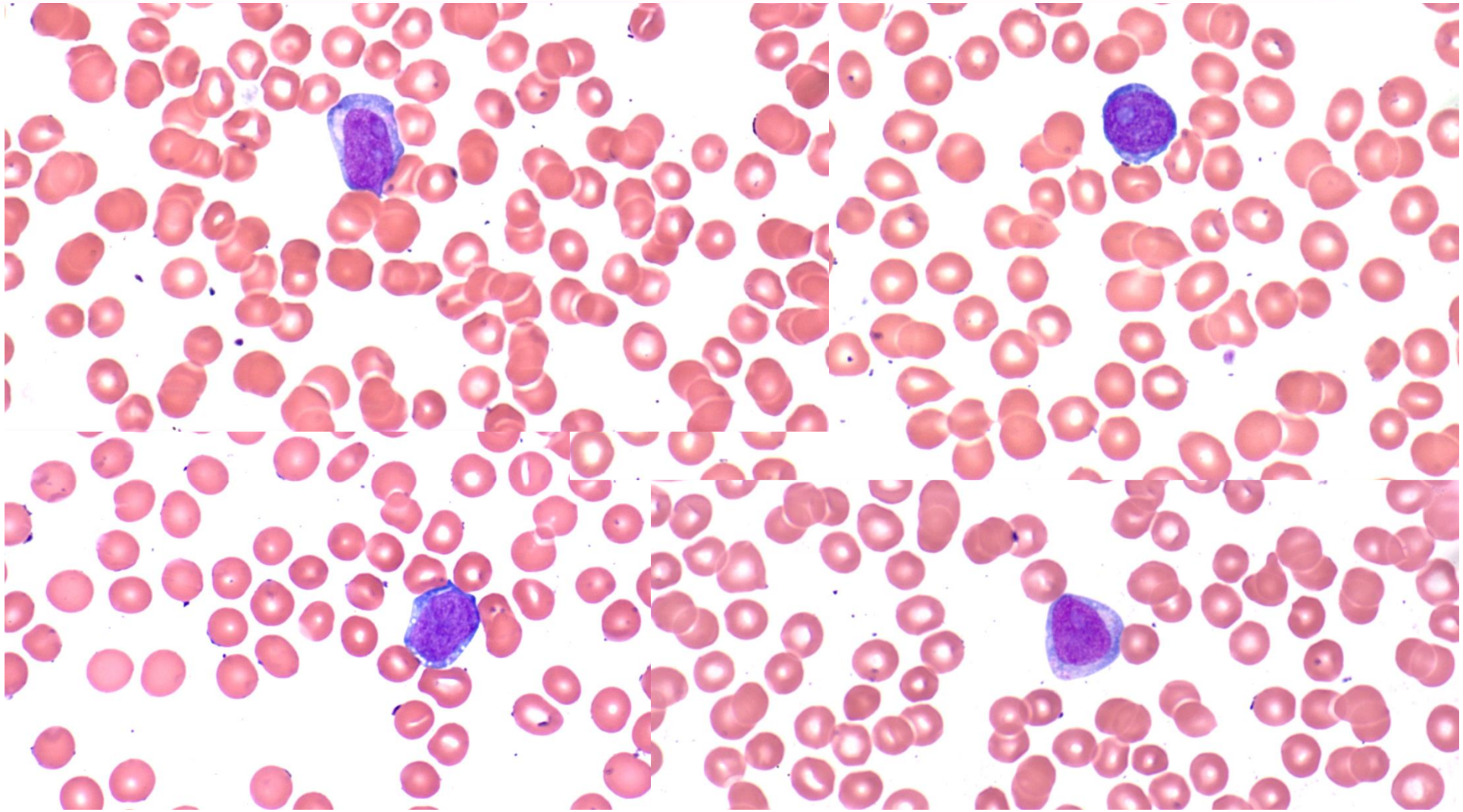
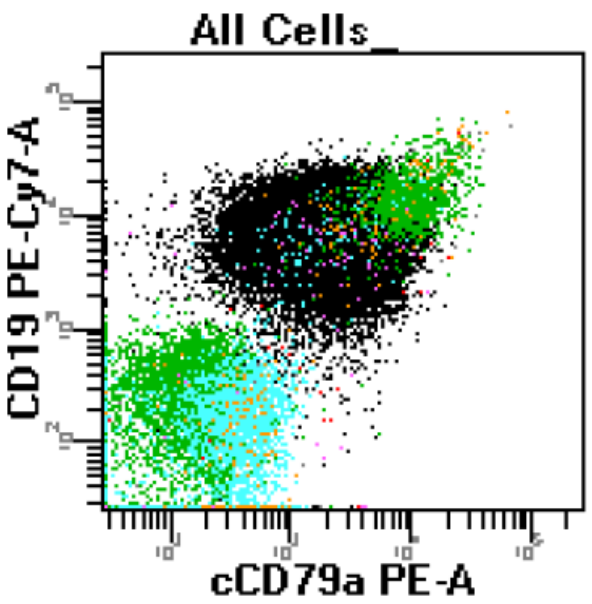
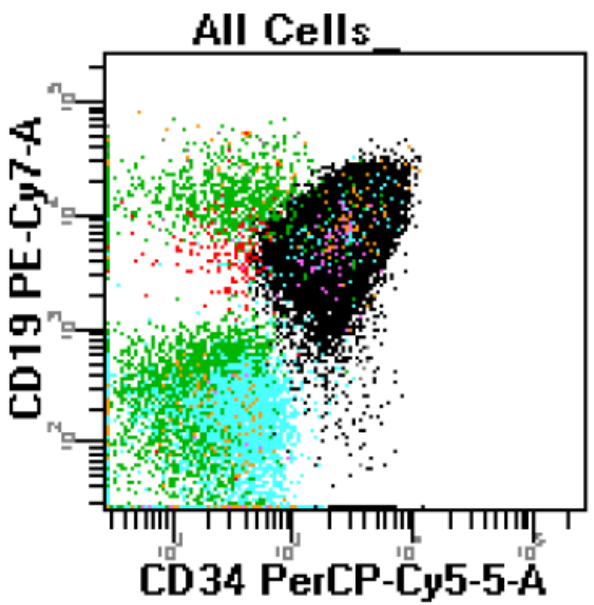
APOYO

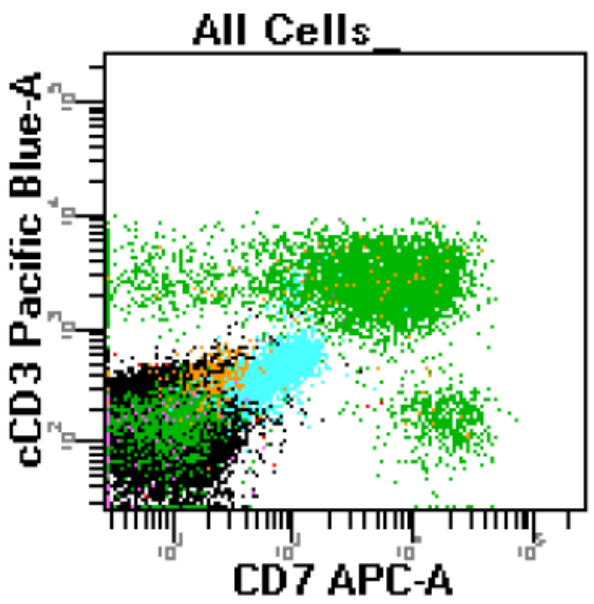
Morphology ~1 hour

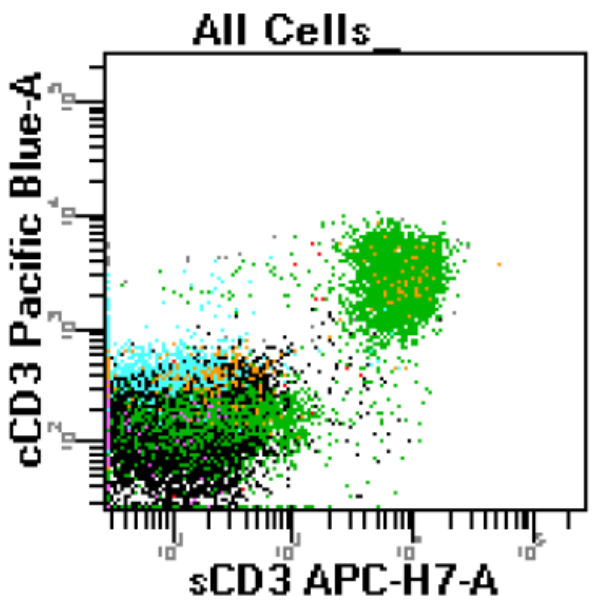


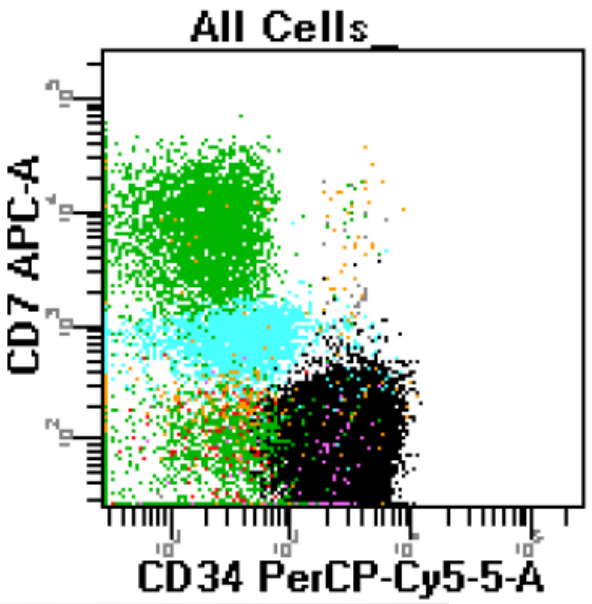
Flow cytometry ~4-5 hours

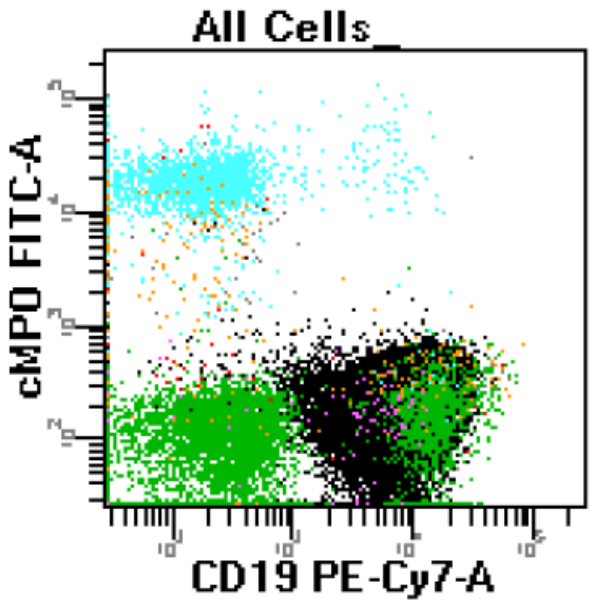


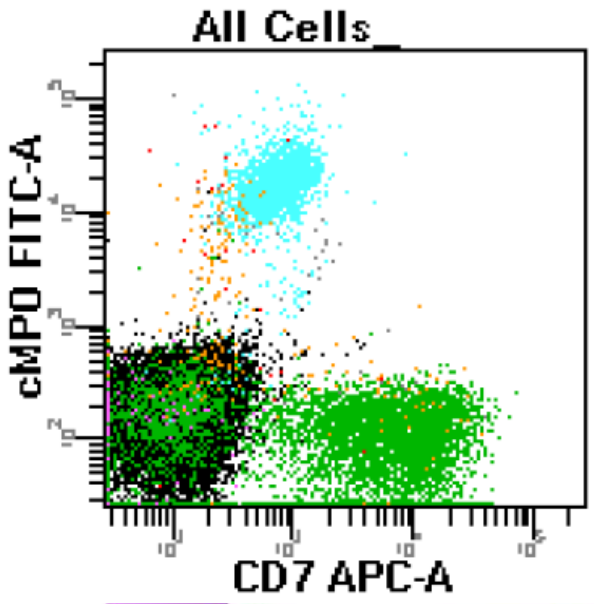


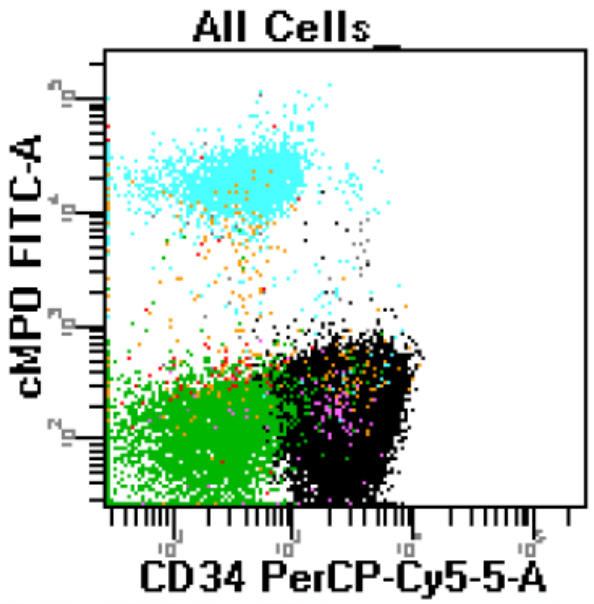




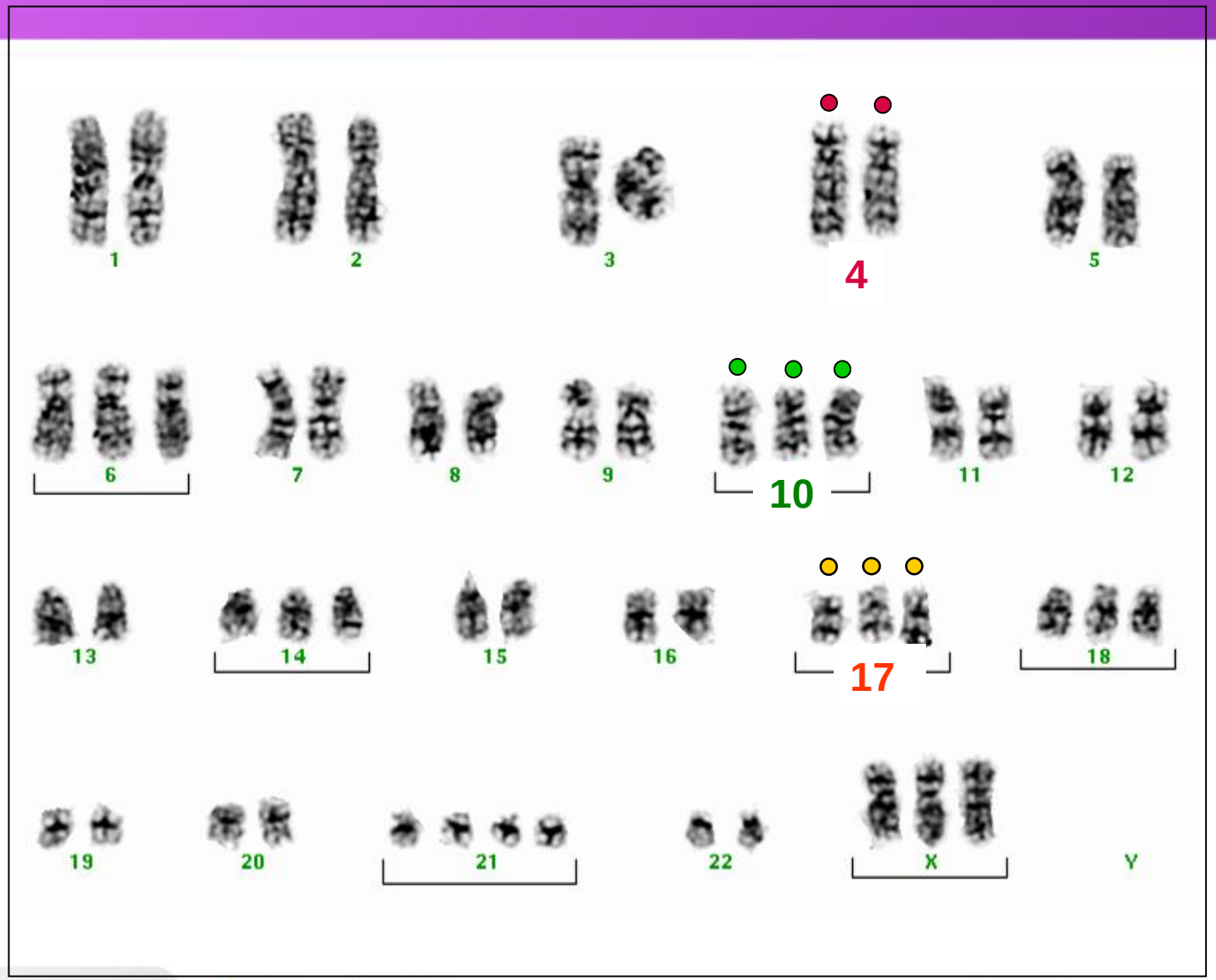


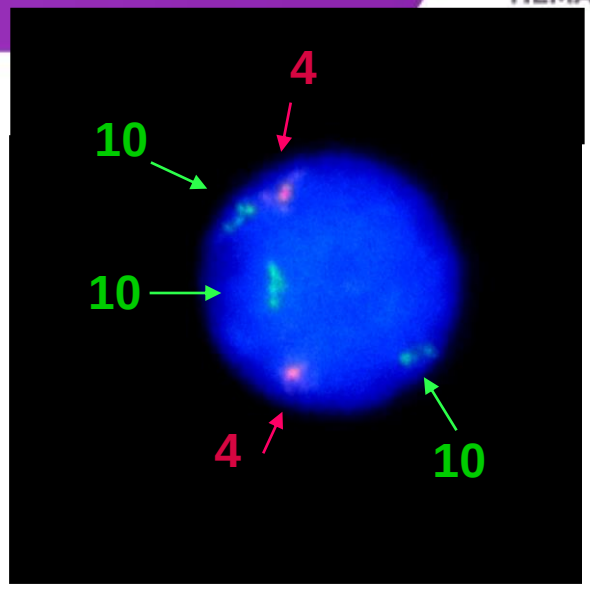


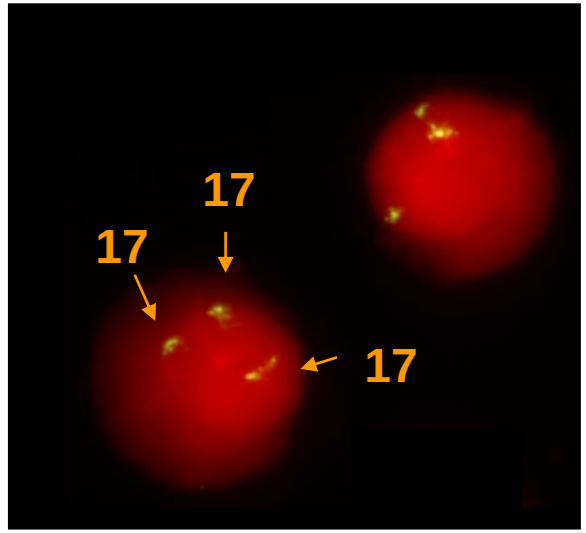




FISH (1-2 days) and karyotype (1 week)







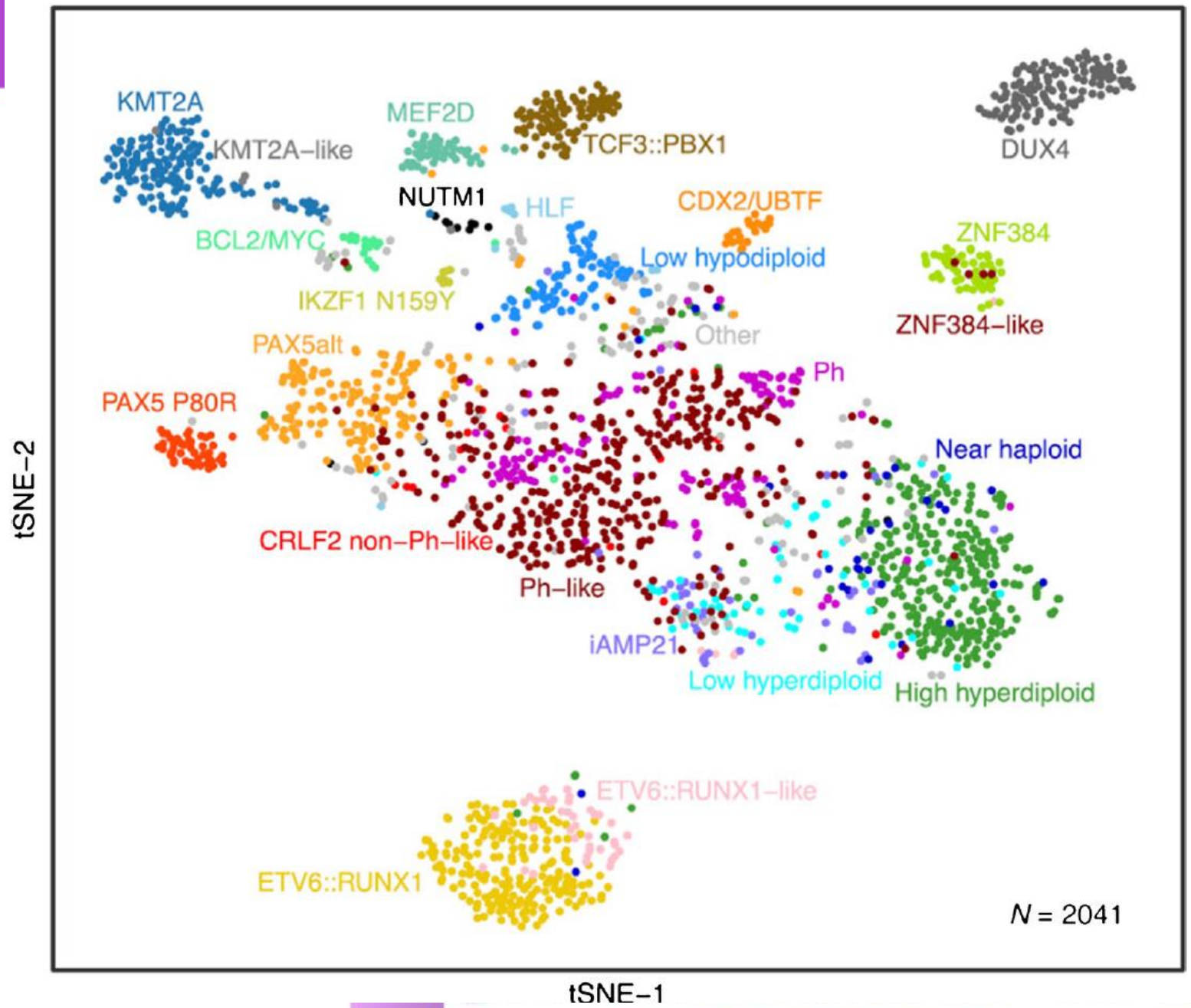
Gene-expression profiling and genetic alteration analysis (RNA-seq)

Heme Fusion RNA NGS 10 days

RNAseq 1-2 weeks

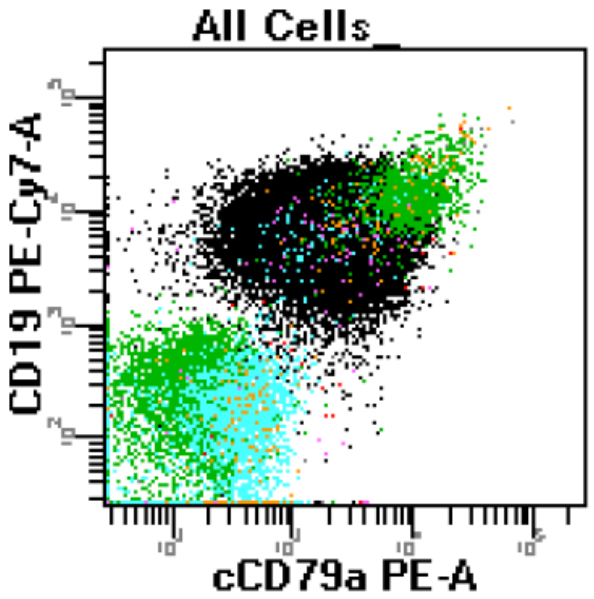
Targeted DNA NGS panel 2-3 weeks

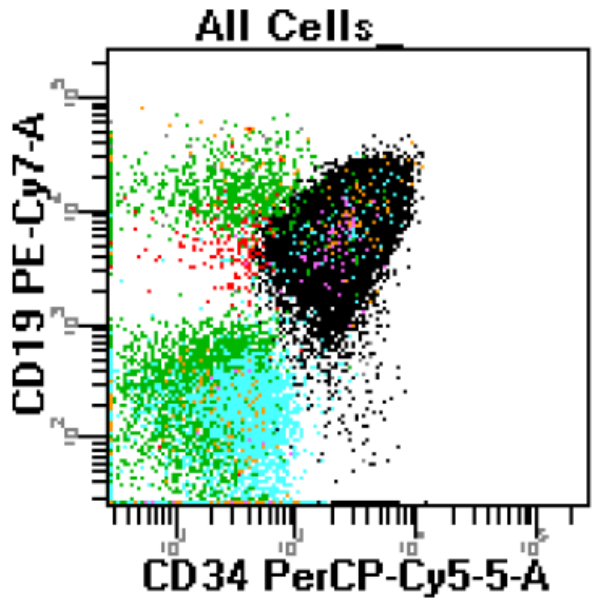
WGS/WES 1-2 months

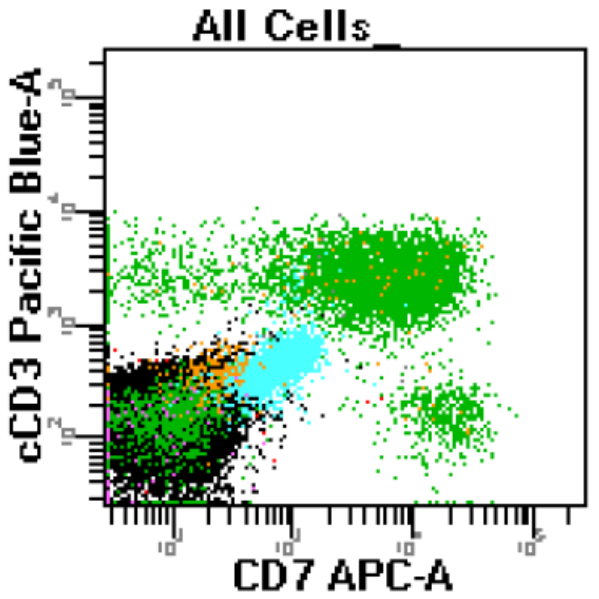


B-LYMPHOBLASTIC LEUKEMIA/LYMPHOMA

- Primarily in children (75% in <6-year-olds)
- B-ALL comprises ~80 of all ALLs, but only ~10% of lymphoblastic lymphomas
  - Leukemia vs lymphoma distinction is based on the site of involvement and the bone marrow blast count
- Cytopenias, lymphadenopathy, organomegaly, bone pain
  - Most frequent sites of involvement are skin, soft tissue, bone and lymph nodes
- Flow cytometry: CD19+, CD79a+, CD10+, TdT+, no light chain expression
- Immunohistochemistry: PAX5+, CD79a+
- Excellent prognosis in children, improving outcomes in adults


All Cells
CD19 PE-Cy7-A
cCD79a PE-A


All Cells
CD19 PE-Cy7-A
CD34 PerCP-Cy5-5-A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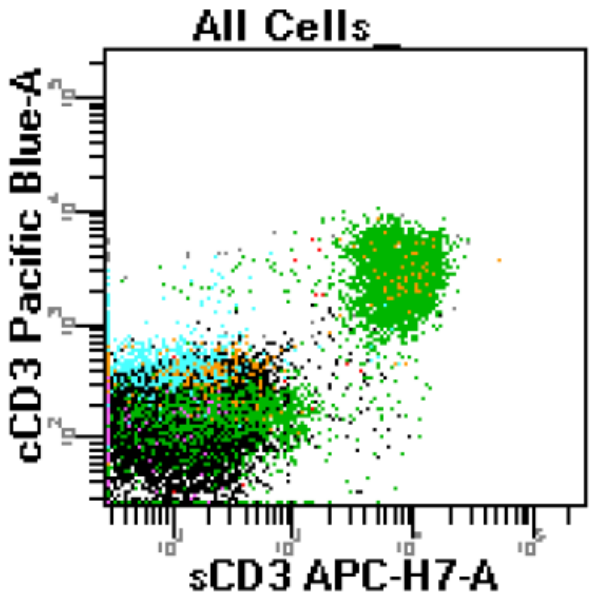
CD7 APC-A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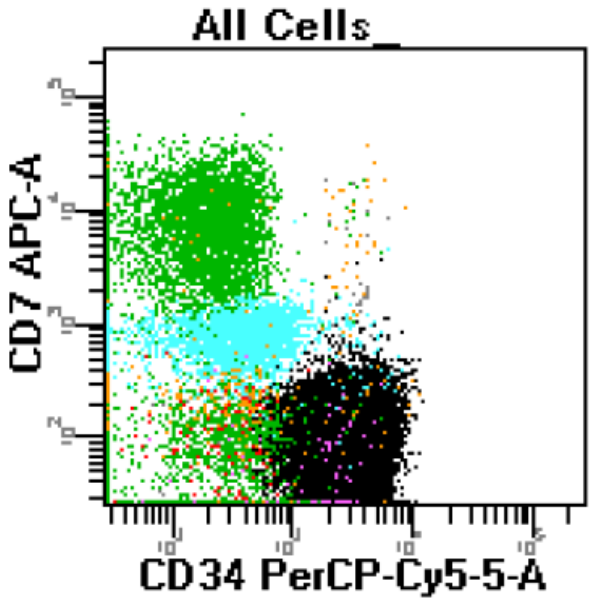
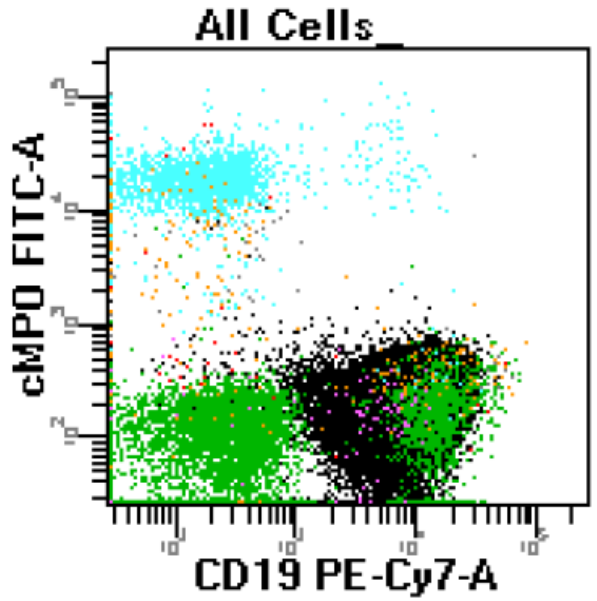

All Cells
CD7 APC-A
CD34 PerCP-Cy5-5-A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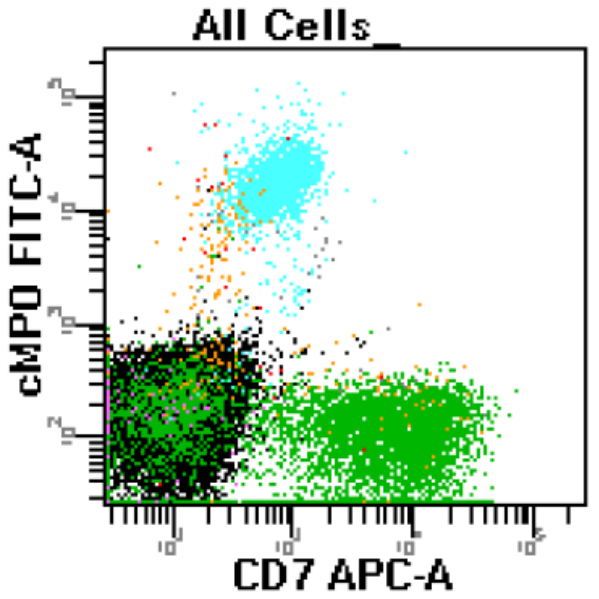
CD19 PE-Cy7-A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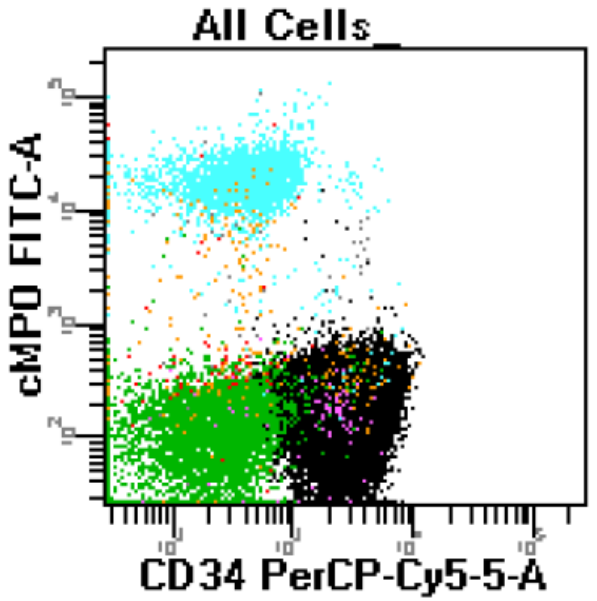
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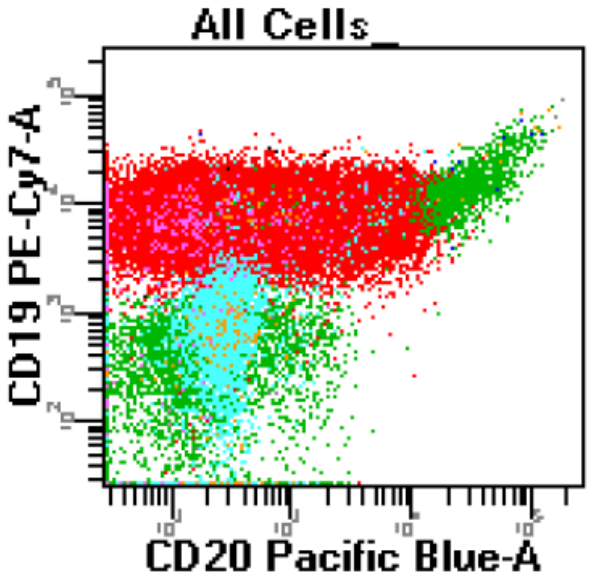
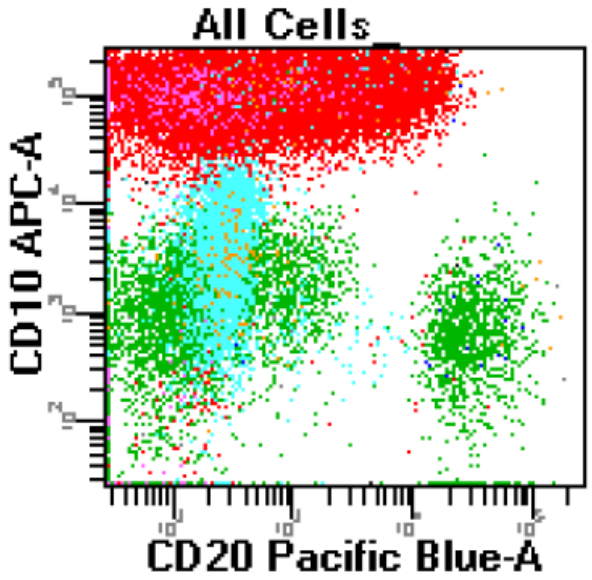
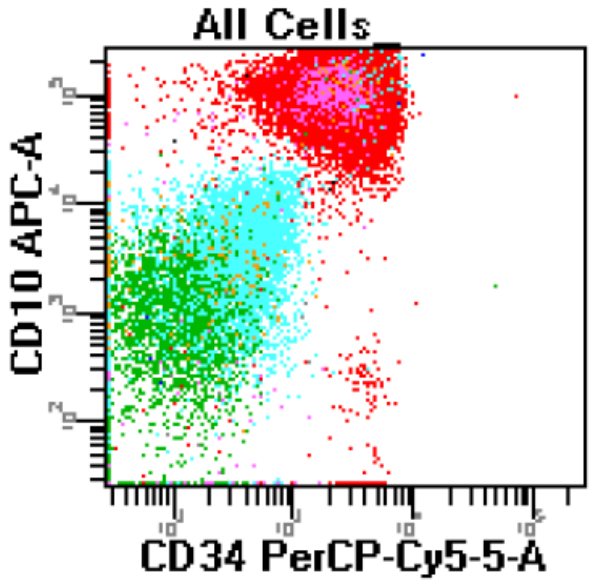
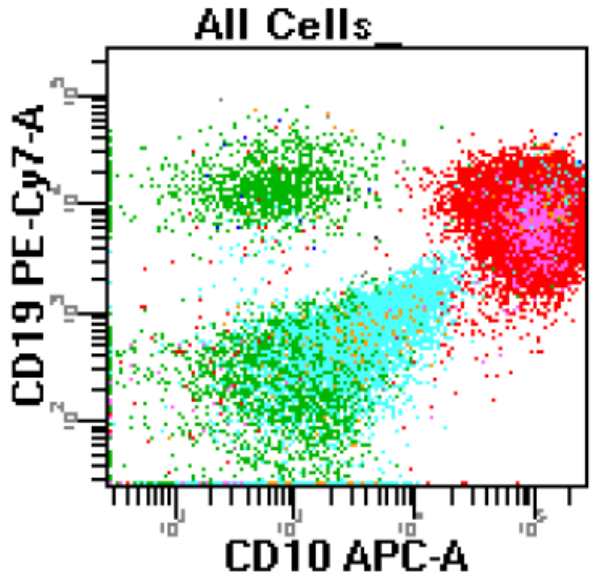
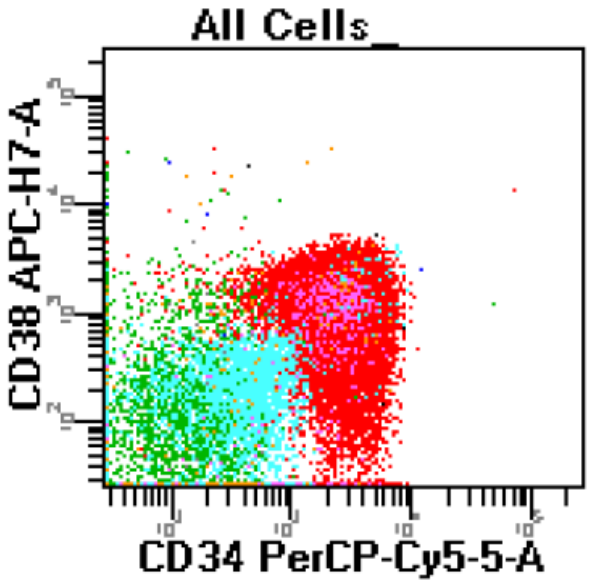
CD34 PerCP-Cy5-5-A

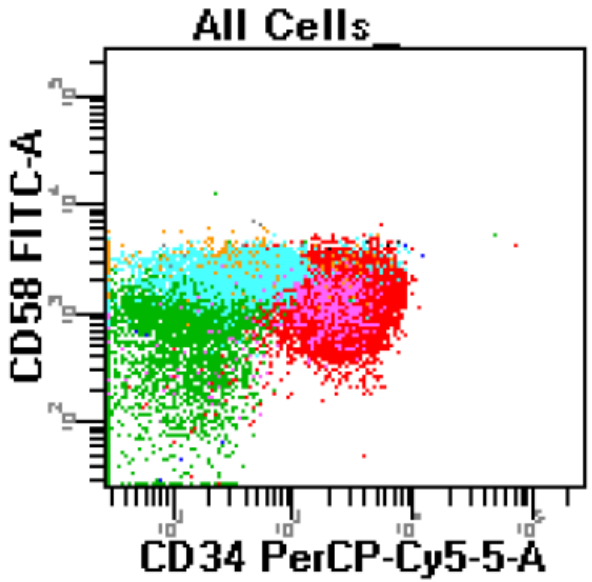


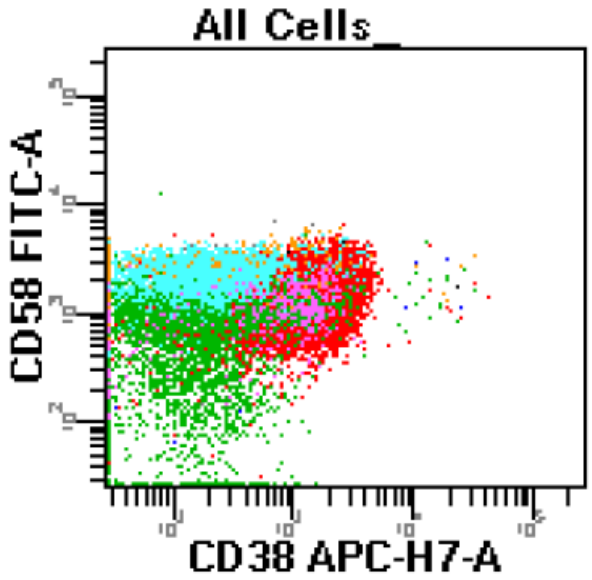


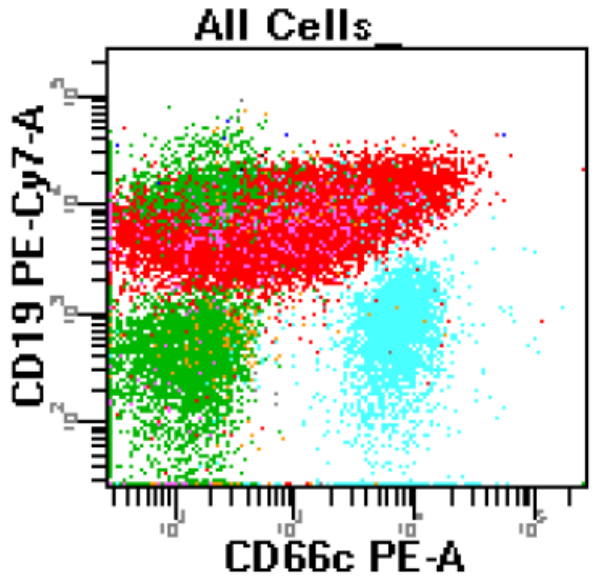






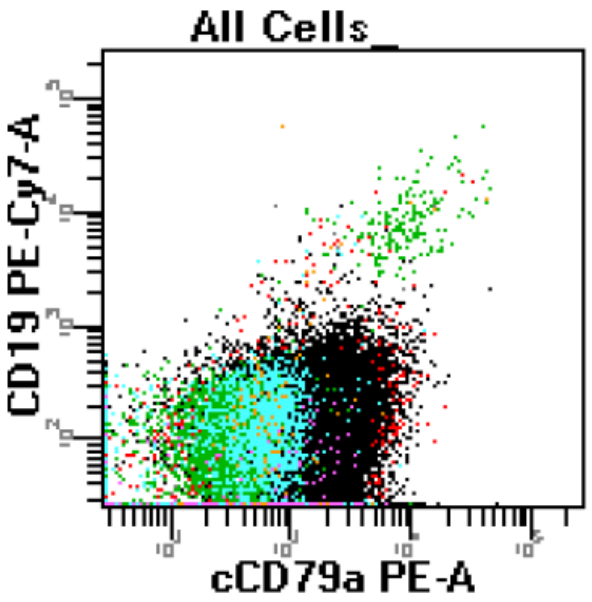


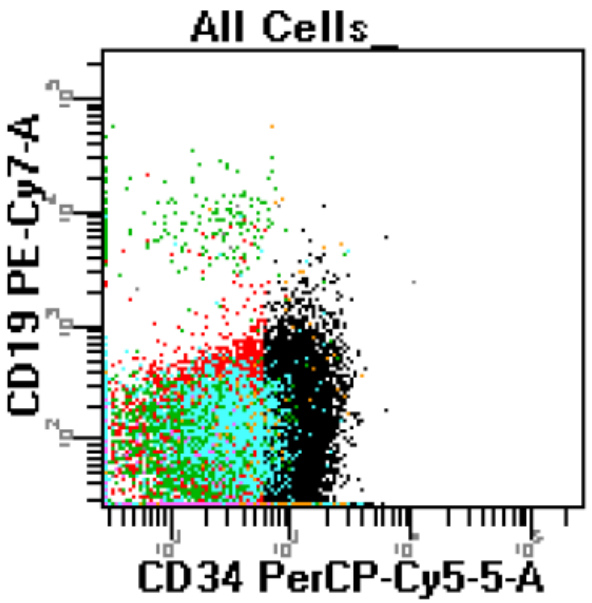


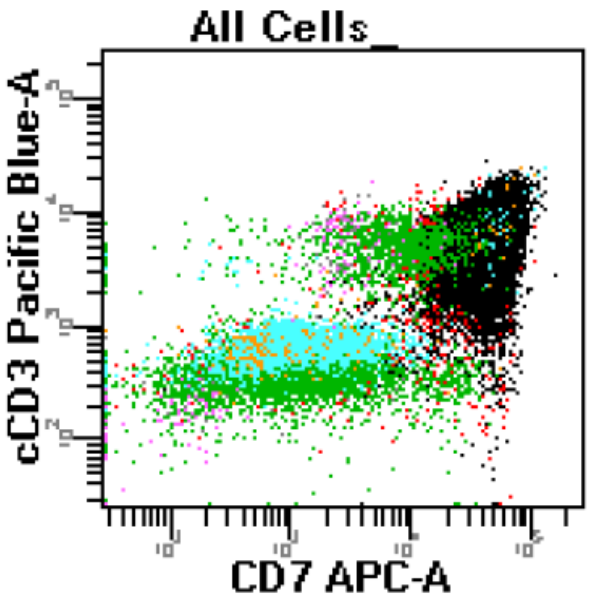


T-LYMPHOBLASTIC LEUKEMIA/LYMPHOMA

- ~15% of childhood ALL and 25% of adult ALL
  - ~90% of lymphoblastic lymphoma
- More common in teenage males
- Clinical presentation: large anterior mediastinal mass, lymphadenopathy, organomegaly, common pleural effusions
- Flow cytometry: TdT+, CD1a+, CD2, CD3, CD5, CD7
- Immunohistochemistry: CD99, CD34, CD1a
- >75% have *NOTCH1* pathway activation
- Long-term response rates approach 85% in children and 60% in adults
- The most significant predictor of outcome is MRD at the end of consolidation


All Cells
CD19 PE-Cy7-A
cCD79a PE-A


All Cells
CD19 PE-Cy7-A
CD34 PerCP-Cy5-5-A


All Cells
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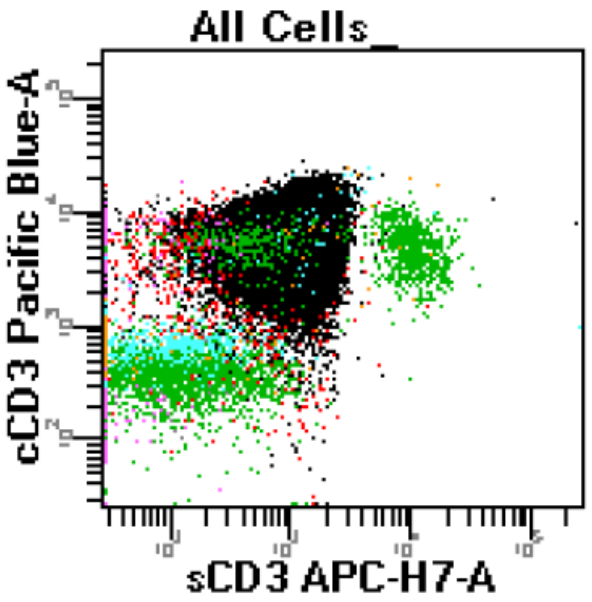
CD7 APC-A


All Cells
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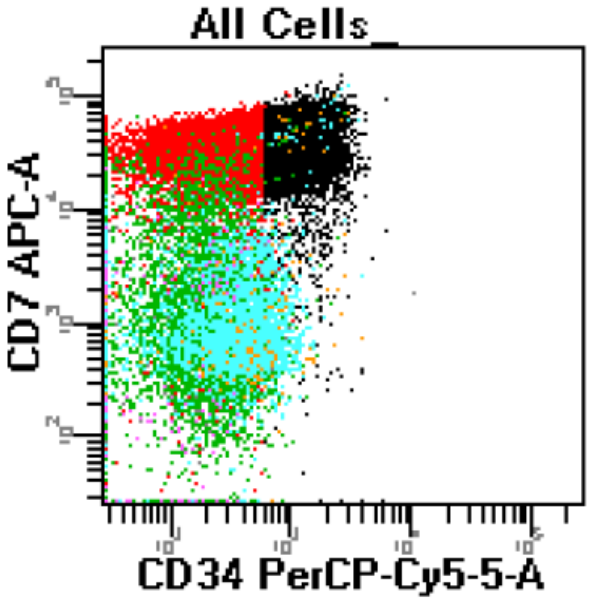
All Cells
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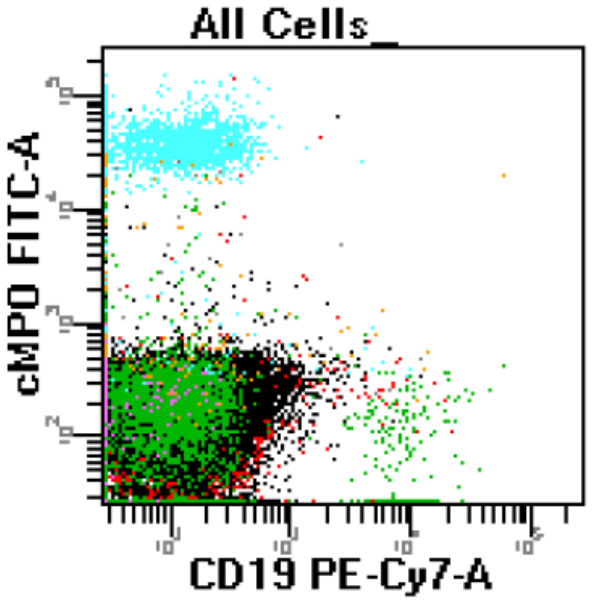
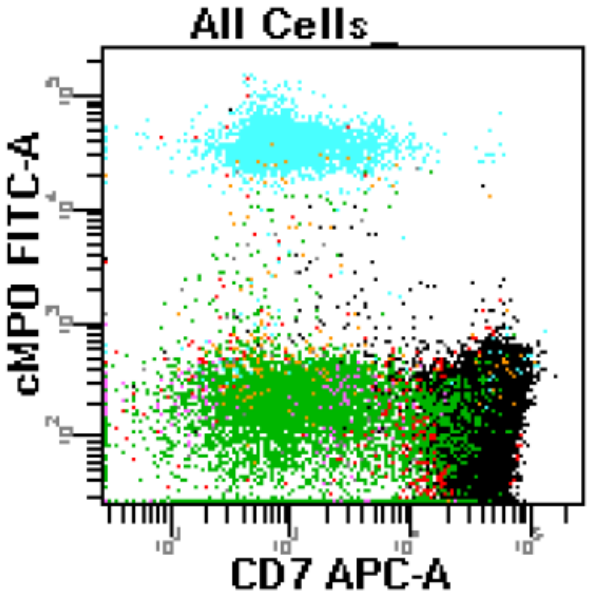
CD34 PerCP-Cy5-5-A


All Cells
cMPO FITC-A
CD19 PE-Cy7-A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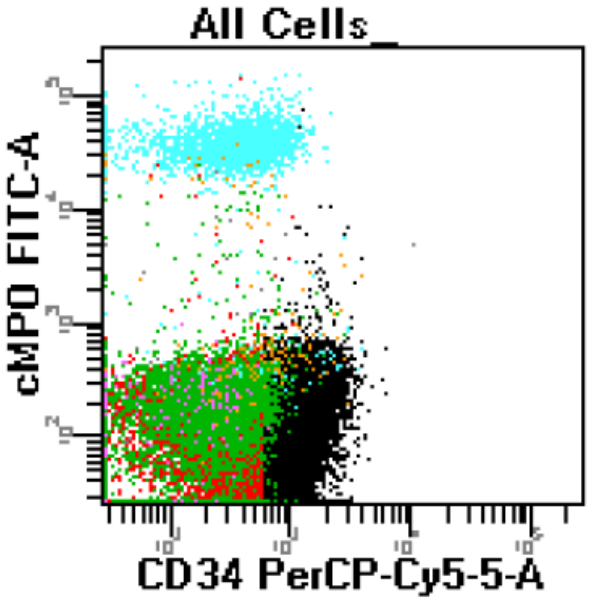
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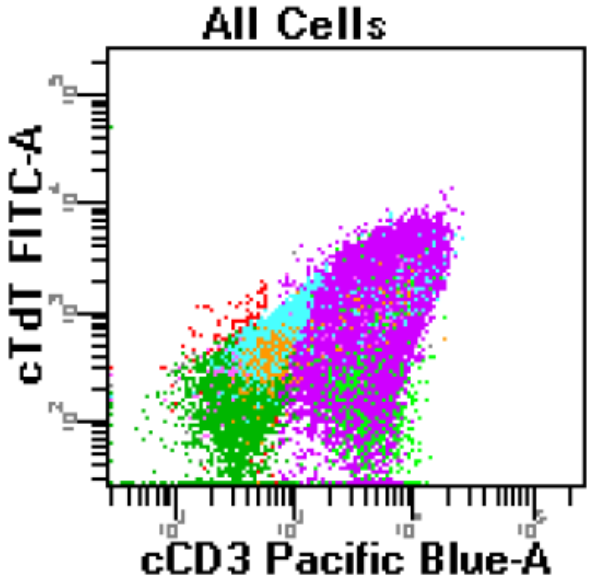
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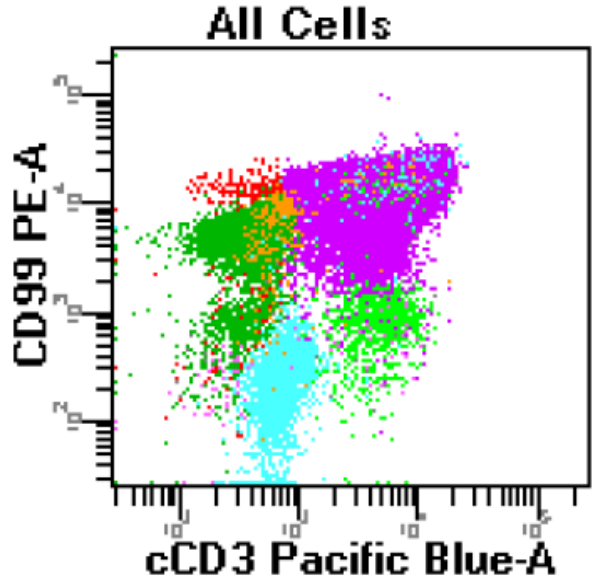
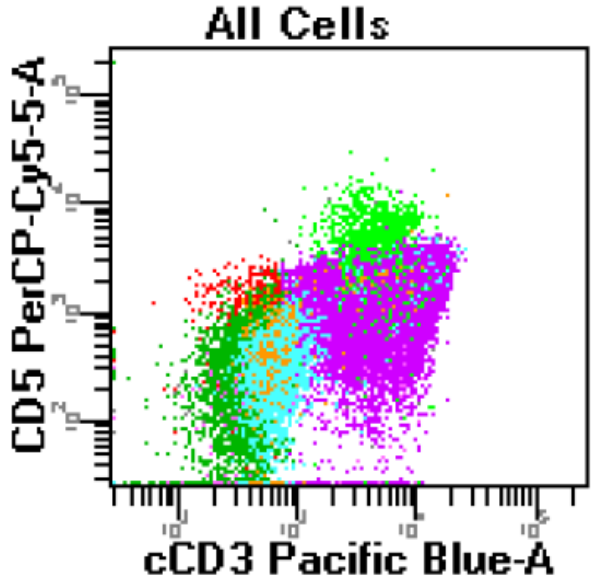

All Cells
CD99 PE-A
cCD3 Pacific Blue-A


All Cells
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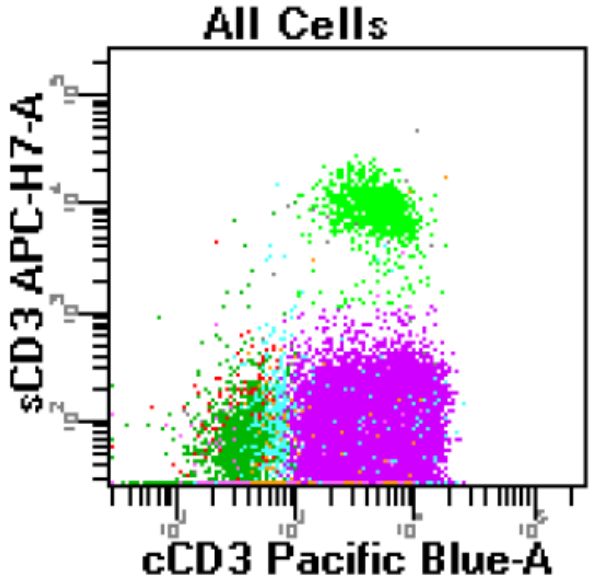
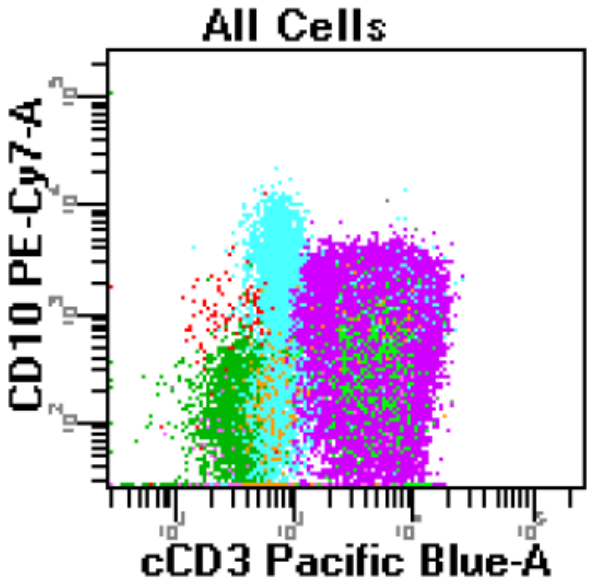

All Cells
sCD3 APC-H7-A
cCD3 Pacific Blue-A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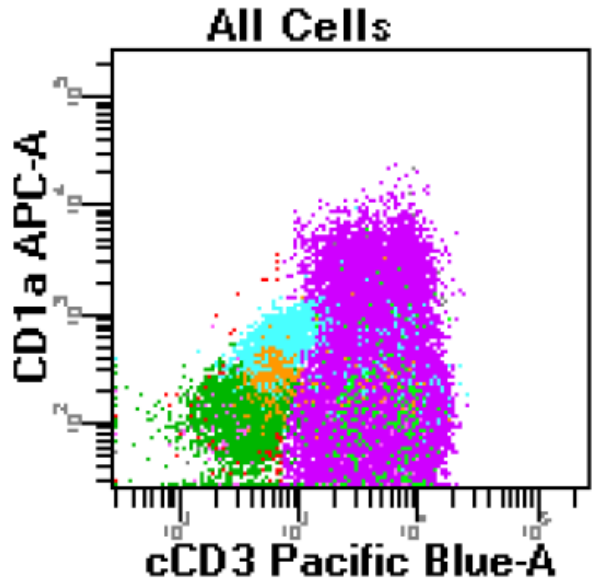
All Cells
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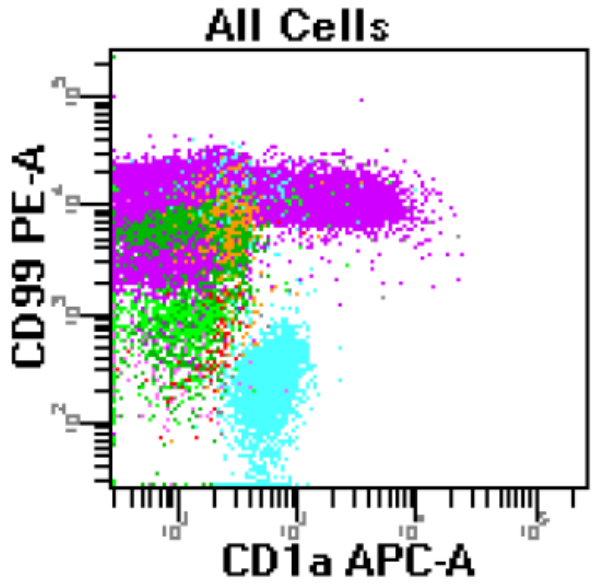
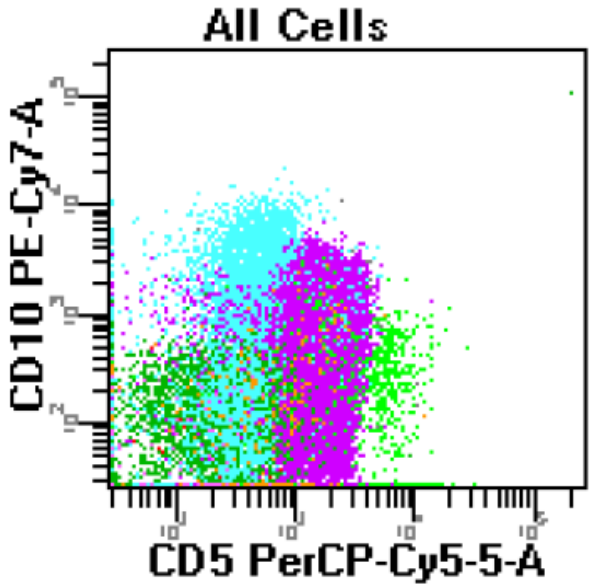

All Cells
CD99 PE-A
CD1a APC-A


All Cells
CD10 PE-Cy7-A
CD5 PerCP-Cy5-5-A

WHO4

10 subtypes

4 provisional

B-lymphoblastic leukemia/lymphoma

- B-lymphoblastic leukemia/lymphoma, NOS
- B-lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
- B-lymphoblastic leukemia/lymphoma with t(9;22)(q34.1;q11.2);*BCR-ABL1*
- B-lymphoblastic leukemia/lymphoma with t(v;11q23.3);*KMT2A* rearranged
- B-lymphoblastic leukemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
- B-lymphoblastic leukemia/lymphoma with hyperdiploidy
- B-lymphoblastic leukemia/lymphoma with hypodiploidy
- B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.3) *IL3-IGH*
- B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3);*TCF3-PBX1*
- *Provisional entity: B-lymphoblastic leukemia/lymphoma, BCR-ABL1–like*
- *Provisional entity: B-lymphoblastic leukemia/lymphoma with iAMP21*

T-lymphoblastic leukemia/lymphoma

- *Provisional entity: Early T-cell precursor lymphoblastic leukemia*
- *Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma*

WHO Classification, 5th edition	WHO Classification, revised 4th edition
B-cell lymphoblastic leukaemias/lymphomas	
B-lymphoblastic leukaemia/lymphoma, NOS	(Same)
B-lymphoblastic leukaemia/lymphoma with high hyperdiploidy	B-lymphoblastic leukaemia/lymphoma with hyperdiploidy
B-lymphoblastic leukaemia/lymphoma with hypodiploidy	(Same)
B-lymphoblastic leukaemia/lymphoma with iAMP21	(Same)
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1* fusion	B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1*-like features	B-lymphoblastic leukaemia/lymphoma, *BCR-ABL1*-like
B-lymphoblastic leukaemia/lymphoma with *KMT2A* rearrangement	B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3); *KMT2A*-rearranged
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1* fusion	B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1*-like features	*Not previously included*
B-lymphoblastic leukaemia/lymphoma with *TCF3::PBX1* fusion	B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3); *TCF3-PBX1*
B-lymphoblastic leukaemia/lymphoma with *IGH::IL3 fusion*	B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.1); *IGH/IL3*
B-lymphoblastic leukaemia/lymphoma with *TCF3::HLF* fusion	*Not previously included*
B-lymphoblastic leukaemia/lymphoma with other defined genetic abnormalities	(Same)
Precursor T-cell neoplasms	
T-lymphoblastic leukaemia/lymphoma	
T-lymphoblastic leukaemia / lymphoma, NOS	T-lymphoblastic leukaemia/lymphoma
Early T-precursor lymphoblastic leukaemia / lymphoma	Early T-cell precursor lymphoblastic leukaemia
(Entity deleted)	NK-lymphoblastic leukaemia/lymphoma

WHO Classification, 5th edition	WHO Classification, revised 4th edition
B-cell lymphoblastic leukaemia/lymphomas	
B-lymphoblastic leukaemia/lym	(Same)
B-lymphoblastic leukaemia/lymphoma with high hyperdiploidy	B-lymphoblastic leukaemia/lymphoma with hyperdiploidy
B-lymphoblastic leukaemia/lym	(Same)
B-lymphoblastic leukaemia/lym	(Same)
B-lymphoblastic leukaemia/lym	B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34;q11.2); *BCR-ABL1*
B-lymphoblastic leukaemia/lym features	B-lymphoblastic leukaemia/lymphoma, *BCR-ABL1*-like
B-lymphoblastic leukaemia/lymphoma with *KMT2A* rearrangement	B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3); *KMT2A*-rearranged
B-lymphoblastic leukaemia/lymphoma with *ETV6*::*RUNX1* fusion	B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
B-lymphoblastic leukaemia/lymphoma with *ETV6*::*RUNX1*-like features	*Not previously included*
B-lymphoblastic leukaemia/lymphoma with *TCF3*::*PBX1* fusion	B-lymphoblastic leukaemia/lymphoma with t(1;19)(q23;p13.3); *TCF3-PBX1*
B-lymphoblastic leukaemia/lymphoma with *IGH*::*IL3 fusion*	B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.1); *IGH/IL3*
B-lymphoblastic leukaemia/lymphoma with *TCF3*::*HLF* fusion	*Not previously included*
B-lymphoblastic leukaemia/lymphoma with other defined genetic abnormalities	(Same)
Precursor T-cell neoplasms	
T-lymphoblastic leukaemia/lymphoma	
T-lymphoblastic leukaemia / lymphoma, NOS	T-lymphoblastic leukaemia/lymphoma
Early T-precursor lymphoblastic leukaemia / lymphoma	Early T-cell precursor lymphoblastic leukaemia
(Entity deleted)	NK-lymphoblastic leukaemia/lymphoma

WHO5

15 subtypes
0 provisional

WHO CLASSIFICATION 5TH EDITION

The classification remains largely unchanged from the previous WHO and has similar terminology, ie “B-lymphoblastic leukemia/lymphoma”

- Abbreviated as “B-ALL or T-ALL”

Most entities based on broadly-available cytogenetic testing

The rare B-ALL with TCF3::HLF fusion has been added as it is distinct from B-ALL with TCF3::PBX1 fusion and is characterized by a particularly aggressive behavior

B-ALL with BCR::ABL1-like features is now an entity (previously a provisional entity), as it is prevalent and shows significant benefit from targeted therapies

“B-ALL with other defined genetic abnormalities” incorporates several novel genetic drivers identified by recent gene expression and sequencing studies

- B-ALL with DUX4, MEF2D, ZNF384 or NUTM1 rearrangements; IG::MYC fusion; PAX5alt or PAX5 p.P80R abnormalities
- May be separated in the future as potential novel entities

WHO CLASSIFICATION 5TH EDITION

- Genetic abnormalities are now the fundamental basis for subclassification of B-ALL
- The category B-ALL/LBL, NOS should only be used for cases lacking defined genetic abnormalities after **comprehensive testing**
- In the absence of complete testing for genetic abnormalities, definitive diagnosis may not be possible and the category B-ALL/LBL, NFC (not further classified) should be used

ICC

25 subtypes
14 provisional

B-acute lymphoblastic leukemia (B-ALL)
- B-ALL with recurrent genetic abnormalities
  - B-ALLwith t(9;22)(q34.1;q11.2)/*BCR::ABL1*
    - with lymphoid only involvement
    - with multilineage involvement
  - B-ALLwith t(v;11q23.3)/*KMT2A* rearranged
  - B-ALL with t(12;21)(p13.2;q22.1)/*ETV6::RUNX1*
  - B-ALL, hyperdiploid
  - B-ALL, low hypodiploid
  - B-ALL, near haploid
  - B-ALL with t(5;14)(q31.1;q32.3)/*IL3::IGH*
  - B-ALL with t(1;19)(q23.3;p13.3)/*TCF3::PBX1*
  - B-ALL, *BCR*::*ABL1*–like , ABL-1 class rearranged
  - B-ALL, *BCR*::*ABL1*–like , JAK-STAT activated
  - B-ALL, *BCR*::*ABL1*–like , NOS
  - B-ALL with iAMP21
  - B-ALL with *MYC* rearrangement
  - B-ALL with *DUX4* rearrangement
  - B-ALL with *MEF2D* rearrangement
  - B-ALL with *ZNF384(362)* rearrangement
  - B-ALL with *NUTM1* rearrangement
  - B-ALL with *HLF* rearrangement
  - B-ALL with *UBTF::ATXN7L3/PAN3,CDX2* (“CDX2/UBTF”)
  - B-ALL with mutated *IKZF1* N159Y
  - B-ALLwith mutated *PAX5* P80R
    - Provisional entity: B-ALL, *ETV6::RUNX1*-like
    - Provisional entity: B-ALL, with *PAX5* alteration
    - Provisional entity: B-ALL, with mutated *ZEB2* (p.H1038R)/IGH::*CEBPE*
    - Provisional entity: B-ALL, *ZNF384* rearranged-like
    - Provisional entity: B-ALL, *KMT2A* rearranged-like
  - B-ALL, NOS

T-ALL
- Early T-cell precursor ALLwith *BCL11B* rearrangement
- Early T-cell precursor ALL, NOS
- T-ALL, NOS
  - Provisional entities (see Supplemental Table 7)

Provisional entity: Natural killer (NK) cell ALL

ICC

25 subtypes
14 provisional

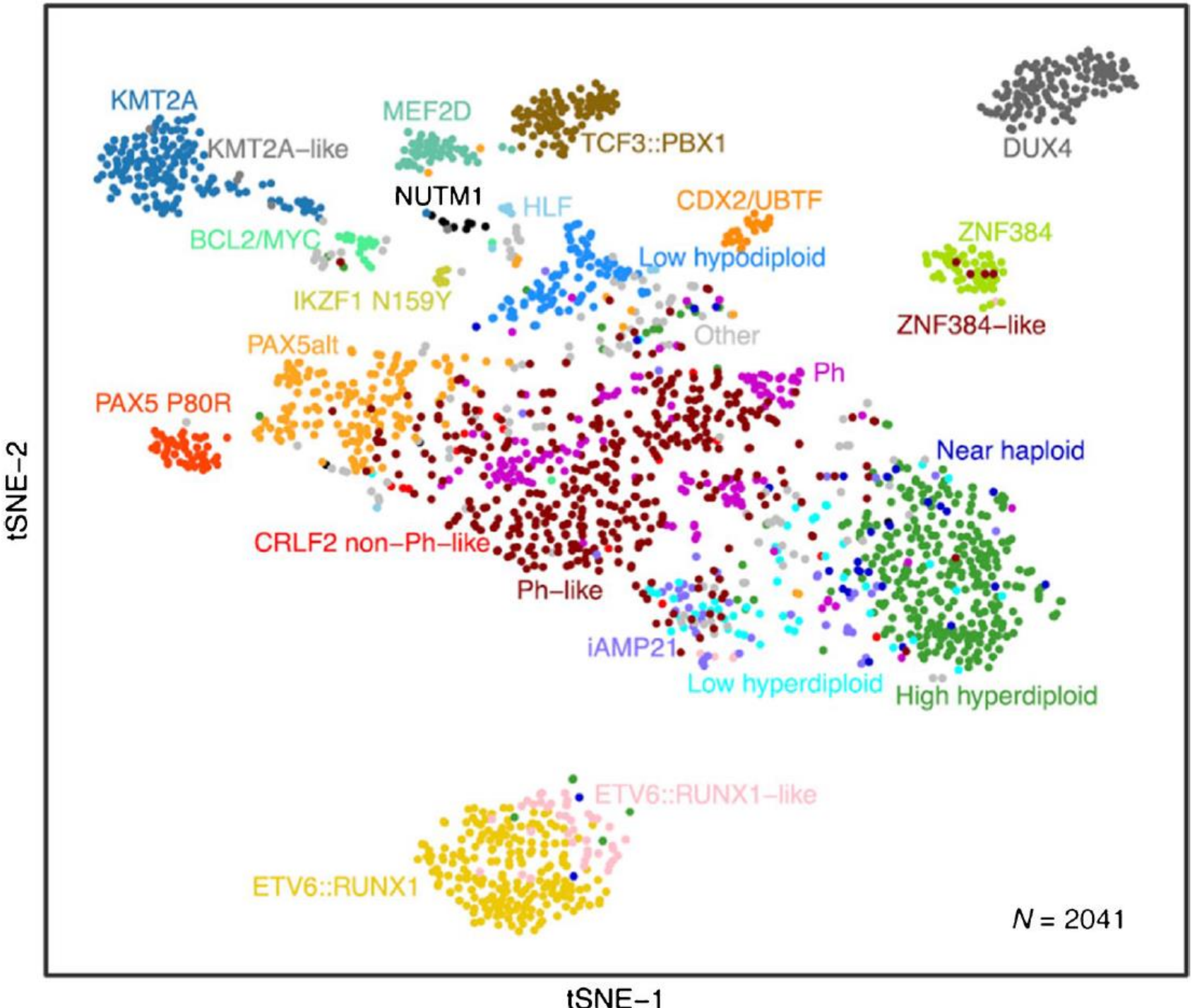
B-acute lymphoblastic leukemia (B-ALL)
- B-ALL with recurrent genetic abnormalities
  - B-ALLwith t(9;22)(q34.1;q11.2)/*BCR::ABL1*
    - with lymphoid only involvement
    - with multilineage involvement
  - B-ALLwith t(v;11q23.3)/*KMT2A* rearranged
  - B-ALL with t(12;21)(p13.2;q22.1)/*ETV6::RUNX1*
  - B-ALL, hyperdiploid
  - B-ALL, low hypodiploid
  - B-ALL, near haploid
  - B-ALL with t(5;14)(q31.1;q32.3)/*IL3::IGH*
  - B-ALL with t(1;19)(q23.3;p13.3)/*TCF3::PBX1*
  - B-ALL, *BCR*::*ABL1*–like , ABL-1 class rearranged
  - B-ALL, *BCR*::*ABL1*–like , JAK-STAT activated
  - B-ALL, *BCR*::*ABL1*–like , NOS
  - B-ALL with iAMP21
  - B-ALL with *MYC* rearrangement
  - B-ALL with *DUX4* rearrangement
  - B-ALL with *MEF2D* rearrangement
  - B-ALL with *ZNF384(362)* rearrangement
  - B-ALL with *NUTM1* rearrangement
  - B-ALL with *HLF* rearrangement
  - B-ALL with *UBTF::ATXN7L3/PAN3,CDX2* ("CDX2/UBTF")
  - B-ALL with mutated *IKZF1* N159Y
  - B-ALLwith mutated *PAX5* P80R
    - Provisional entity: B-ALL, *ETV6::RUNX1*-like
    - Provisional entity: B-ALL, with *PAX5* alteration
    - Provisional entity: B-ALL, with mutated *ZEB2* (p.H1038R)/IGH::*CEBPE*
    - Provisional entity: B-ALL, *ZNF384* rearranged-like
    - Provisional entity: B-ALL, *KMT2A* rearranged-like
  - B-ALL, NOS

T-ALL
- Early T-cell precursor ALLwith *BCL11B* rearrangement
- Early T-cell precursor ALL, NOS
- T-ALL, NOS
  - Provisional entities (see Supplemental Table 7)

Provisional entity: Natural killer (NK) cell ALL

T-ALL/LL provisional entities		
Subtype	Frequency	Partner genes/other re
HOXA dysregulated	15-25%	*HOXA*::TRB/TRG; *KMT2* *PICALM*::*MLLT10*; *SET*::
SPI1 rearrangement	<5%, children	*STMN1*; *TCF7*; *BCL11B*
TLX1 rearrangement	5-10% children; near 30% adult	TCR
TLX3 rearrangement	20-25% children <5% adult	TCR; *BCL11B*; *CDK6*
NKX2 rearrangement	<5% children	*NKX2.1/NKX2.2/NKX2.*
TAL1-2 rearrangement	30-40% (TAL2 rare)	TRA/D; TRB (*TAL2*); 1p3 intergenic SNV (super e
LMO1-2 rearrangement	*LMO1*-R -5% *LMO2*-R 10%	TCR; cryptic deletion; e mutations
BHLH, other	<2%	*LYL1*::TRB *OLIG2/BHLHB1*::TCR

Fig. 1 Gene expression clustering of B-ALL cases. The figure depicts two dimensional clustering using t-distributed stochastic neighbor embedding of whole transcriptome sequencing data from 2041 leukemia samples collected at diagnosis from children or adults with ALL. Cases are color coded by subtype. This approach, and much of the data, was first reported by Gu, et al. [3], and has been updated to include additional *CDX2/UBTF* cases following recent definition of this subtype



Duffield AS, et al. Virchows Archiv 2022
Gu Z, et al. Nat Genet 2019

INTERNATIONAL CONSENSUS CLASSIFICATION (ICC)

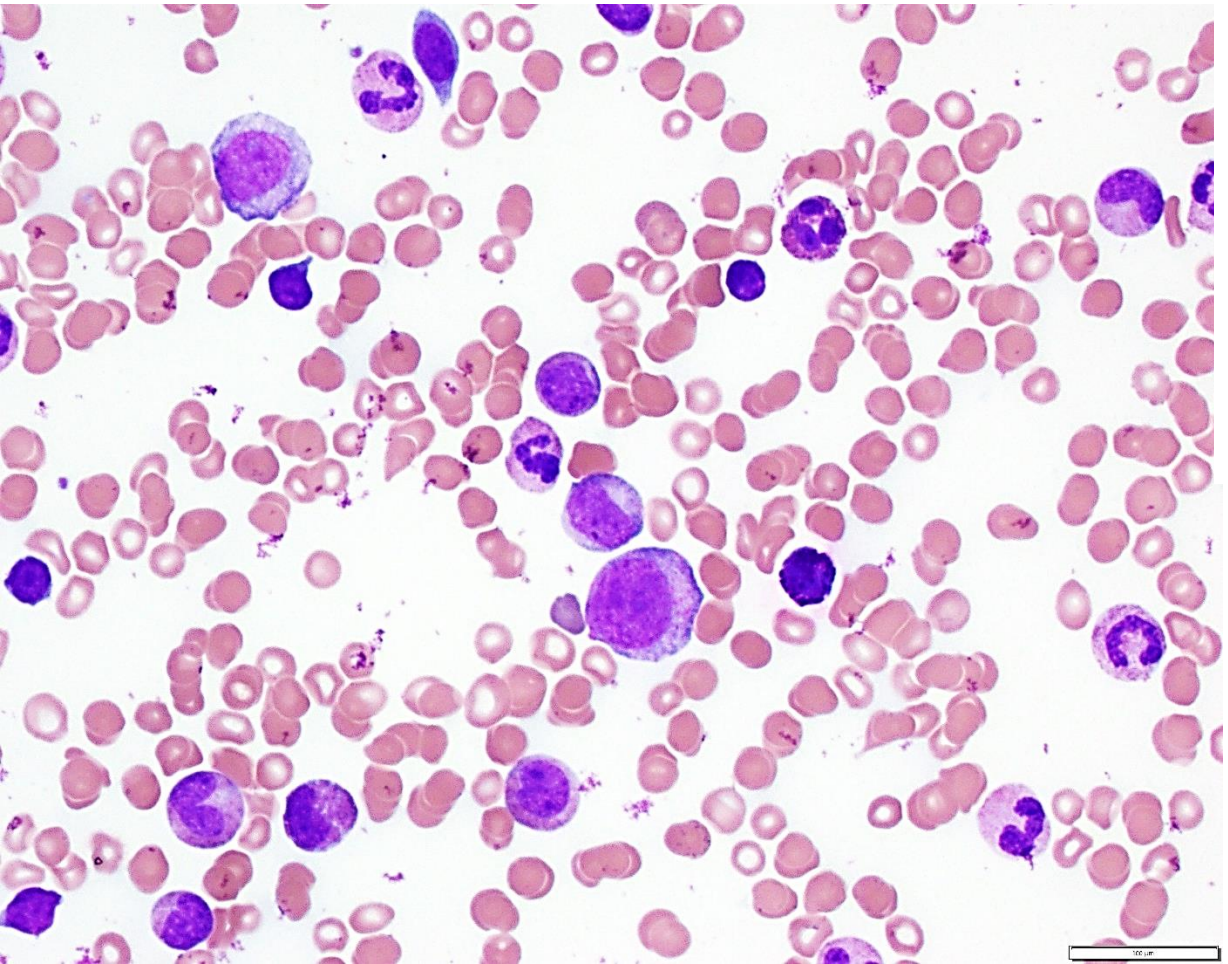
- Incorporates recent clinical, cytogenetic, and molecular data, with a particular emphasis on whole transcriptome analysis and gene expression clustering studies
- Renamed “B- and T-acute lymphoblastic leukemia”
    - Abbreviated as B-ALL or T-ALL, same as the WHO
- 9 new B-ALL categories, including 7/9 with distinguishing gene rearrangements, and 2/9 characterized by a specific single gene mutation
- 4 provisional B-ALL entities are included, that require gene expression studies
- 1 new subtype of early T-cell precursor ALL
- 8 new provisional T-ALL entities are added

B-ALL with t(9;22)(q34;q11.2) / *BCR::ABL1*

- The incidence increases with age, more common in adults
- The main differential diagnosis is with blast phase CML
  - Most childhood ALLs have a p190kd *BCR-ABL1* protein product, while most adults with CML have a p210kd fusion protein
- ICC divides into 2 distinct subsets distinguished by FISH
  - With lymphoid only involvement (translocation only in lymphoblasts) DE NOVO
  - With multilineage involvement (translocation detected in neutrophils) akin to CML in blast phase
  - Prognosis and treatment may differ
- Immunophenotype: frequently express myeloid-associated markers, such as CD13, CD33, CD66c and expression of CD25 is common in adults
- Molecular diagnosis: karyotyping, FISH, PCR, DNA or RNA sequencing is required
- Prognosis: historically low, but improving with use of targeted therapies

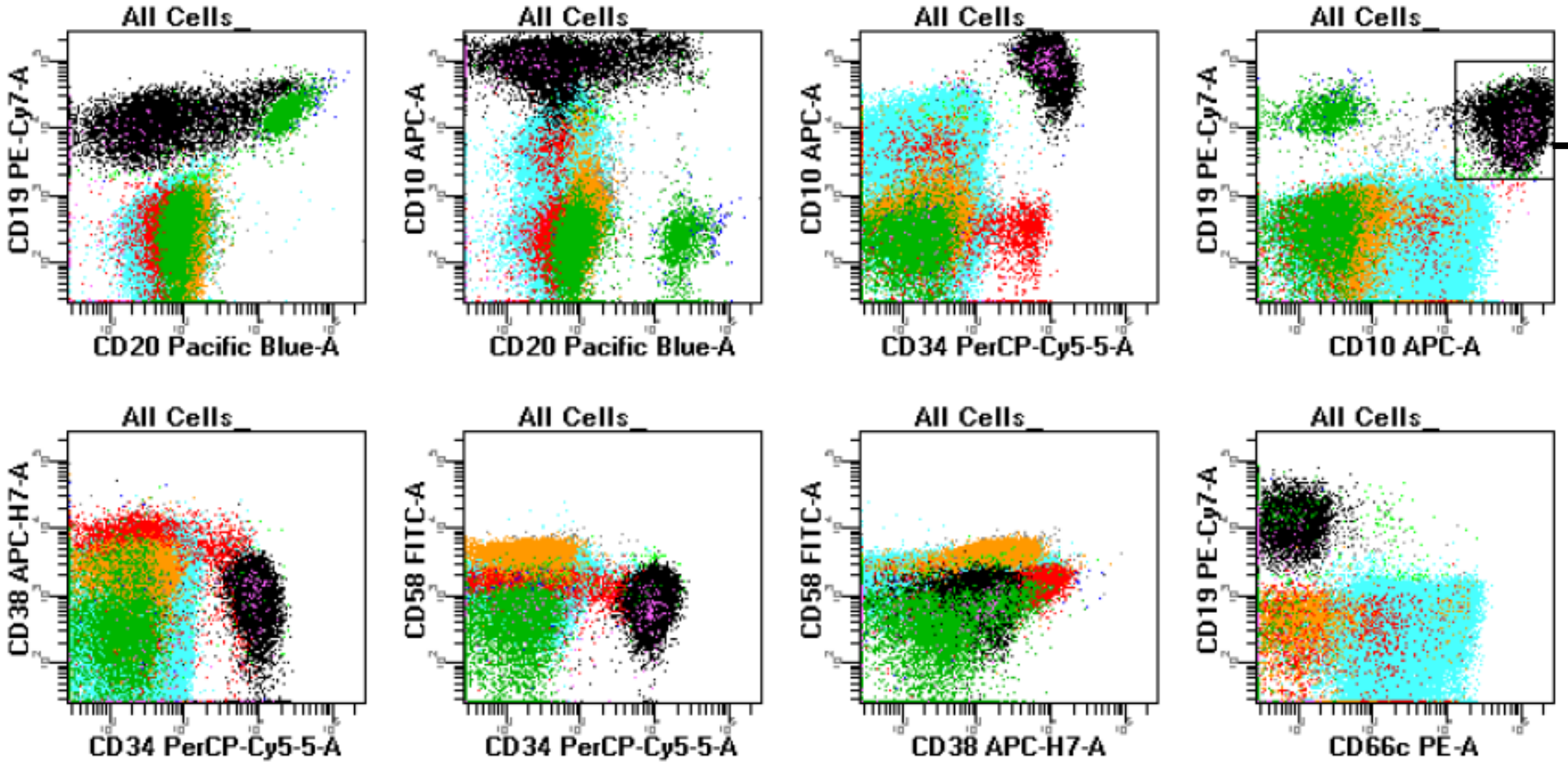
CLINICAL EXAMPLE

21-year-old, previously healthy woman



PERIPHERAL BLOOD DIFFERENTIAL CELL COUNT: 300 CELLS:

			%		%
WBC	77.43 $10^3/\mu L$	NEUTROPHILS	58	LYMPHOCYTES	5
RBC	2.97 $10^6/\mu L$	BAND	8	MONOCYTES	2
HGB	8.6 g/dL	META	8	EOSINOPHILS	2
HCT	26.6 %	MYELOCYTE	11	BASOPHILS	1
MCV	89.6 fL	PROMYELOCYTE	1		
PLATELETS	37 $10^3/\mu L$	BLAST	4		
NRBC	2/100 WBC				

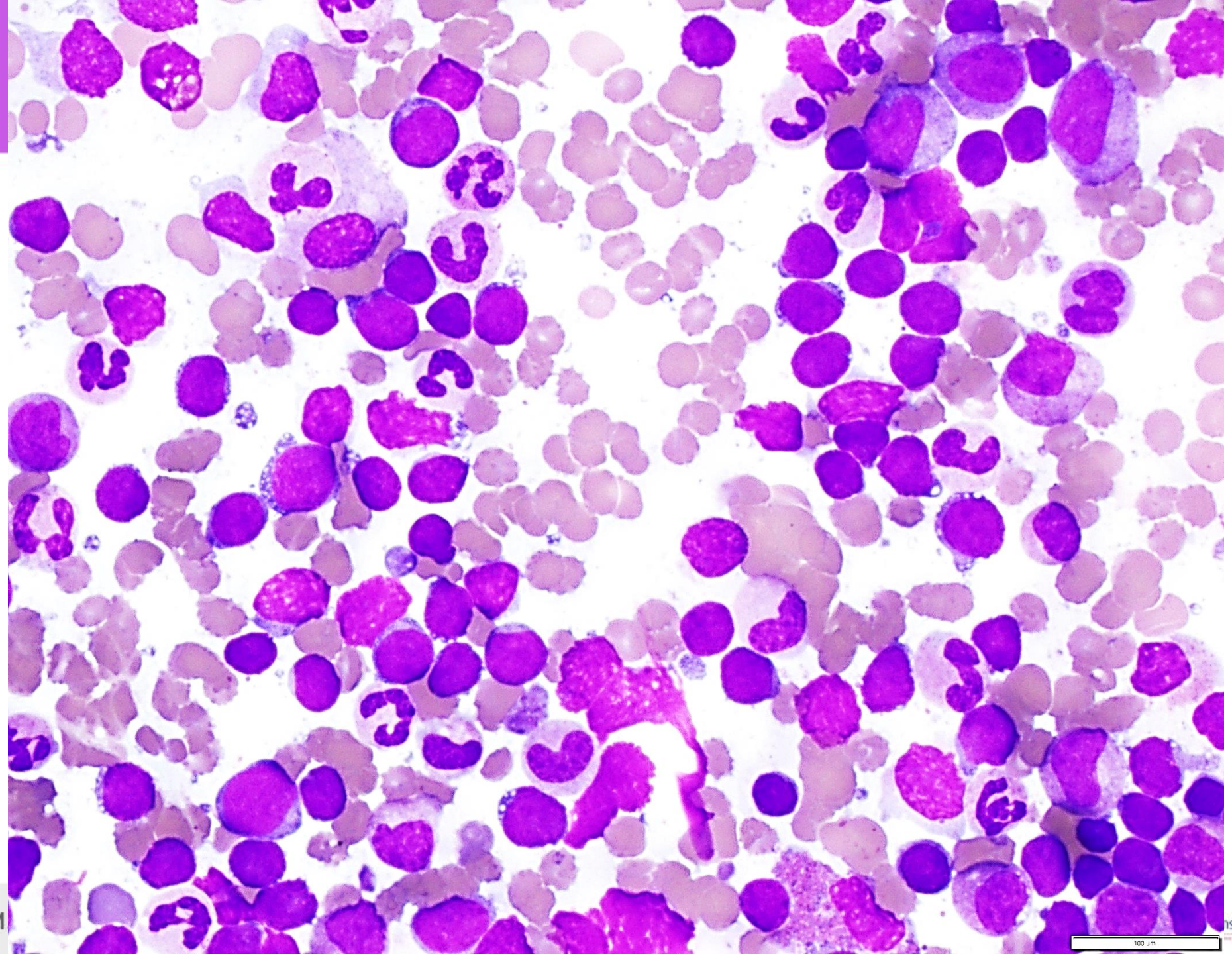


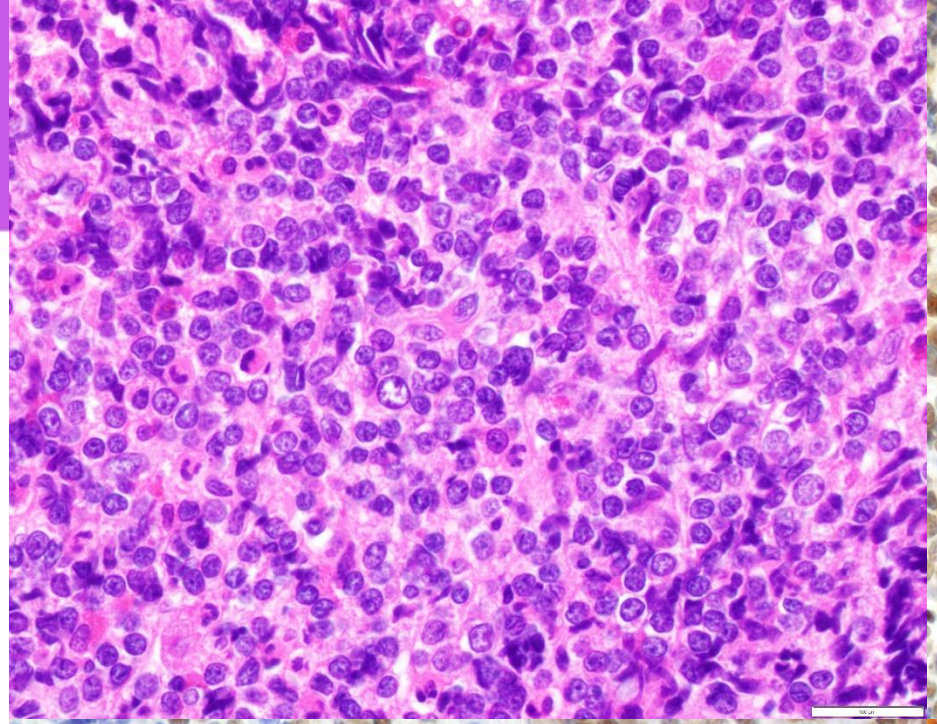
3% of all events

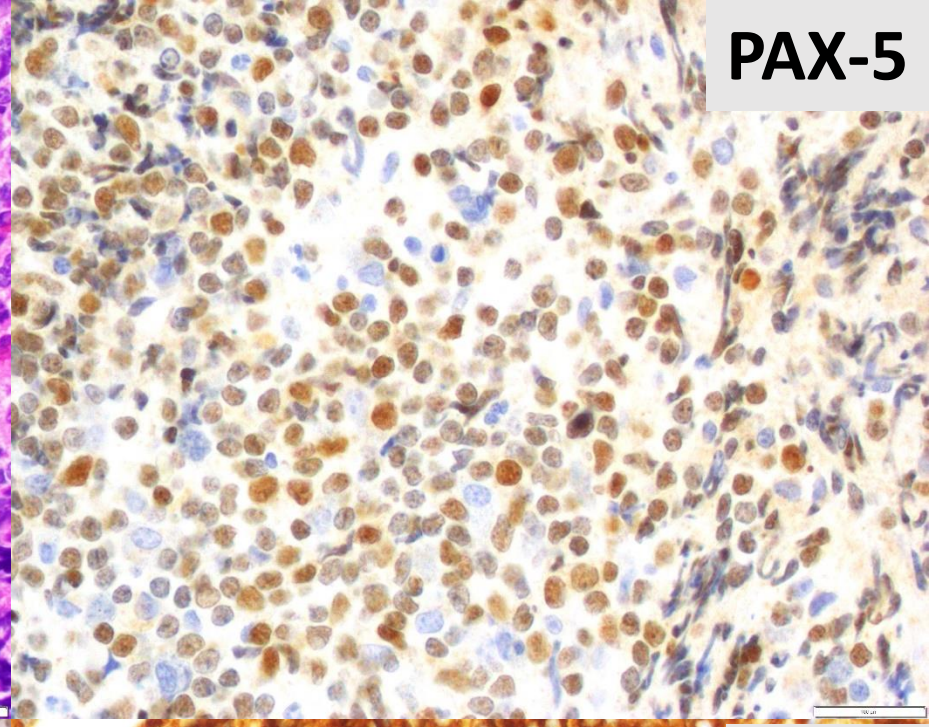
Karyotype on peripheral blood: 46,XX,t(9;22)(q34;q11.2)[20]

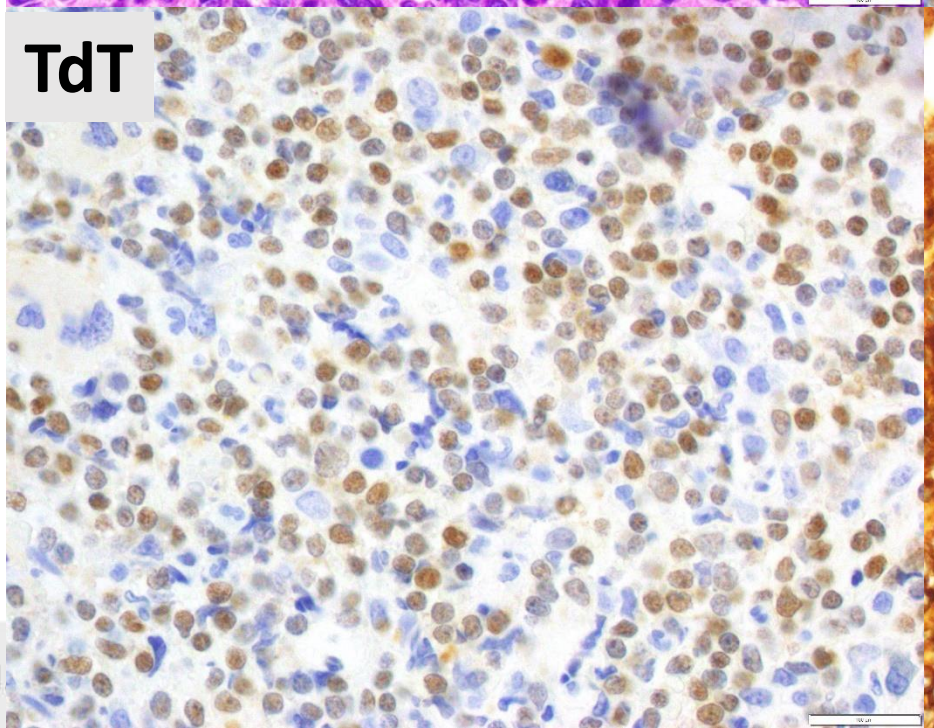
FISH on peripheral blood: BCR::ABL1 gene rearrangements in 92% of 200 nuclei

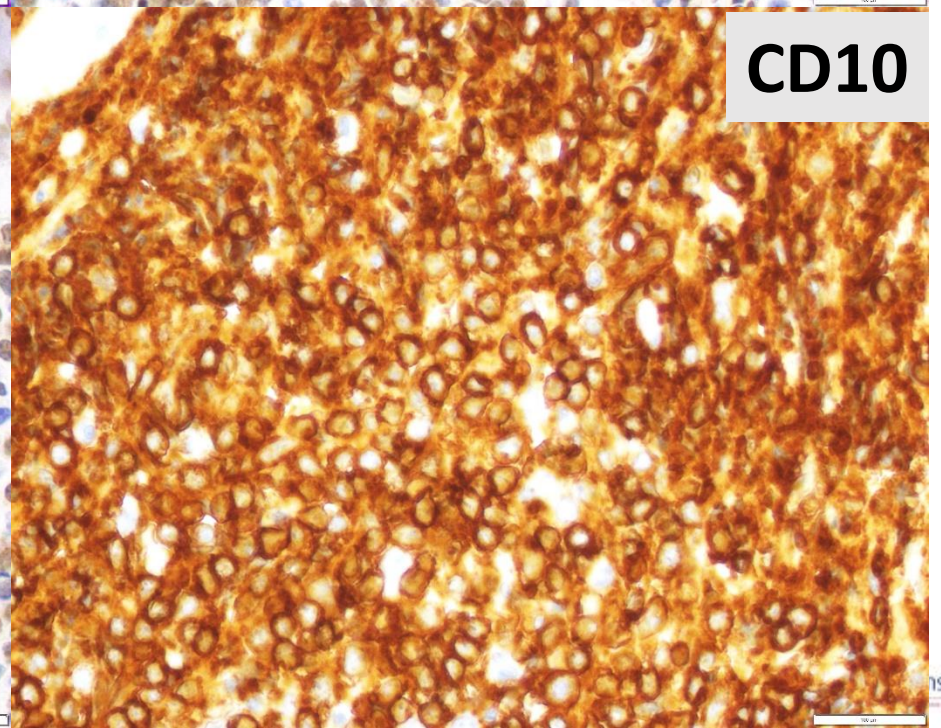
PCR: *BCR-ABL1* (e13a2 or e14a2) fusion transcripts coding for p210 positive at 84.98%

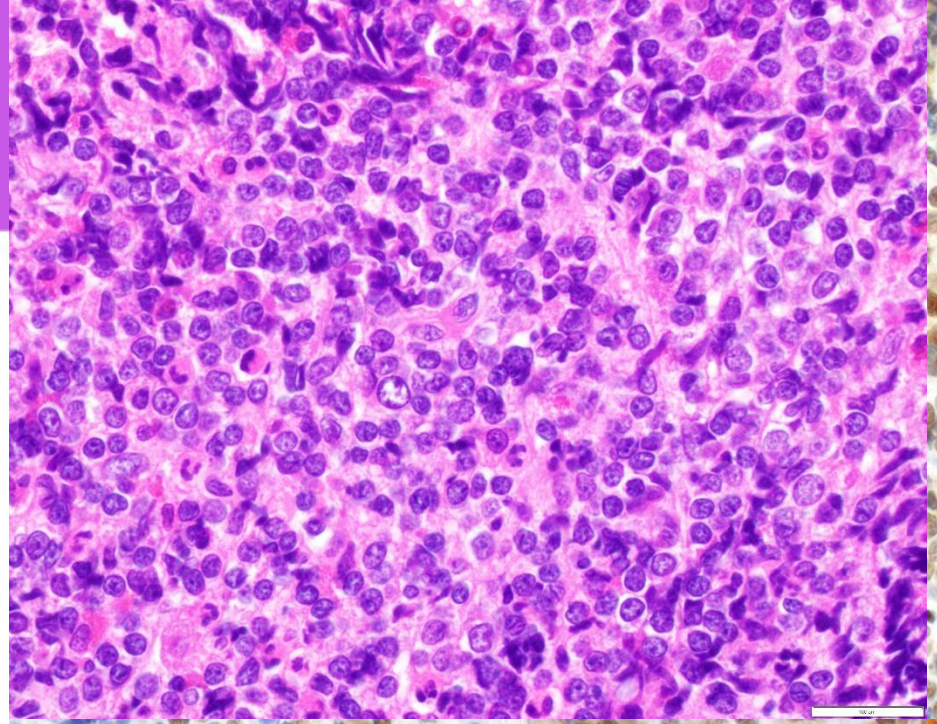


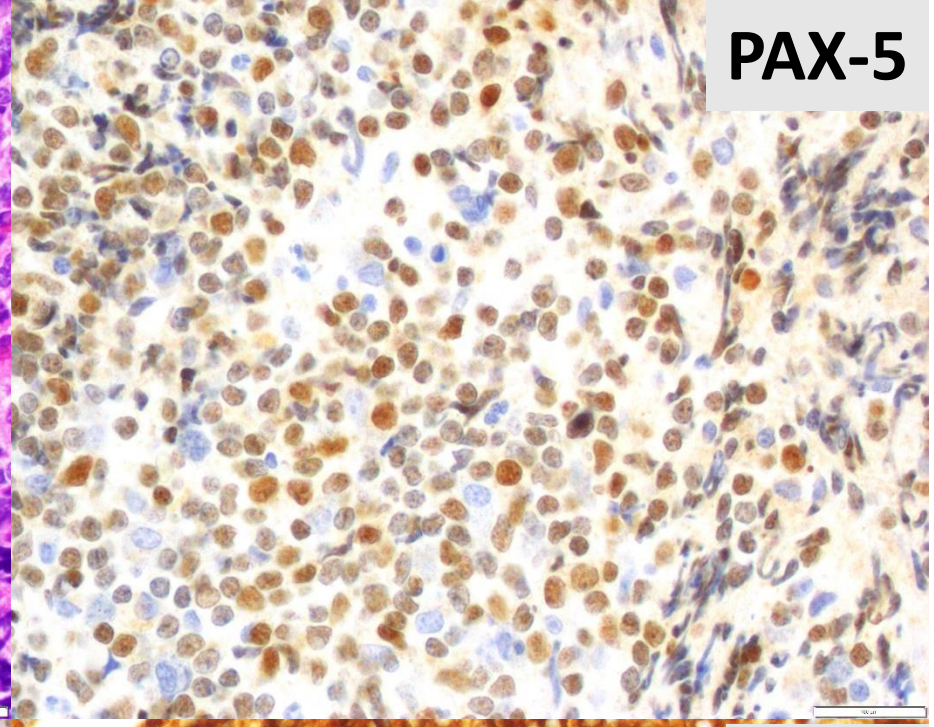


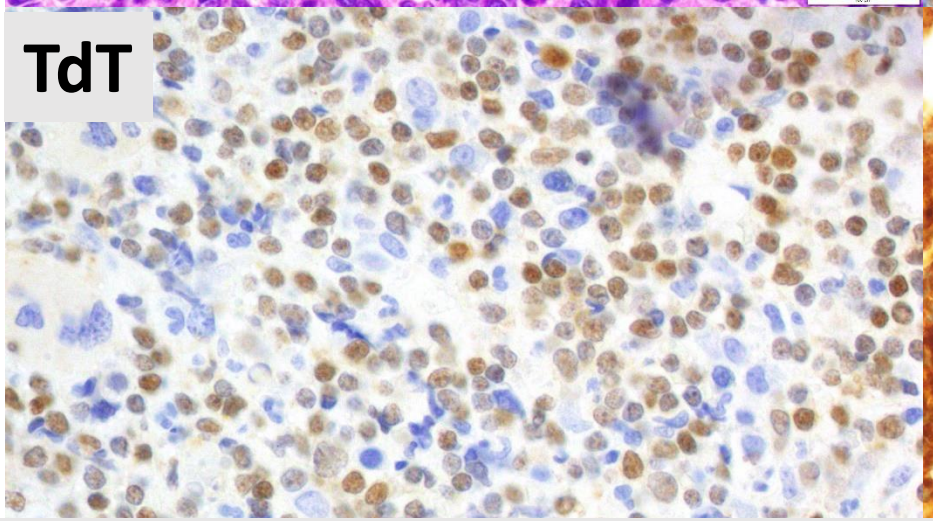


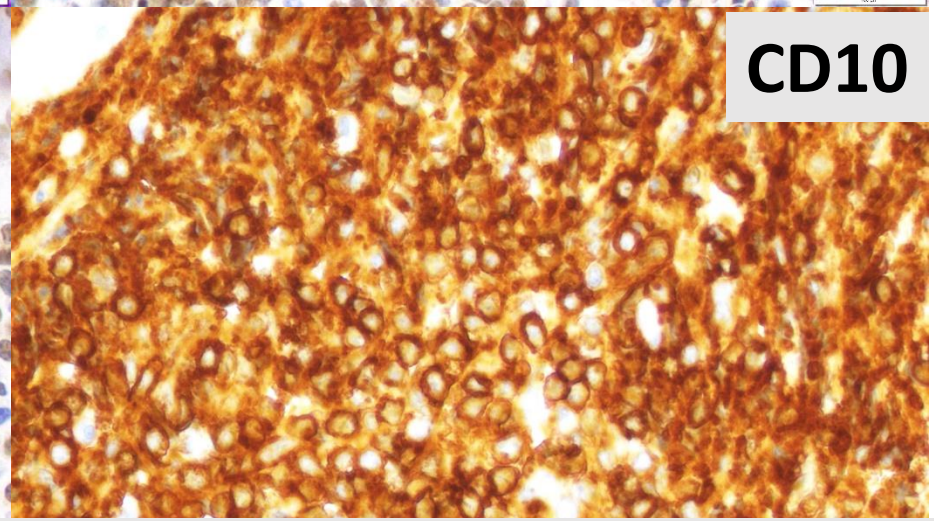






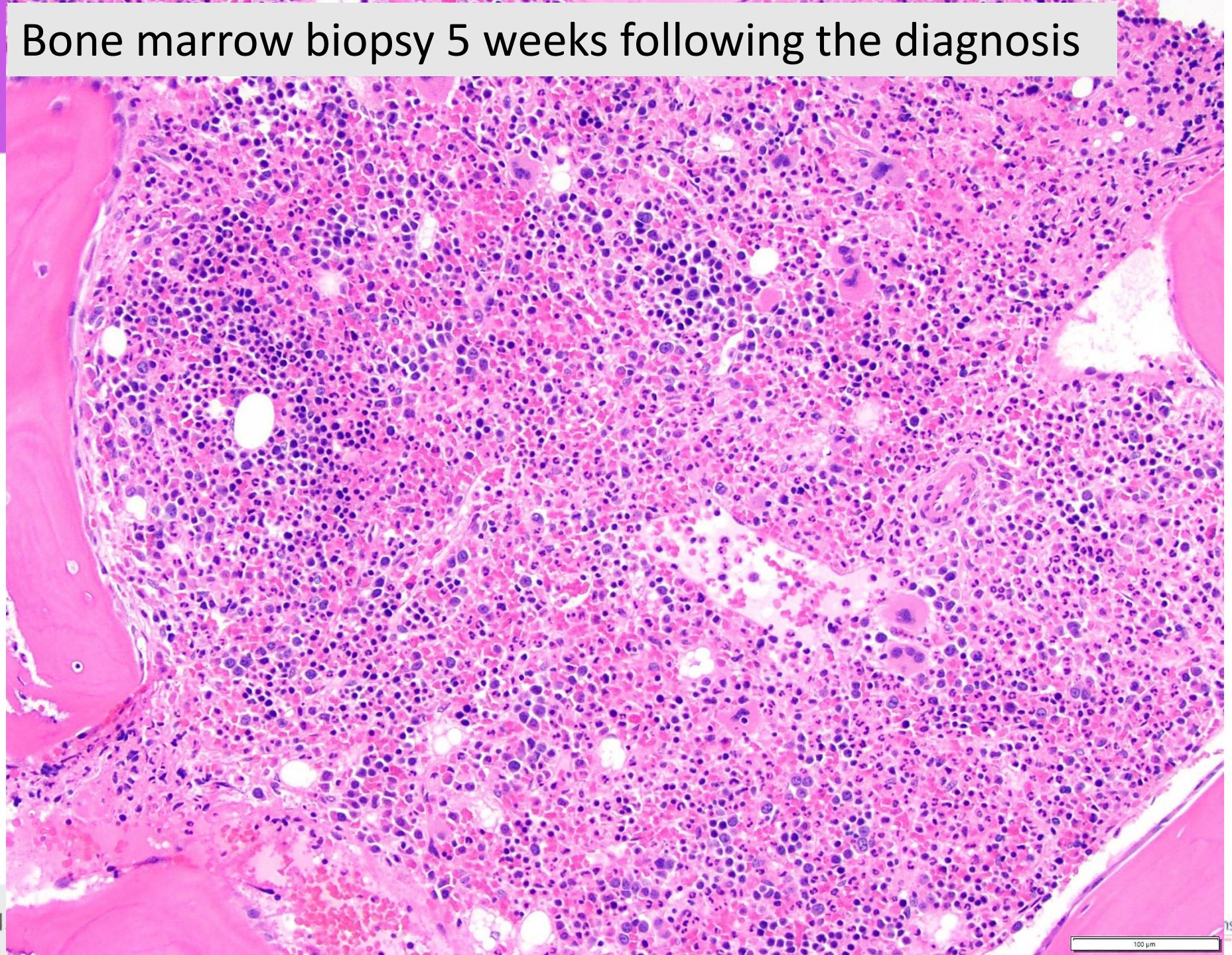


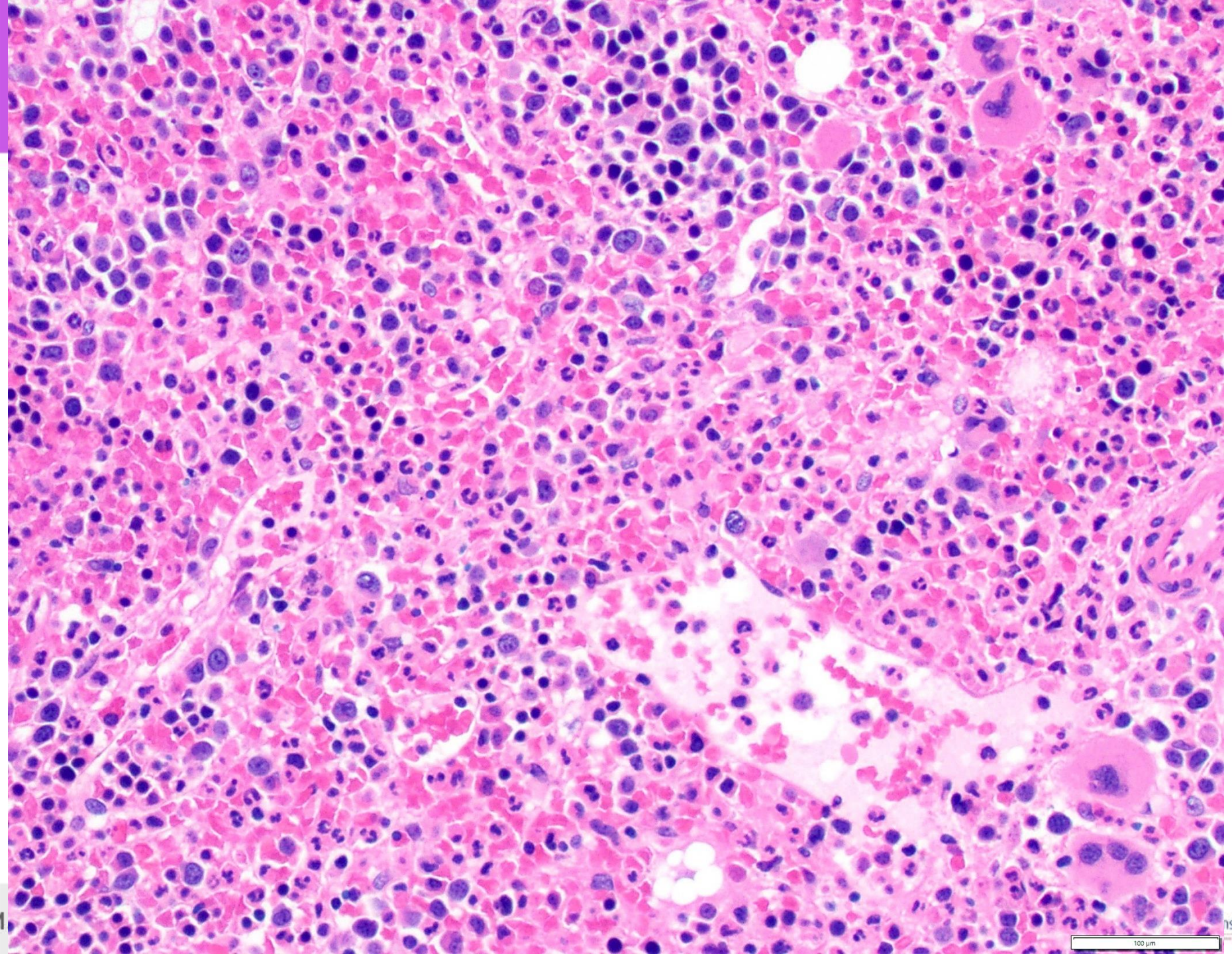


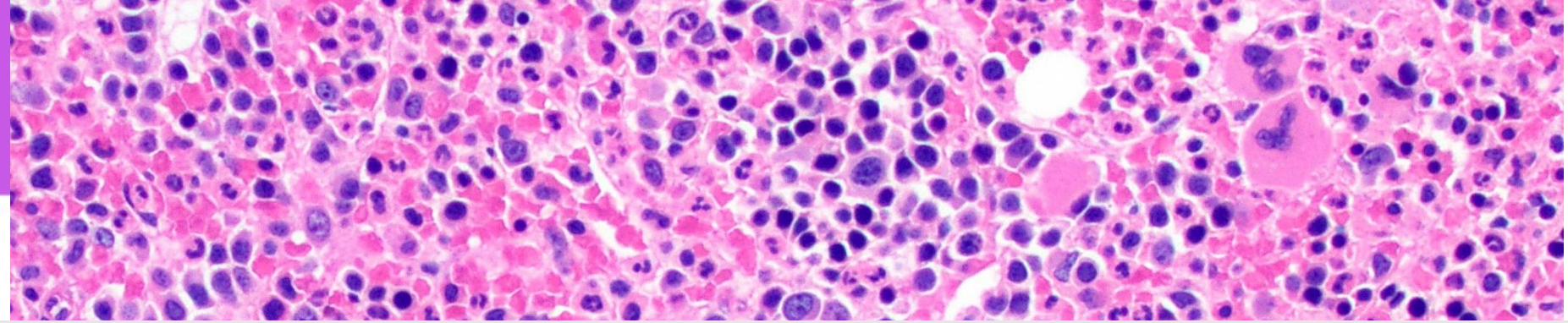


The patient was treated with prednisone/ponatinib/blinatumumab per MDACC protocol

Bone marrow biopsy 5 weeks following the diagnosis







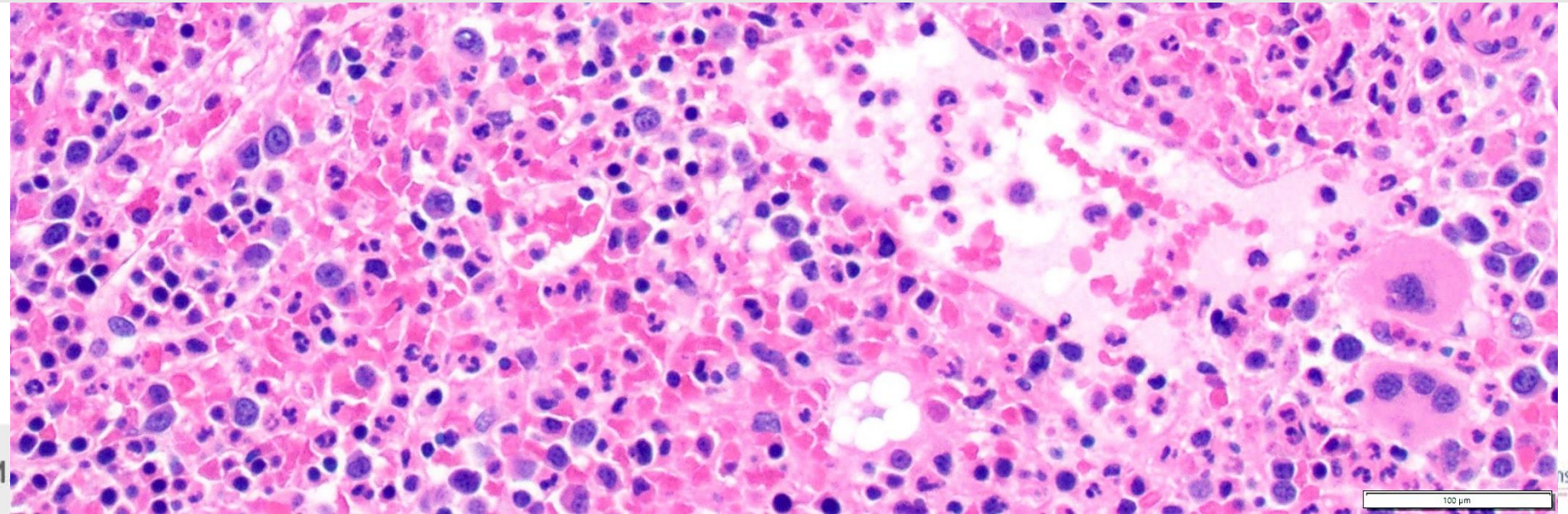
3º CONGRESO LATINOAMERICANO DE HEMATOPATOLOGÍA
SÃO PAULO | 2023

No evidence of residual B-ALL by MRD flow cytometry analysis

Karyotype: 46,XX,t(9;22)(q34;q11.2)[14]/46,XX[6]

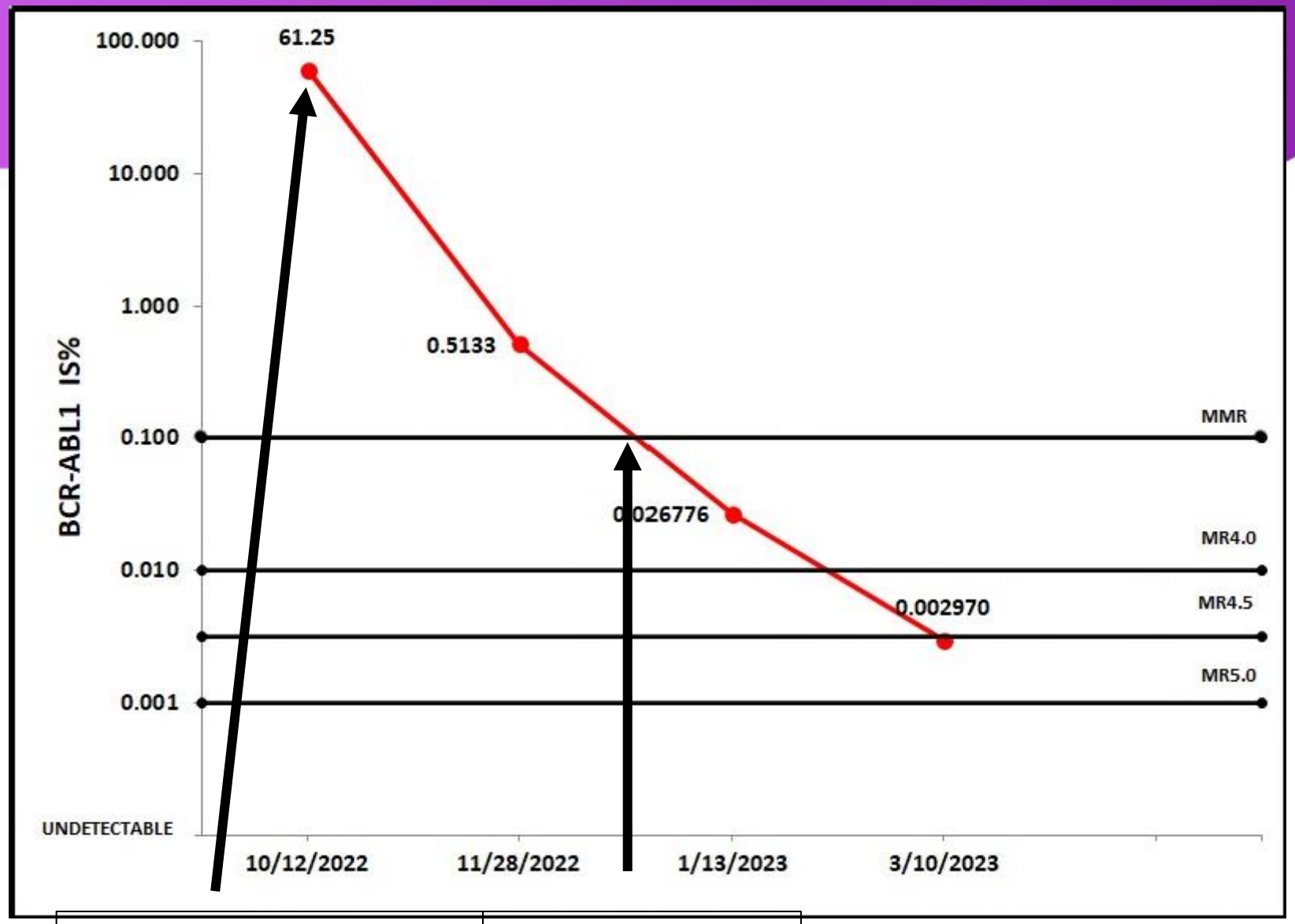
FISH: BCR::ABL1 gene rearrangements in 75% of 200 nuclei

PCR: *BCR-ABL1* (e13a2 or e14a2) fusion transcripts coding for p210 positive at 64.25%



CLINICAL EXAMPLE

- Treated with HyperCVAD and ponatinib→ bone marrow morphology completely normal and no evidence of MRD by flow cytometry
  - Karyotype on peripheral blood: 46,XX[20]
  - FISH on peripheral blood: BCR::ABL1 gene rearrangements in 75% of 200 nuclei
  - PCR: *BCR-ABL1* (e13a2 or e14a2) fusion transcripts coding for p210 positive at 0.51%
- The patient underwent a bone marrow transplant and is currently in complete remission

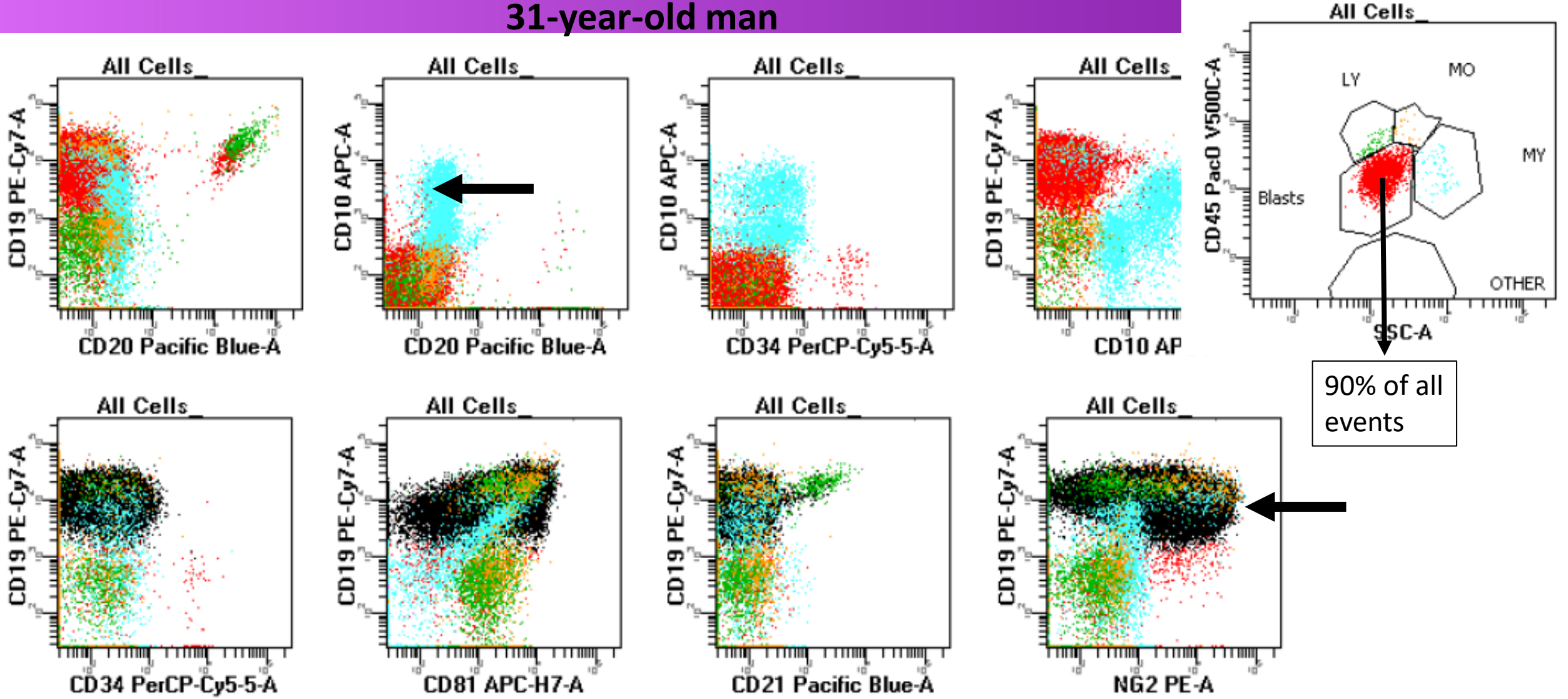


Bone marrow after the induction	Bone marrow transplant

B-ALL with t(v;11q23.3) / *KMT2A* rearranged

- May occur in utero, since this ALL presents in very young infants (most common leukemia in infants)
- The incidence decreases in childhood, but then becomes more common again in adults
- Immunophenotype: the blasts are typically CD19+, CD10-, CD24-, and are often positive for the myeloid markers CD15 and CD65s as well as the neural/glial antigen NG2; TdT is frequently negative
- Molecular diagnosis: suspected by translocations/inversions at 11q23 but requires verification by FISH, RT-PCR or NGS
  - *KMT2A* rearrangements can be “cryptic” by karyotype and/or FISH studies and require NGS evaluation
- Prognosis: poor

31-year-old man



Karyotype: 46,XY,t(4;11)(q21;q23)[3]/47~48,idem,+X,+1[cp7]/46,XY[10]

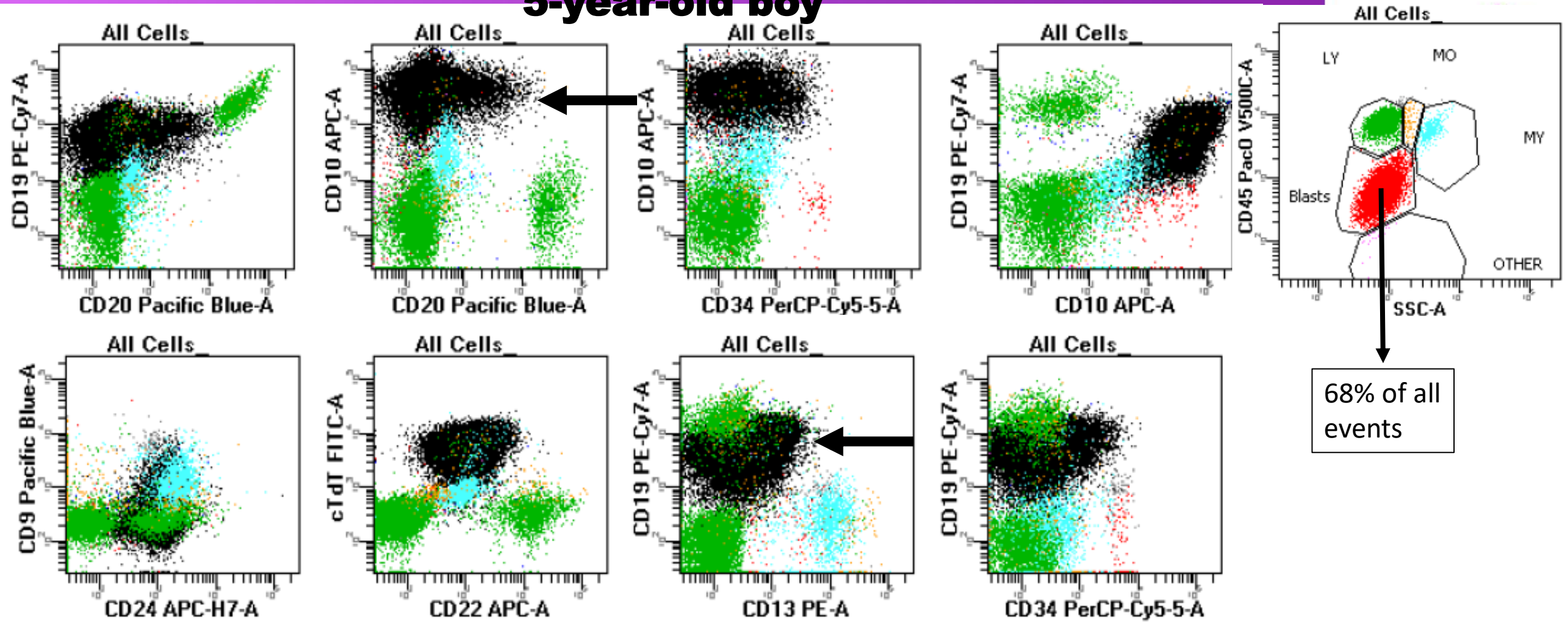
FISH: KMT2A (MLL) gene rearrangement was observed in 85% of 200 interphase nuclei

Received a stem cell transplant, in remission after 3-year follow-up

B-ALL with t(12;21)(p13;q22) / *ETV6::RUNX1*

- The most common recurrent translocation in children
  - not seen in babies, very rare in adults
- Immunophenotype: positive for CD10, CD19 and CD34; near complete absence of CD9, CD20 and CD66c; myeloid-associated antigens (CD13 and CD33) are frequently expressed
- Molecular diagnosis: identifiable by FISH, RT-PCR, and RNA sequencing methods
  - Usually NOT detected by standard cytogenetics
- Prognosis: excellent

5-year-old boy

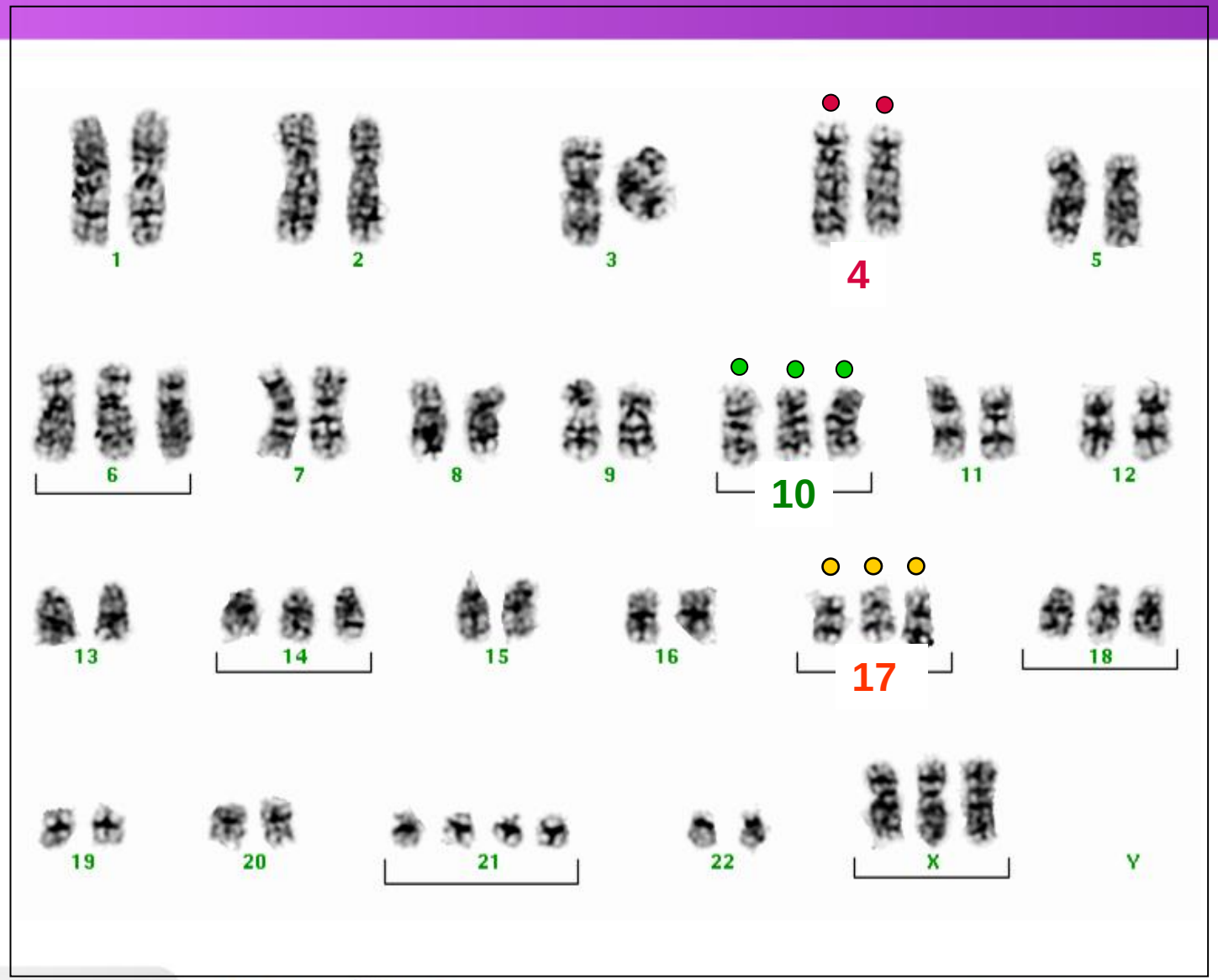


- Karyotype: 46,XY[20]
- FISH: ETV6-RUNX1 rearrangement in 94% of 200 interphase nuclei, indicating the presence of a cryptic t(12;21) translocation
- MRD positive at end of induction; relapsed after 2 years of consolidation therapy. Subsequently achieved MRD-negative CR2 following CD19-directed CAR T-cell therapy, followed by HLA-matched stem cell transplant. In remission 3 years after initial diagnosis

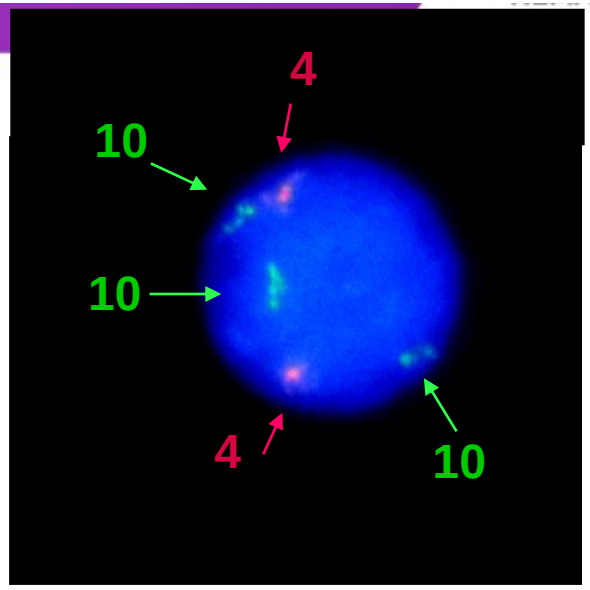
B-ALL, HYPERDIPLOID (ICC)
B-ALL WITH HIGH HYPERDIPLOIDY (WHO)

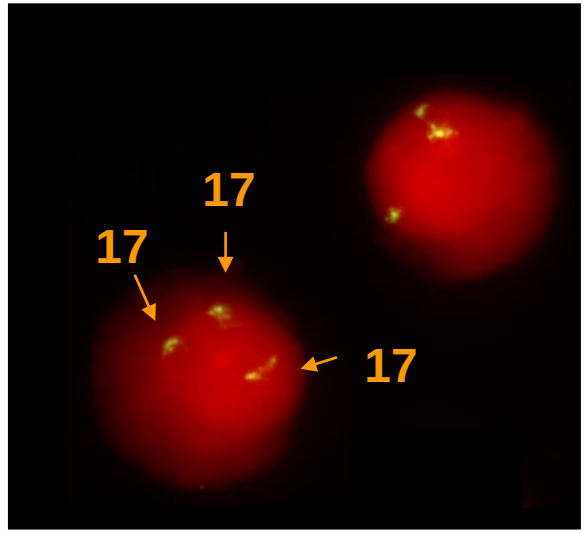
- Hyperdiploidy (51-65 chromosomes) occurs usually in children
  - recurrent, non-random gains of one or more copies of entire chromosomes, usually chromosomes X, 4, 6, 10, 14, 17, 18 and 21
- Common in children, uncommon in adults
- Immunophenotype: no unique features
- Molecular diagnosis: identifiable by karyotype, FISH, flow cytometric DNA index or SNP arrays
- Prognosis: very favorable

54,XX,+X,+6,+10,+14,+17,+18,+21,+21 (hyperdiploid)



FISH for 4, 10, and 17





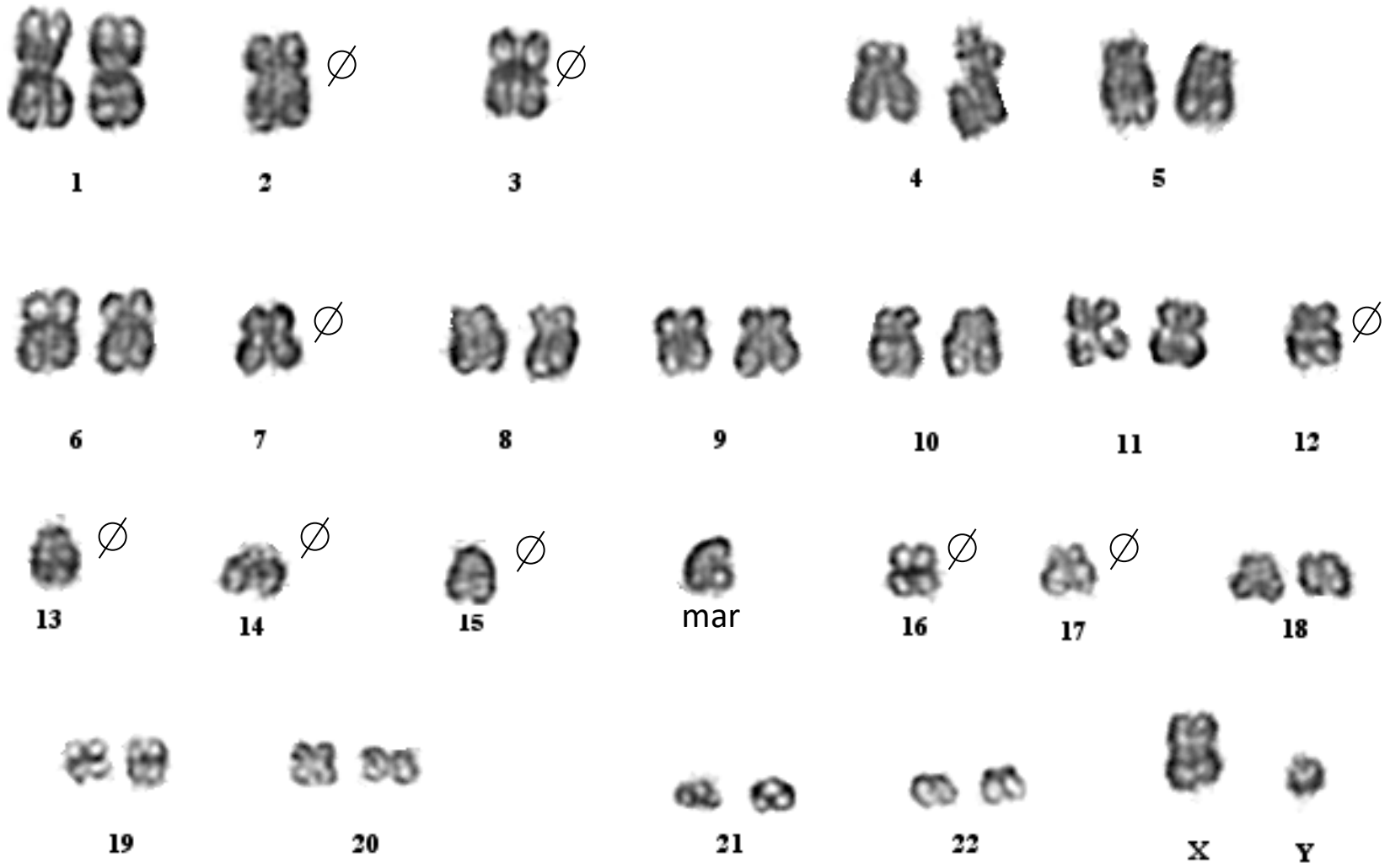
Slide courtesy of Dr. Liming Bao, Division of Cytogenetics, Weill Cornell Medicine

B-ALL, LOW HYPODIPLOID AND NEAR HAPLOID (ICC)
B-ALL WITH HYPODIPLOIDY (WHO)

- Hypodiploidy is defined by the WHO5 as ≤43 chromosomes
  - Near-haploid (24-31 chromosomes)
  - Low-hypodiploid (32-39 chromosomes)
  - High-hypodiploid (40-43 chromosomes)
- ICC formally separates 2 categories:
  - B-ALL, near haploid (24-31 chromosomes): more common in children
  - B-ALL, low hypodiploid (32-39 chromosomes): more common in adults, associated with IKZF2 deletions and TP53 mutations
- Immunophenotype: no unique features
- Molecular diagnosis: karyotype, flow cytometry DNA index, FISH, and SNP arrays
- Prognosis: poor for all subtypes

Hypodiploid B-ALL

38,XY,-2,-3,-7,-12,-13,-14,-16,-17,+mar



Slide courtesy of Dr. Liming Bao, Division of Cytogenetics, Weill Cornell Medicine

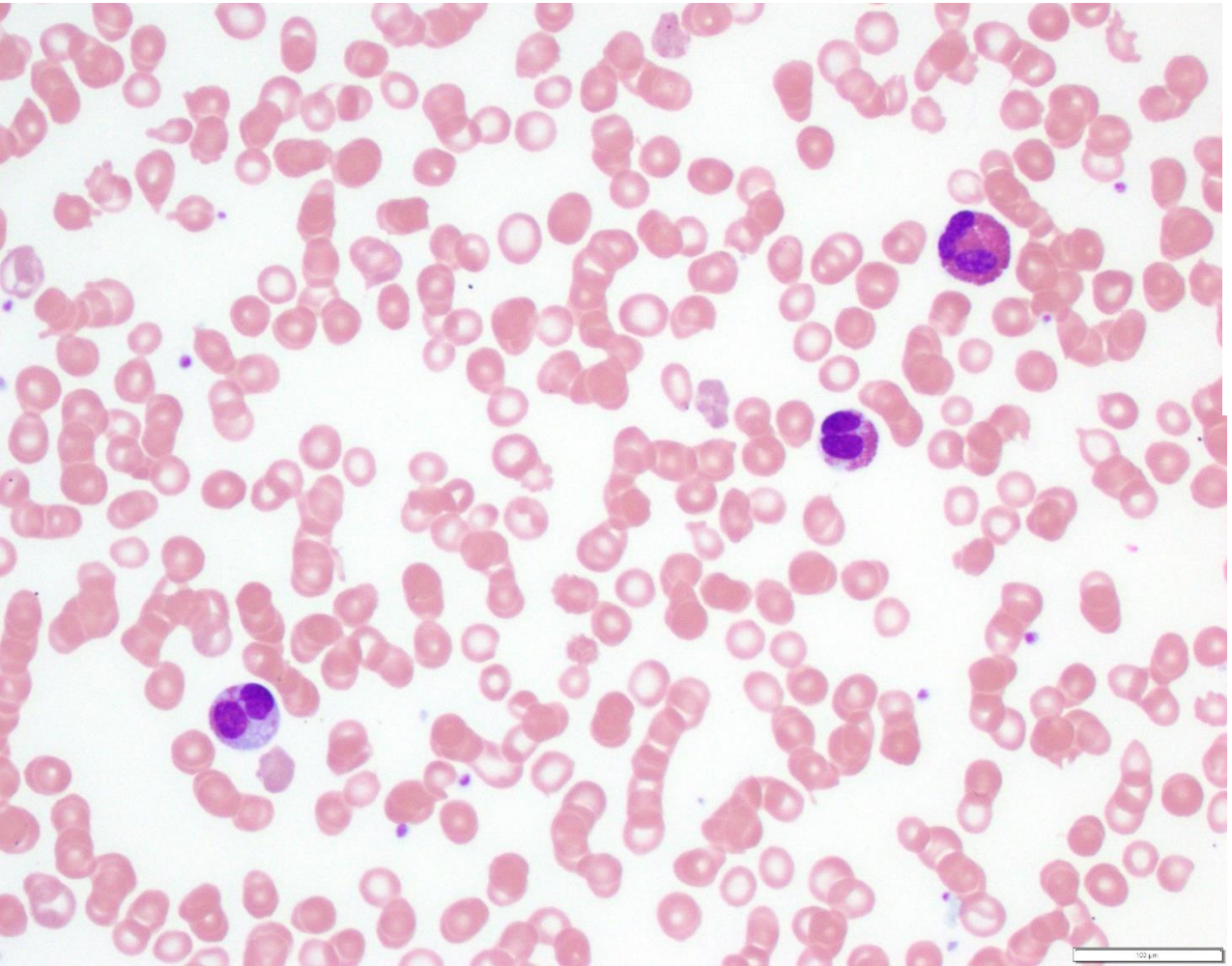
B-ALL with t(5;14)(q31.1;q32.3) / *IL3::IGH*

- t(5;14)(q31;q32); *IGH-IL3* is characterized by marked bone marrow and peripheral blood reactive eosinophilia
- Very rare disease, most patients are children and young adults
- Clinical presentation: neoplastic blasts can be low in number and obscured by the eosinophilic proliferation
- Immunophenotype: no unique features
- Molecular diagnosis: identifiable by karyotype and FISH
- Prognosis: uncertain due to low number of reported cases

CLINICAL CASE

3º CONGRESO LATINOAMERICANO DE HEMATOPATOLOGÍA
SÃO PAULO | 2023

57-year-old man who presented in July 2021 with myocardial infarction and was found to have marked eosinophilia



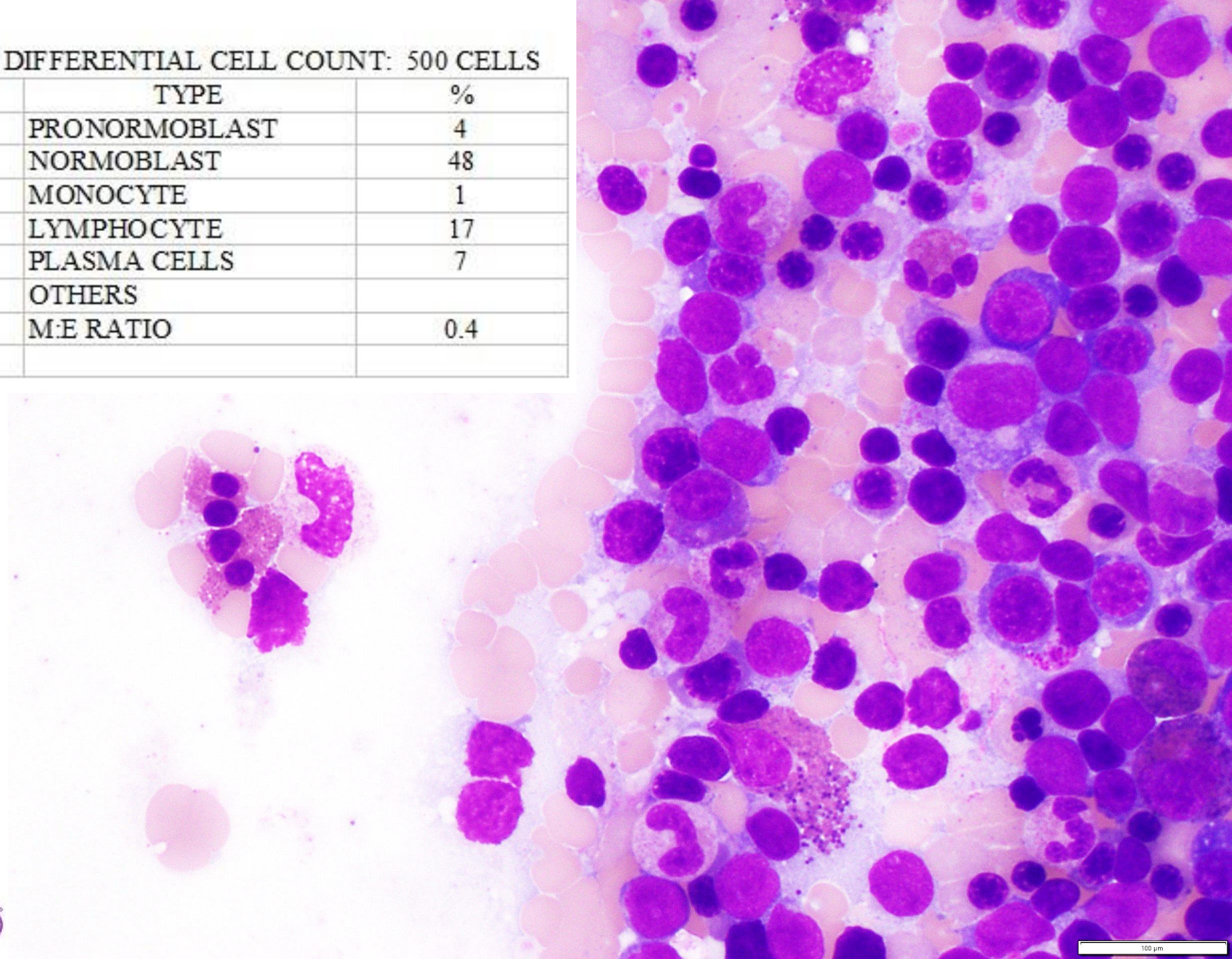
PERIPHERAL BLOOD DIFFERENTIAL CELL COUNT: 200 CELLS:

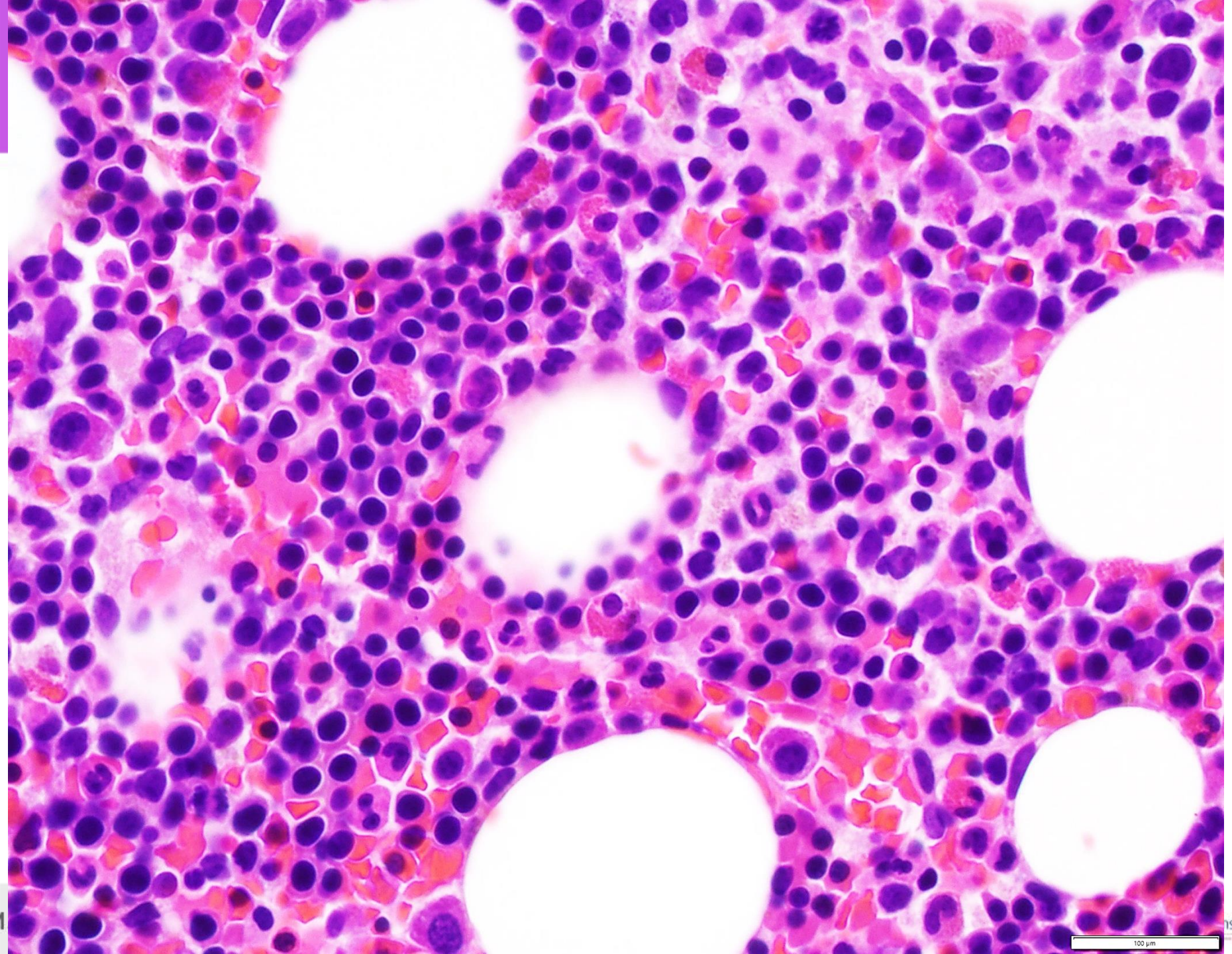
			%		%
WBC	8.91 $10^3/\mu L$	NEUTROPHILS	14	LYMPHOCYTES	35
RBC	4.49 $10^6/\mu L$	BAND		MONOCYTES	2
HGB	9.0 g/dL	META		EOSINOPHILS	49
HCT	28.3 %	MYELOCYTE		BASOPHILS	
MCV	63.0 fL	PROMYELOCYTE			
PLATELETS	196 $10^3/\mu L$	BLAST			
NRBC	0/100 WBC				

Bone Marrow Aspirate Smear:

BONE MARROW ASPIRATE SMEAR DIFFERENTIAL CELL COUNT: 500 CELLS

TYPE	%	TYPE	%
BLAST	5	PRONORMOBLAST	4
PROMYELOCYTE	0	NORMOBLAST	48
MYELOCYTE	2	MONOCYTE	1
METAMYELOCYTE	1	LYMPHOCYTE	17
BAND / NEUTROPHIL	5	PLASMA CELLS	7
EOSINOPHIL	10	OTHERS	
BASOPHIL	0	M:E RATIO	0.4





3º CONGRESO
LATINOAMERICANO DE
HEMATOPATOLOGÍA
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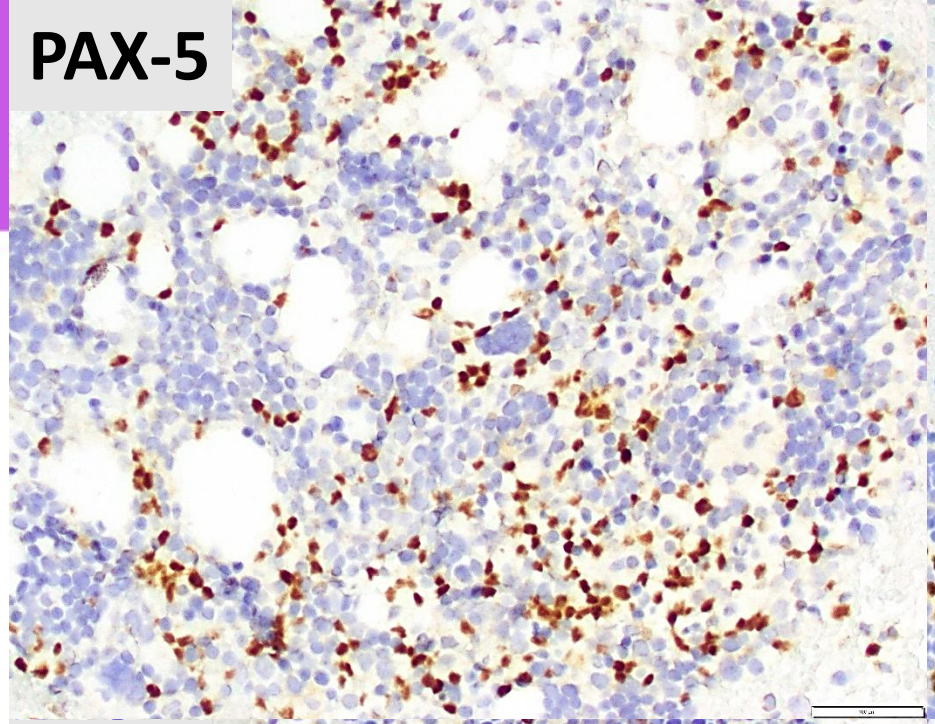
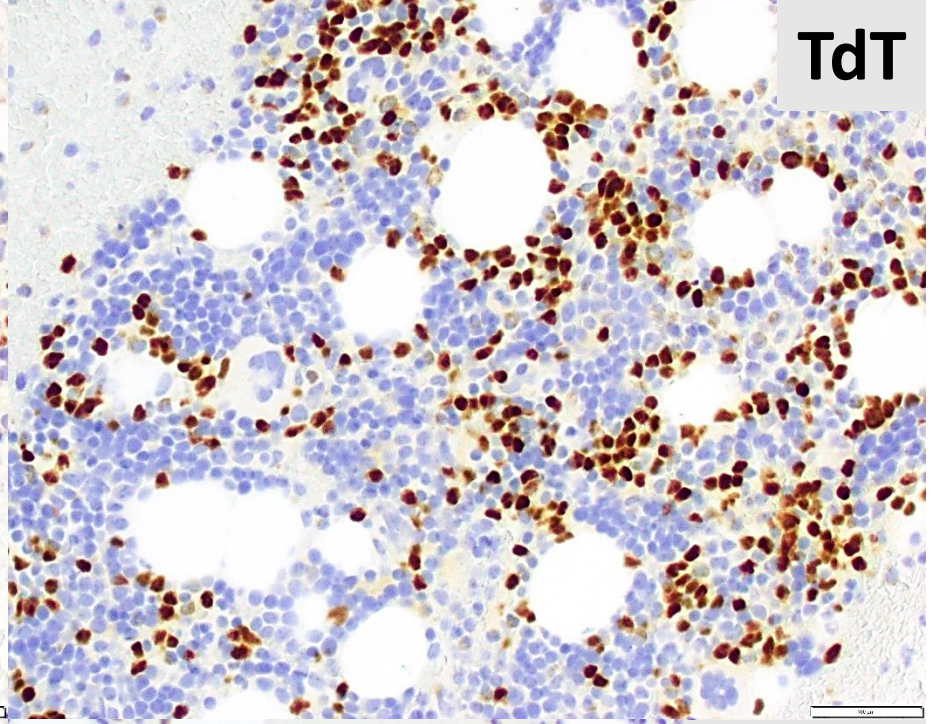
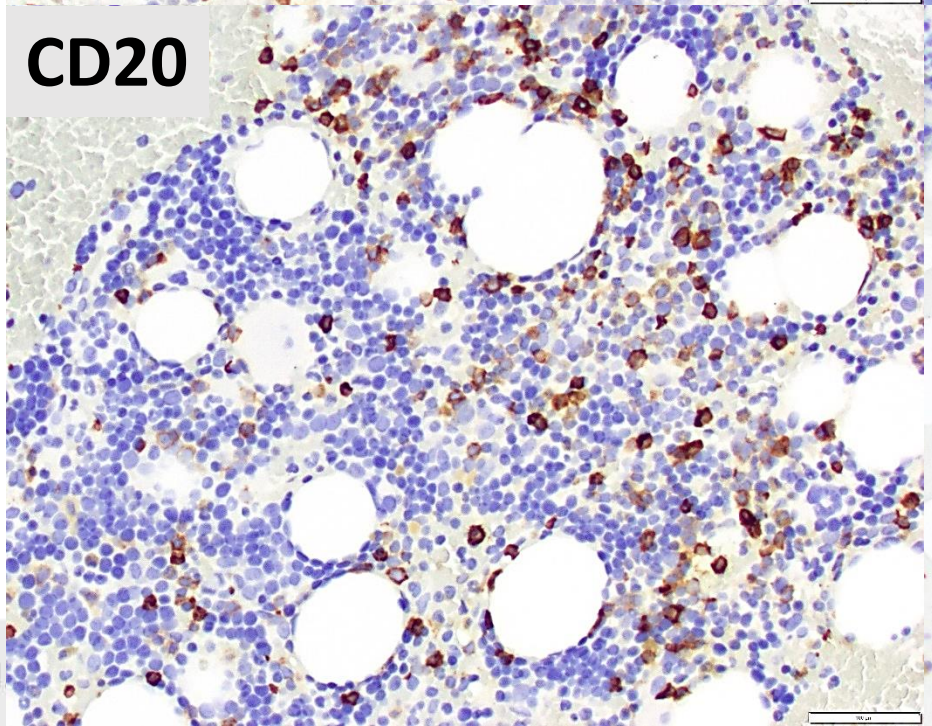
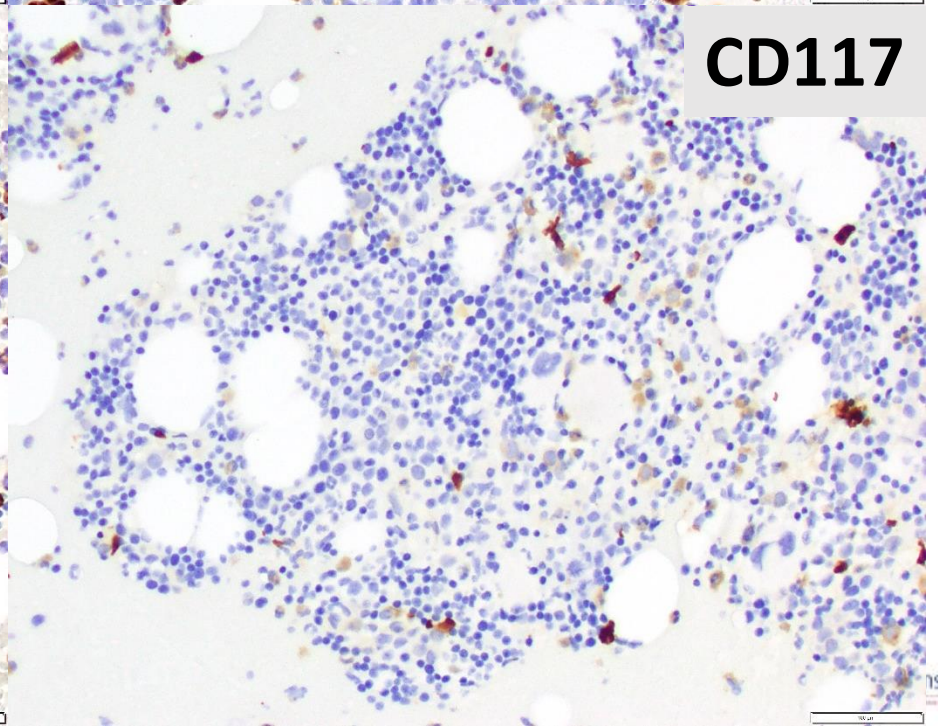
A.C.Camargo Cancer Center

Agilent Dako

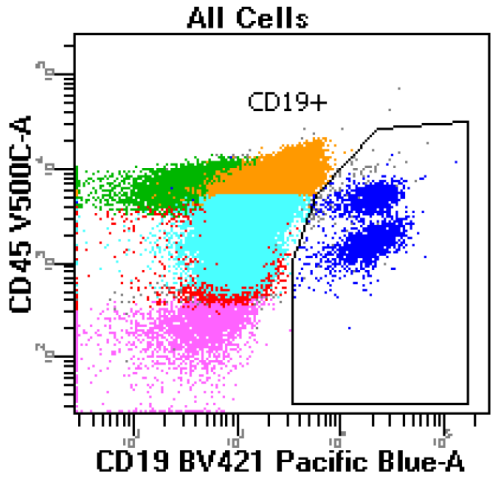

PAX-5

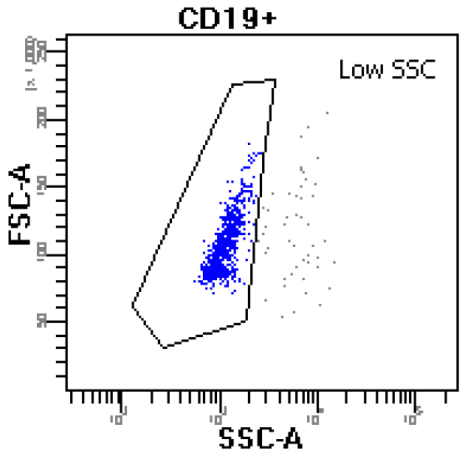

TdT

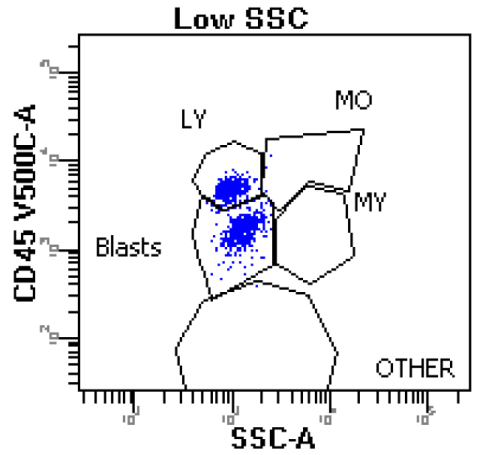

CD20

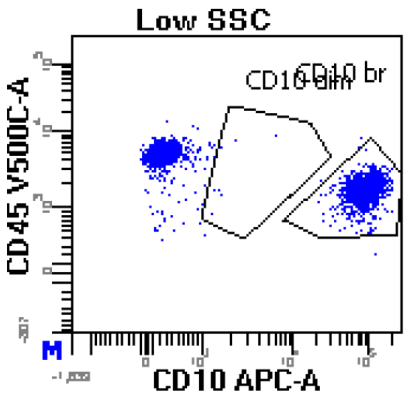

CD117

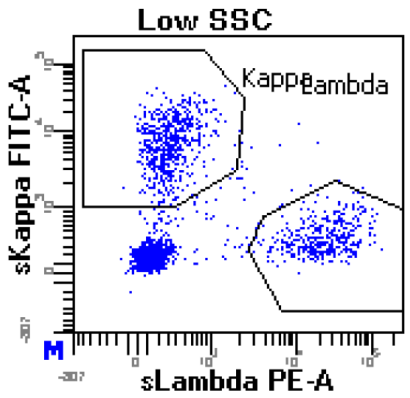
Bone marrow biopsy flow cytometry B cell evaluation

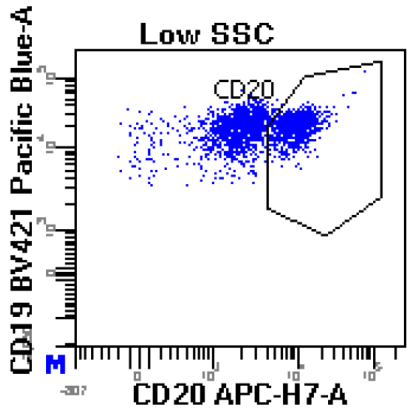


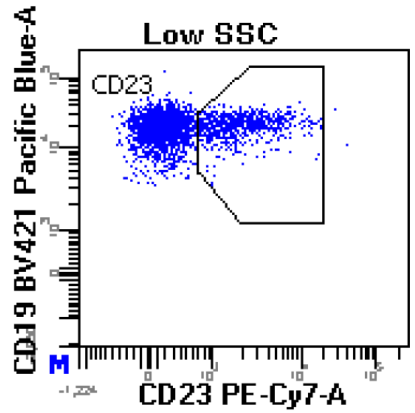


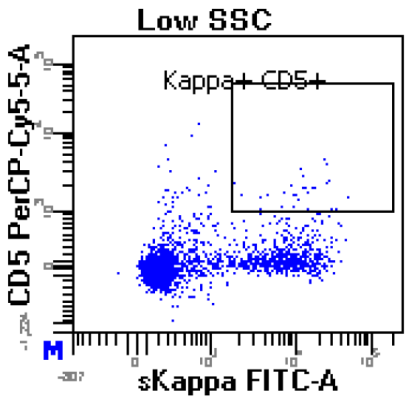


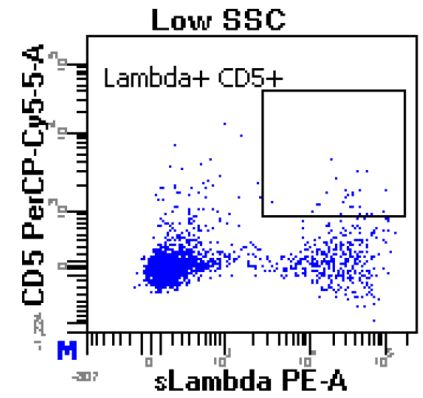


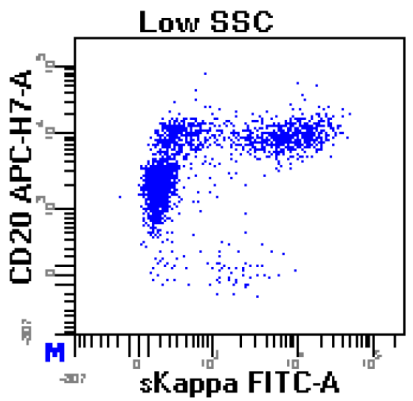


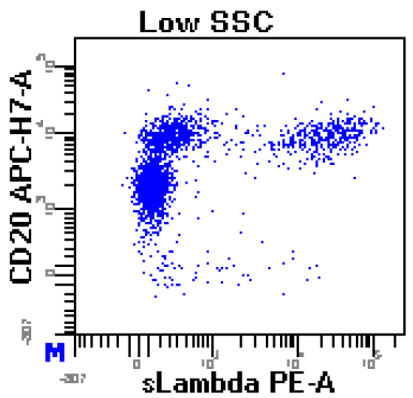


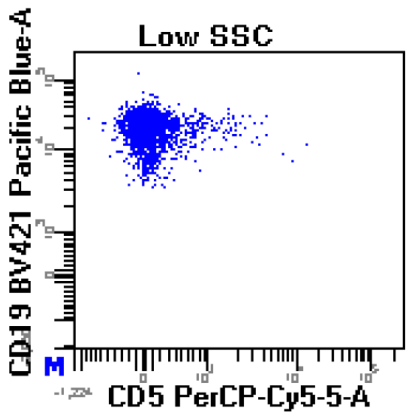


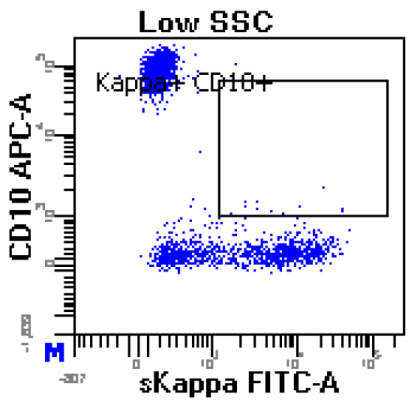


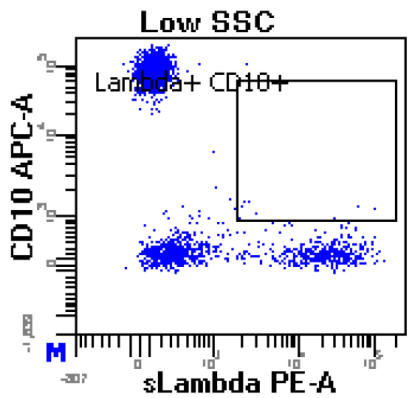




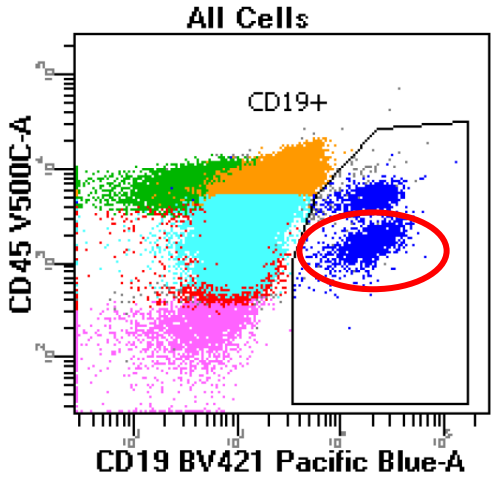


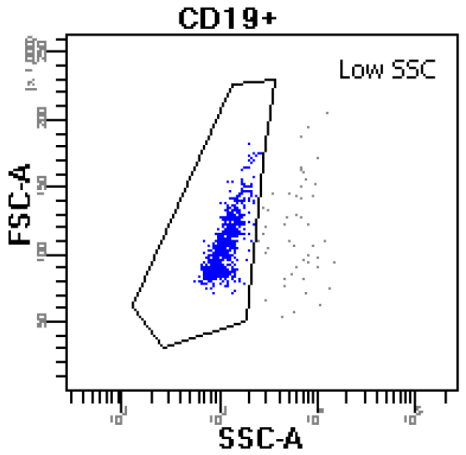


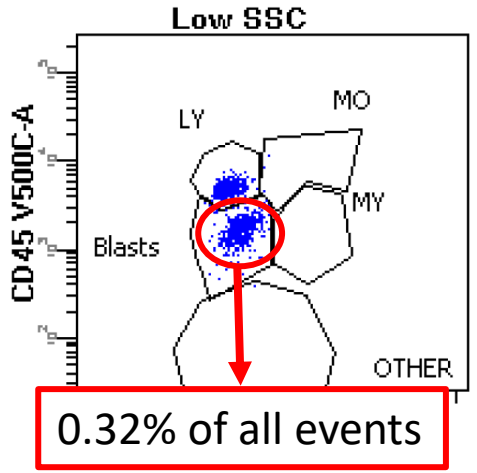




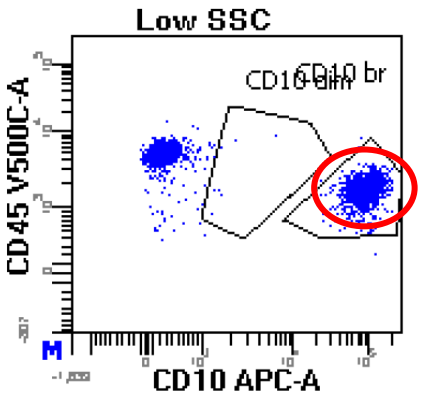
Bone marrow biopsy flow cytometry B cell evaluation

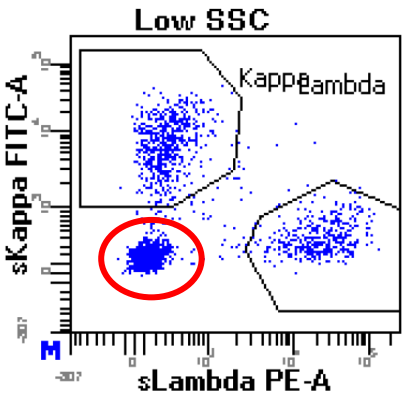


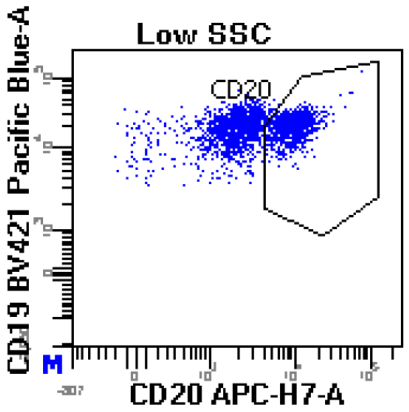


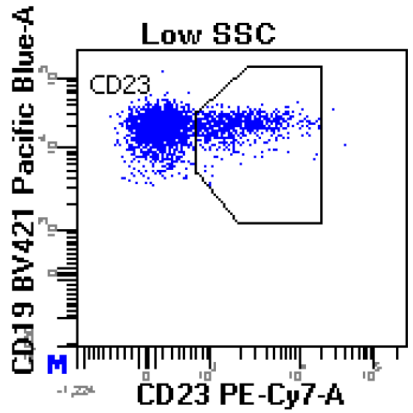


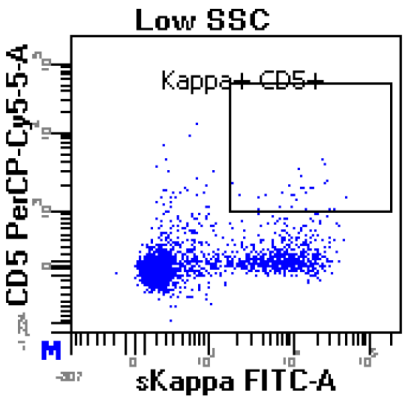
0.32% of all events

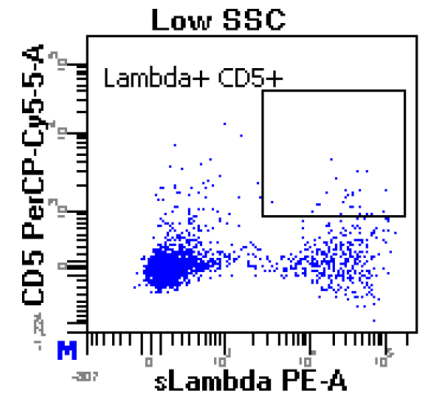


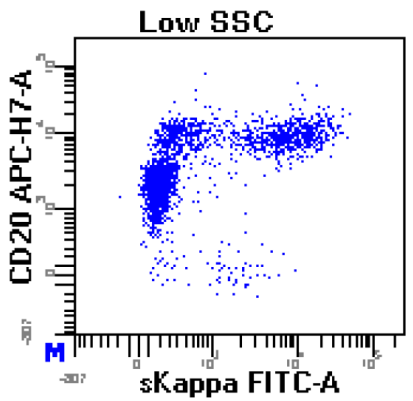


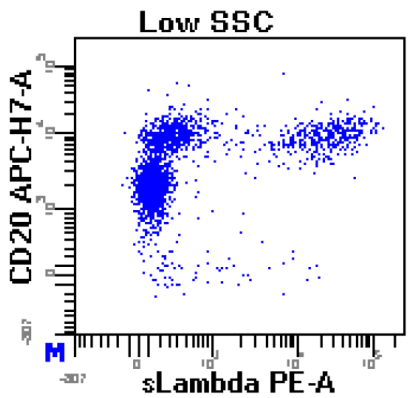


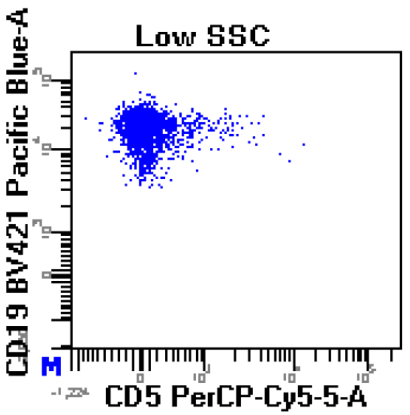


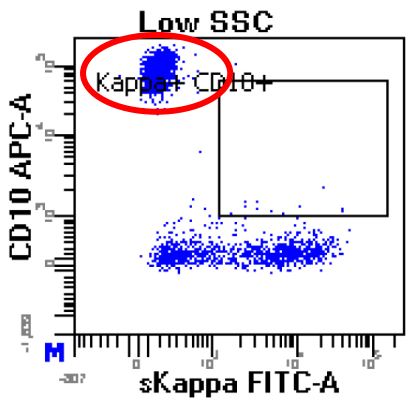


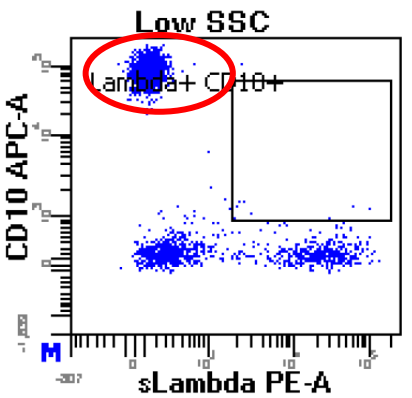












CLINICAL CASE

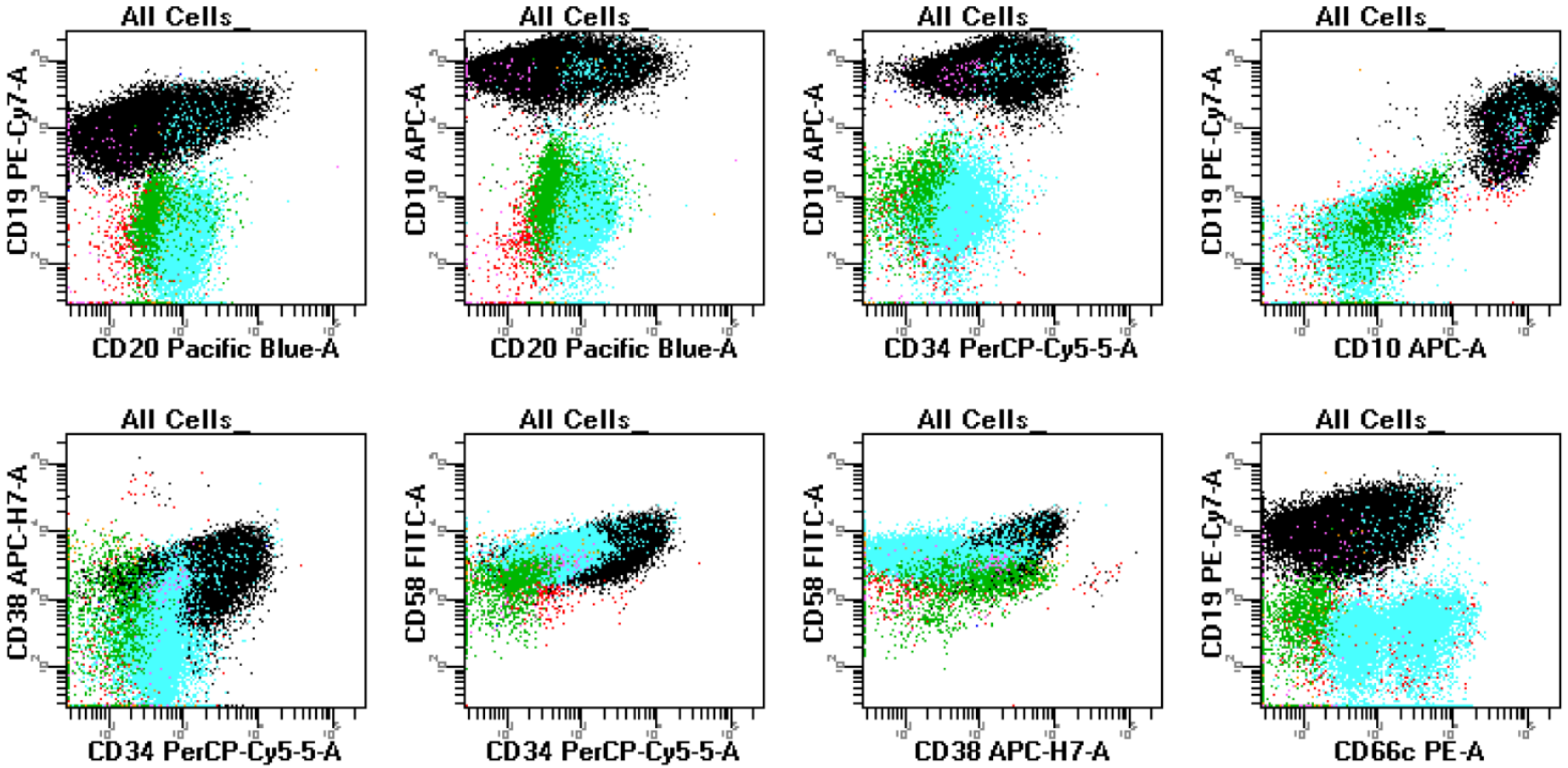
- Myeloid NGS panel showed IDH2 (R140Q, MAF 5.3%) and SRSF2 (P95H, MAF 10.6%)
- Normal male karyotype
- FISH studies did not detect rearrangements in BCR-ABL1, PDGFRA, PDGFRB, FGFR1 and JAK2
    - Interphase FISH showed IGH gene rearrangement in 2.5% of 200 cells analyzed (below the cut off limit of reporting an abnormal result (4.43%))
- Patient clinically assumed to have chronic eosinophilic leukemia and treated with prednisone, however eosinophilia did not resolve
- Following the bone marrow review the patient was treated with HyperCVAD and achieved complete remission

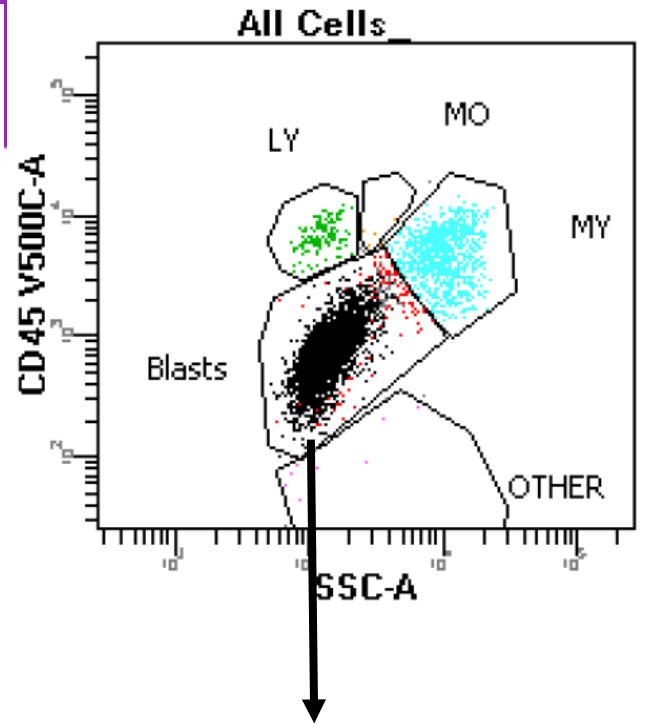
CLINICAL CASE

The patient relapsed with overt B-ALL eight months following the initial presentation

PERIPHERAL BLOOD DIFFERENTIAL CELL COUNT: 100 CELLS:

			%		%
WBC	8.14 $10^3/\mu L$	NEUTROPHILS	40	LYMPHOCYTES	20
RBC	3.67 $10^6/\mu L$	BAND	8	MONOCYTES	2
HGB	8.7 g/dL	META		EOSINOPHILS	24
HCT	28.2 %	MYELOCYTE		BASOPHILS	2
MCV	76.8 fL	PROMYELOCYTE			
PLATELETS	64 $10^3/\mu L$	BLAST	4		
NRBC	3/100 WBC				





75% of all events

Bone marrow karyotype: t(5;14)(q31.1;q32.3)[7]/46,XY[13]
NGS: SRSF2 mutation (VAF 3.4%); CREBBP (VAF 6.2%)

B-ALL, *BCR::ABL1*-LIKE

- Gene expression profile is similar to *BCR-ABL1*+ ALL, but lacking the *BCR::ABL1* gene rearrangement
- Accounts for 10% of pediatric and 25% of adult ALL
- Very high incidence in children with Down syndrome
- ICC separates the entity into 3 subtypes:
    - ABL-1 class rearranged, includes fusions between *ABL1, ABL2, CSF1R* or *PDGFRB* and may respond to TKI inhibitors
    - JAK-STAT activated, includes CRLF2 rearrangement and diverse mutations in JAK or RAS pathway genes; may be considered for JAK inhibitors
    - NOS, other kinases and cytokine receptor abnormalities, many seem to respond to TKI inhibitors
- Immunophenotype: TSLPR (encoded by *CRLF2*) overexpression by is a surrogate for CRLF2 rearrangement

B-ALL, *BCR::ABL1*-LIKE

- Molecular diagnosis: very complex and highly specialized!
  - Cytogenetics and FISH used to rule out known molecular subgroups, which are usually mutually exclusive.
  - The majority of *BCR::ABL1*-like ALL rearrangements are cytogenetically cryptic
  - Break-apart FISH probes are available to identify the major rearrangements (*ABL1, ABL2, CRLF2, EPOR, JAK2* and *PDGFRB*)
  - DNA and RNA sequencing
  - Whole transcriptome analysis / targeted RNA sequencing are the most reliable methods
- Prognosis: overall poor (resistant to standard chemotherapy), but many ongoing clinical trials

CLINICAL CASE

75-year-old man with a new diagnosis of B-ALL

PERIPHERAL BLOOD DIFFERENTIAL CELL COUNT: 200 CELLS:

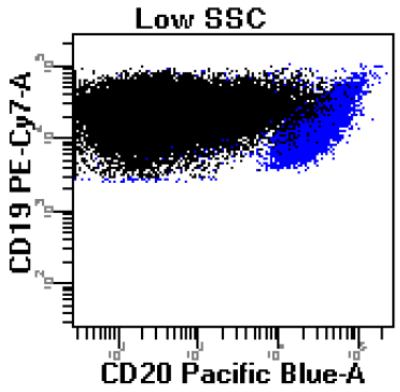
			%		%
WBC	3.32 $10^3/\mu L$	NEUTROPHILS	34	LYMPHOCYTES	30
RBC	3.85 $10^6/\mu L$	BAND	4	MONOCYTES	3
HGB	10.5 g/dL	META		EOSINOPHILS	
HCT	30.4 %	MYELOCYTE		BASOPHILS	
MCV	79.0 fL	PROMYELOCYTE		PLASMA CELLS	1
PLATELETS	8 $10^3/\mu L$	BLAST	28		
NRBC	0 /100 WBC				

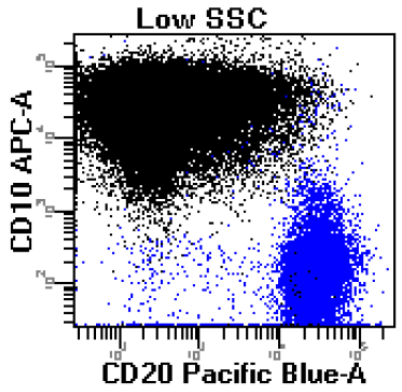
CLINICAL CASE

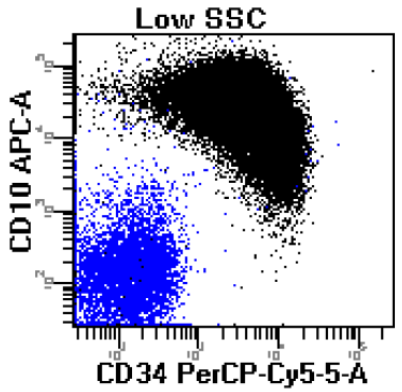
75-year-old man with a new diagnosis of B-ALL

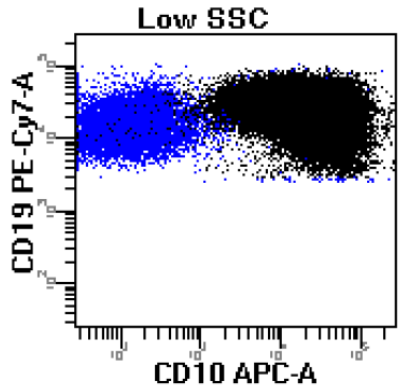
PERIPHERAL BLOOD DIFFERENTIAL CELL COUNT: 200 CELLS:

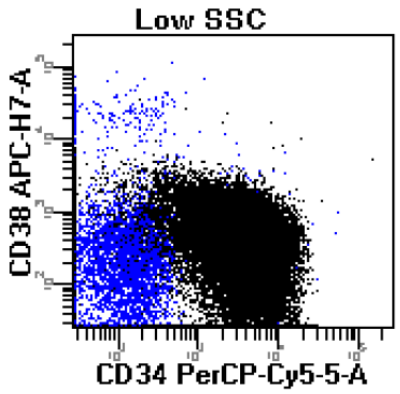
			%		%
WBC	3.32 $10^3/\mu L$	NEUTROPHILS	34	LYMPHOCYTES	30
RBC	3.85 $10^6/\mu L$	BAND	4	MONOCYTES	3
HGB	10.5 g/dL	META		EOSINOPHILS	
HCT	30.4 %	MYELOCYTE		BASOPHILS	
MCV	79.0 fL	PROMYELOCYTE		PLASMA CELLS	1
PLATELETS	8 $10^3/\mu L$	BLAST	28		
NRBC	0 /100 WBC				

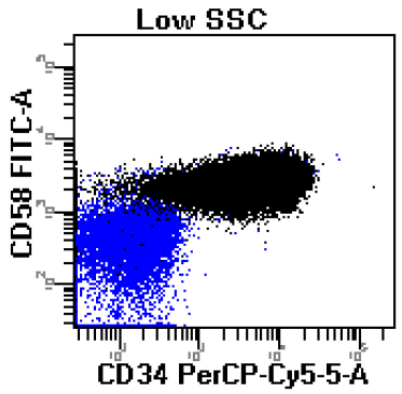


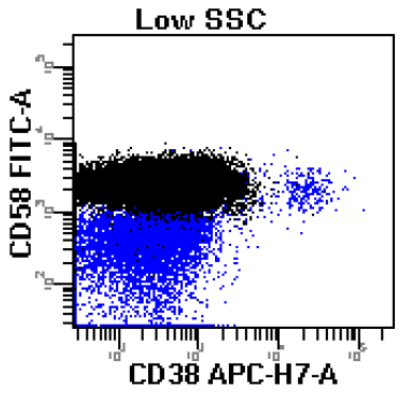


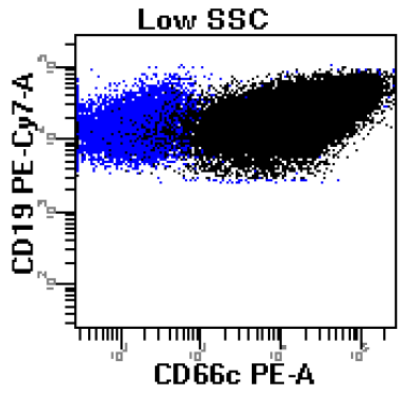


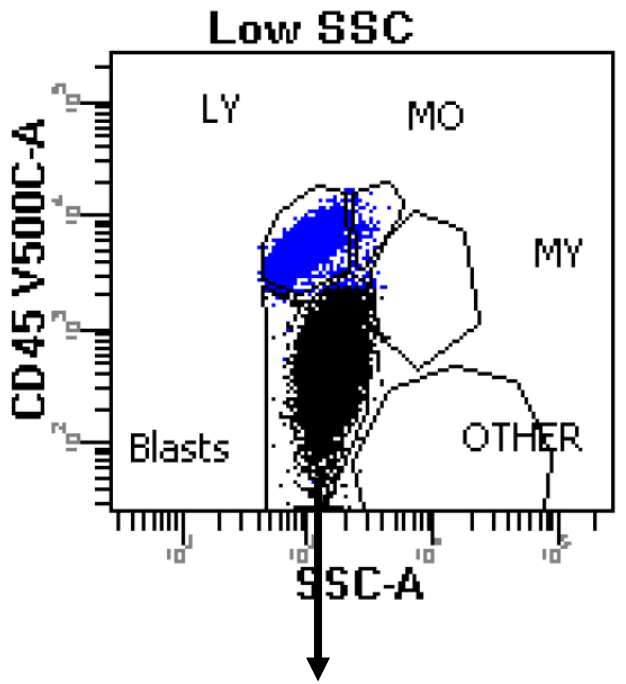












30% of all events

CLINICAL CASE

- Karyotype: 48,XY,+X,add(14)(q32),+22[1]/46,XY[13]
- FISH: positive for rearrangements of IGH, CRLF2, P2RY8 and PDGFRB; negative for BCR::ABL1 gene fusion or KMT2A rearrangement
- Heme fusion RNA NGS panel (Archer): CD74-PDGFRB fusion transcript (96% of reads); P2RY8-IGH rearrangement was detected in this sample (96% of reads); marked increase in CRLF2 transcript expression
- Myeloid NGS panel: mutations in JAK2 R683G (VAF 10%) and KRAS G13D (VAF 7%)

CLINICAL CASE

- The patient was treated with mini hyperCVD (lower intensity than hyper-CVAD) + inotuzumab (anti-CD22 antibody) and achieved complete remission
- Followed by blinatumomab (anti-CD19 bispecific T-cell engager)
- In remission 6 months following the initial presentation

EARLY T-CELL PRECURSOR ALL

- T-ALL with a unique immunophenotype of immature T cell precursor with myeloid differentiation
- About 15% of pediatric and 20-40% of adult T-ALL cases
- They are characterized by multilineage differentiation potential and expression of lymphoid, myeloid and stem cell markers
- Genetically heterogeneous
- Mutation profiles are more similar to AML than T-ALL
- Prognosis of this entity is controversial

DIAGNOSTIC CRITERIA (WHO 5TH EDITION)

ETP-ALL/LBL: Must meet all 5 criteria of antigen expression
1. CD3 (cytoplasmic +/surface-[a])
2. Absent myeloperoxidase (<10% by flow cytometry, <3% by cytochemistry)
3. Absent CD1a and CD8
4. ≥25% of blasts with ≥1 of stem cell or myeloid markers: CD34, CD117, CD13, CD33, CD65, CD11b, HLA-DR
5. Dim to negative CD5 (<75% of blasts positive)[b]
Near ETP-ALL: Criteria 1-4 met, however ≥75% blasts are CD5 positive

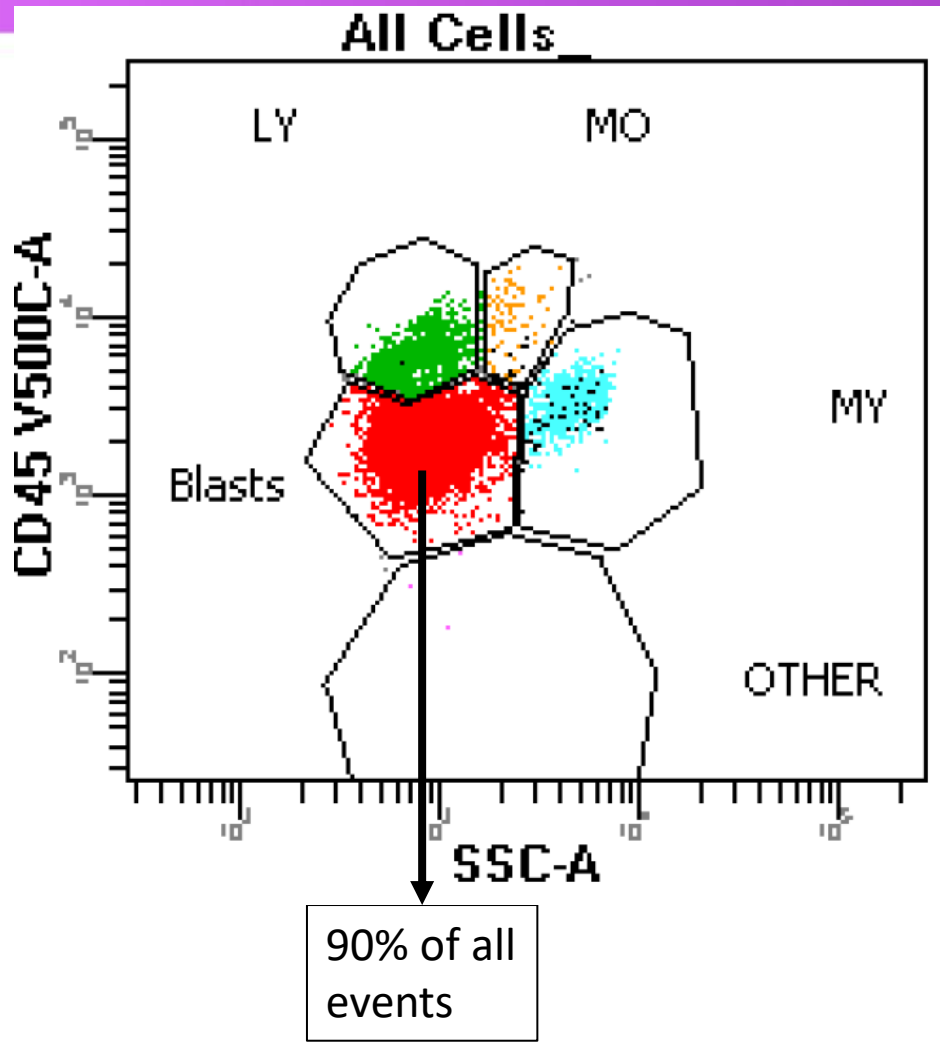
a. Expression of surface CD3 is rare

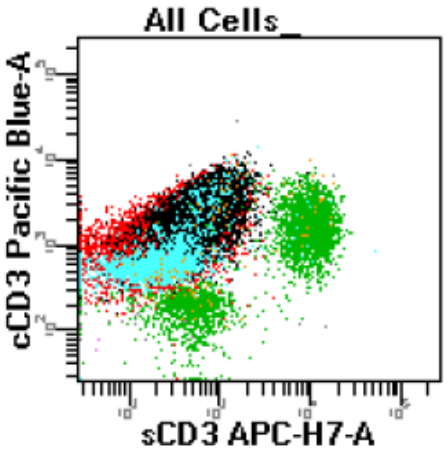
b. Dim CD5 can be also defined as mean fluorescent intensity (MFI) that is at least 1 log less than that of normal T cells; with this approach, MFI of T cells should be at least two logs greater than that of negative control.

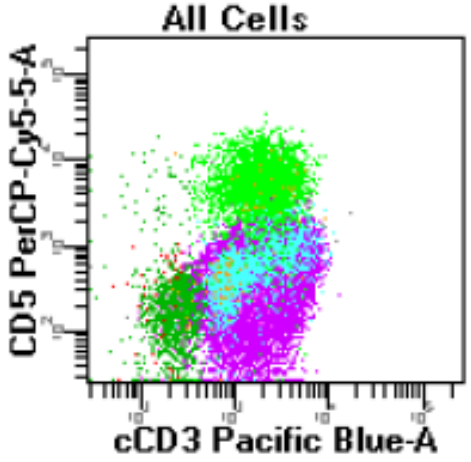
Near ETP-ALL has different genetic lesions than ETP-ALL

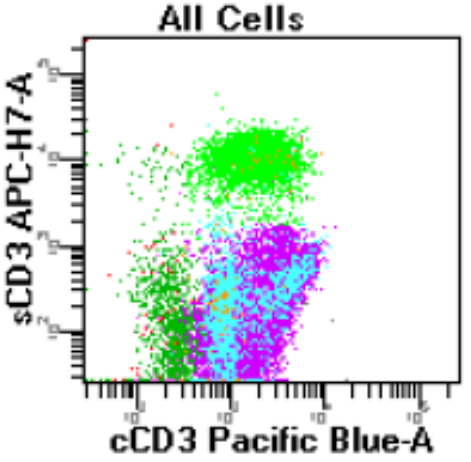
Minor differences in clinical presentation and response to therapy

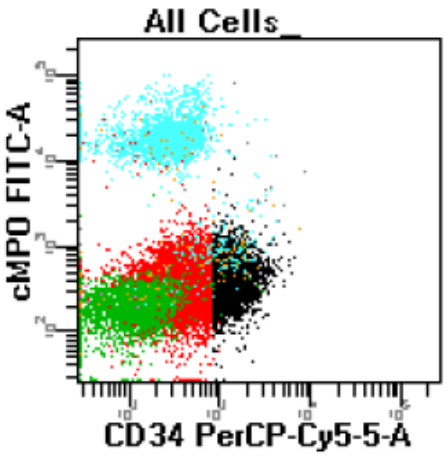
Overall, clinical implications of neat ETP-ALL are still unclear

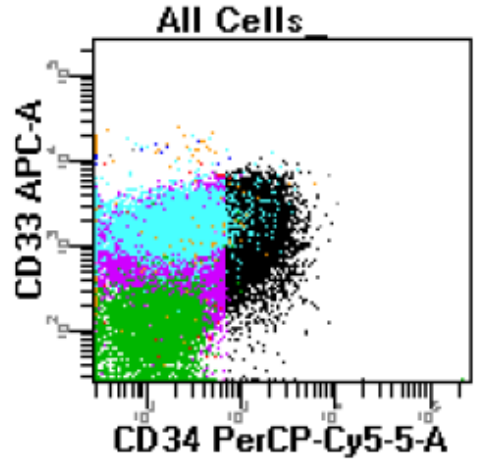

All Cells
LY
MO
MY
Blasts
OTHER
CD45 V500C-A
SSC-A
90% of all events

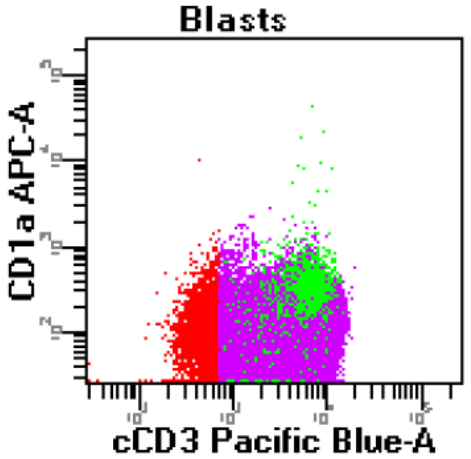

All Cells
cCD3 Pacific Blue-A
sCD3 APC-H7-A


All Cells
CD5 PerCP-Cy5-5-A
cCD3 Pacific Blue-A

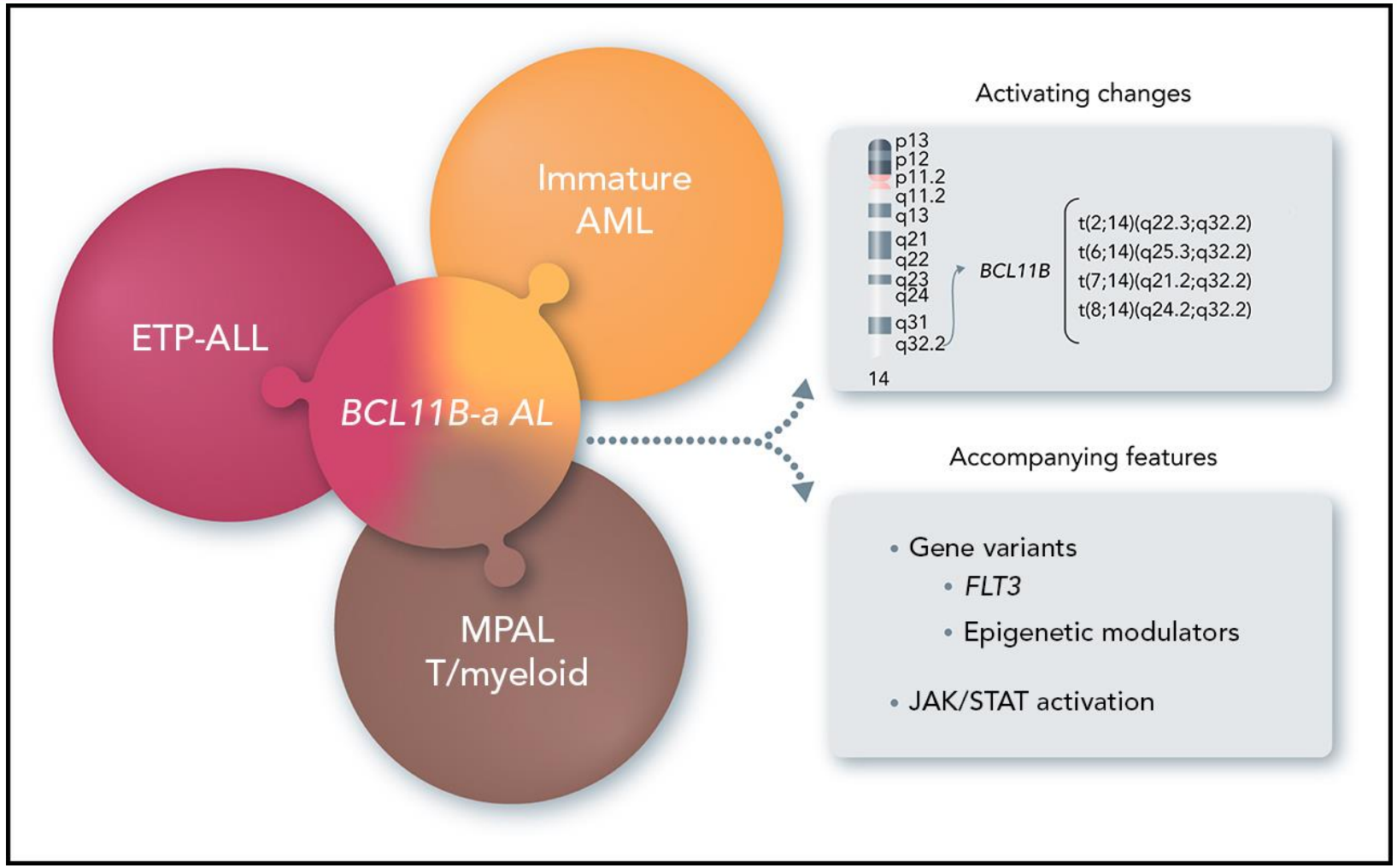

All Cells
sCD3 APC-H7-A
cCD3 Pacific Blue-A


All Cells
cMPO FITC-A
CD34 PerCP-Cy5-5-A


All Cells
CD33 APC-A
CD34 PerCP-Cy5-5-A


Blasts
CD1a APC-A
cCD3 Pacific Blue-A

EARLY T-CELL PRECURSOR ALL, *BCL11B*-ACTIVATED (ICC)



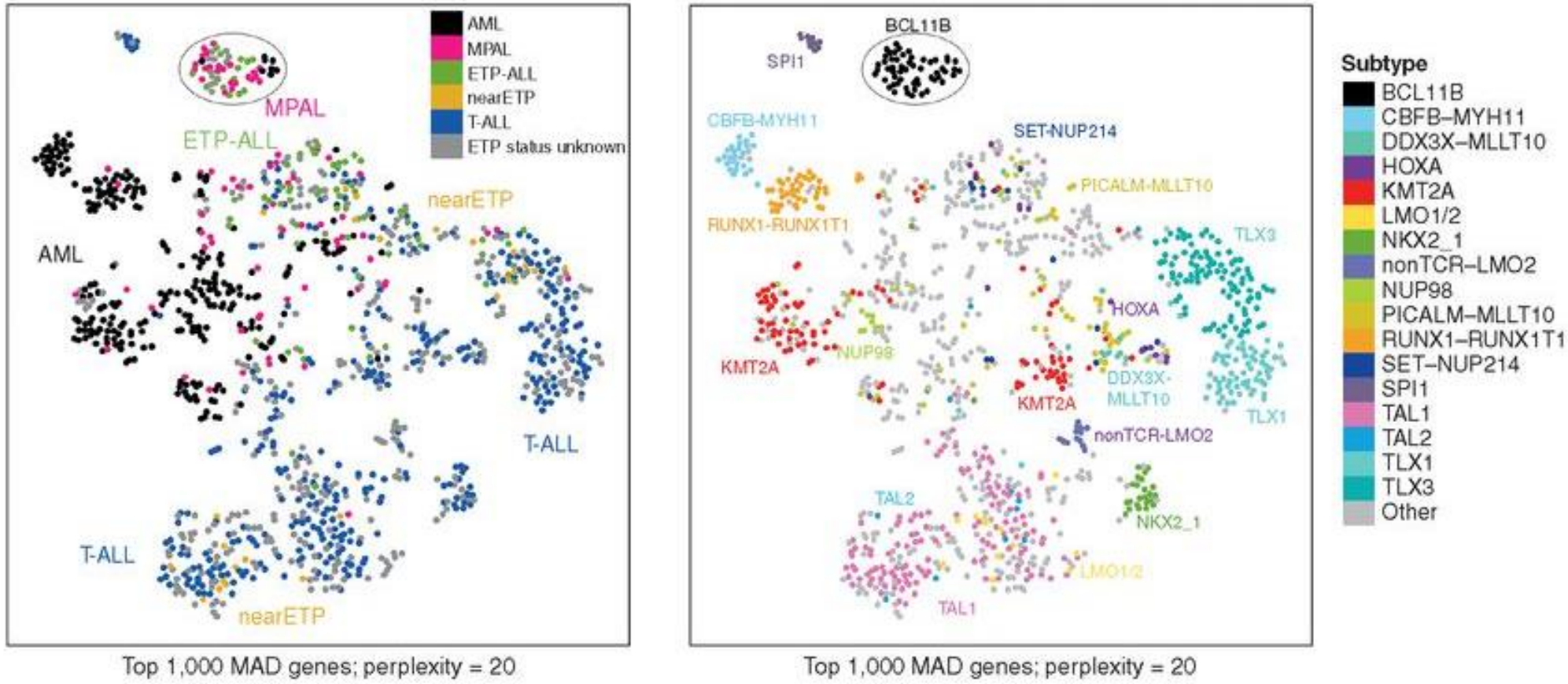
A distinct subgroup of immature acute leukemias covering AML, T/myeloid mixed-phenotype acute leukemia (T/M MPAL), and early T-cell precursor acute lymphoblastic leukemia (ETP-ALL) are characterized by rearrangements at 14q32/*BCL11B*

Di Giacomo D, et al. Blood (2021) 138 (9): 773–784

EARLY T-CELL PRECURSOR ALL, *BCL11B*-ACTIVATED (ICC)

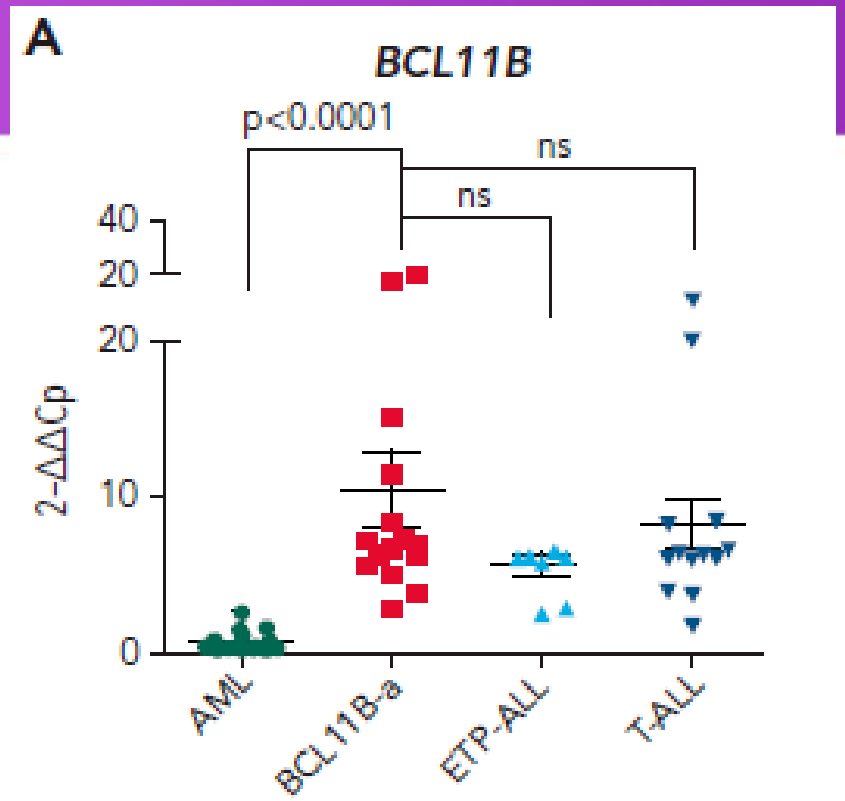
- About 1/3 of ETP-ALL cases is characterized by rearrangement and deregulation of the T-lineage transcription factor gene BCL11B in the stem cells
- The BCL11B protein is a critical transcription factor in regulating thymic T lineage commitment and specification, and its overexpression leads to the inhibition of the T cell differentiation and activation of the JAK/STAT transduction pathway
- >80% of the cases have activating FLT3 mutations
- All rearrangements relocate super-enhancers to positions near the *BCL11B* gene at 14q32.2, leading to increased *BCL11B* expression
- Most of the cases can be detected by FISH analysis (14q32 break apart probe)
  - several *BCL11B*-related rearrangements, including t(2;14), t(3;14), t(6;14), t(7;14), t(8;14), t(12;14) and t(14;21)
  - diverse fusion partners (*ZEB2* at 2q22.3, *SATB1* at 3p24.3, *ARID1* at 6q25.3, *CDK6* at 7q22.1, *CCDC26*/*MYC* at 8q24.2, *ETV6* at 12p13.2, and *RUNX1* at 21q22.1)

Enhancer Hijacking Drives Oncogenic BCL11B Expression in Lineage-Ambiguous Stem Cell Leukemia



tSNE projection analysis of 1,114 leukemia transcriptomes.
Left panel shows samples colored by original diagnosis; right panel shows samples colored by driver genomic alteration. Samples belonging to the BCL11B group are circled.

Montefiori LE, et al Cancer Discov. 2021;11(11):2846-2867



In *BCL11B*-a acute leukemia cases, *BCL11B* was activated, showing a significant upregulation compared with AML (P < .0001) and similar levels of expression compared with cases of T-ALL and ETP-ALL.

Di Giacomo D, et al. Blood (2021) 138 (9): 773–784

EARLY T-CELL PRECURSOR ALL, *BCL11B*-ACTIVATED (ICC)

- Comparing the specific drug sensitivities to an unrelated cohort of T-ALL cases, in *BCL11B*-a acute leukemia cases, there was a striking decrease in activity for the genotoxic agents used in both AML and T-ALL, such as docetaxel, mitoxantrone, idarubicin, etoposide, doxorubicin, cytarabine, gemcitabine, and topotecan
- There was higher sensitivity to tyrosine kinases (sunitinib, crenolanib) and JAK/STAT inhibitors (NVPBVB808, momelotinib, fedratinib, and NVP-BSK805) in *BCL11B*-aAL cases compared with other T-ALL samples
- Of note, the tyrosine kinase inhibitor midostaurin showed low activity in *BCL11B*-a acute leukemia cases, despite the presence of *FLT3* mutations, indicating that drug sensitivities may depend on more complex factors than candidate gene mutations

Di Giacomo D, et al. Blood (2021) 138 (9): 773–784

3° CONGRESO
LATINOAMERICANO DE
HEMATOPATOLOGÍA
SÃO PAULO | 2023

REALIZACIÓN

SOLAHP Sociedad Latinoamericana de Hematopatología

Sociedade Brasileira de PATOLOGIA

EA4HP European Association for Haematopathology

APOYO

RECORDATI RARE DISEASES GROUP

AstraZeneca

NOVARTIS

Janssen PHARMACEUTICAL COMPANIES OF Johnson & Johnson

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Agilent Dako