Neoplastic bone marrow eosinophilias and their differential diagnosis

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Definitions

Eosinophilia: Increased eosinophils above the normal range

- >6% of WBCs
- Absolute count (AEC) >0.5 x 10^9/L.

Hypereosinophilia (HE)

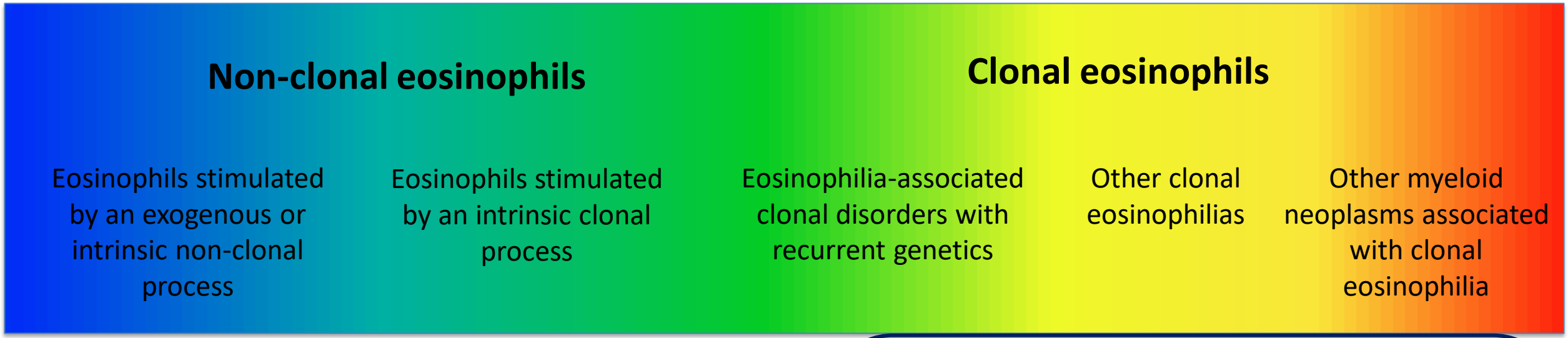
- AEC ≥ 1.5 x 10^9/L and ≥10% of WBCs

Tissue hypereosinophilia

- Eosinophils >20% of nucleated cells in bone marrow
- Extensive tissue infiltration of target organ by histology; or histologic evidence of eosinophil degranulation

Valent P J Allergy Clin Immunol 2012;130:607-612.

The spectrum of eosinophilias



Allergen

Drug

Autoimmune

Aberrant T-cell clone
T-cell lymphoma
Hodgkin RS cells
B-ALL with t(5;14)
Mastocytosis

PDGFRA
PDGFRB
FGFR1
JAK2
FLT3

CEL, NOS

CML
Mastocytosis
AML inv(16)

Idiopathic hypereosinophilic syndrome

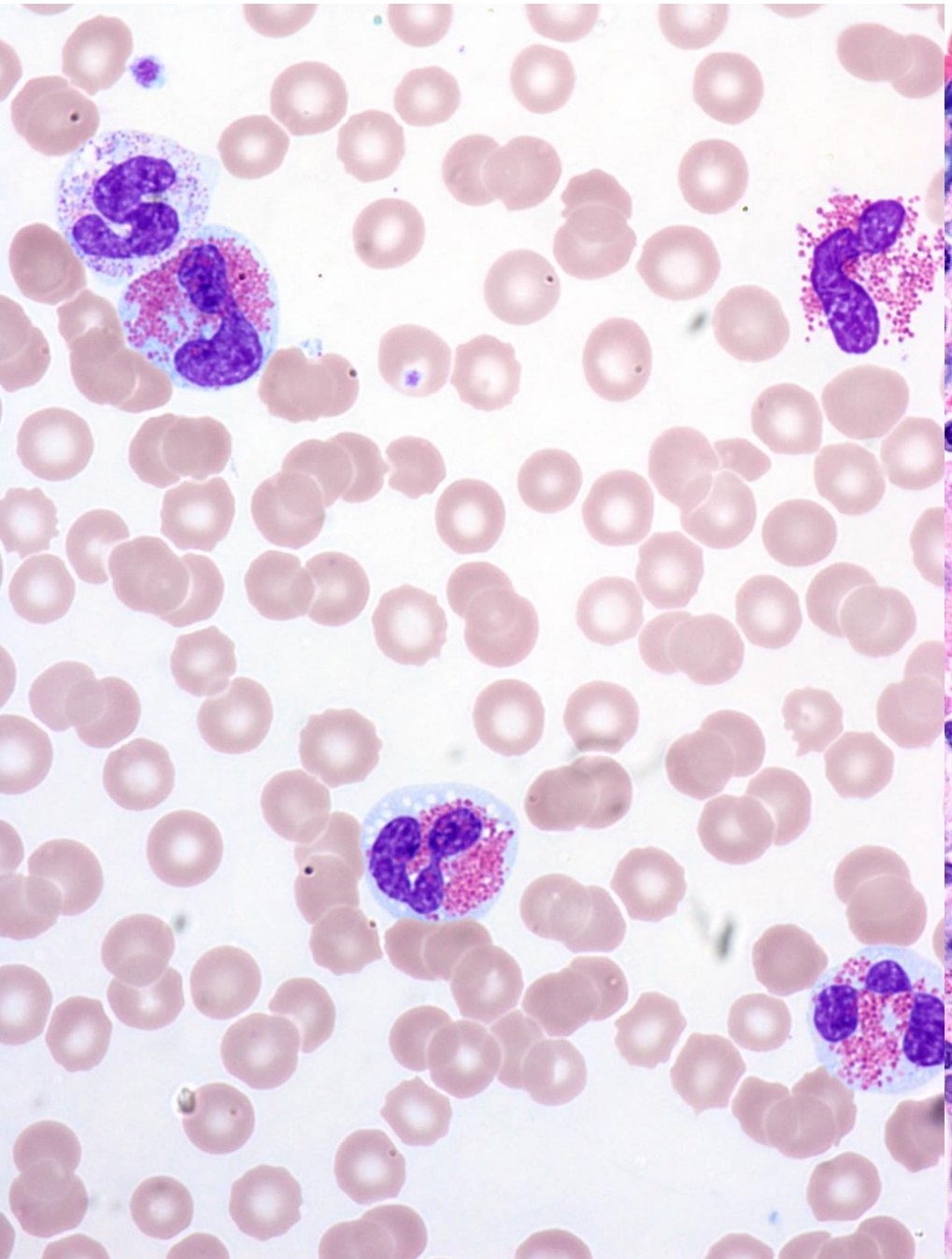
First steps in working up eosinophilias:

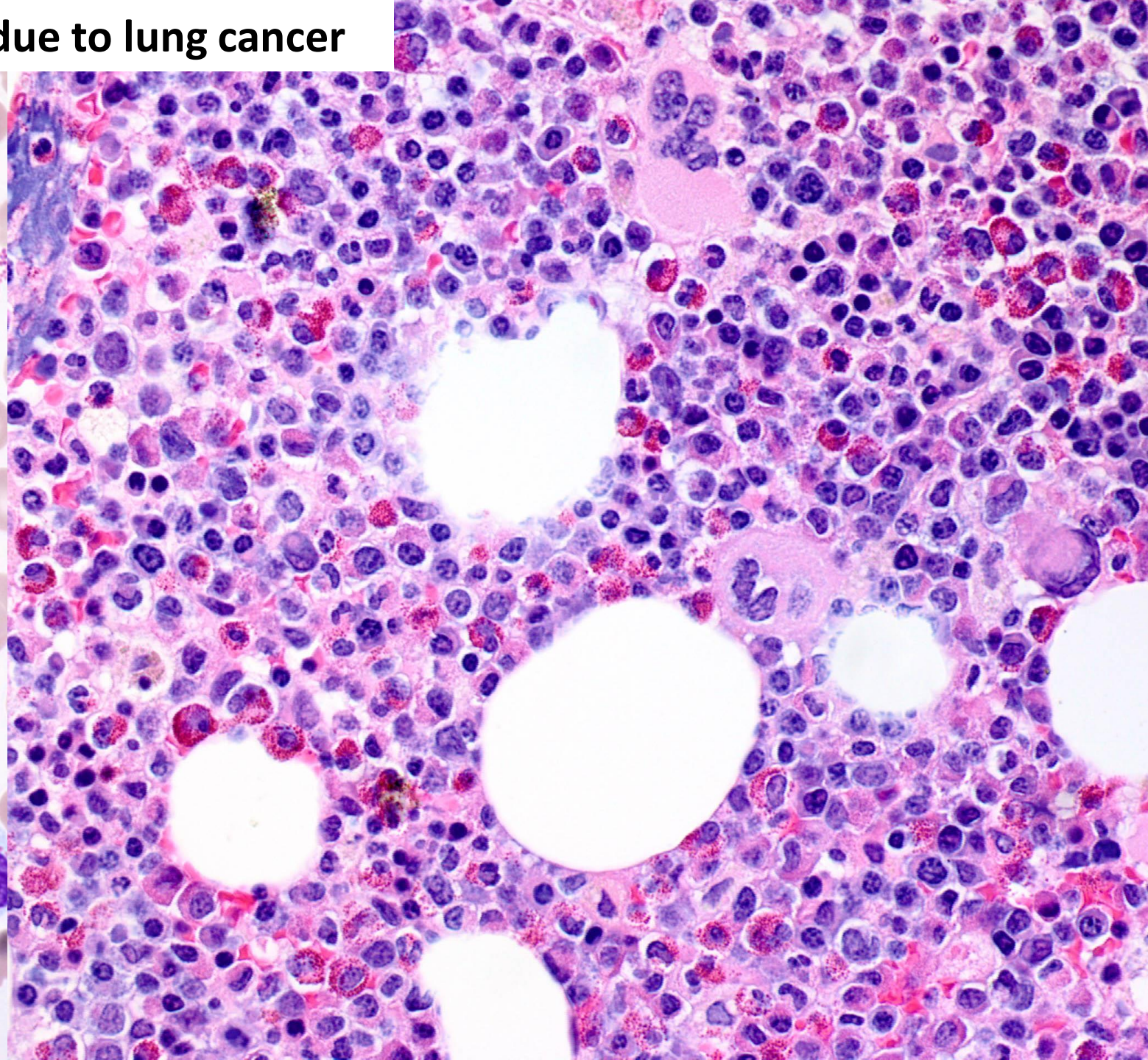
Exclude secondary reactive causes!

Exclude CML, AML (and ALL)!

- Secondary eosinophilias due to drugs, parasites, paraneoplastic phenomena, or inflammatory conditions may have striking leukocytosis and eosinophilia
  - ”Benign” eosinophilias can cause significant organ damage
  - Reactive eosinophils can sometimes display abnormalities of granulation and nuclear lobation
  - Treatment is directed against underlying cause of the eosinophilia
- CML often has absolute eosinophilia and occasionally presents with eosinophil-predominant leukocytosis
- AML with inv(16) has increased marrow, but not blood eosinophils

Paraneoplastic reactive eosinophilia due to lung cancer





Once CML and reactive eosinophilias are excluded:

Perform genetic testing!

Carefully evaluate the bone marrow morphology!

- Complete conventional karyotype to investigate for a clonal cytogenetic aberration
- FISH/RT-PCR for *PDGFRA* rearrangement (usually cryptic)
- Consider FISH/RT-PCR for *PDGFRB* and *FGFR1* rearrangements (rarely cryptic)
- Consider next-generation sequencing
  - Presence of pathogenic mutations can help establish clonality
- Consider RNA-based fusion assays
- Morphologic assessment for abnormal morphology and/or increased blasts

Myeloid/lymphoid neoplasms with eosinophilia and tyrosine-kinase gene rearrangements (MLN-TKR)

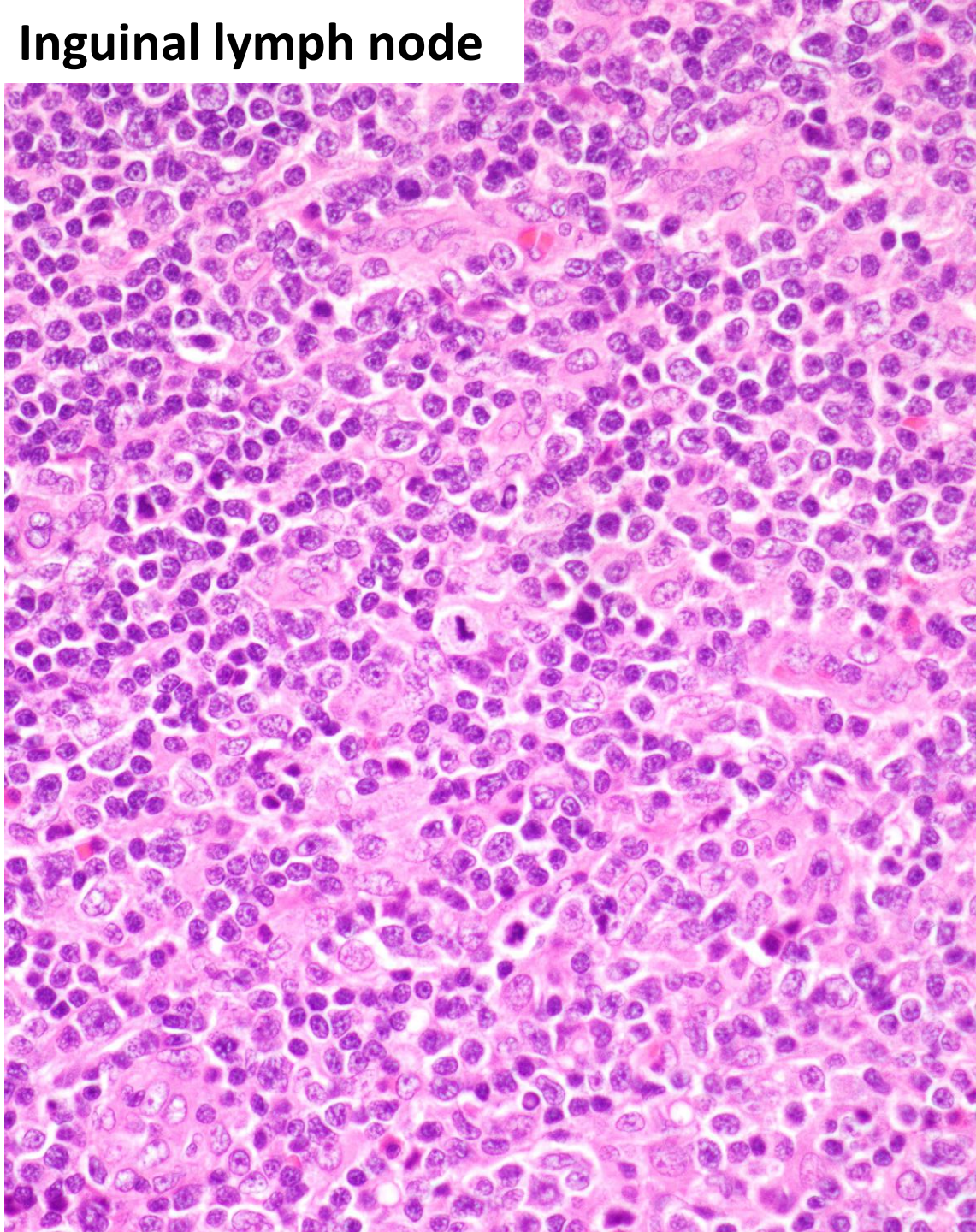
- Share several genetic and biologic features analogous to CML
  - Originate from a pluripotent stem cell with both lymphoid and myeloid differentiation capacity
  - Rearrangements activate genes encoding tyrosine kinases
  - Eosinophilia is characteristic (but not ubiquitous)
- Highly variable presentations that can mimic other myeloid neoplasms
  - MPN-like, MDS/MPN-like, or acute leukemias/lymphoblastic lymphoma
- Very rare diseases, but important treatment implications mandate that we do not miss them!

Myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions

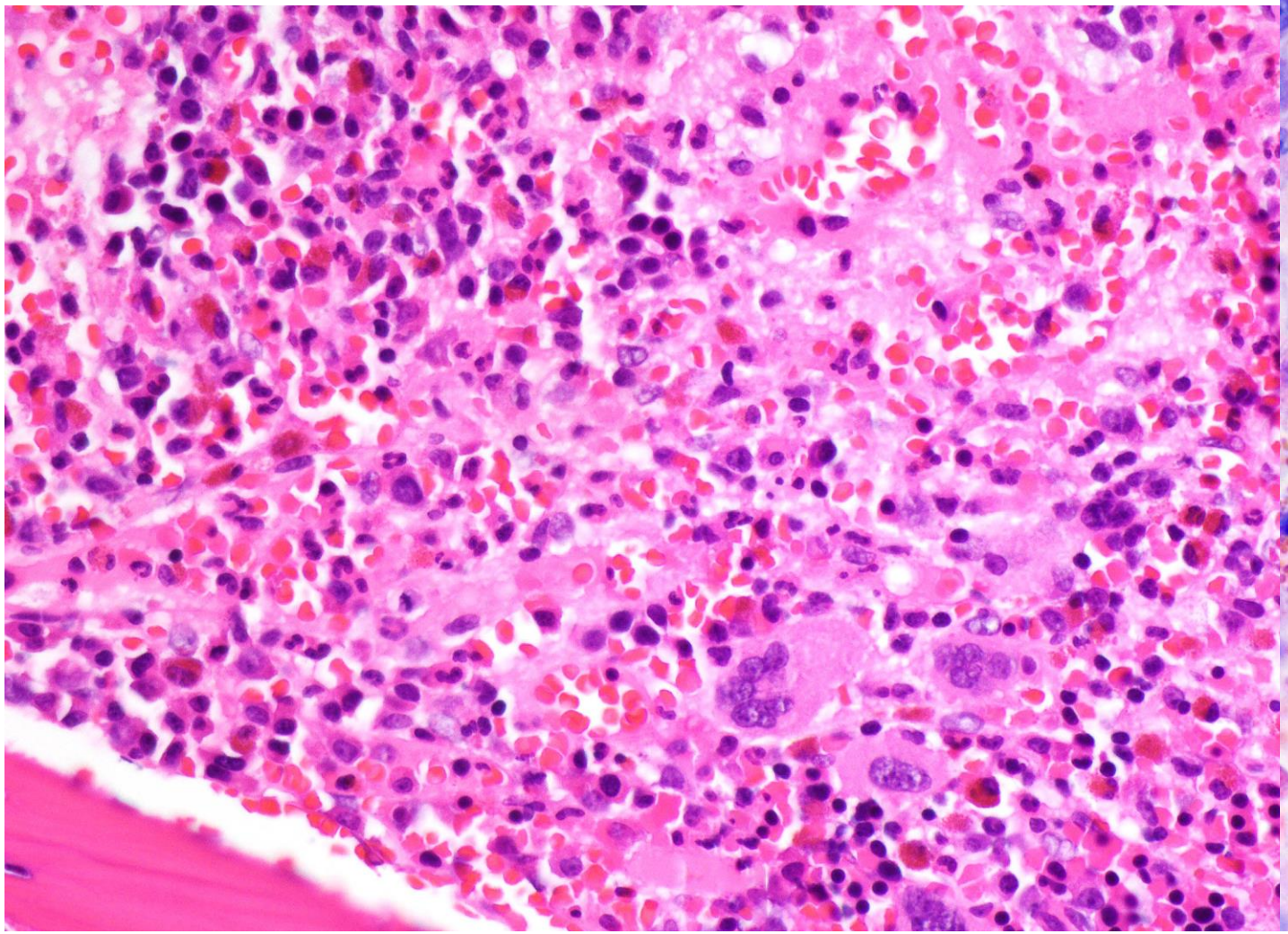
ICC		WHO5	2017 WHO Equivalents
with *PGFRA* rearrangement	=	with *PGFRA* rearrangement	with *PGFRA* rearrangement
with *PGFRB* rearrangement	=	with *PGFRB* rearrangement	with *PGFRB* rearrangement
with *FGFR1* rearrangement	=	with *FGFR1* rearrangement	with *FGFR1* rearrangement
with *JAK2* rearrangement	=	with *JAK2* rearrangement	with *PCM1-JAK2*
with *FLT3* rearrangement	=	**with *FLT3* rearrangement**	
with *ETV6::ABL1*	=	**with *ETV6::ABL1* fusion**	
		with other defined tyrosine kinase fusions *ETV6::FGFR2, ETV6::LYN, ETV6::NTRK3, RANBP2::ALK BCR::RET, FGFR1OP::RET*	

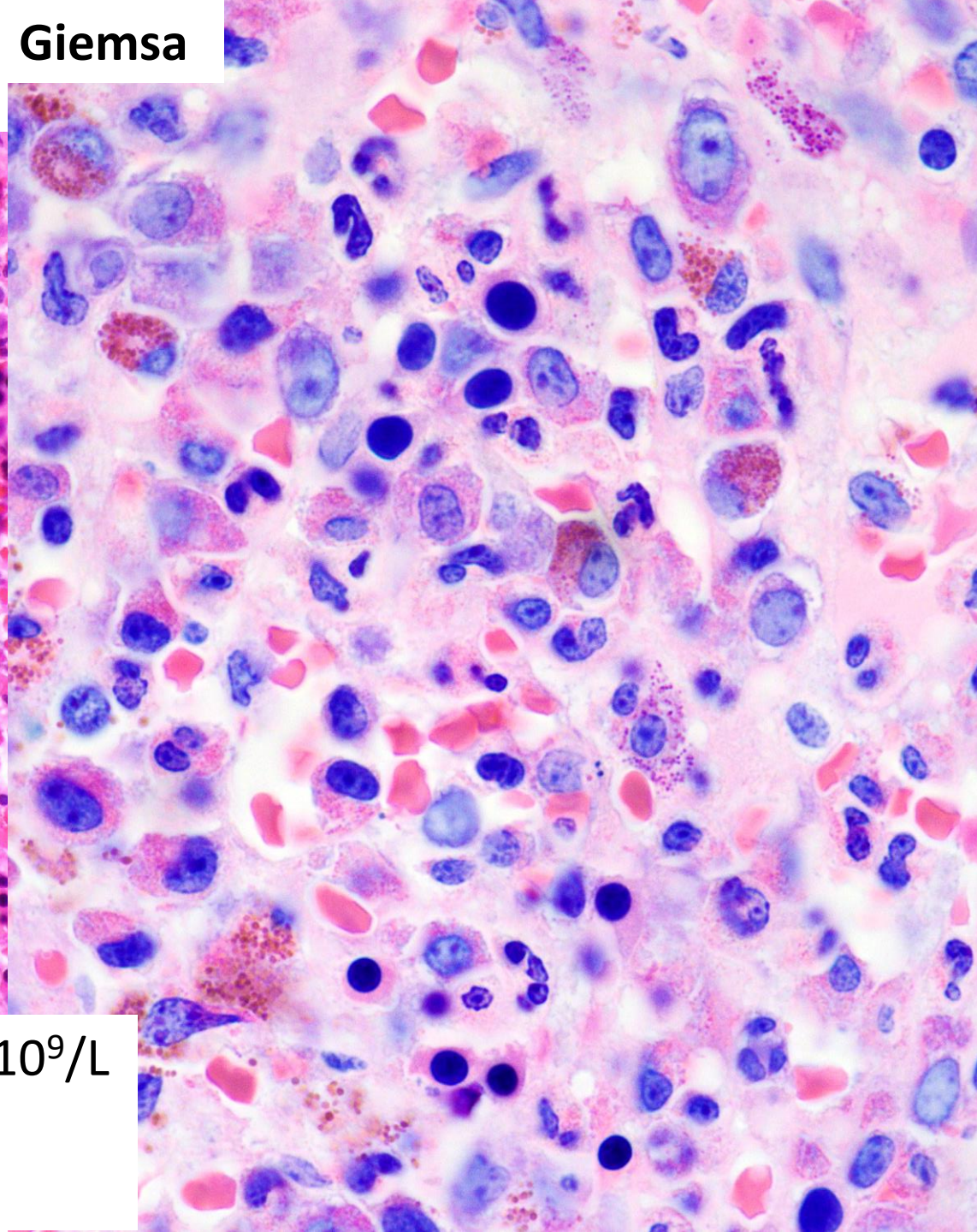
Let's start with a mystery case. . .

- 29 year-old man presented with inguinal lymphadenopathy
- Flow cytometry and IHC confirmed T-lymphoblastic lymphoma
  - CD2+, CD7+, cyto CD3+, TdT+, CD1a+, CD4+, CD8dim, CD5dim
  - Negative for myeloid, B-cell markers
  - Clonal TCR gamma rearrangement
- "Staging" bone marrow biopsy performed



Staging marrow for T-LBL



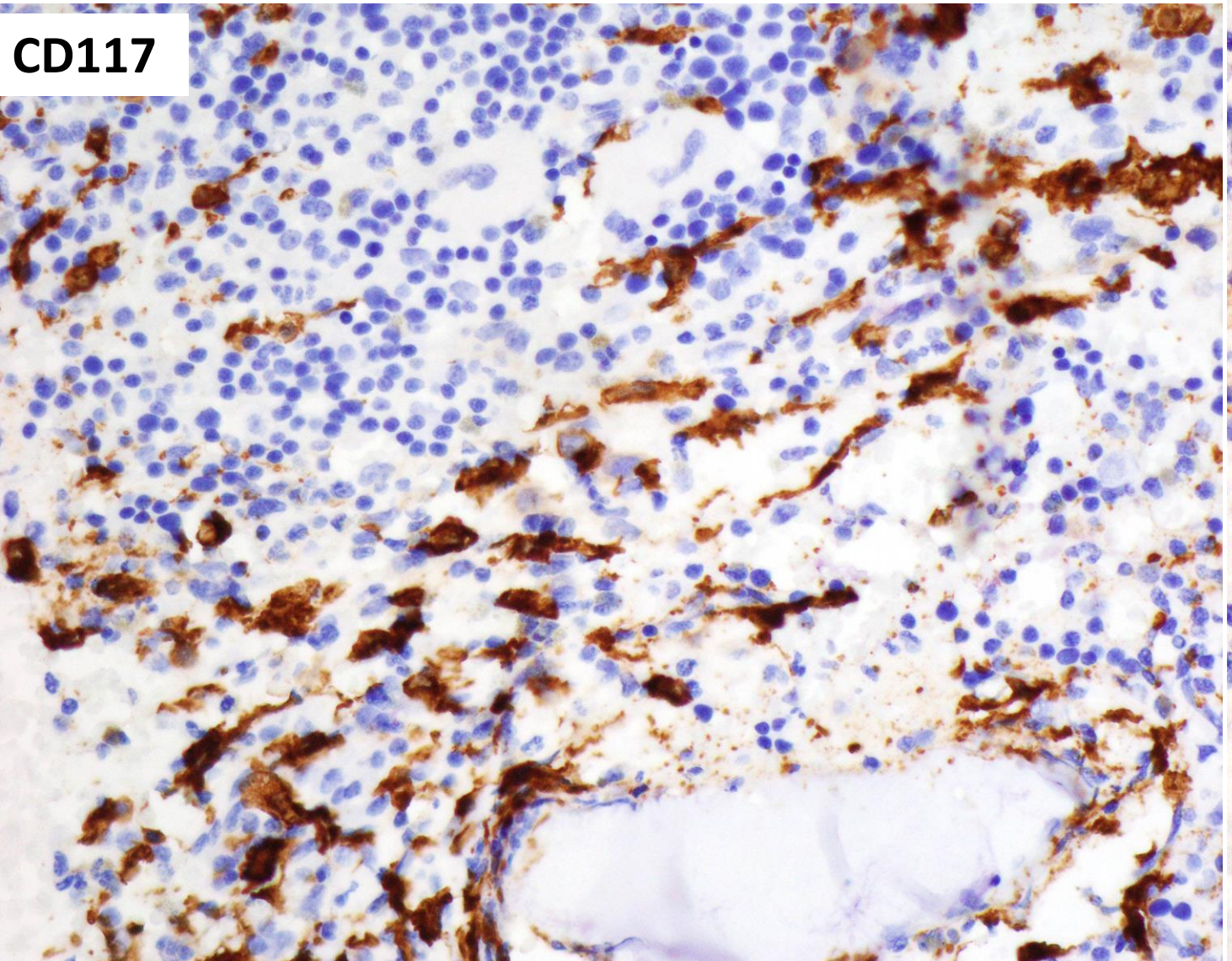


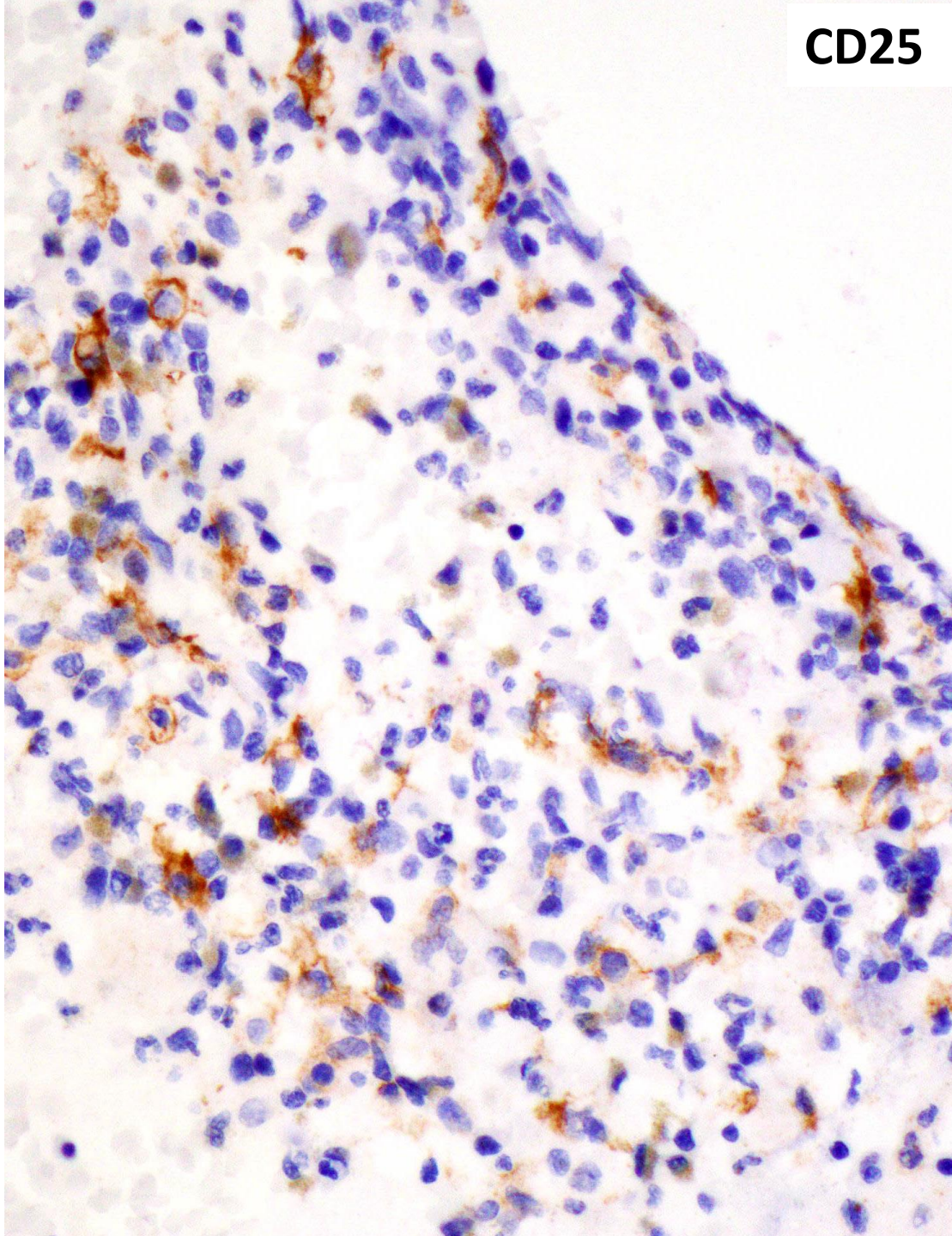
WBC 4.8 x 10^9/L (13% eos), HGB 15.2 g/dL, PLT 151 x 10^9/L

18% eosinophils in bone marrow aspirate

No T-lymphoblasts detected by flow cytometry

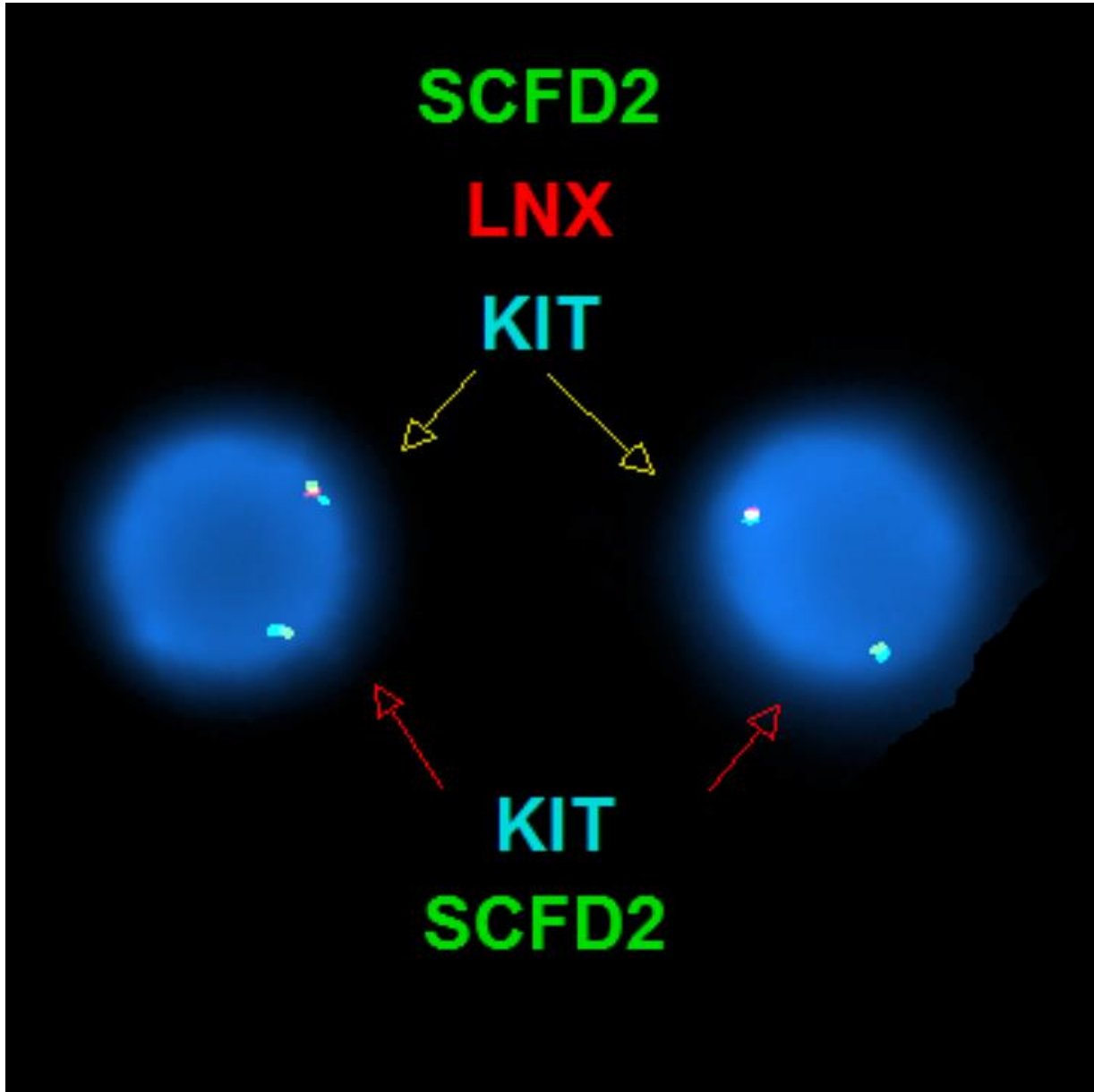
Staging marrow for T-LBL





Further studies resolved the mystery

- Cytogenetics on marrow: normal karyotype
- FISH on marrow: 4q12 deletion (LNX/CHIC2 loci deleted), consistent with *FIP1L1::PDGFRA* rearrangement
- FISH performed on touch-prep taken from inguinal lymph node: also positive for *FIP1L1::PDGFRA* rearrangement



One neoplasm with two simultaneous disparate manifestations

- Myeloid/lymphoid neoplasm with *PDGFRA* rearrangement, presenting as T-LBL and bone marrow eosinophilic neoplasm
- Patient was treated with standard ALL induction and consolidation chemotherapy
  - Developed absolute eosinophilia upon marrow recovery after induction, treated for 3 months with imatinib which resolved the eosinophilia
- Received matched-unrelated hematopoietic stem cell transplant
- Alive in complete remission 11 years after diagnosis

Myeloid/lymphoid neoplasm with *PDGFRA* rearrangement

- Patients present with peripheral blood eosinophilia (95% >1 x 10^9/L), bone marrow eosinophilia, and often increased fibrosis (65%)
  - "Chronic-phase disease": CEL
  - "Blast-phase disease": T-ALL/LBL, AML

  } May be synchronous or metachronous
- Increased bone marrow mast cells: interstitial, not aggregated
  - Often have mildly elevated serum tryptase, but no *KIT* mutation
- Small cryptic interstitial deletion at 4q12 fuses *FIP1L1* to *PDGRFA*
  - Cannot be detected by conventional karyotype: detect by FISH or RT-PCR
  - Should be sought in all cases of idiopathic hypereosinophilia

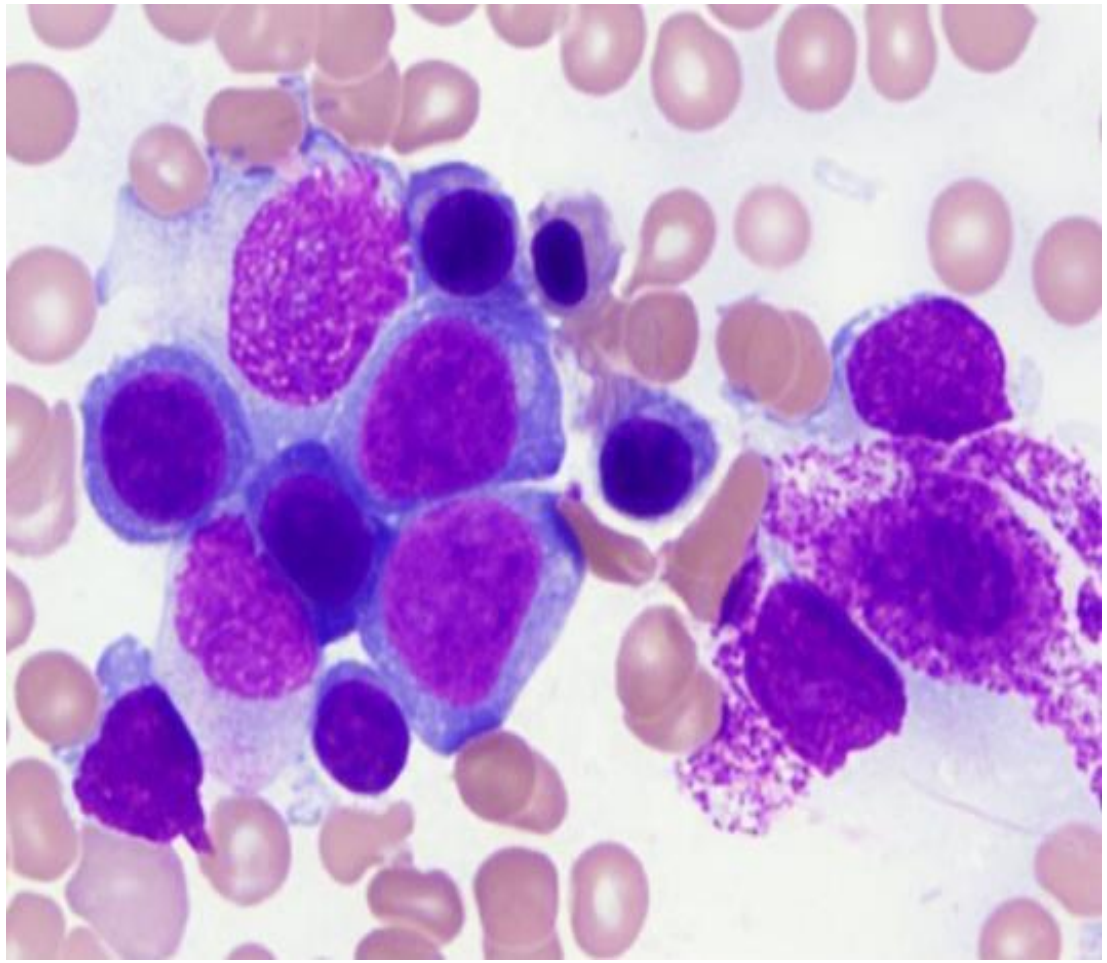
Reiter A, German Registry on Disorders of Eosinophils and Mast Cells

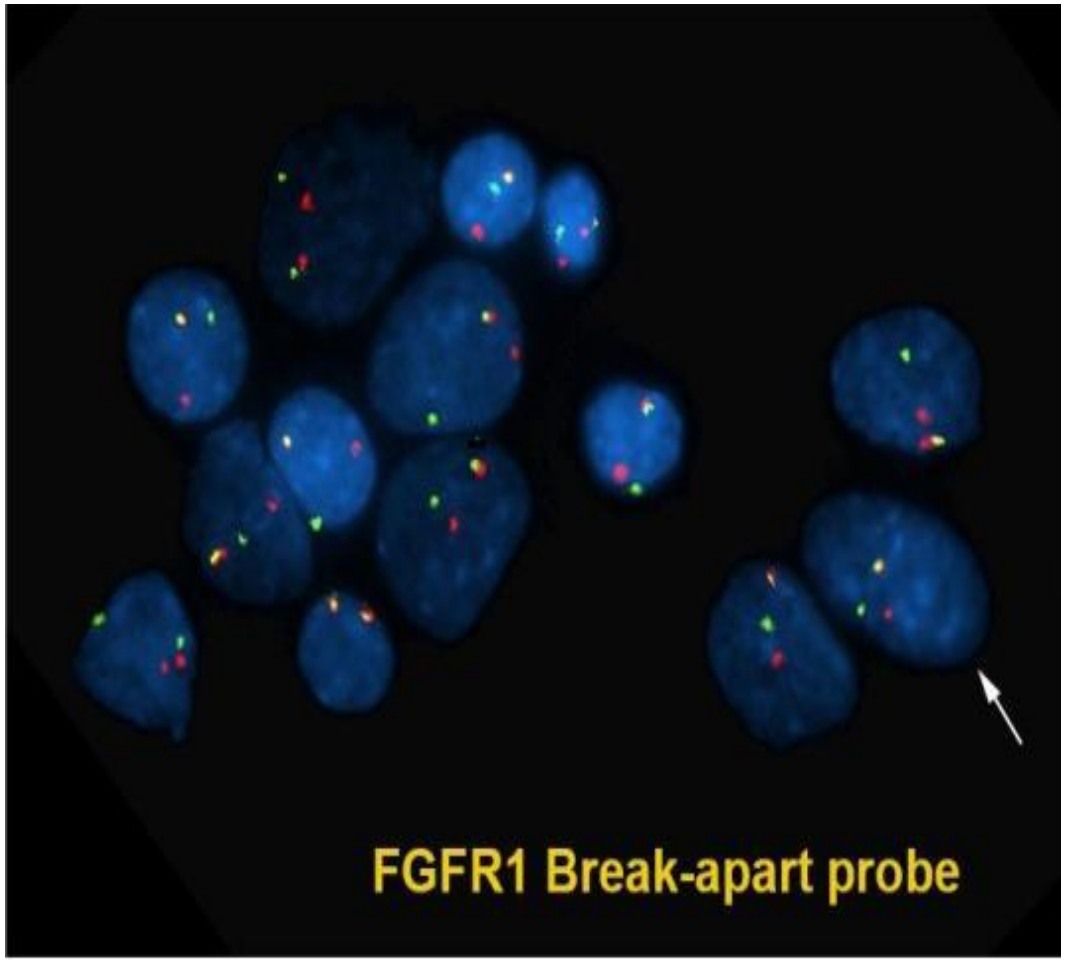
MLN with *PDGFRA* versus systemic mastocytosis

	MLN with *PDGFRA* rearrangement	Systemic mastocytosis
BM mast cell aggregates	Interstitial or loose clusters	Dense aggregates
Absolute eosinophil count/serum tryptase ratio	>100	≤100
Vitamin B12 level	Elevated	Normal
***KIT* gene**	Wild type	D816V mutation
Treatment	Imatinib-sensitive	Imatinib-resistant; other TKIs effective

Gotlib and Akin. Semin Hematol. 2012;49:128-37, Maric et al. J Allergy Clin Immunol. 2007;120:680-7.
Courtesy of Tracy George, ARUP Laboratories

Atypical mast cells carry the same tyrosine-kinase rearrangements as the hematopoietic cells





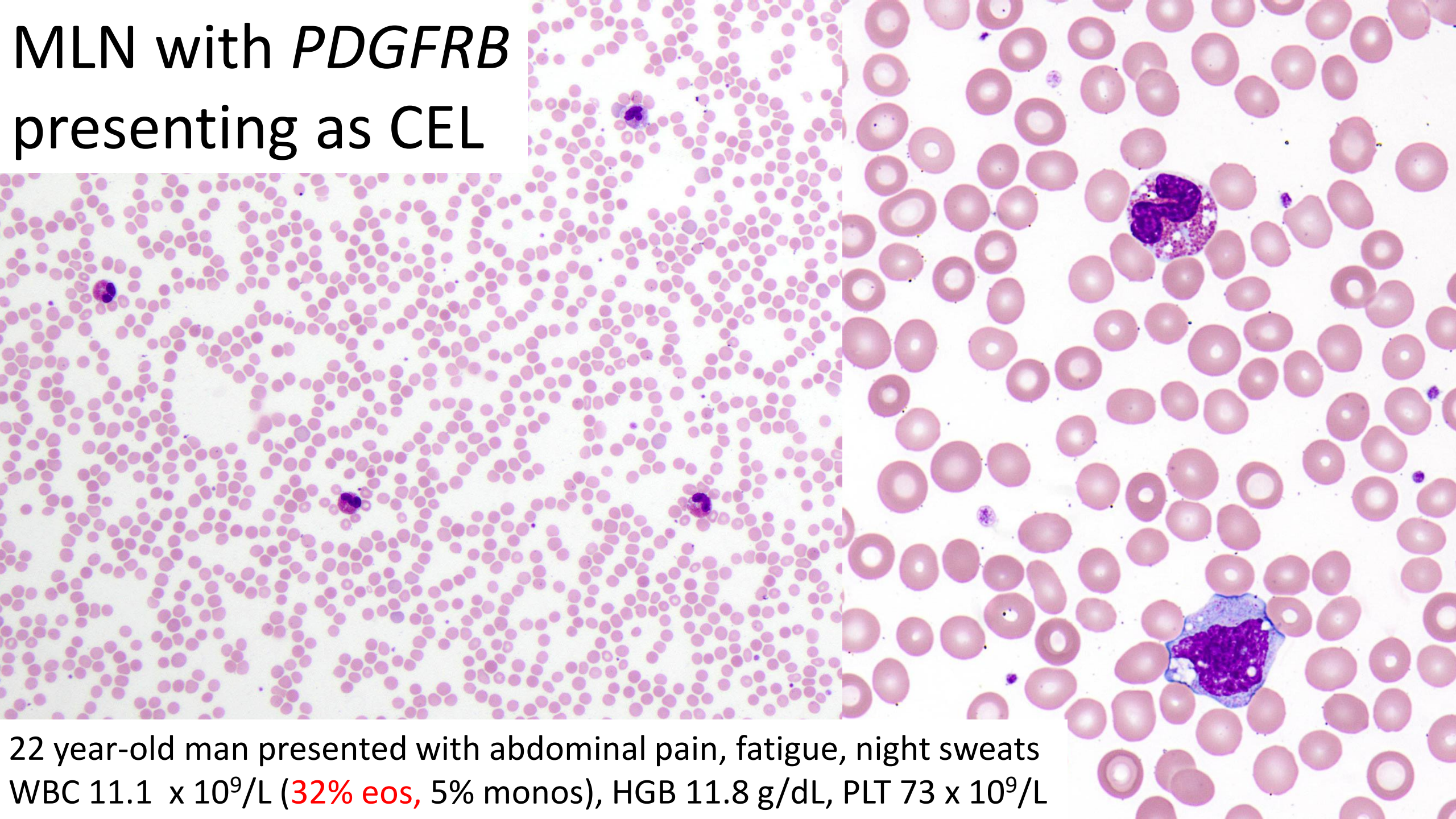
Courtesy of Guilin Tang and Sa Wang, MDACC

Myeloid/lymphoid neoplasms with *PDGFRB* rearrangement

- Patients present with blood eosinophilia (80%), monocytosis, increased marrow fibrosis; morphologic dysplasia is often seen
  - "Chronic-phase disease": CMML, CEL
  - "Blast-phase disease": B-ALL/LBL, AML
    - Cases presenting as de novo B-ALL are best classified as Ph-like B-ALL
  - Bone marrow mast cells often increased, similar to *PDGFRA* disease
- Rearranged *PDGFRB* at 5q33 (multiple partners)
  - t(5;12)(q32;p13.2); *ETV6::PDGFRB* most common
  - Rarely can be cryptic; *PDGFRB* FISH should be considered
  - Consider RNA-based fusion panel to detect rearrangement with any partner

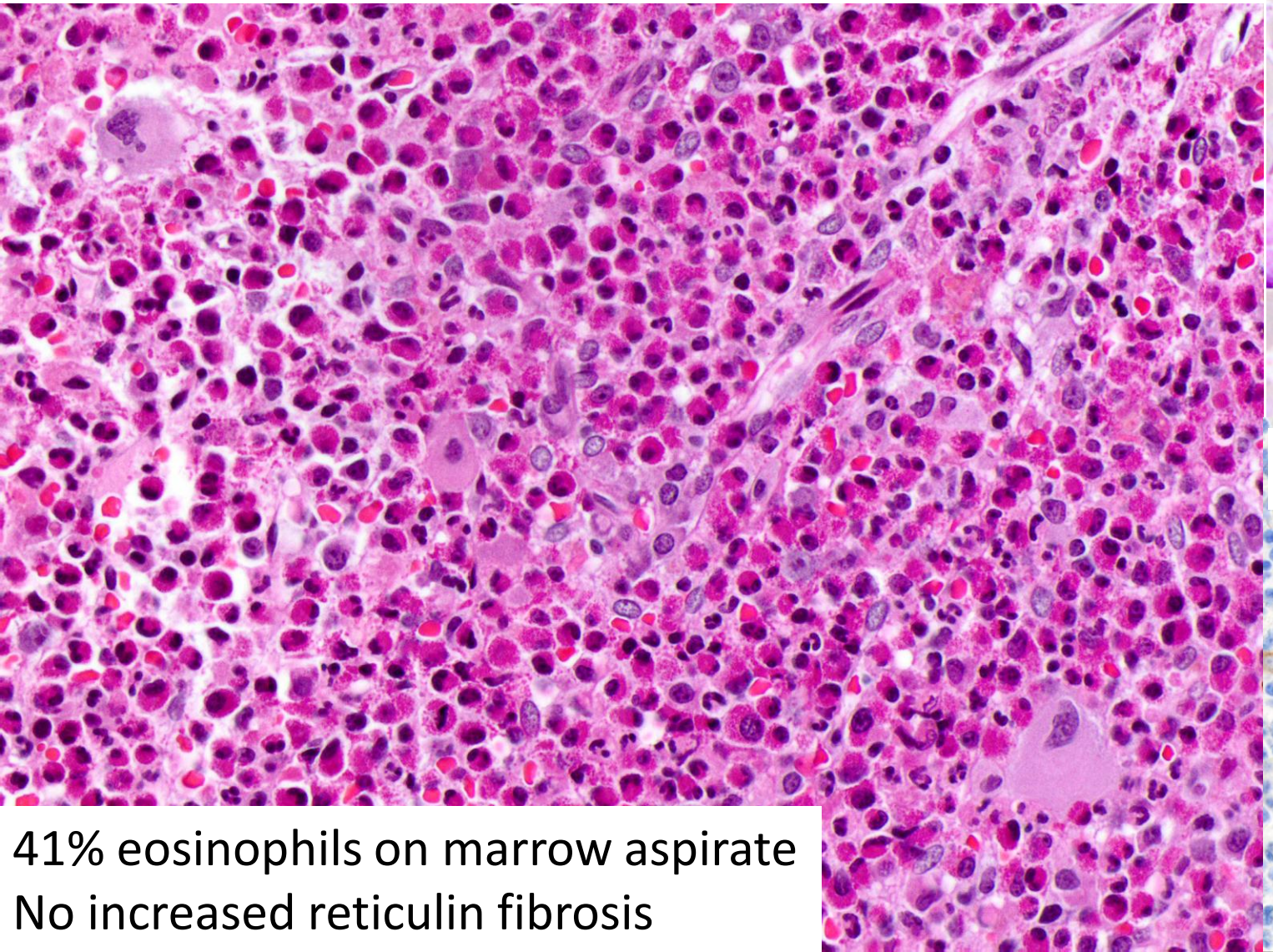
Jawhar M et al. Leukemia 2017;31:2771, Reiter A and Gotlib J. Blood 2017; 129:704

MLN with *PDGFRB* presenting as CEL

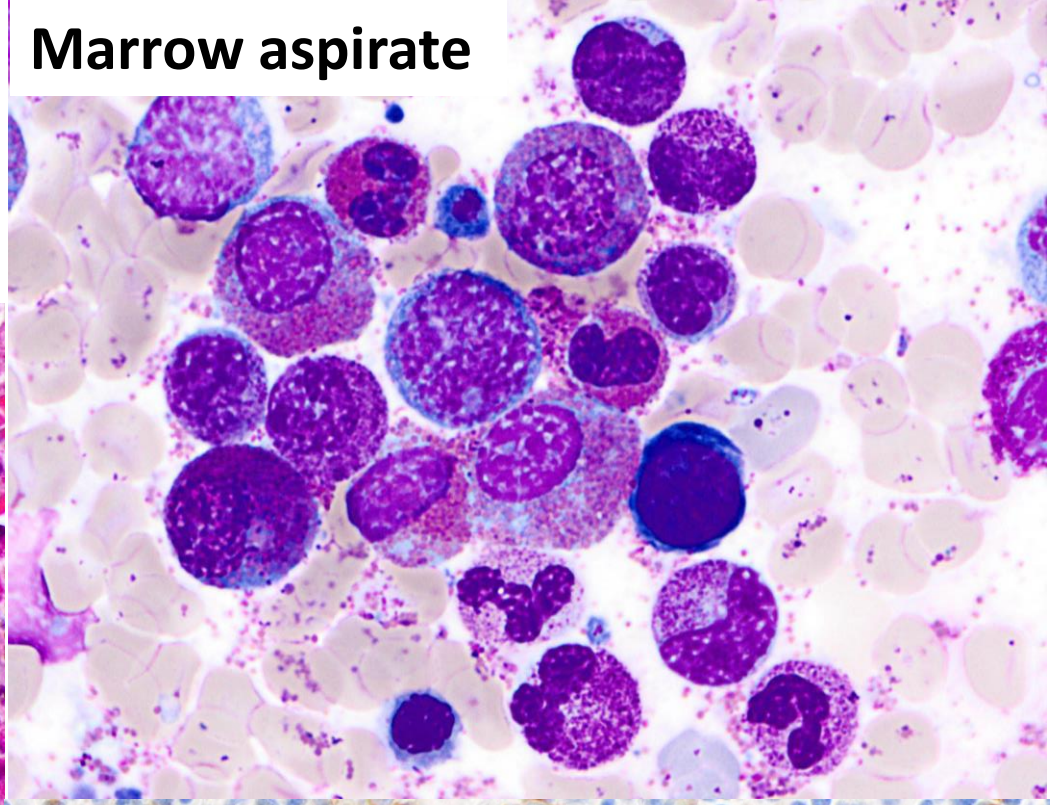


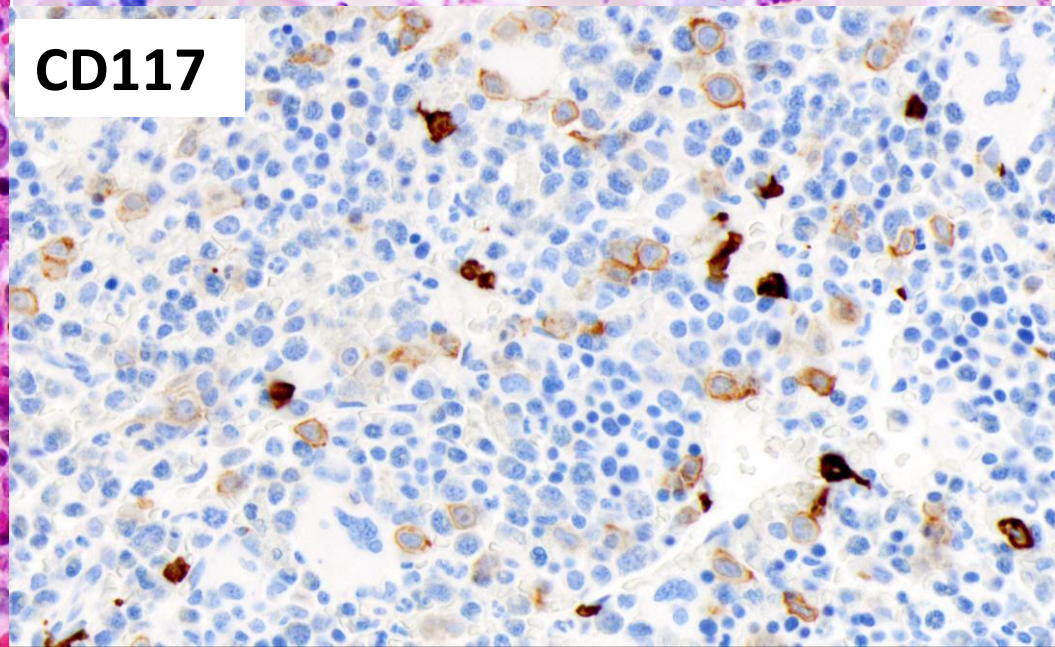
22 year-old man presented with abdominal pain, fatigue, night sweats
WBC 11.1 x 10^9/L (32% eos, 5% monos), HGB 11.8 g/dL, PLT 73 x 10^9/L

MLN with *PDGFRB* presenting as CEL

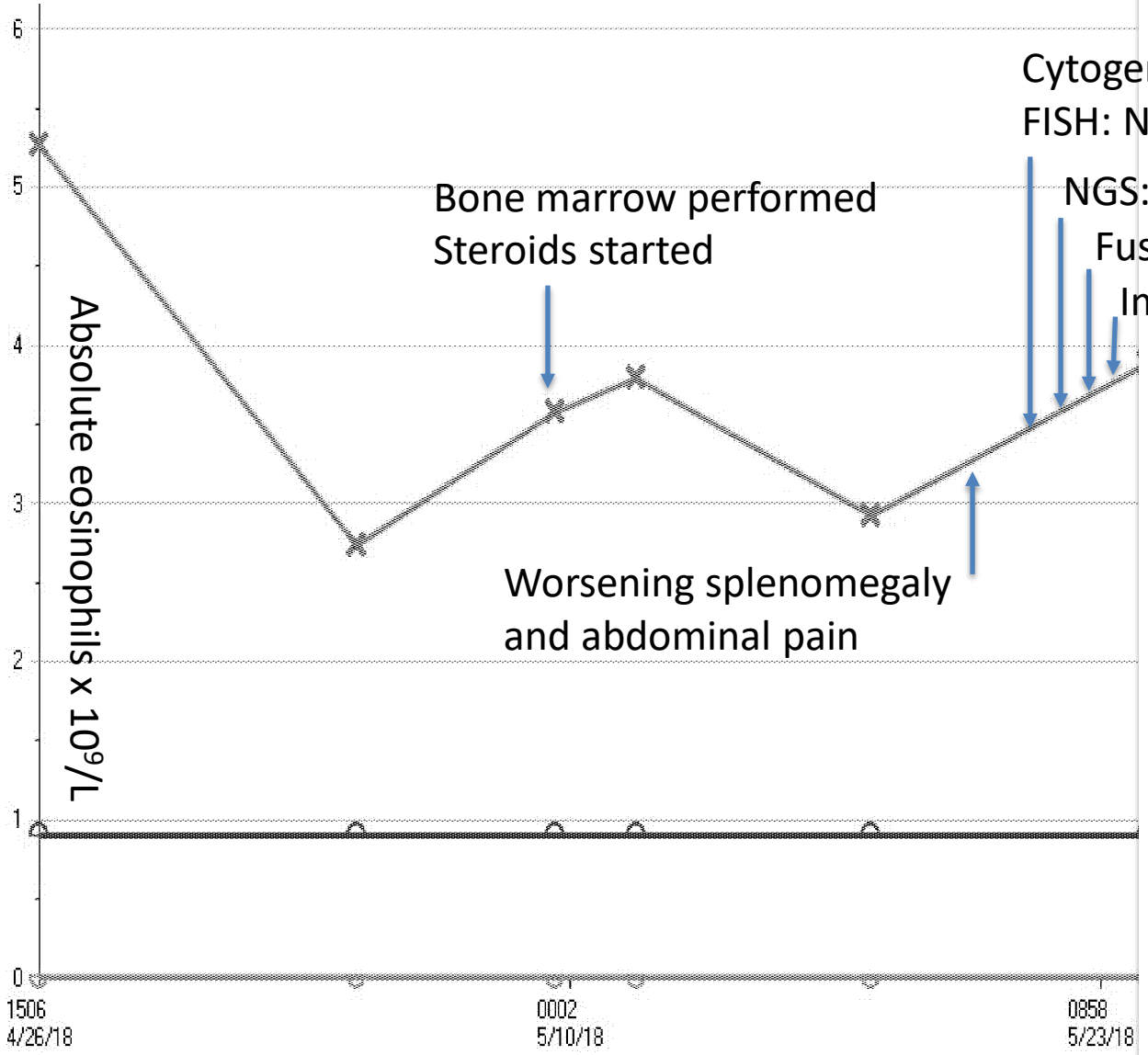


41% eosinophils on marrow aspirate
No increased reticulin fibrosis





Genetic tests and followup



Patient being maintained on imatinib; now asymptomatic with normal spleen size 2 years after diagnosis

Findings from the 2019 SH workshop on *PDGFRB*-rearranged cases

- ~70% of cases present with PB eosinophilia
  - In a significant number of cases, abnormal eosinophils are present; associated monocytosis is another feature
- Bone marrow:
  - BM all abnormal, with various morphological changes
  - Atypical mast cell proliferation (when present) can be a clue
- 42% of cases were cryptic, warranting FISH and molecular tests in patients with suspected disease

Pozdnyakova O AJCP 2021;155:160. Courtesy of Sa Wang, MDACC

“Heme Fusion Assay” at MGH (Archer): detects fusions involving 86 genes (with any potential partner) using targeted RNA sequencing

ABL1	CHD1	FLT3	MLLT10	PAG1	SEMA6A
ABL2	CHIC2	GLIS2	MLLT4	PAX5	SETD2
ALK	CIITA	IKZF1	MYC	PDCD1LG2	STIL
BCL11B	CREBBP	IKZF2	MYH11	PDGFRA	SYK
BCL2	CRLF2	IKZF3	NF1	PDGFRB	TAL1
BCL6	CSF1R	IL2RB	NFKB2	PICALM	TCF3
BCR	CTLA4	JAK2	NOTCH1	PML	TFG
BIRC3	DEK	KAT6A	NOTCH2	PRDM16	TP63
CBFB	DUSP22	KLF2	NOTCH3	PTK2B	TYK2
CCND1	EBF1	KMT2A	NOTCH4	RARA	VAV
CCND3	EIF4A1	MALT1	NTRK3	RBM15	ZCCHC7
CD19	ERG	MECOM	NUP214	ROS1	ZNF384
CD28	ETV6	MEF2D	NUP98	RUNX1	LYN
CDK6	FGFR1	MKL1	P2RY8	RUNX1T1	TSLP

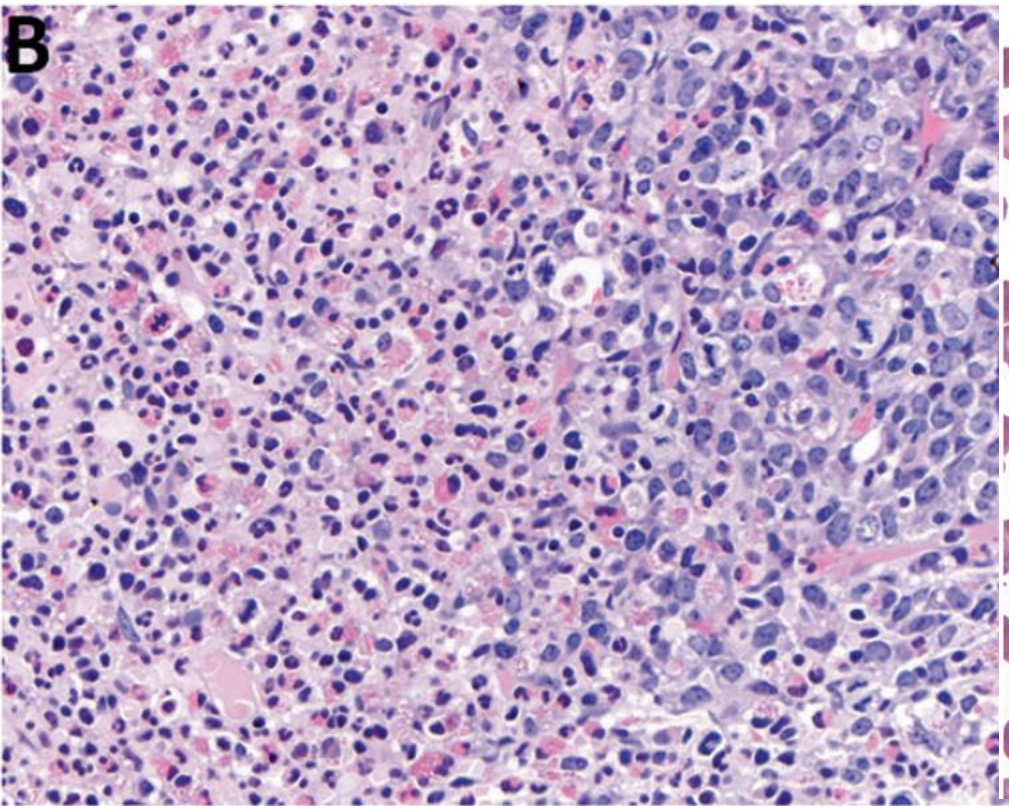
Zheng Z et al. Nat Med 2014;20:1479, Courtesy of Dr Valentina Nardi, MGH

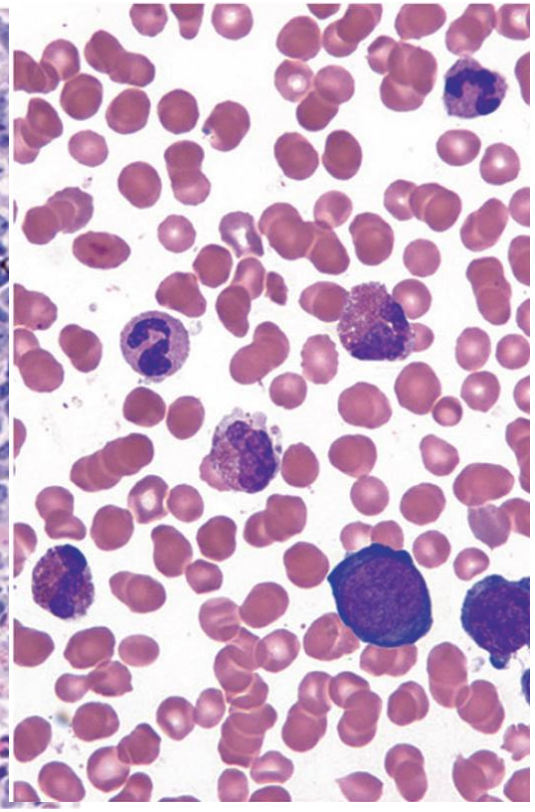
Myeloid/lymphoid neoplasms with *PCM1::JAK2*

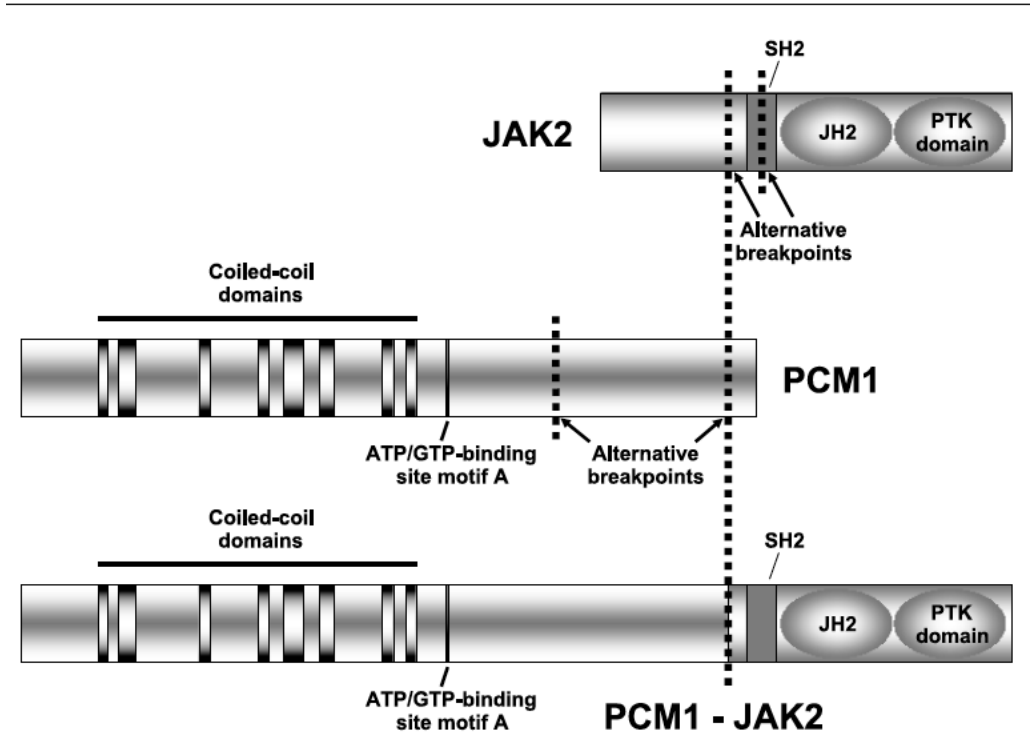
- Usually present with eosinophilia
  - "Chronic-phase disease": CEL, MPN, MDS/MPN, rarely MDS
  - "Blast-phase disease" (rare): B-ALL/LBL, AML
    - Cases presenting as de novo B-ALL may be best classified as Ph-like B-ALL
- Erythroid predominance with left-shifted erythroid forms, often prominent lymphoid aggregates and increased fibrosis
- Added to the group of genetically-defined eosinophilic leukemias as a provisional entity in 2016 revised WHO
- Rearrangements of JAK2 with other partners (e.g. *BCR::JAK2* and *ETV6::JAK2)* now included in ICC and WHO5

Reiter A et al. Cancer Res 2005;65:2662, Bousquet M et al. Oncogene 2005;24:7248, Dargent JL et al. Eur J Haematol 2011;86:87, Masselli E et al. Br J Haematol 2013;162:563, Tang G et al. Mod Pathol 2019;32:490, Bain BJ and Ahmad S. Br J Haematol 2014;166:809

Myeloid/lymphoid neoplasm with *PCM1::JAK2*







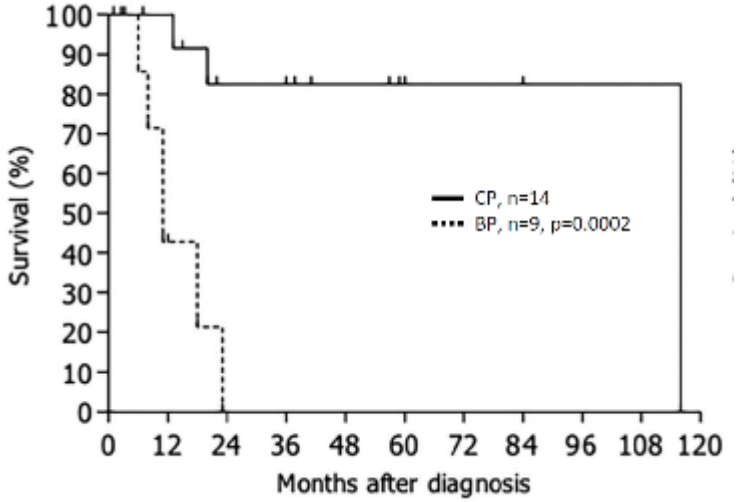
71 year-old man with eosinophilia
Karyotype: 46,XY,t(8;9)(p22;p24)[19]
FISH confirms *JAK2* rearrangement

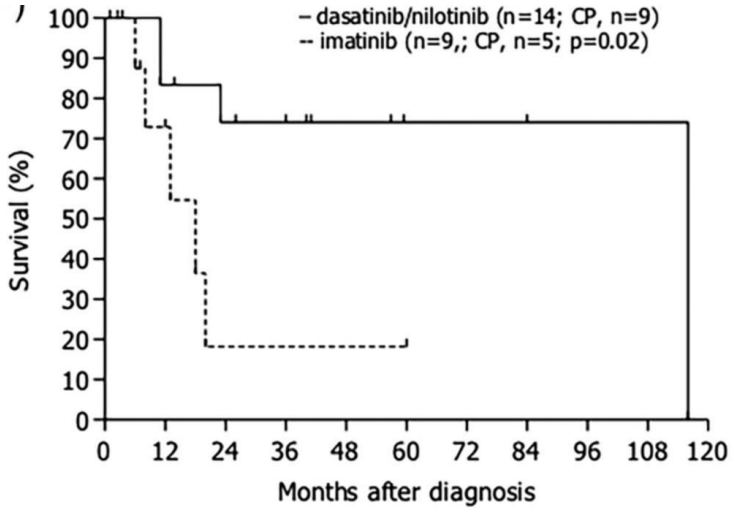
Series included 2 cases of de novo B-ALL that showed subsequent evidence of an underlying MPN

Tang G et al. Mod Pathol 2019;32:490

New MLN-TKR entity: *ETV6::ABL1*

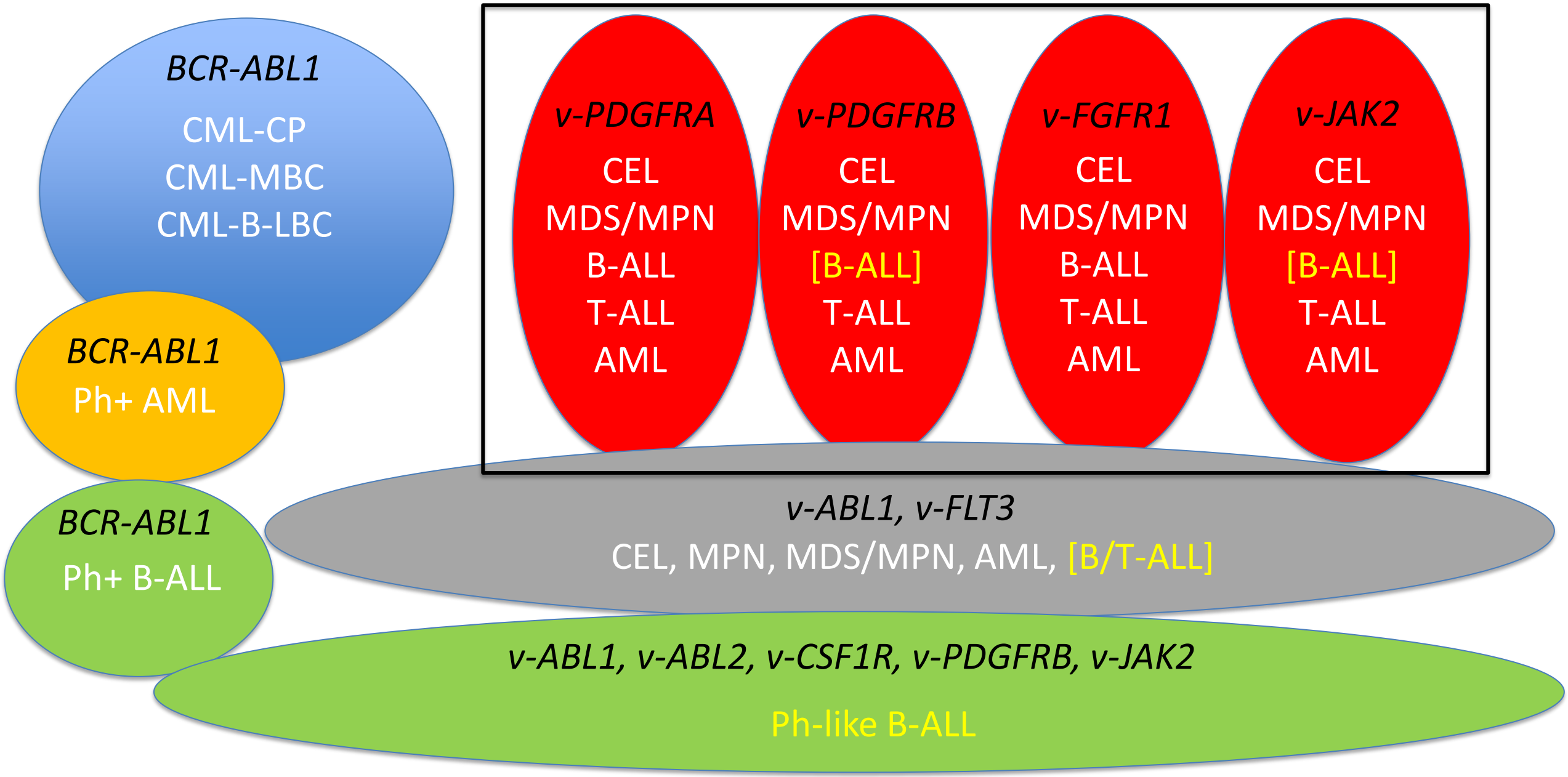
- *ETV6::ABL1* in children usually presents as de novo T-ALL, may not belong to this category
- Cases presenting in lymphoblastic/myeloblastic have significantly worse prognosis
- Often cytogenetically cryptic, requiring RT-PCR or RNAseq is needed
- 2nd and 3rd generation TKI may be treatment option





Schwaab J Am J Hematol. 2020;95:82. Courtesy of Sa Wang, MDACC

The family of tyrosine-kinase rearranged diseases



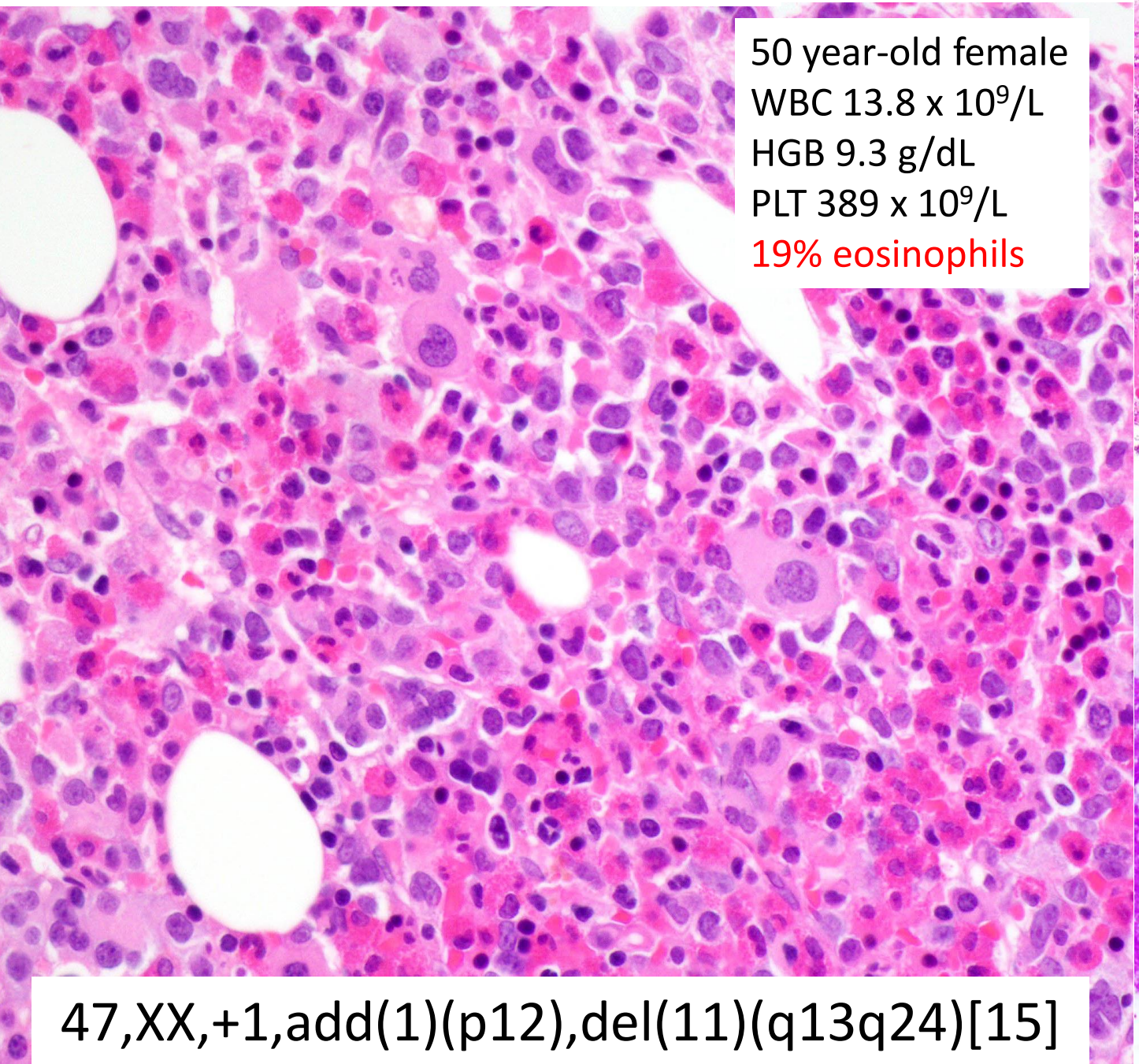
TK gene	Most common fusion	Other Partner genes/variants	Typical clinical and bone marrow (BM) manifestations	Accompanying mutations	Targeted therapy
PDGFRA	**Cryptic deletion** 4q12/ FIP1L1::PDGFRA	BCR; CDK5RAP2; ETV6; FOXP1; KIF5B; STRN; TNKS2	Most common M/LN-eo-TK with a male-to-female ratio: 17:1, median age in the late 40s, eosinophilia in >95% patients. Most commonly present as CEL-like with extramedullary** involvement.	20-50%, including ASXL1, BCOR, DNMT3A, RUNX1, SRSF2, TET2	Excellent response to TKI, imatinib
PDGFRB	t(5;12)(q32;p13.2)/ ETV6::PDGFRB	>30 partners, cryptic	Male-to-female ratio: 2:1, median age in the late 40s, PB eosinophilia in around 80% patients. Age at presentation: late 40s Common presentations are CEL-like or CMML, aCML-like neoplasm, less commonly MDS	30-50%, including ASXL1, BCOR, DNMT3A, NRAS, STAG2, STAT5B, TET2, ZSRS2	Excellent response to TKI, imatinib
FGFR1	t(8;13)(p11.1;q12.1)/ ZMYM2::FGFR1	15 other partners, including BCR	Male-to-female ratio: 1.5:1; median age in the late 30s; PB eosinophilia in about 70% Commonly present with nodal T-ALL/LBL with MPN-like features or blast phase (myeloid, B-lymphoblastic or mixed phenotype)	70-80% including RUNX1, ASXL1, CSFR3, STAG2,	Responsive to FGFR1 inhibition by pemigatinib, 3rd generation TKI ponatinib, especially in chronic phase
JAK2	t(8;9)(p22;p24.1)/ PCM1::JAK2	ETV6 and BCR, other rarely reported RPN1, NF-E2, RUNX1, PEX14	Male-to-female ratio: 5.5:1, median age in the late 40s; PB eosinophilia: about 70-80% Commonly present as MPN or MDN/MPN-like BM with eosinophilia. Rarely present in blast phase (B- and T-ALL/LBL, myeloid)	14-50% including ASXL1, BCOR, BCORL1, CD36, EP300, ETV6, RUNX1, SRSF2, TET2, TP53	Limited response to ruxolitinib, resistant to imatinib and dasatinib
FLT3	t(12;13)(p13.2;q12.2)/ ETV6::FLT3	BCR; CCDC88C; GOLGB1; MYO18A; SPTBN1; TRIP11; ZMYM2	Male-to-female ratio: 2.2:1, median age in the mid 40s. PB eosinophilia: about 70-80% Commonly present with T-ALL/LBL or myeloid sarcoma with CEL-, MPN- or MDS/MPN-like BM	50%, including ASXL1, RUNX1, STAT5B, SRSF2, TET2, TP53, U2AF1	Various responses to specific FLT3 inhibitors
ETV6::ABL1	t(9;12)(q34.1;p13.2)/ ETV6::ABL1	Unknown	Male-to-female ratio: 3:1, median age in the late 40s. PB eosinophilia: about 90-100% Commonly present as CML-like with eosinophilia in chronic or blast phase	40-50% including ARID2, CDKN1B, TP53, SMC1A	Various responses to 2nd or 3rd generation TKI

Adapted Wang S AJH 2023 (in press), Tsankov A Virchow Archiv 2023;482:85.

Chronic eosinophilic leukemia (CEL), not otherwise specified

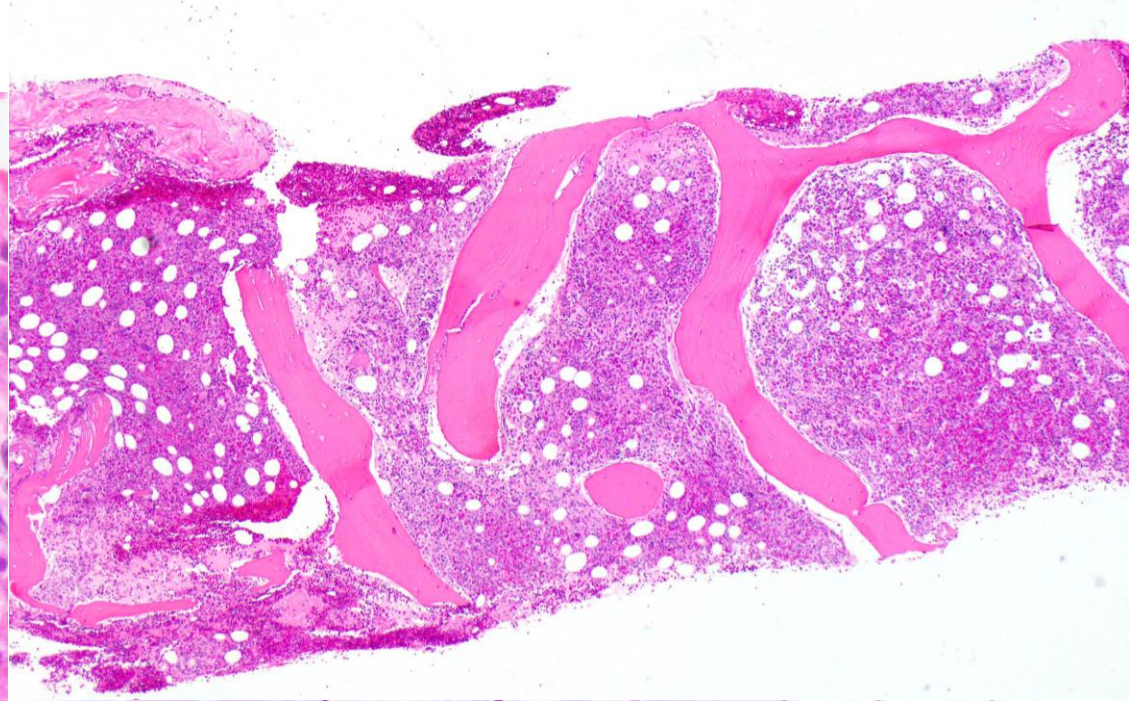
- Persistent blood eosinophilia >1,500/mm^3 and increased bone marrow eosinophils
- Exclusion of all secondary/reactive causes of eosinophilia
- Exclusion of *BCR::ABL1* and MLN-TKR
- Evidence of clonality
  - *Proven* by demonstrating clonal cytogenetic abnormality or pathogenic mutation(s)
  - *Implied* by increased bone marrow (≥5%) or peripheral blood (≥2%) blasts
  - Abnormal bone marrow morphology

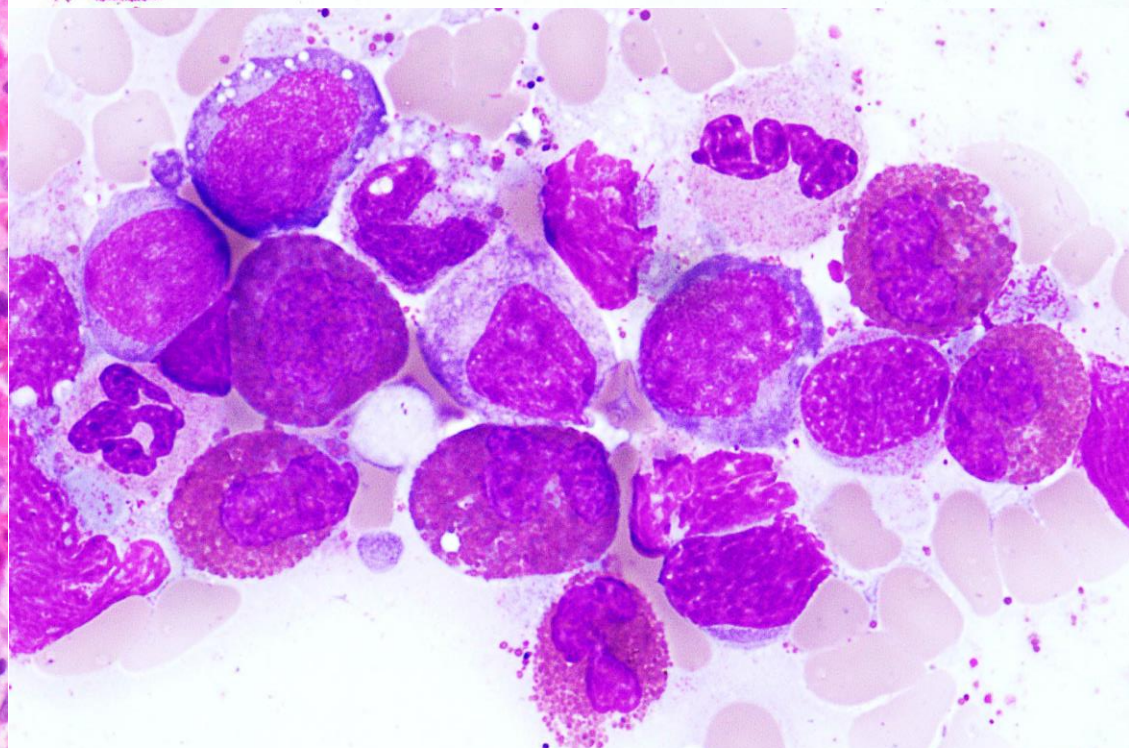
Chronic eosinophilic leukemia, NOS



50 year-old female
WBC 13.8 x 10^9/L
HGB 9.3 g/dL
PLT 389 x 10^9/L
19% eosinophils

47,XX,+1,add(1)(p12),del(11)(q13q24)[15]

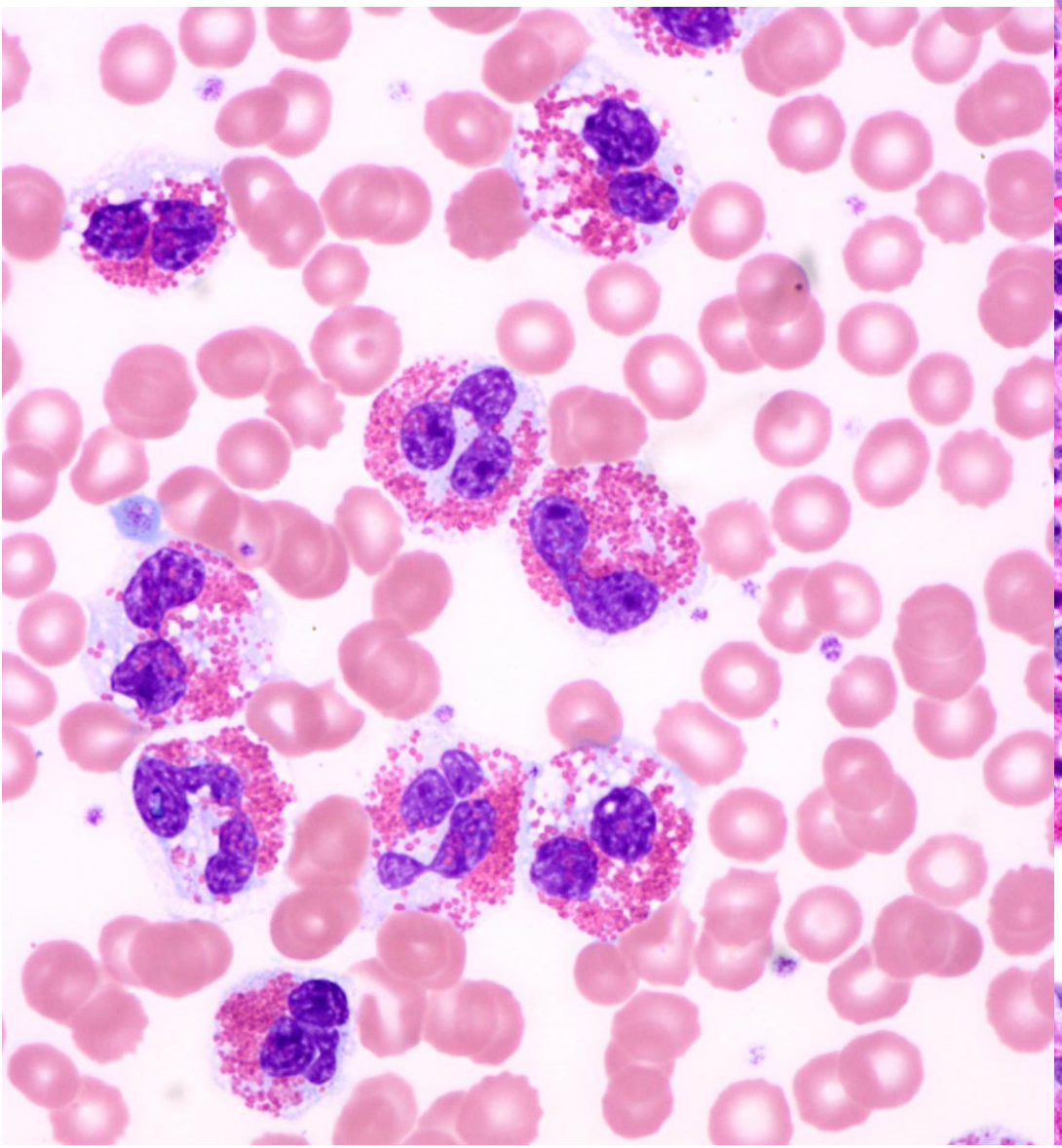




Idiopathic hypereosinophilic syndrome (HES)

- Persistent blood eosinophilia ≥1.5 x 10^9/L with increased bone marrow eosinophils
- Symptoms or signs of tissue damage or infiltration by eosinophils
- Exclusion of all secondary/reactive causes of eosinophilia, *BCR::ABL1* and genetically-defined eosinophilias
- Unable to establish clonality by genetics or blast increase, overall normal bone marrow morphology
- "Lymphocytic variant" HES
  - Hypereosinophilia driven by an abnormal clonal T-cell population
  - T cells are immunophenotypically aberrant, but criteria for a peripheral T-cell lymphoma are not met

Idiopathic hypereosinophilic syndrome



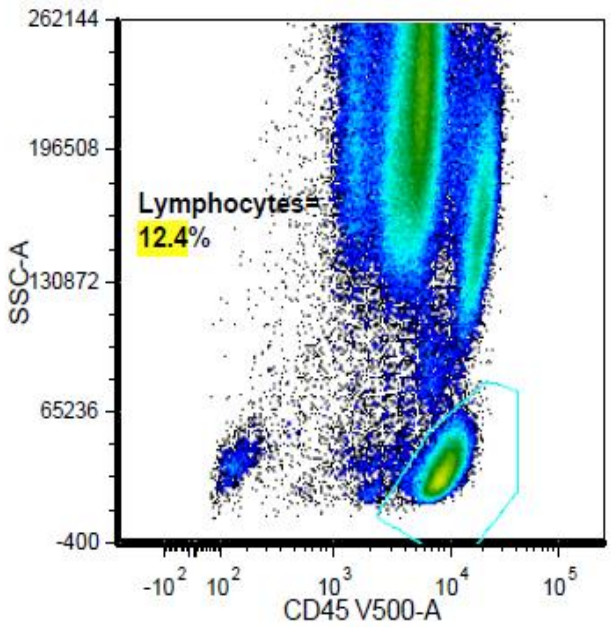
Normal karyotype
No *FIP1L1::PDGFRA* rearrangement
No mutations on NGS
No abnormal T-cell population

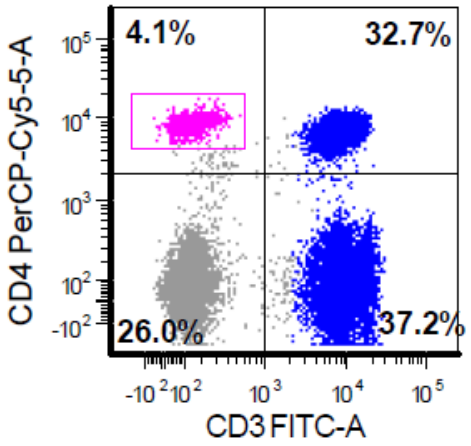
Eosinophilia uncontrolled by steroids
Cardiac LVEF 71% -> 26%

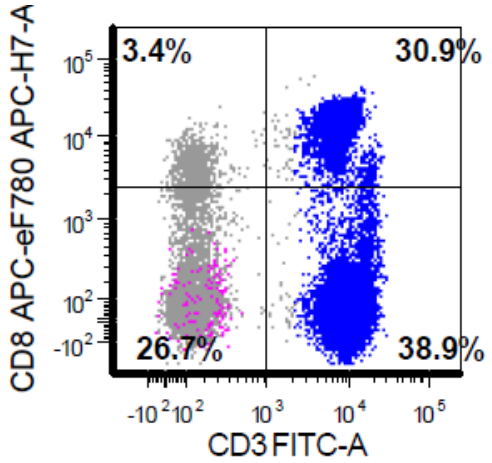
Treated with vincristine followed by allo-SCT, now alive and in complete remission 5 years later.

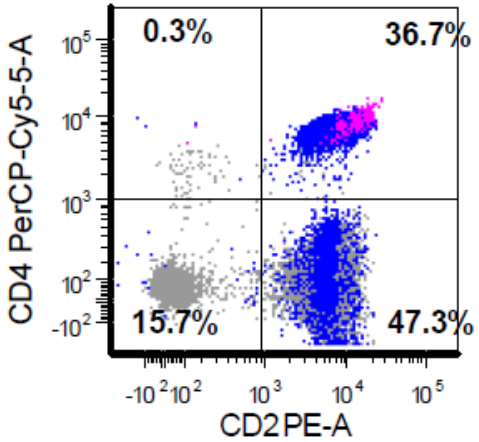
27 year-old man presented with fatigue and diarrhea
WBC 145.0 x 10^9/L (83% eos), HGB 14.9 g/dL, PLT 147 x 10^9/L

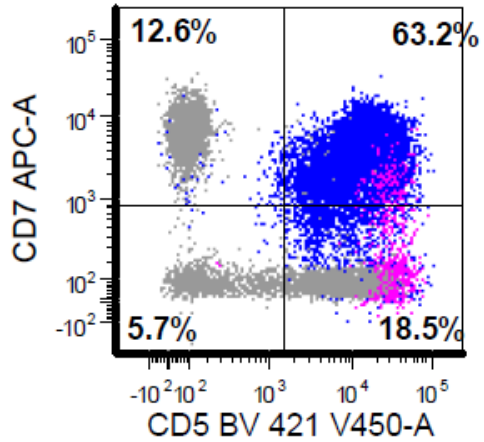
Lymphocytic variant HES











Abnormal T-cell population: CD3-CD4+CD5bright+CD2+CD7-/+

Hu Z Cytometry B Clin Cytom. 2021;100:352. Courtesy of Sa Wang, MDACC

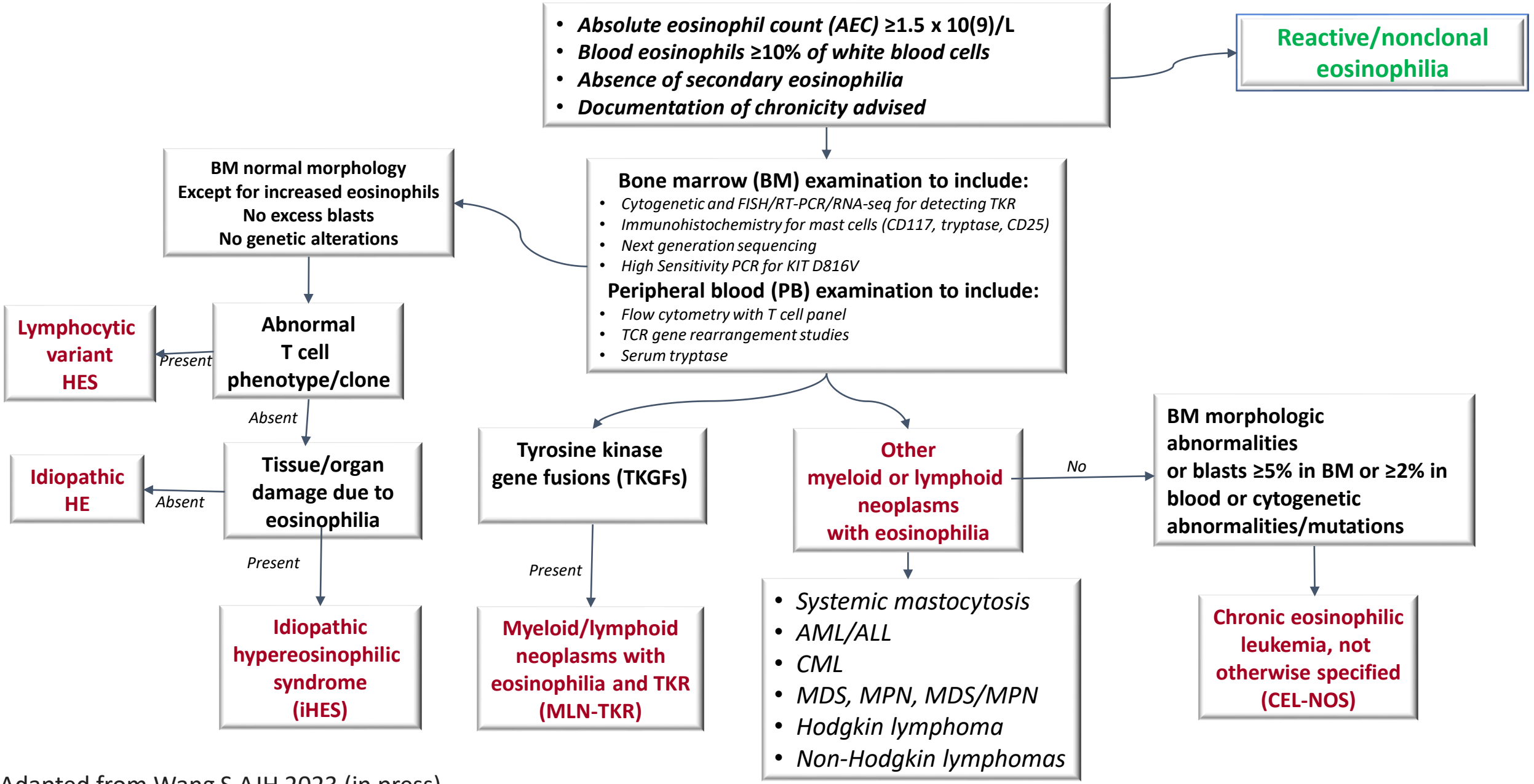
Distinguishing HES from CEL

Feature	HES	CEL
Peripheral blood eosinophils	Sustained ≥ 1.5 x 10^9/L, ≥10% WBCs	Sustained ≥ 1.5 x 10^9/L, ≥10% WBCs
Symptoms attributable to the increased eosinophils	Present	Not required
Bone marrow eosinophils	Increased	Increased
Cytogenetics	Normal	Abnormal (if no increased blasts)
Bone marrow/blood blasts	Not increased	Increased (if karyotype normal)
Mutations	Present in 28-53% of cases (*ASXL1, TET2, EZH2, SETBP1, NOTCH1, STAG2, SH2B3, STAT5B*)	Present in 50% of cases
Bone marrow/blood morphology	Generally normal	Abnormal

Abnormal morphology defined as: increased (≥5%) blasts, significant dysplasia in any lineage (usually MDS-like megakaryocytes), hypercellularity for age, MF2 or MF3 fibrosis, abnormal eosinophils, M:E ratio >10, markedly decreased/absent megakaryocytes

Wang SA Mod Pathol 2016;19:854, Lee J-S PLOSOne 2017;12:e e0185602, Wang SA Haematologica 2017;102:1352, Cross NCP Leukemia 2019;33:415

Suggested workup for hypereosinophilia



Adapted from Wang S AJH 2023 (in press).