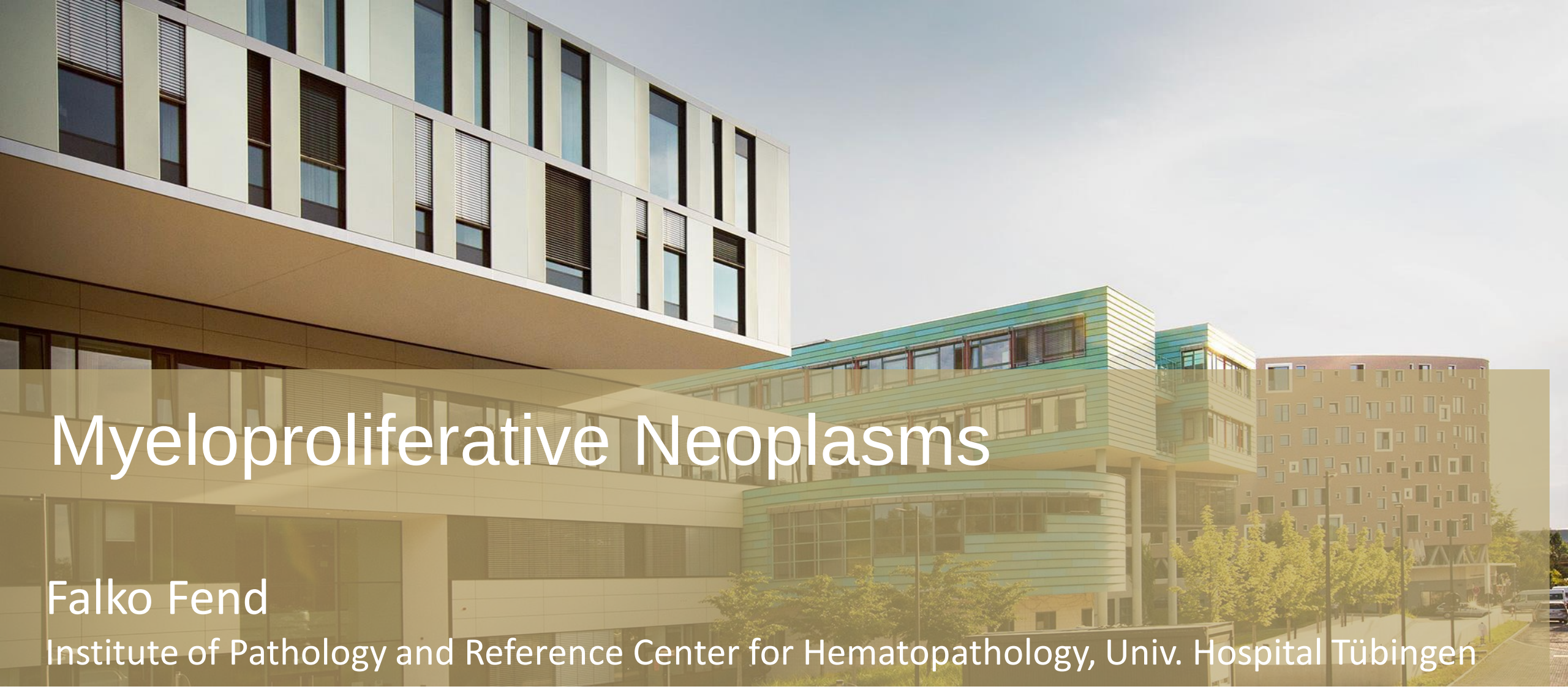


Myeloproliferative Neoplasms

Falko Fend
Institute of Pathology and Reference Center for Hematopathology, Univ. Hospital Tübingen

Universitätsklinikum
Tübingen

Definition

MPN show effective overproduction of mature **(=functional)** hematopoietic elements

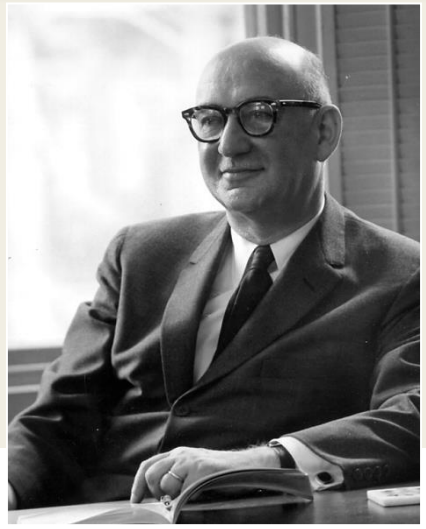
Clinical symptoms related to

- functional disturbances due to **inappropriate cell mass**
- Blood cell sequestration and re-emergence of **extramedullary hematopoiesis**
  - **Marrow fibrosis**
  - Blastic transformation or other forms of disease progression

clonal disorder of hematopoietic stem cell

Myeloproliferative/myelodysplastic neoplasms show overlapping features of MPN with cytopenia

"It is possible that these various conditions—'myeloproliferative disorders'—are all somewhat variable manifestations of proliferative activity of the bone marrow cells, perhaps due to a hitherto undiscovered stimulus"



W. Dameshek, Blood 1951

MPN in the new classifications

Overall only minor changes compared to WHO 4th Edition

Large diagnostic categories unchanged

Increased emphasis on mutation profile

ICC 2022	WHO 5th Edition
Chronic myeloid leukemia	Chronic myeloid leukemia
Polycythemia vera	Polycythemia vera
Essential thrombocythemia	Essential thrombocythemia
Primary myelofibrosis - early/prefibrotic phase - Overt primary myelofibrosis	Primary myelofibrosis
Chronic neutrophilic leukemia	Chronic neutrophilic leukemia
Chronic eosinophilic leukemia, NOS	Chronic eosinophilic leukemia
MPN unclassifiable	Juvenile myelomonocytic leukemia*
	MPN NOS

Included in pediatric and/or germline mutation-associated disorders in ICC

Chronic myeloid leukemia, ~~BCR/ABL1+~~

Defined by presence of *BCR::ABL1*

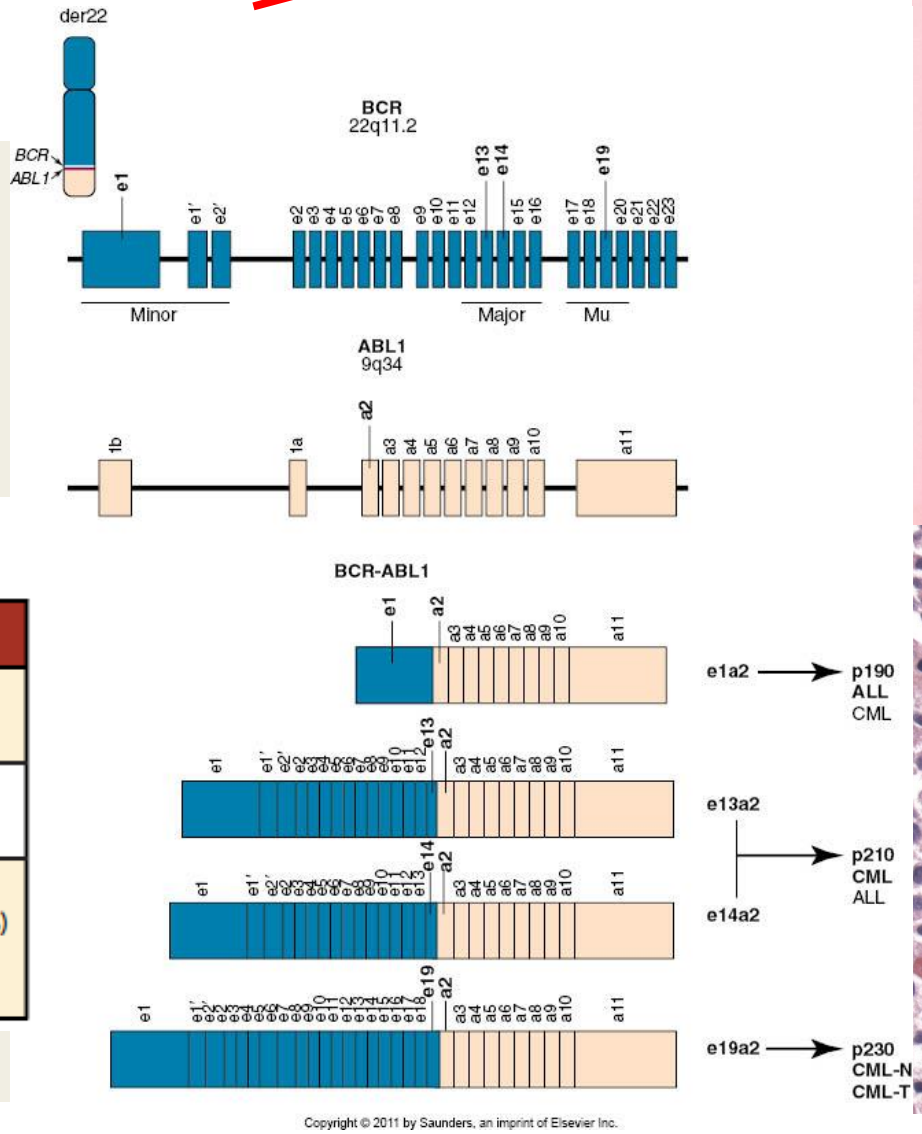
Almost always **p210** transcript

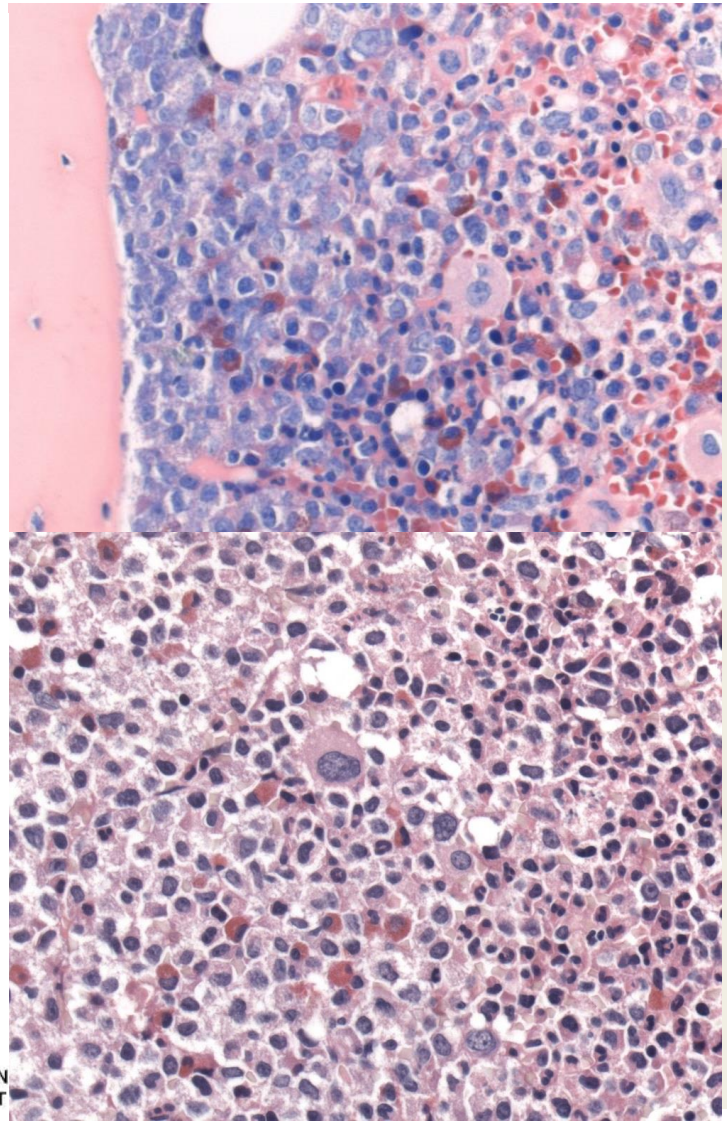
p230 with neutrophilia or thrombocytosis

Table 2. Diagnostic criteria for accelerated and blast phase chronic myeloid leukemia (CML)

Accelerated phase	Blast phase
Bone marrow or peripheral blood blasts 10%-19%	Bone marrow or peripheral blood blasts ≥ 20%
Peripheral blood basophils ≥ 20%	Myeloid sarcoma†
Presence of additional clonal cytogenetic abnormality in Ph+ cells (ACA)*	Presence of morphologically apparent lymphoblasts (>5%) warrants consideration of lymphoblastic crisis‡

AP deleted in WHO 5th



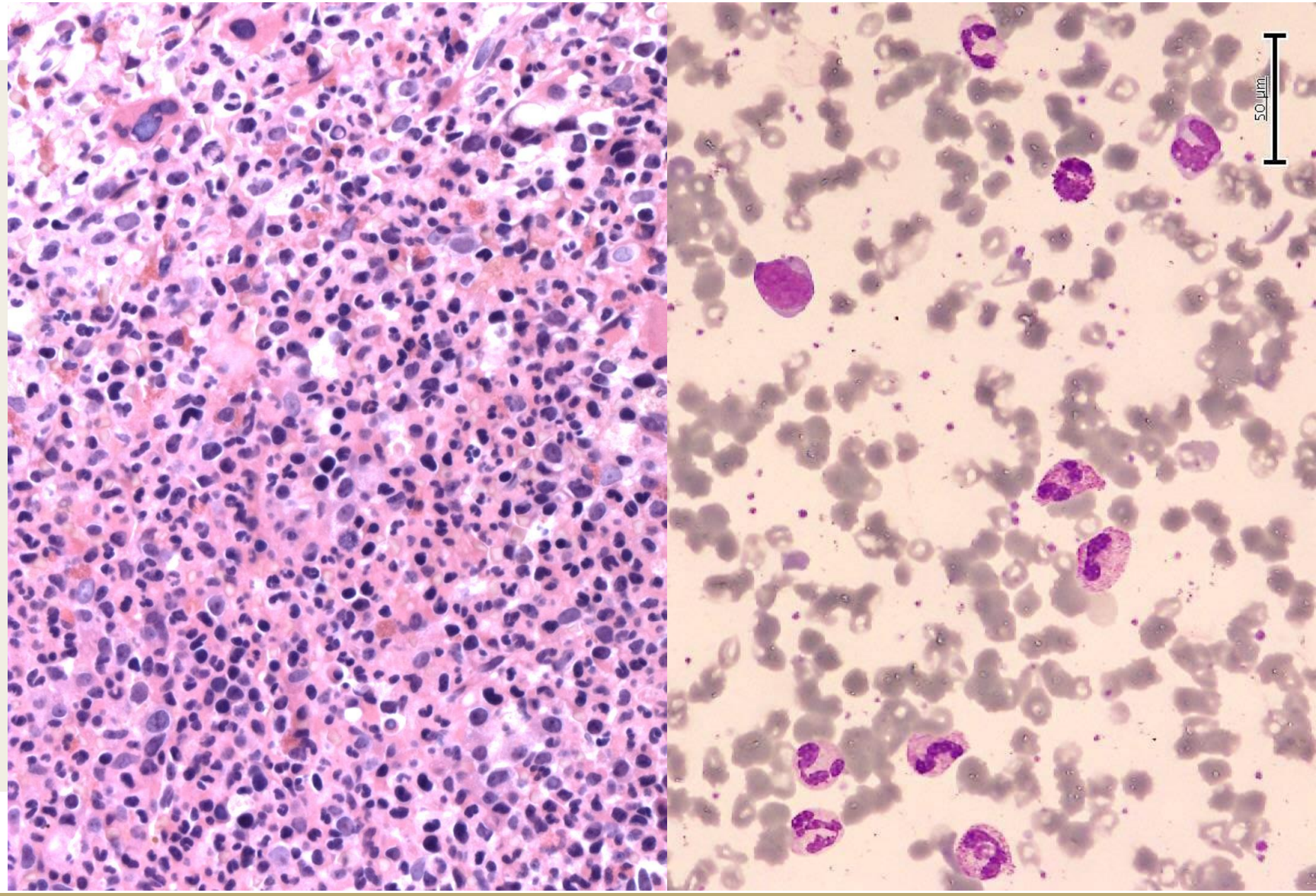


Chronic neutrophilic leukemia

BCR::ABL1-negative leukemia with increase in mature granulocytes

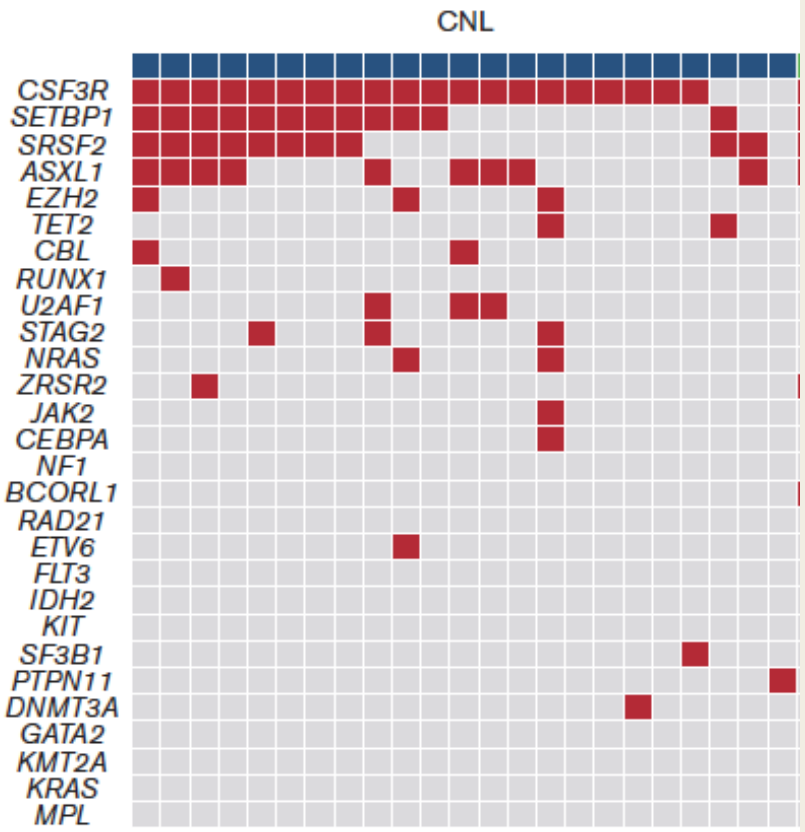
Lack of dysplastic features and significant left shift (DD aCML)

80% show *CSF3R* mutations encoding G-CSF receptor



CNL diagnostic criteria

ICC 2022
>**13.000** leukocytes, >80% neutrophils and band forms, <10% immature forms or monocytes >25.000 leukocytes in WHO 5th Ed
Hypercellular marrow with dominant granulopoiesis, normal maturation
Activating *CSF3R* (T618I) or persistence of leukocytosis >3 mo, splenomegaly, no reactive cause or plasma cell neoplasm, or clonal marker
Exclusion of other MPN



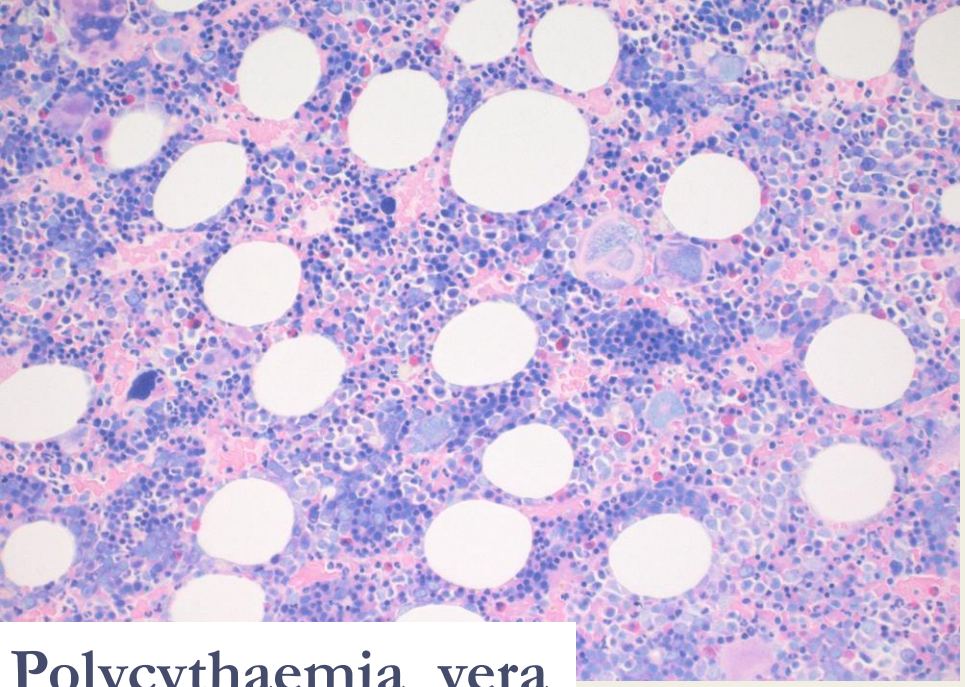
>80% of CNL have activating mutations of *CSF3R*

Frequent mutations in SETBP1, ASXL1 and other myeloid genes

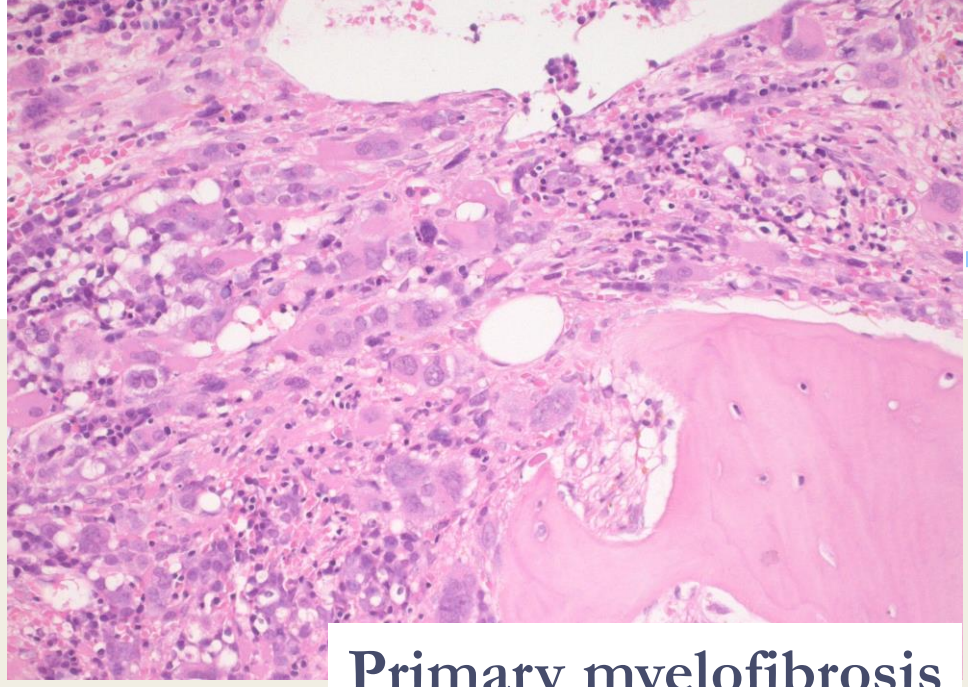
Overlapping profile with aCML

Poor prognosis

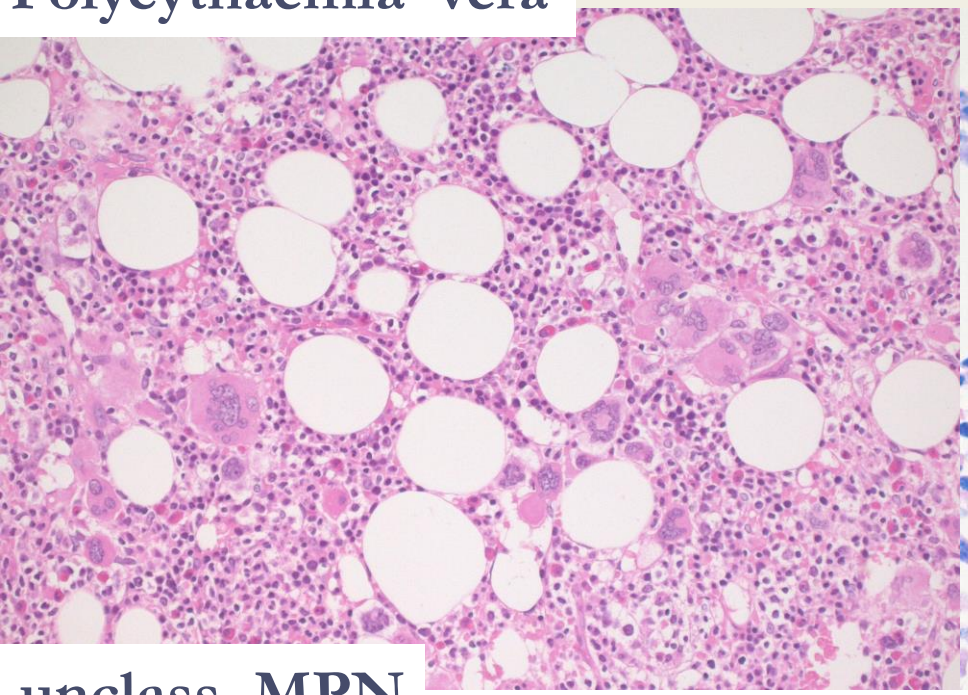
Carreno-Tarragona et al, Blood Adv 2023



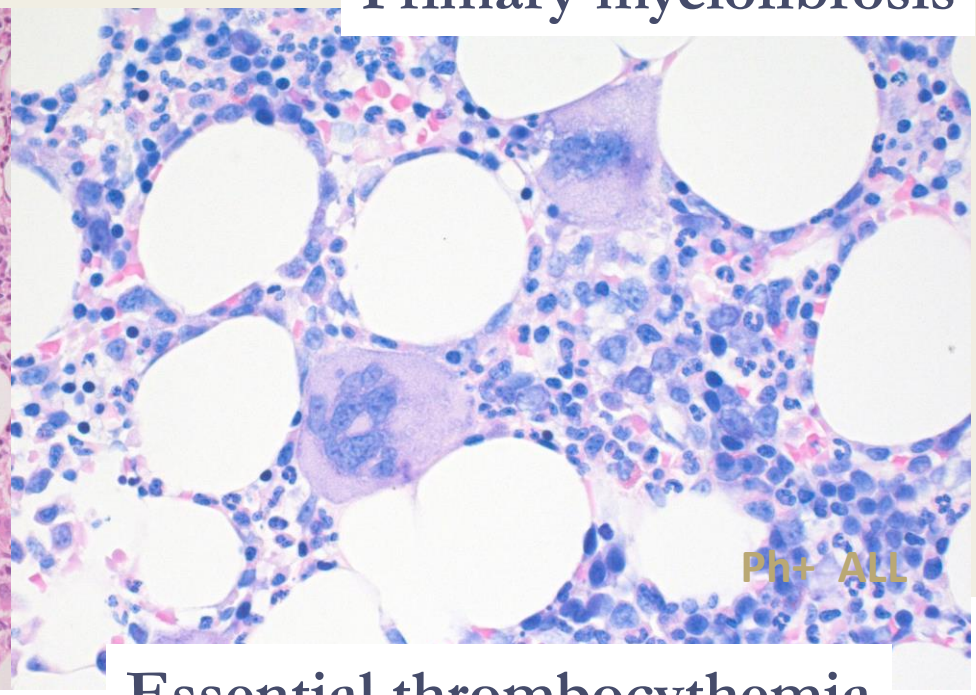
Polycythaemia vera



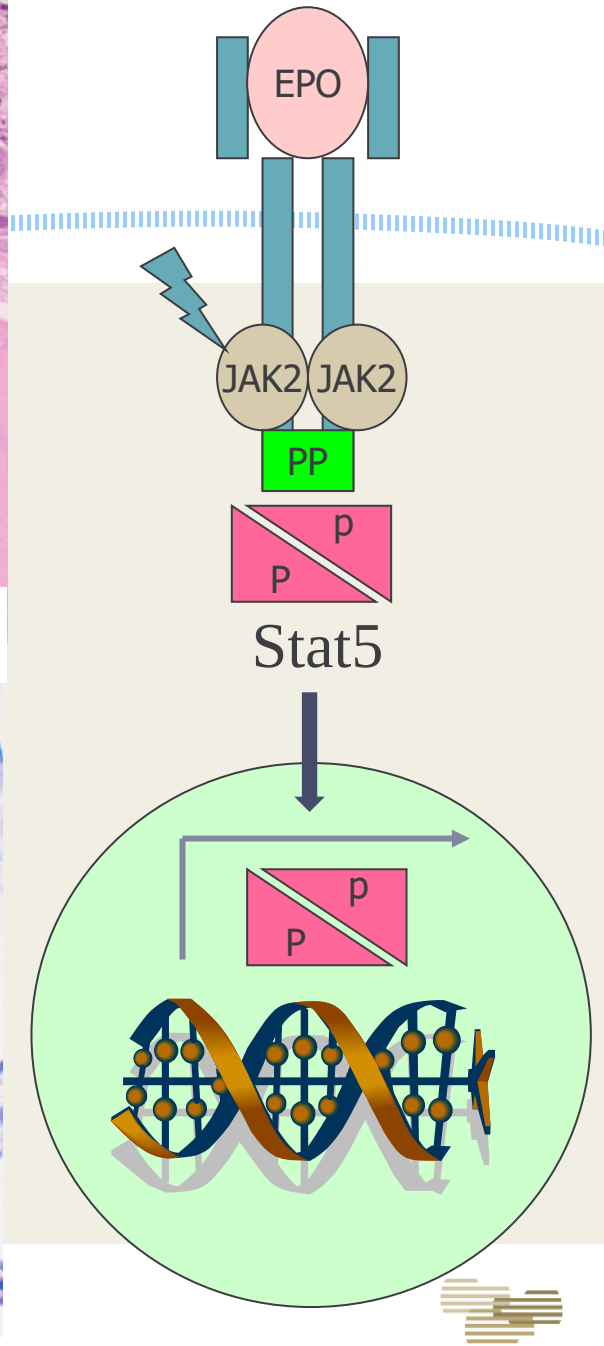
Primary myelofibrosis



unclass. MPN



Essential thrombocythemia



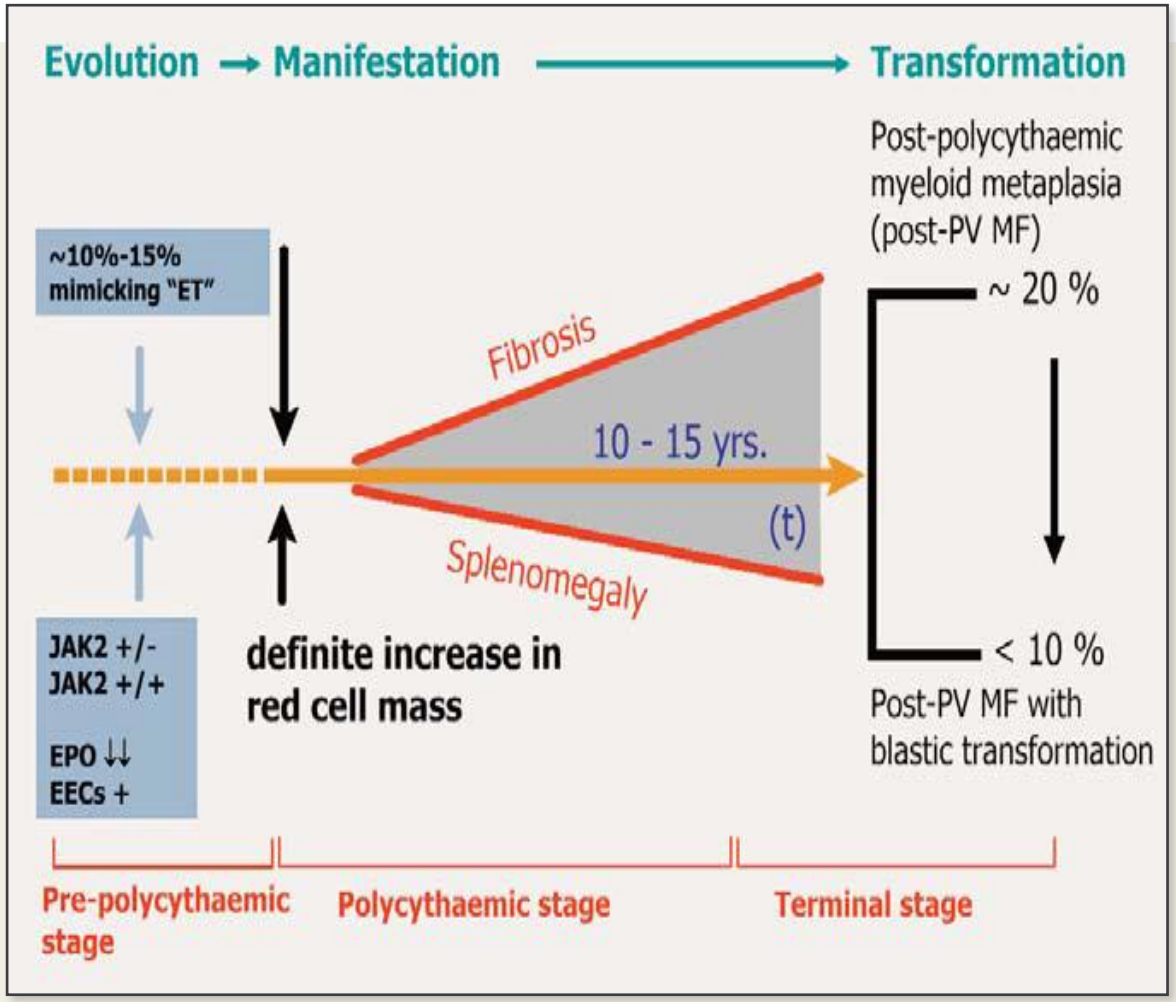
Polycythemia vera

Chronic myeloproliferative disorder characterized by uncontrolled overproduction of red cells and **mutated *JAK2***

PV may be preceded by JAK2+ pre-polycythemic phase

JAK2 allele burden plays mayor role in risk for complications and progression

Post-PV myelofibrosis usually homozygous due to uniparental disomy of Chr 9



Thiele J, Kvasnicka HM, Orazi A et al. WHO 2008

Polycythemia vera

PV

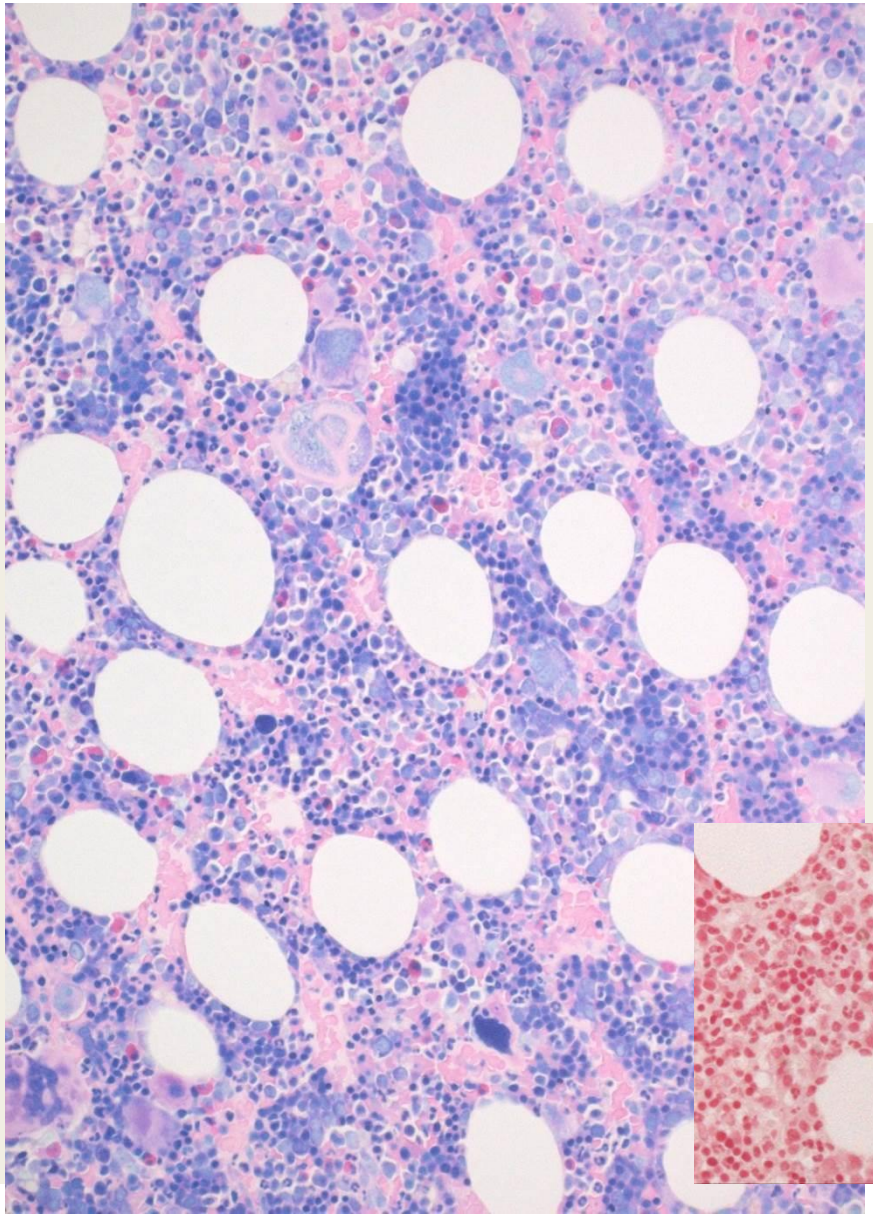
Major criteria

1. Elevated hemoglobin concentration or elevated hematocrit or increased red blood cell mass*
2. Presence of *JAK2* V617F or *JAK2* exon 12 mutation†
3. Bone marrow biopsy showing age-adjusted hypercellularity with trilineage proliferation (panmyelosis), including prominent erythroid, granulocytic, and increase in pleomorphic, mature megakaryocytes without atypia

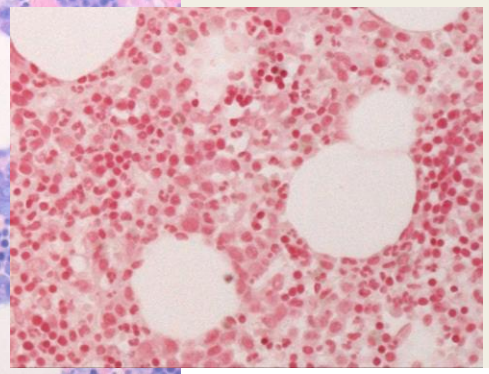
Minor criterion

- Subnormal serum erythropoietin level

The diagnosis of PV requires either all 3 major criteria or the first 2 major criteria plus the minor criterion‡



Negative iron stain in BM



Essential Thrombocythemia

ET

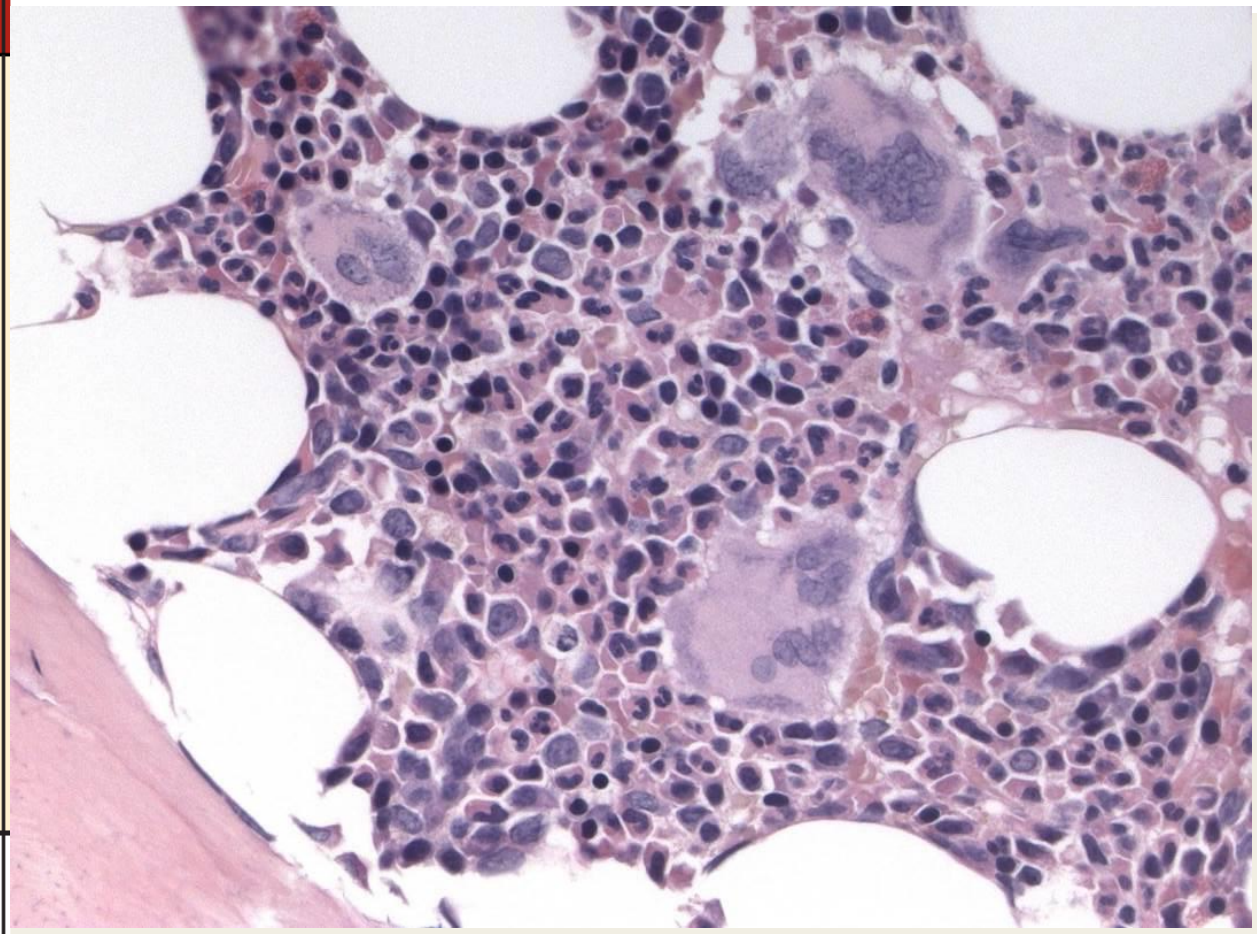
Major criteria

1. Platelet count ≥ 450 × 10^9/L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocytic lineage, with increased numbers of enlarged, mature megakaryocytes with hyperlobulated staghorn-like nuclei, infrequently dense clusters*; no significant increase or left shift in neutrophil granulopoiesis or erythropoiesis; no relevant BM fibrosis†
3. Diagnostic criteria for *BCR::ABL1*-positive CML, PV, PMF, or other myeloid neoplasms are not met
4. *JAK2*, *CALR*, or *MPL* mutation‡

Minor criteria

- Presence of a clonal marker§ or absence of evidence of reactive thrombocytosis||

The diagnosis of ET requires either all major criteria or the first 3 major criteria plus the minor criteria



Arber et al, ICC Blood 2022

Prefibrotic primary myelofibrosis

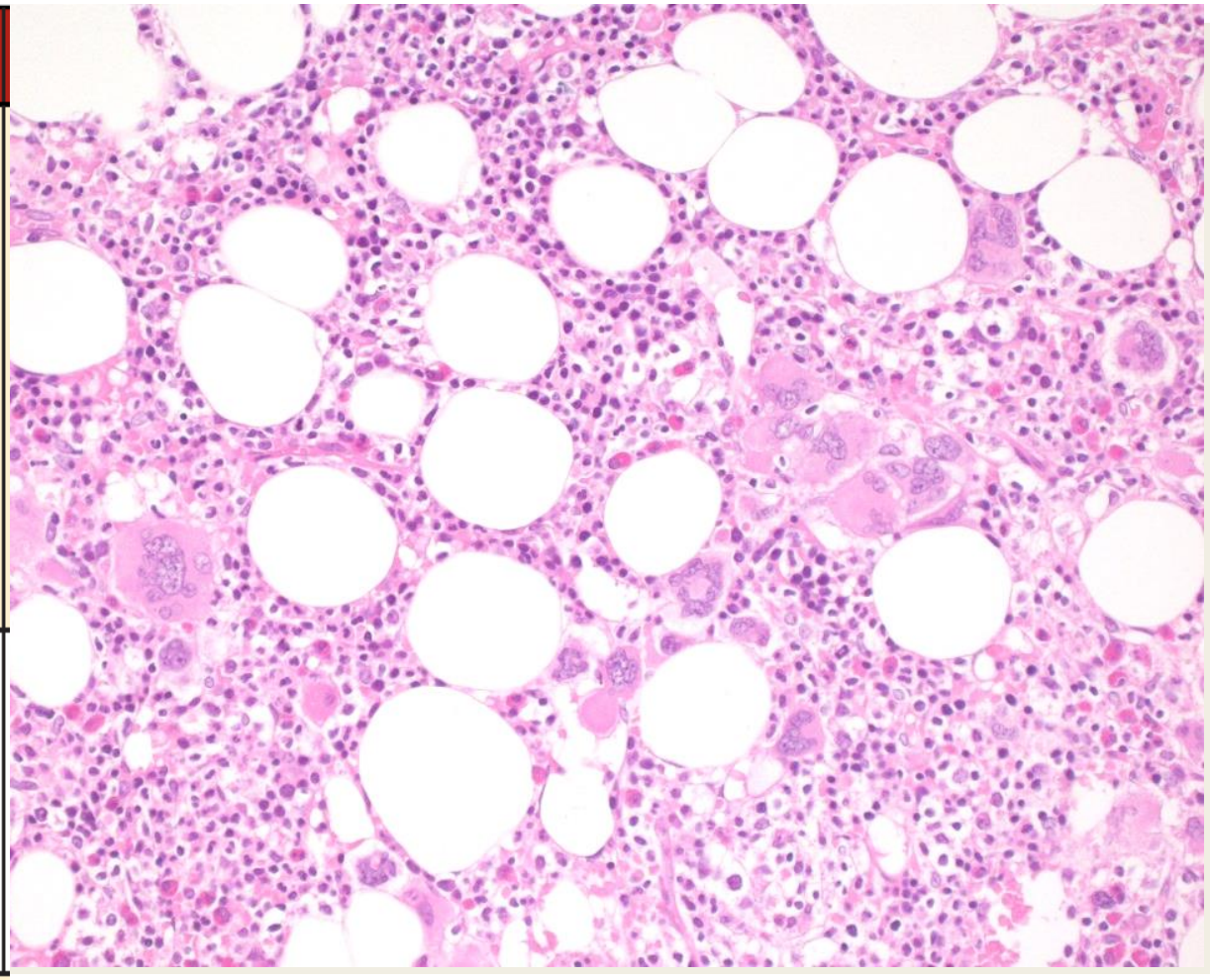
PMF, early/prefibrotic stage (pre-PMF)

Major criteria

1. Bone marrow biopsy showing megakaryocytic proliferation and atypia,* bone marrow fibrosis grade < 2, increased age-adjusted BM cellularity, granulocytic proliferation, and (often) decreased erythropoiesis
2. *JAK2*, *CALR*, or *MPL* mutation† or presence of another clonal marker‡ or absence of reactive bone marrow reticulin fibrosis§
3. Diagnostic criteria for *BCR::ABL1*-positive CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms are not met

Minor criteria

- Anemia not attributed to a comorbid condition
- Leukocytosis $\geq 11 \times 10^9$/L
- Palpable splenomegaly
- Lactate dehydrogenase level above the above the reference range



All 3 major and at least one minor criterion in at least 2 consecutive exams

Distinction between prePMF and ET – an ongoing controversy

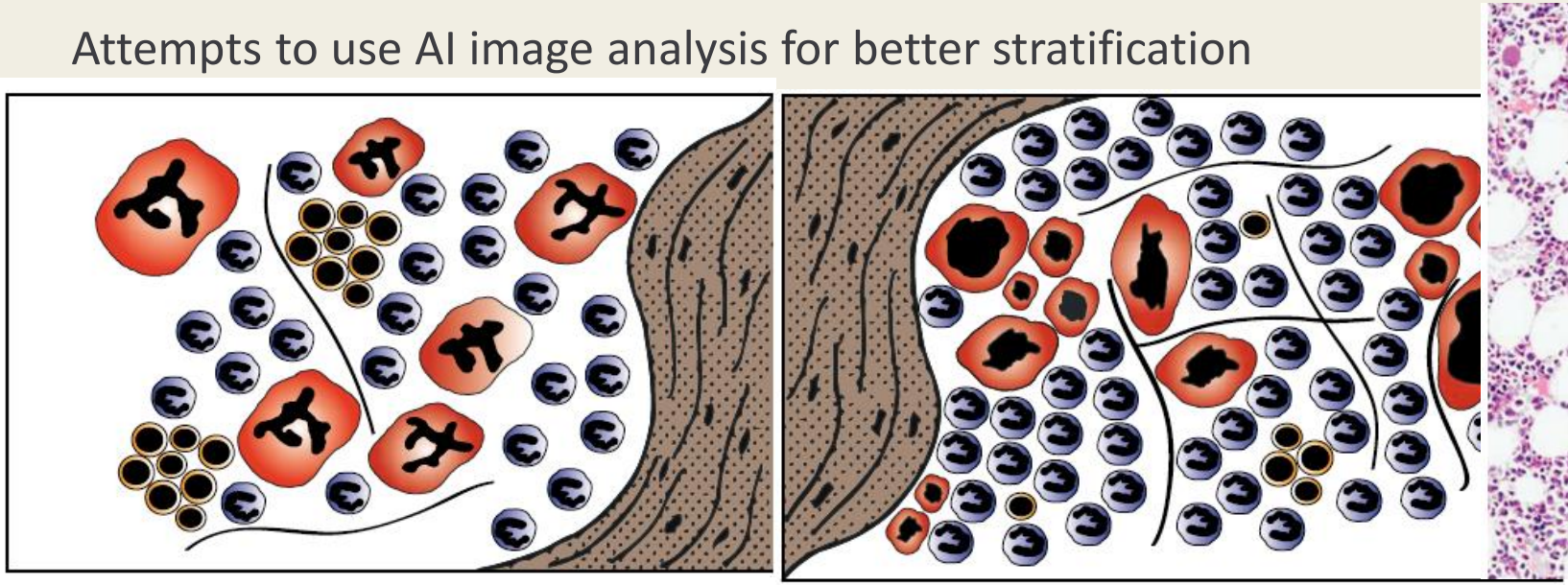
Role of BM biopsy in Ph- MPN controversial, reproducibility in some studies low

Prognosis of both entities good, long term follow-up required

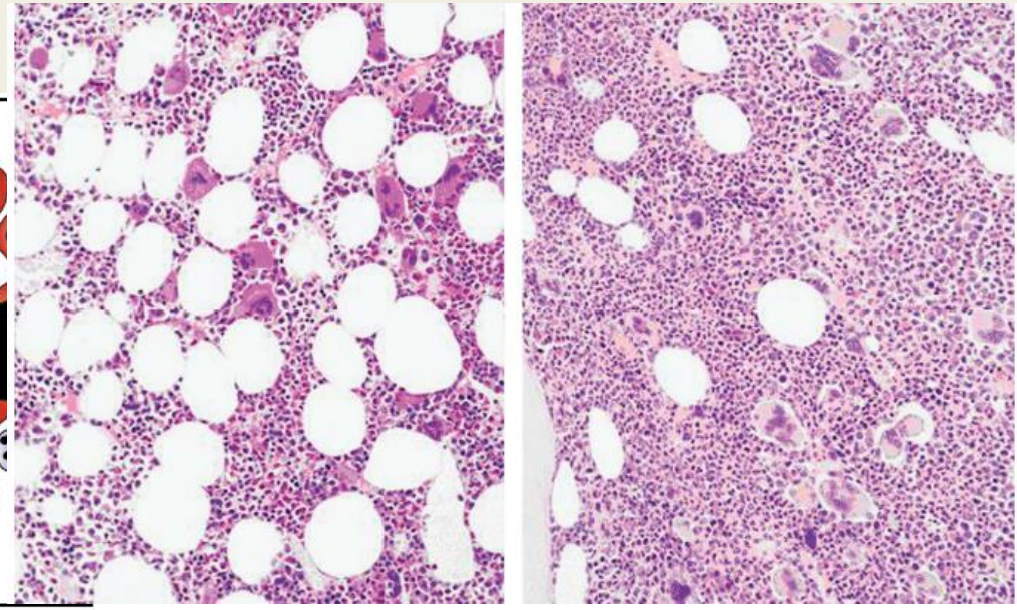
Both ICC and WHO retain the distinction between prePMF and ET

Hypercellularity, tight clustering and atypia of MEGs most relevant

Attempts to use AI image analysis for better stratification

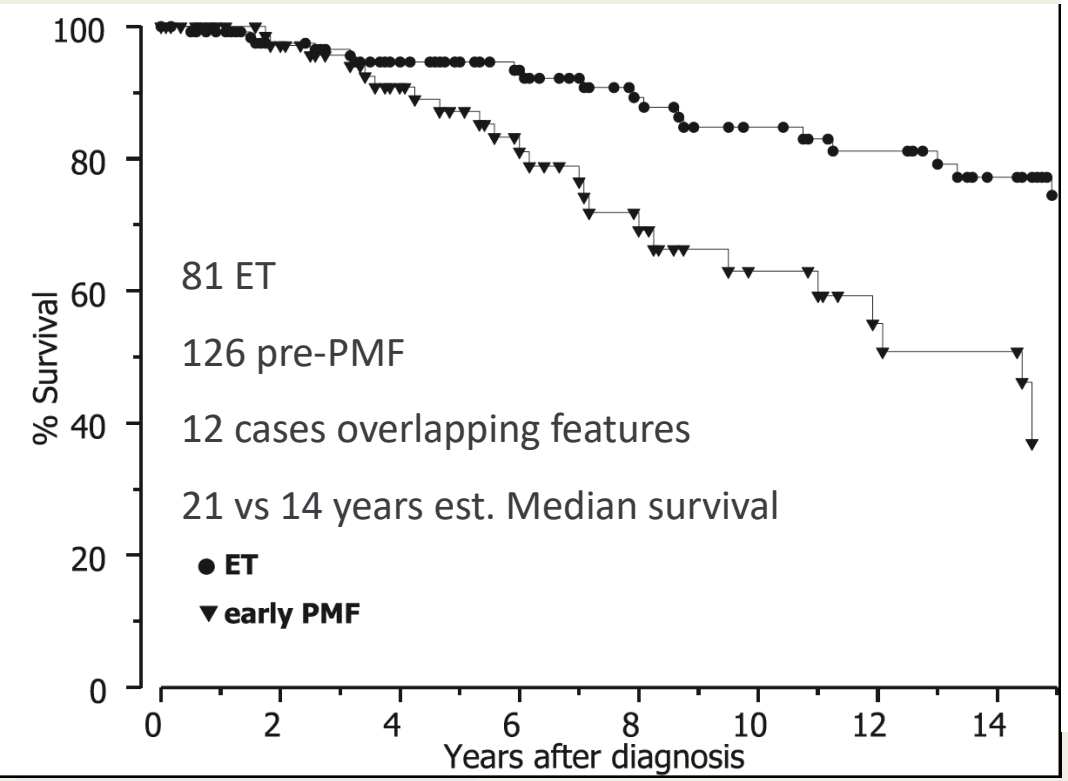


Thiele J et al, *Blood*. 2011;117(21):5710-5718)



Barbui et al., Leukemia (2013) 27, 1953–1958

BM morphology renders additional prognostic information



Thiele et al, Blood 2011

Table 3. Differentiation and comparison of the BSCH-defined cohort of ET patients[1,2] by applying the diagnostic criteria of the WHO classification[3]

	BCSH-defined ET (n = 238)	*WHO-defined ET (n = 232)*
ET	141 (59.2%)	232 (100%)
prePMF	77 (32.4%)	0
PV	16 (6.7%)	0
PMF	4 (1.7%)	0

Abbreviations: BCSH, British Committee of Standards in Haematology; ET, essential thrombocythemia; PMF, advanced PMF; prePMF, prefibrotic primary myelofibrosis; PV, polycythemia vera.

Gisslinger et al, Leukemia 2016

Comparison of British Committee of Standards in Hematology versus WHO

Primary Myelofibrosis

Primarily disease of elderly

Leukoerythroblastic PB, anemia and leuko- and frequently thrombocytosis

Increasing splenomegaly and extramedullary hematopoiesis

Constitutional symptoms

Poor prognosis and more frequent transformation to acute leukemia

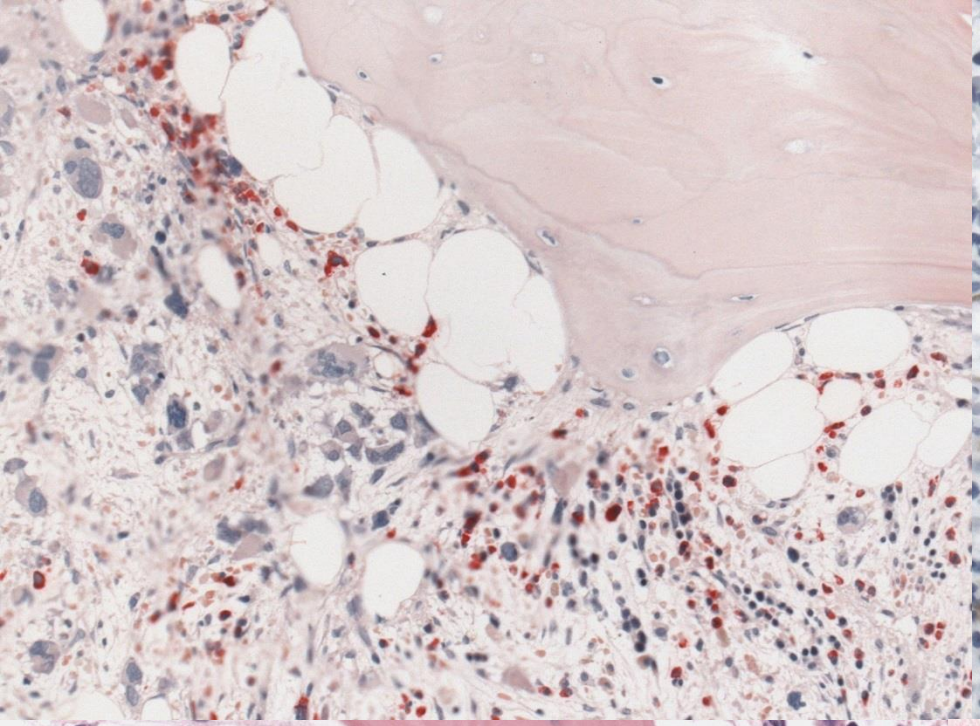
PMF, overt fibrotic stage

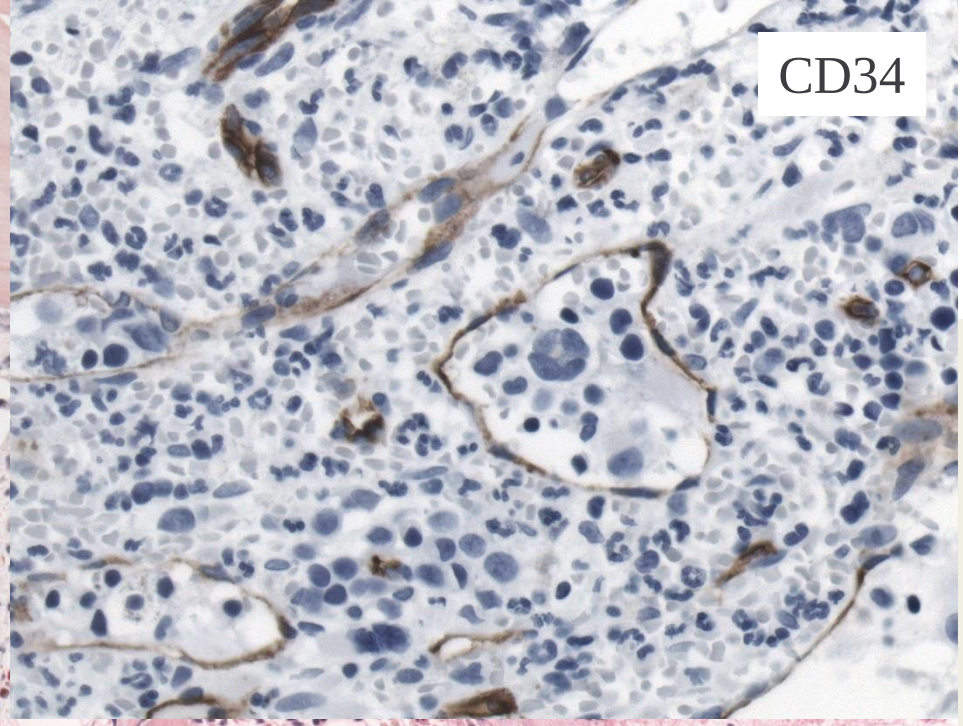
Major criteria

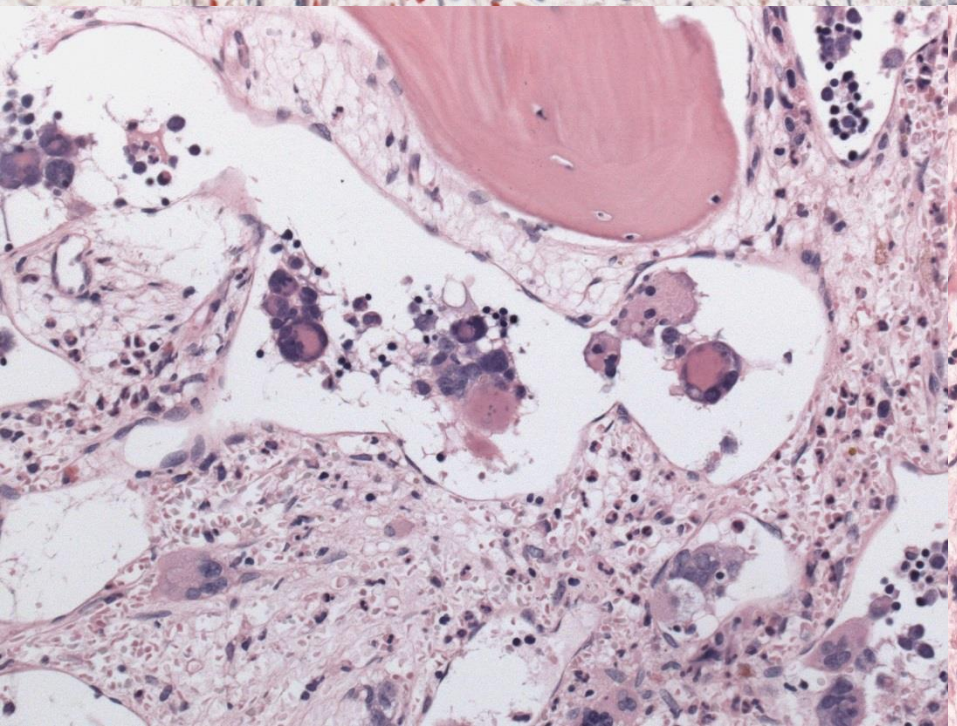
1. Bone marrow biopsy showing megakaryocytic proliferation and atypia,* accompanied by reticulin and/or collagen fibrosis grades 2 or 3
2. *JAK2*, *CALR*, or *MPL* mutation† or presence of another clonal marker‡ or absence of reactive myelofibrosis§
3. Diagnostic criteria for ET, PV, *BCR::ABL1*-positive CML, myelodysplastic syndrome, or other myeloid neoplasms|| are not met

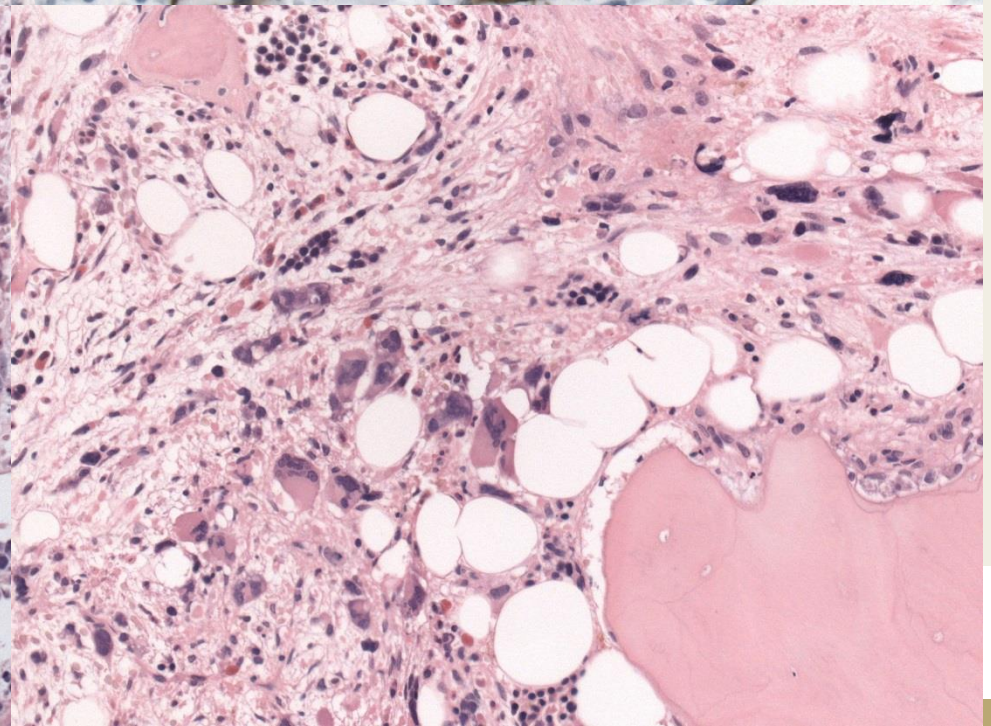
Minor criteria

- Anemia not attributed to a comorbid condition
- Leukocytosis $\geq 11 \times 10^9$/L
- Palpable splenomegaly
- Lactate dehydrogenase level above the above the reference range
- Leukoerythroblastosis









Overt PMF

Fibrosis ≥ grade 2
Normo- to hypocellular, often patchy distribution

peritrabecular fat

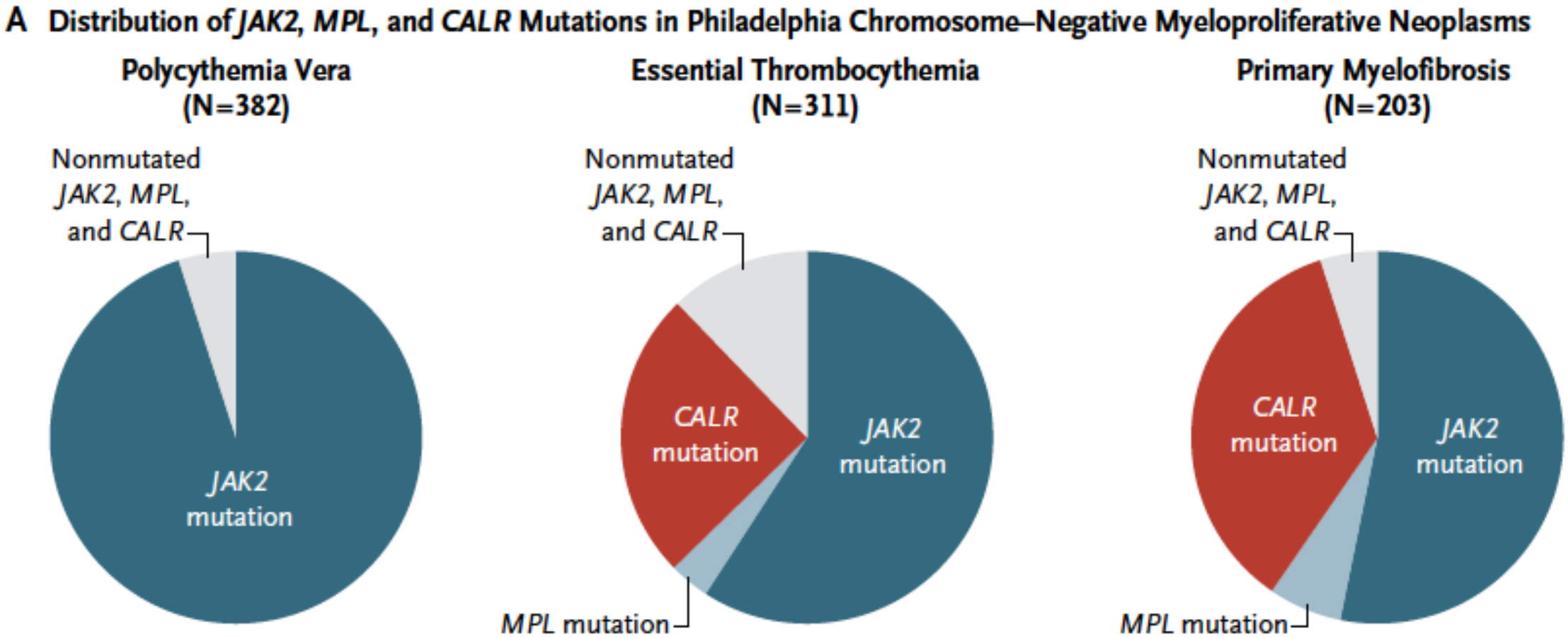
Intrasinusoidal hematopoiesis

Increase in atypical meg clusters related to ectatic sinuses

Increasing osteosclerosis with osteoblast rimming and buds

No increase in blasts

Common driver mutations in Ph- MPN



50% of ET and PV and 20% of PMF lack additional mutations

Klampfl T, et al. N Engl J Med. 2013;369:2379-90

How does mutant Calreticulin induce myeloproliferation?

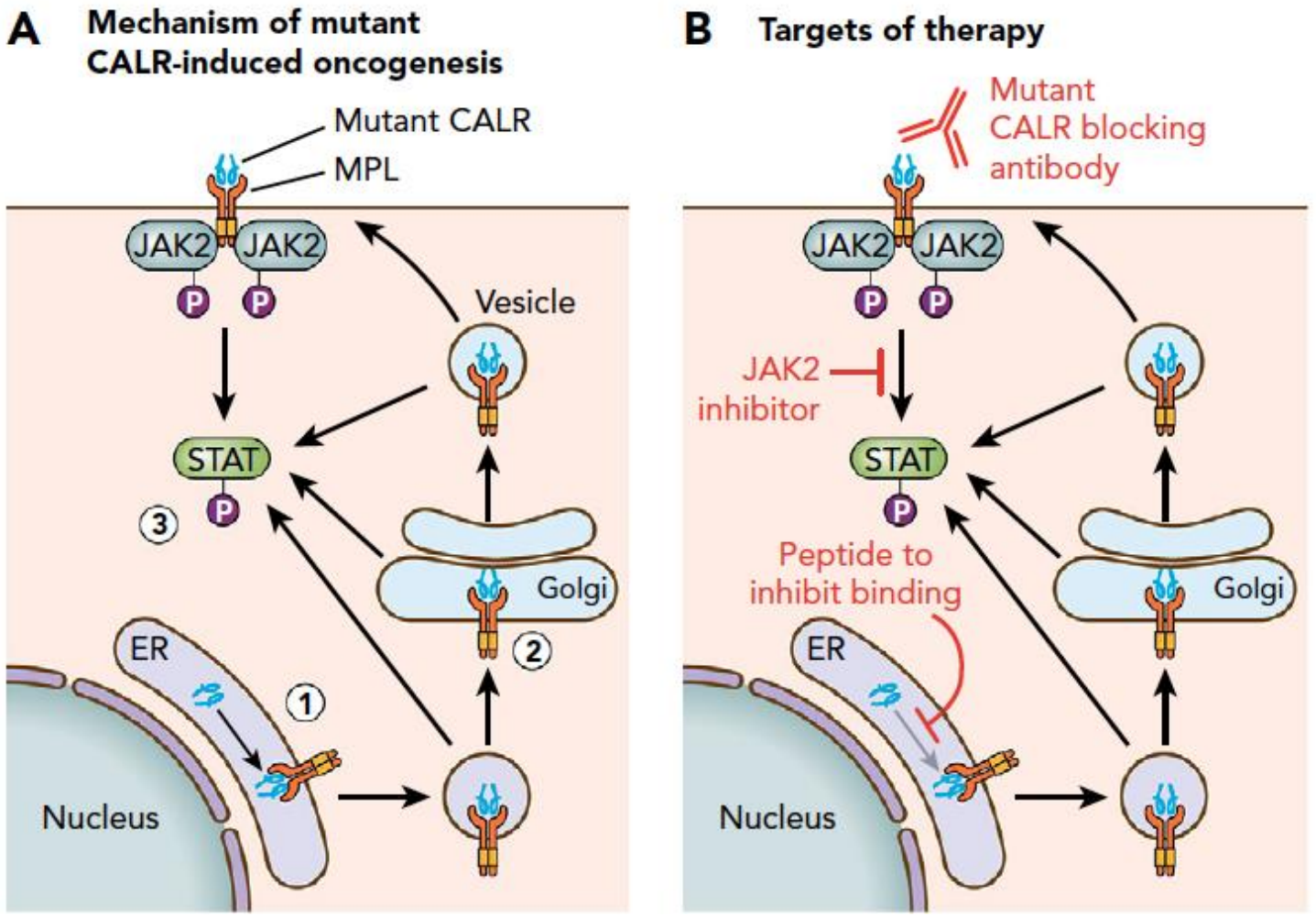
CALR is ER chaperone protein

52-bp deletion (L367fs*46) type I and 5-bp insertion (K385fs*47) (type II) delete negatively charged AA and create a new C-terminus

Type II more common in MF

Mutant CALR binds to MPL in the ER and is transferred to the surface

Results in ligand-independent receptor activation in stem cells and megakaryocytes



Impact of different driver mutations in Ph- MPN

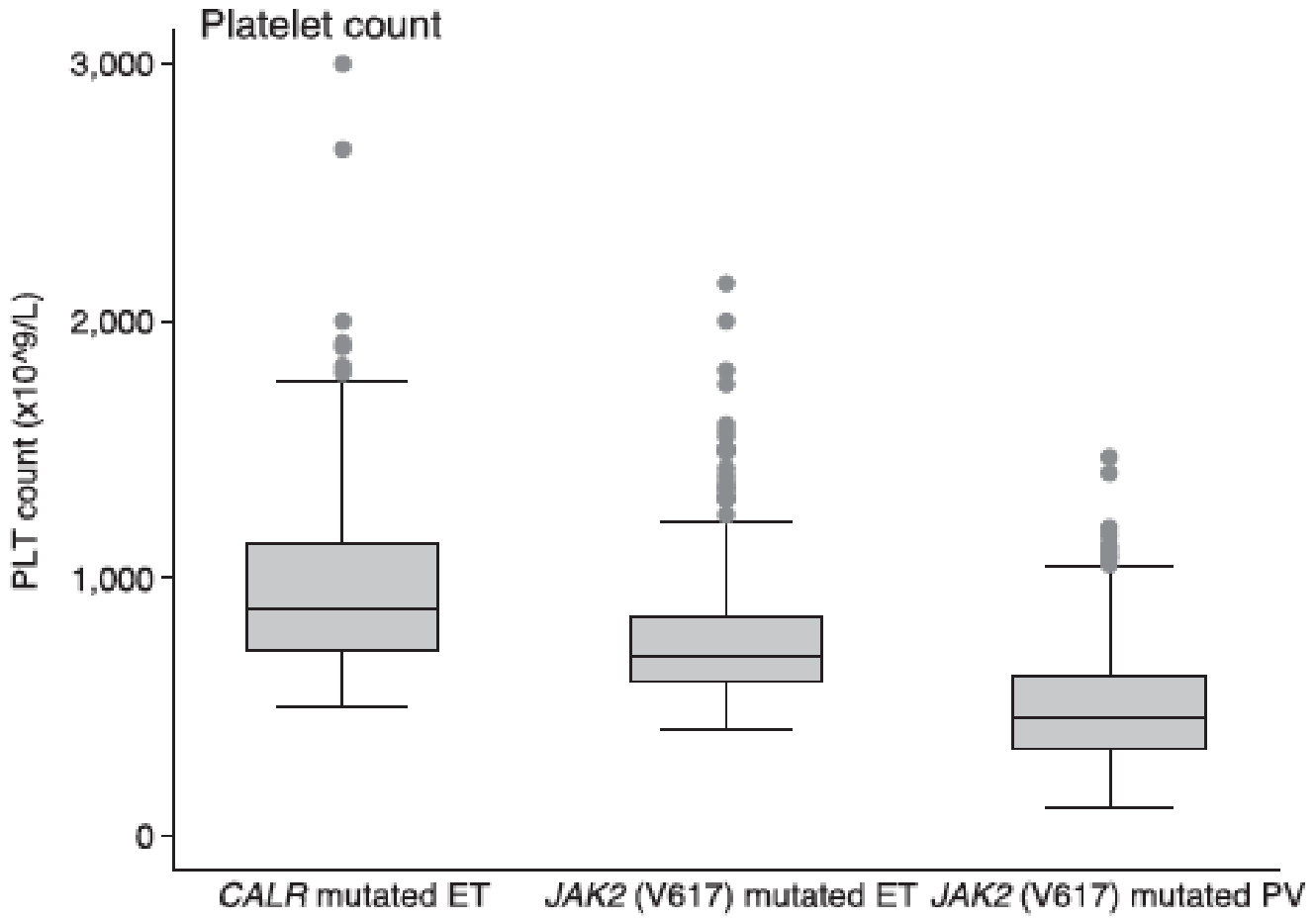
CALR-mutated ET has higher platelet and lower leukocyte counts

Lower risk of thrombosis

No transformation to PV (vs. 29% at 15 years for JAK2+ ET)

Younger age of patients

CALR+ ET is different nosological entity



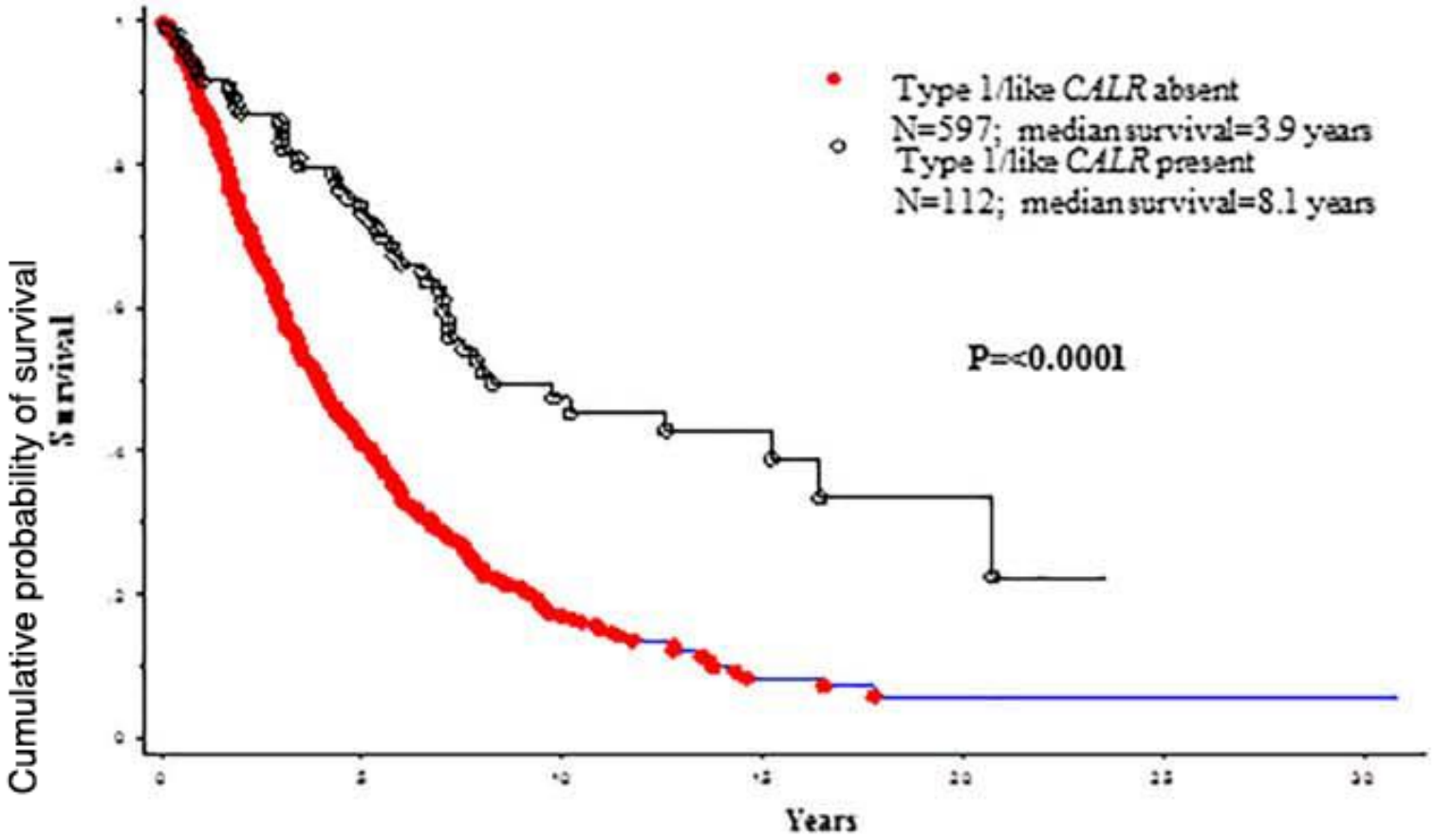
Rumi et al, Blood 2014

Impact of mutations in PMF

CALR mutated PMF has superior diagnosis

Less development of anemia, leukocytosis and transformation
Independent from IPSS scoring

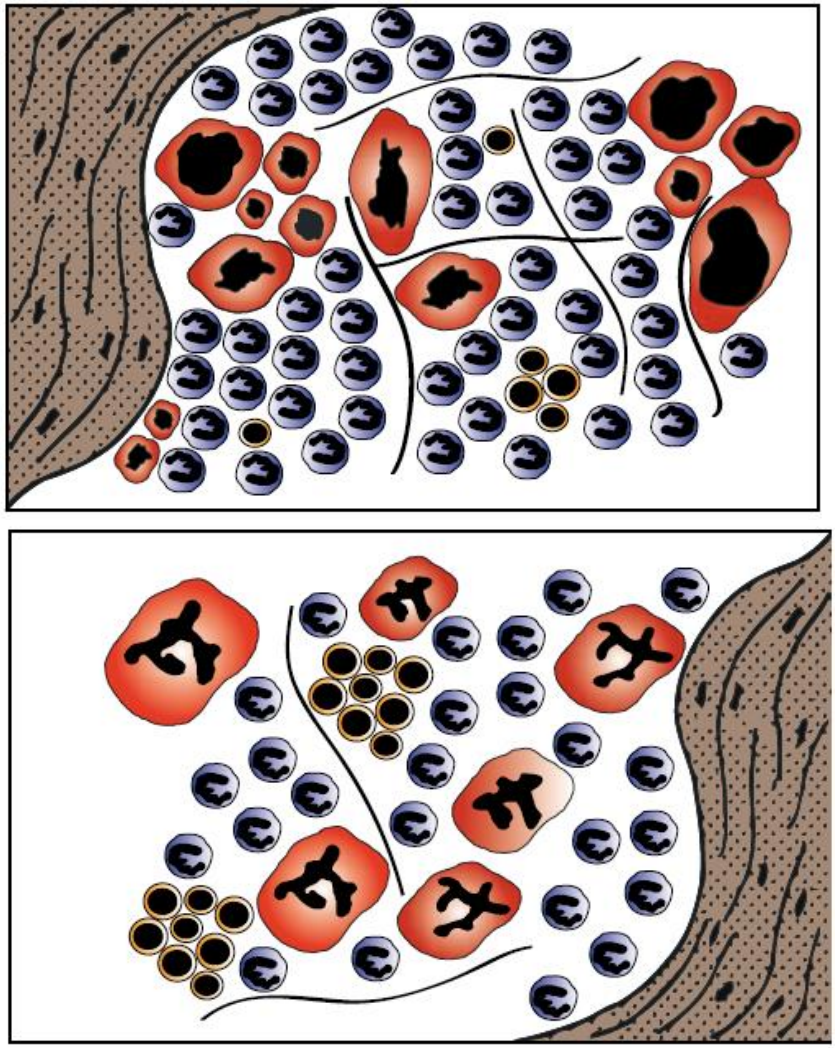
“triple-negative” PMF the worst prognosis, even if corrected for age



a: Overall survival of 709 primary myelofibrosis patients from the Mayo Clinic, stratified by presence or absence of type 1/like *CALR* mutations

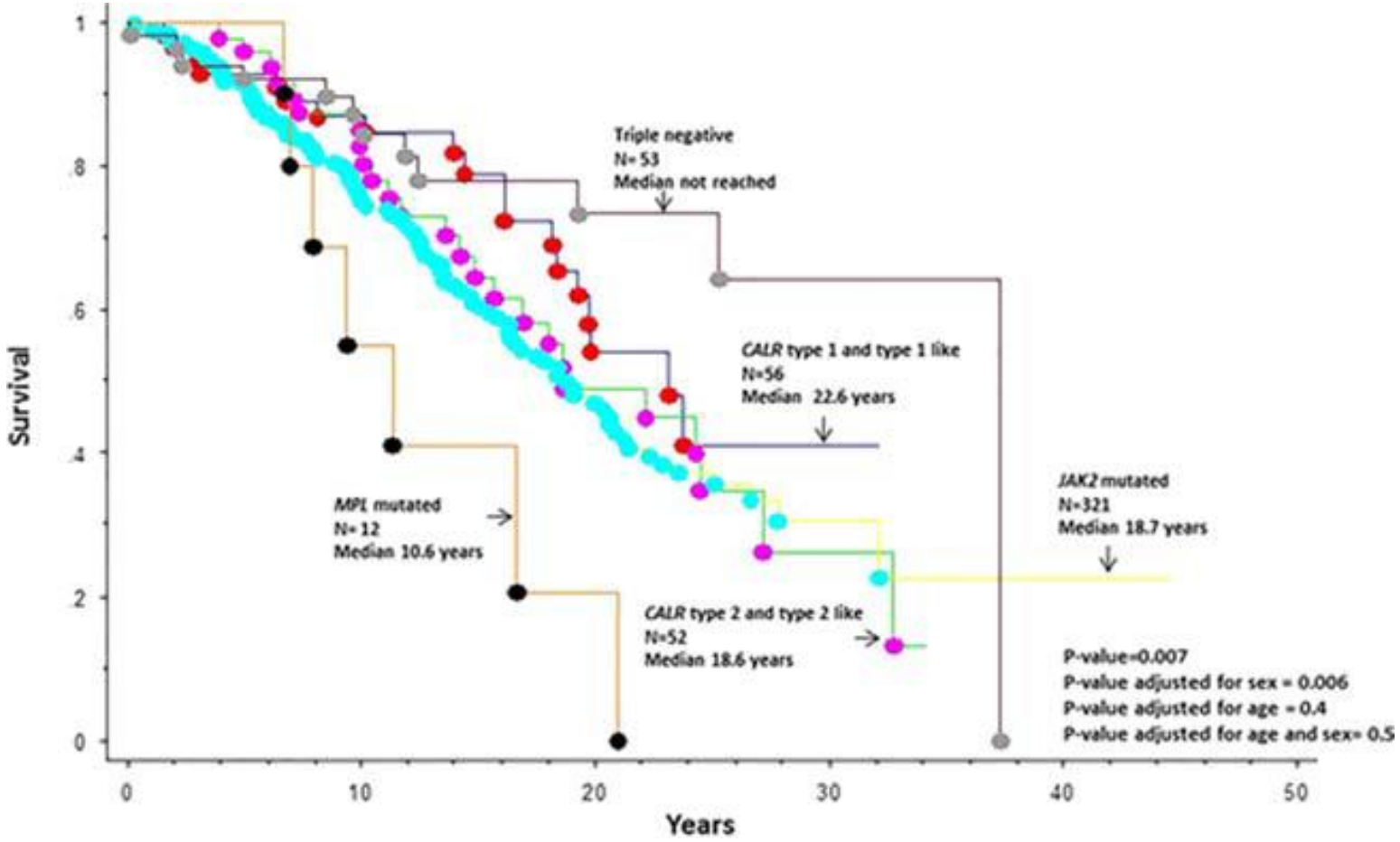
Tefferi et al, Am J Hematol 2018

Is mutational profiling enough?



Thiele J et al, *Blood*. 2011;117(21):5710-5718)

Elala et al, Am. J. Hematol. 91:503–506, 2016

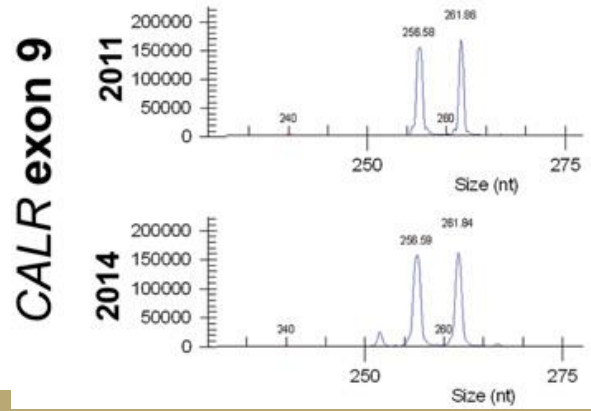


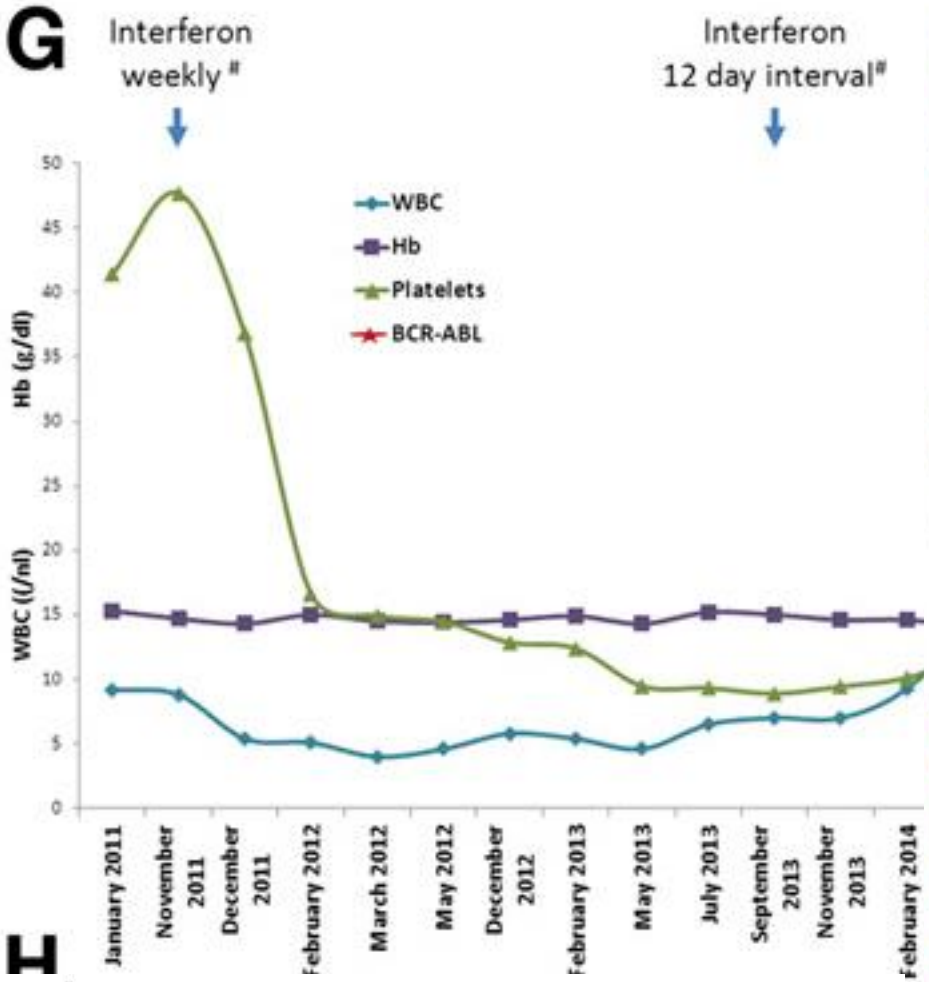
In essential thrombocythemia, triple negative cases show the best prognosis

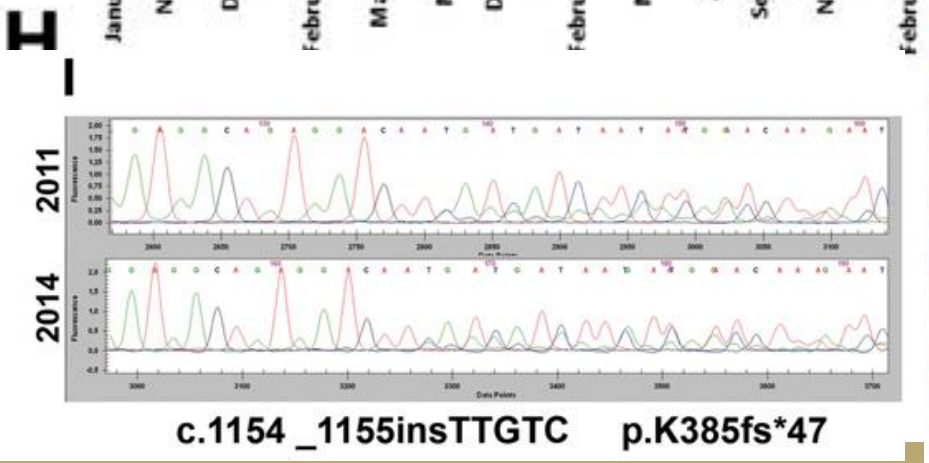
Diagnostic pitfalls…

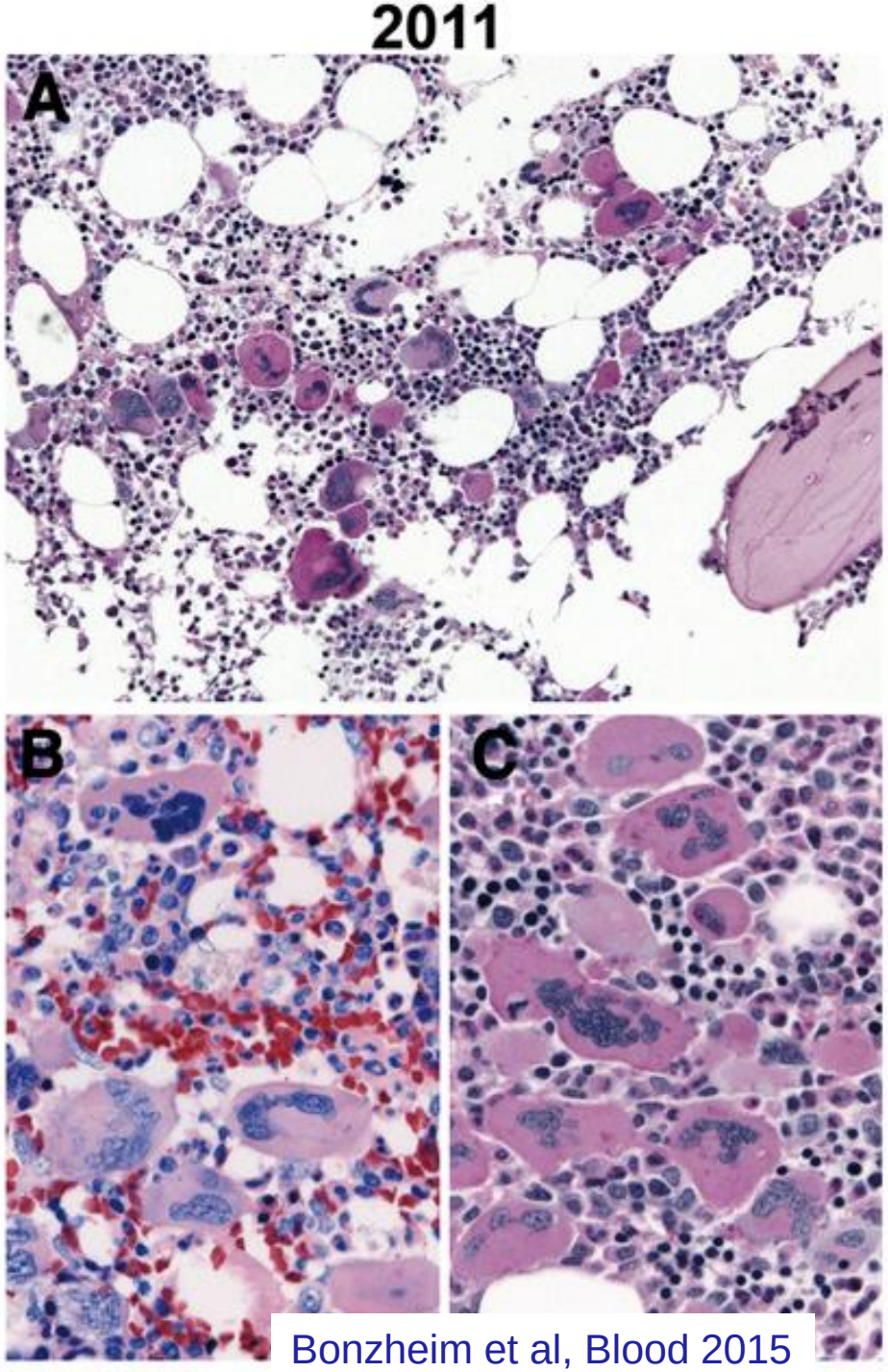
26-year-old male with new onset thrombocytosis

JAK2-negative ET Diagnosis in 2011

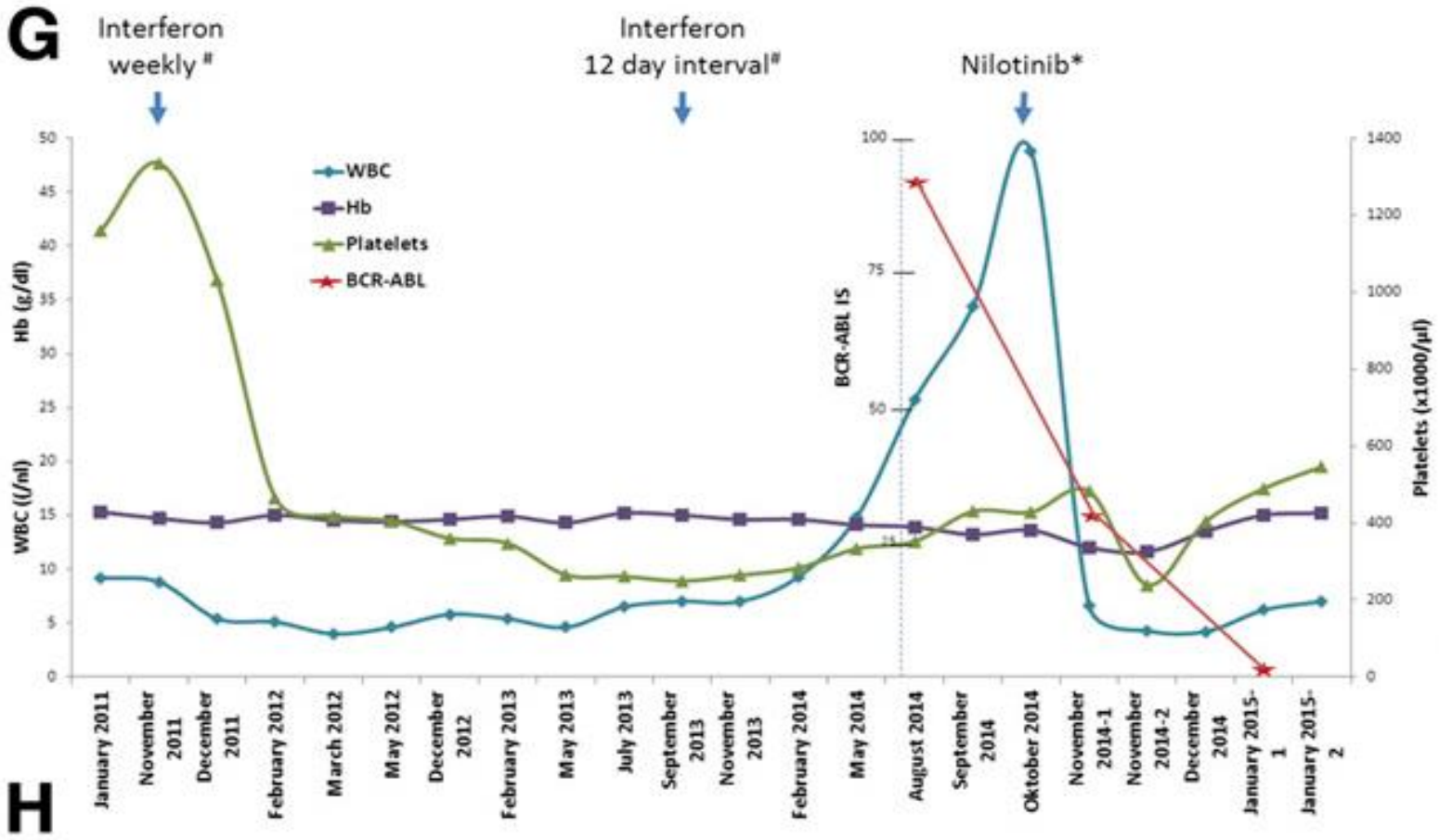


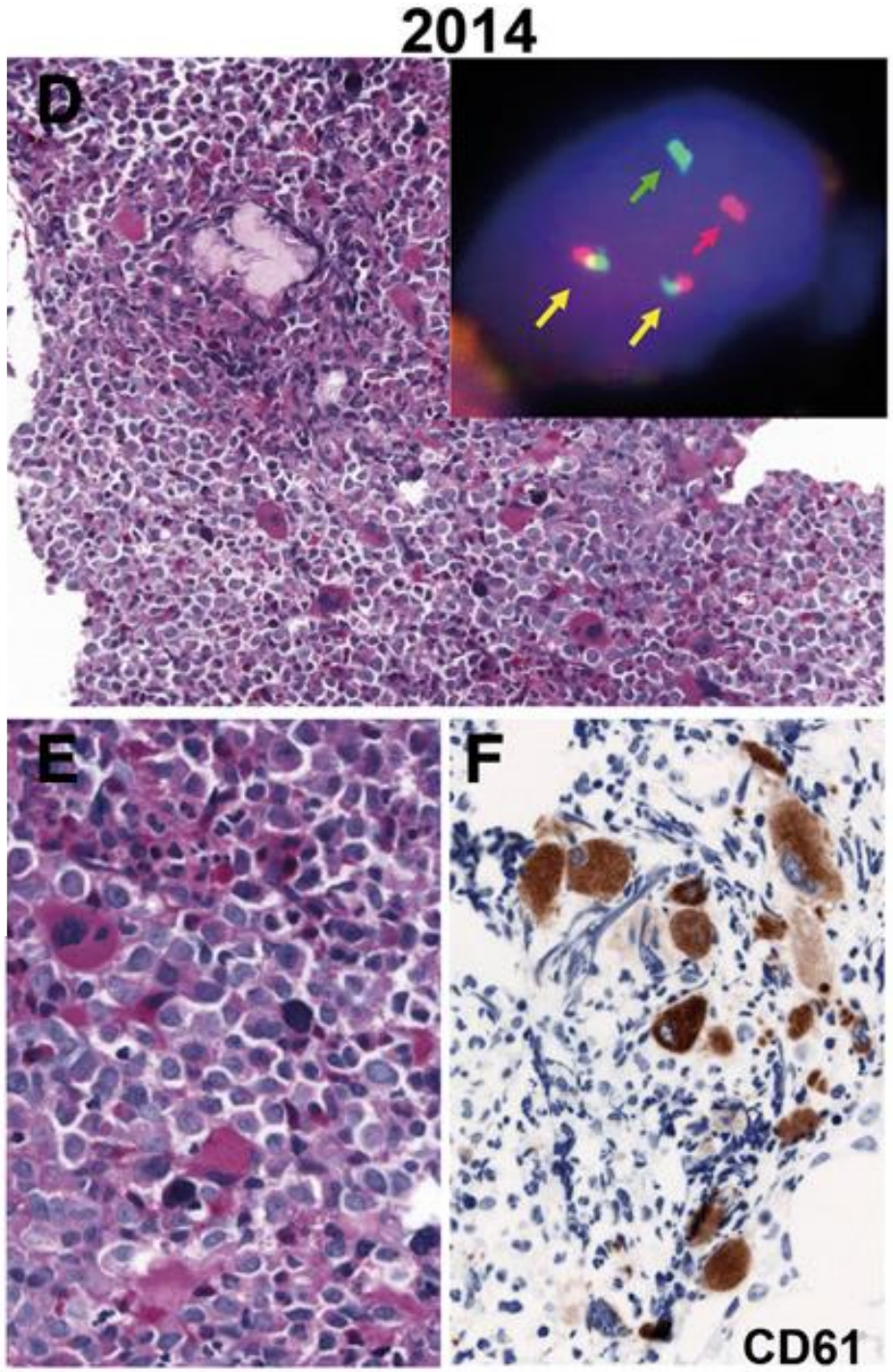






Bonzheim et al, Blood 2015





New onset leukocytosis in 2014, detection of *BCR::ABL1*

CML arising in the background of ET

1. Bonzheim et al, Blood 2015

Ph+ CML and MPN with *JAK2* or *CALR* mutation

Probably both present in same population as well as separate clones

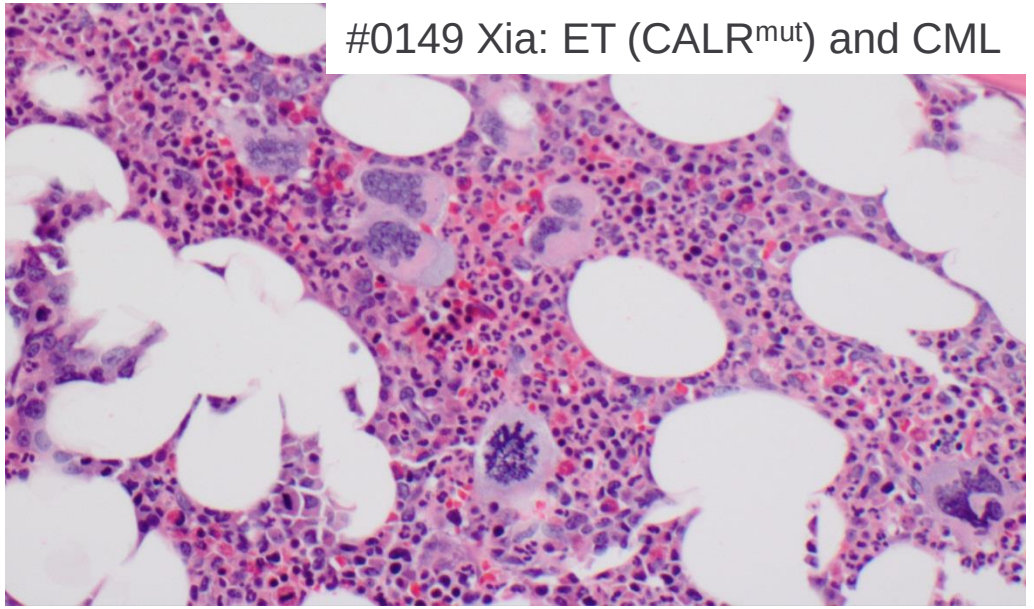
Temporal sequence of mutations variable:

- CML may appear at later time
- Treatment with TKI unmasks second clone

Clinical and morphological changes suggestive of second MPN

BM biopsy and molecular studies necessary to discern from TKI resistance or CML/CNL-like progression

#0149 Xia: ET ($CALR^{mut}$) and CML



0231 Soderquist: CML and ET $JAK2^{mut}$

Cases of SH Workshop Chicago 2017

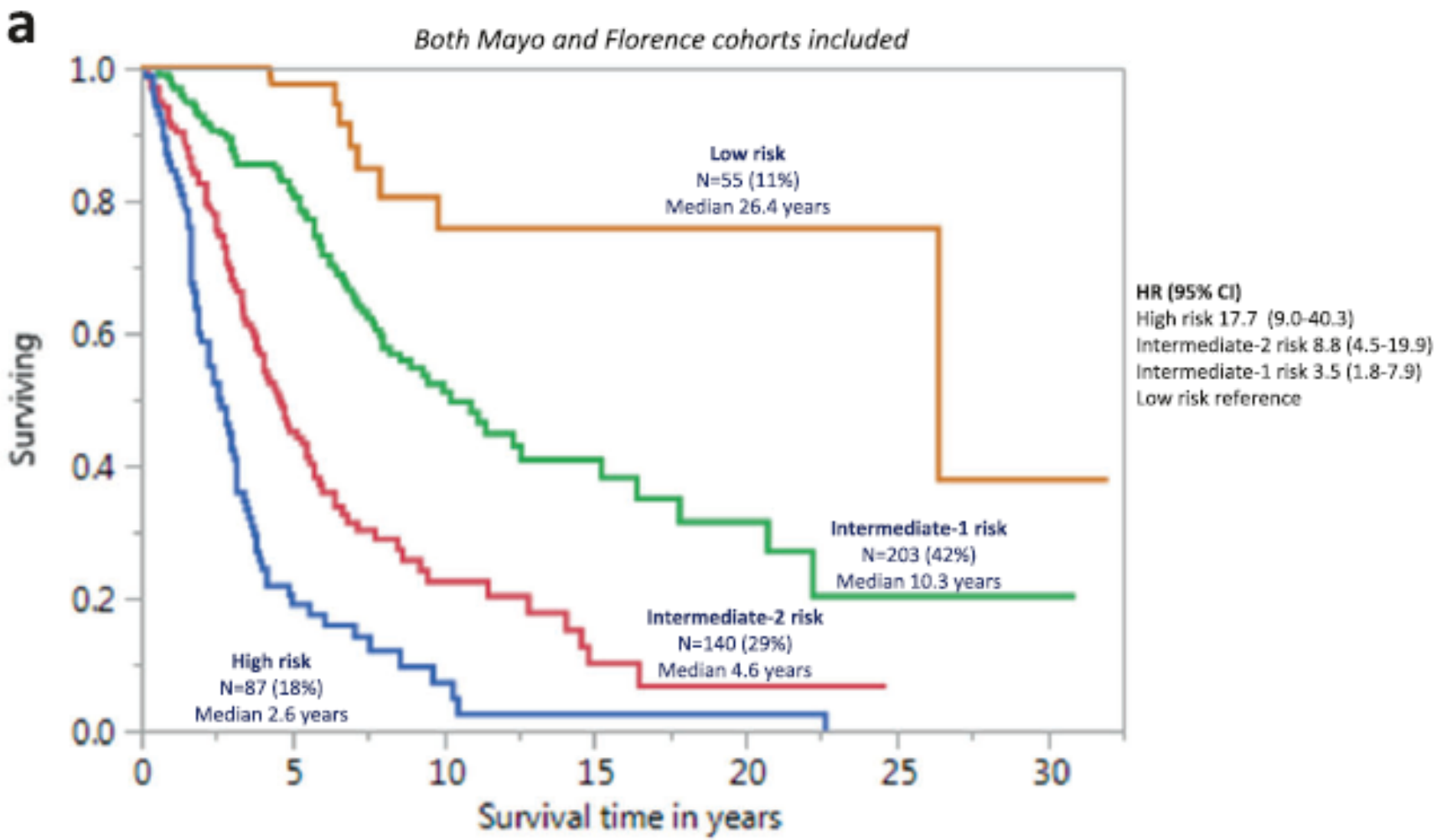
The impact of secondary mutations in PMF

- Integration of
  - Clinical variables
  - Cytogenetics
  - Additonal driver mutations (80% of patients)
  - *ASXL1, SRSF2, U2AF1, IDH2, EZH2* - unfavorable
  - Presence and number of additional mutations have significant prognostic impact

GIPSS: genetically inspired prognostic scoring system for primary myelofibrosis

Leukemia (2018) 32:1631–1642

Ayalew Tefferi[1] · Paola Guglielmelli[2] · Maura Nicolosi[1] · Francesco Mannelli[2] · Mythri Mudireddy[1] · Niccolo Bartalucci[2] · Christy M. Finke[1] · Terra L. Lasho[1] · Curtis A. Hanson[3] · Rhett P. Ketterling[4] · Kebede H. Begna[1] Naseema Gangat[1] · Animesh Pardanani[1] · Alessandro M. Vannucchi[2]



Prognostic scores for PMF

New scores include

clinical features

blood counts (anemia, leukocytosis, blasts)

genetics

Variables (weight)	Score					
	DIPSS	DIPSS+	MIPSS-70	MIPSS-70+ version 2.0	MYSEC-PM	MTSS
Clinical features	Age >65 y (1) Constitutional symptoms (1)	Age >65 y (1) Constitutional symptoms (1) RBC transfusions need (1)	Constitutional symptoms (1)	Constitutional symptoms (2)	Age (0.15 × y of age) Constitutional symptoms (1)	Age ≥57 y (1) Karnofsky <90% (1) MMUD (2)
Complete blood count	Hb <10 g/dL (2) WBC >25 × 10^9/L (1) Blasts ≥1% (1)	Hb <10 g/dL (1) WBC >25 × 10^9/L (1) Blasts ≥1% (1) PLT <100 × 10^9/L (1)	Hb <10 g/dL (1) WBC >25 × 10^9/L (2) Blasts ≥2% (1) PLT <100 × 10^9/L (2)	Severe anemia* (2) Moderate anemia† (1) Blasts ≥2% (1)	Hb <11 g/dL (2) Blasts ≥3% (2) PLT <150 × 10^9/L (1)	WBC >25 × 10^9/L (1) PLT <150 × 10^9/L (1)
Driver mutation status			Absence of *CALR* type 1/like (1)	Absence of *CALR* type 1/like (2)	Absence of *CALR* (2)	Absence of *CALR/MPL* (2)
Additional myeloid-gene mutations			1 HMR (1) ≥2 HMR (2)	1 HMR included *U2AF1*Q157 (2) ≥2 HMR included *U2AF1*Q157 (3)		*ASXL1* (1)
Cytogenetics		Unfavorable‡ (1)		Unfavorable§ (3) Very high-risk‖ (4)		
BM morphology			BMF grade ≥2 (1)			

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MPN evolution

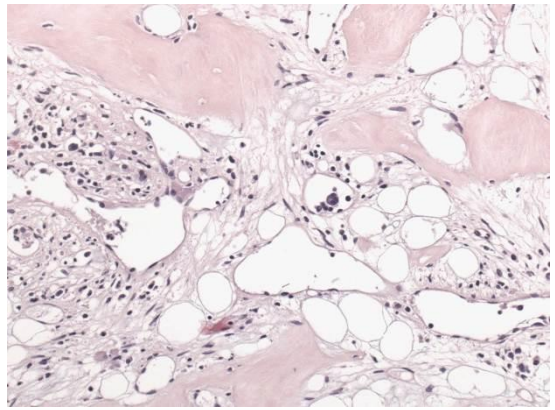
Often long, stable disease phases and low rate of progressison

MPN show low genetic complexity and high genetic stability

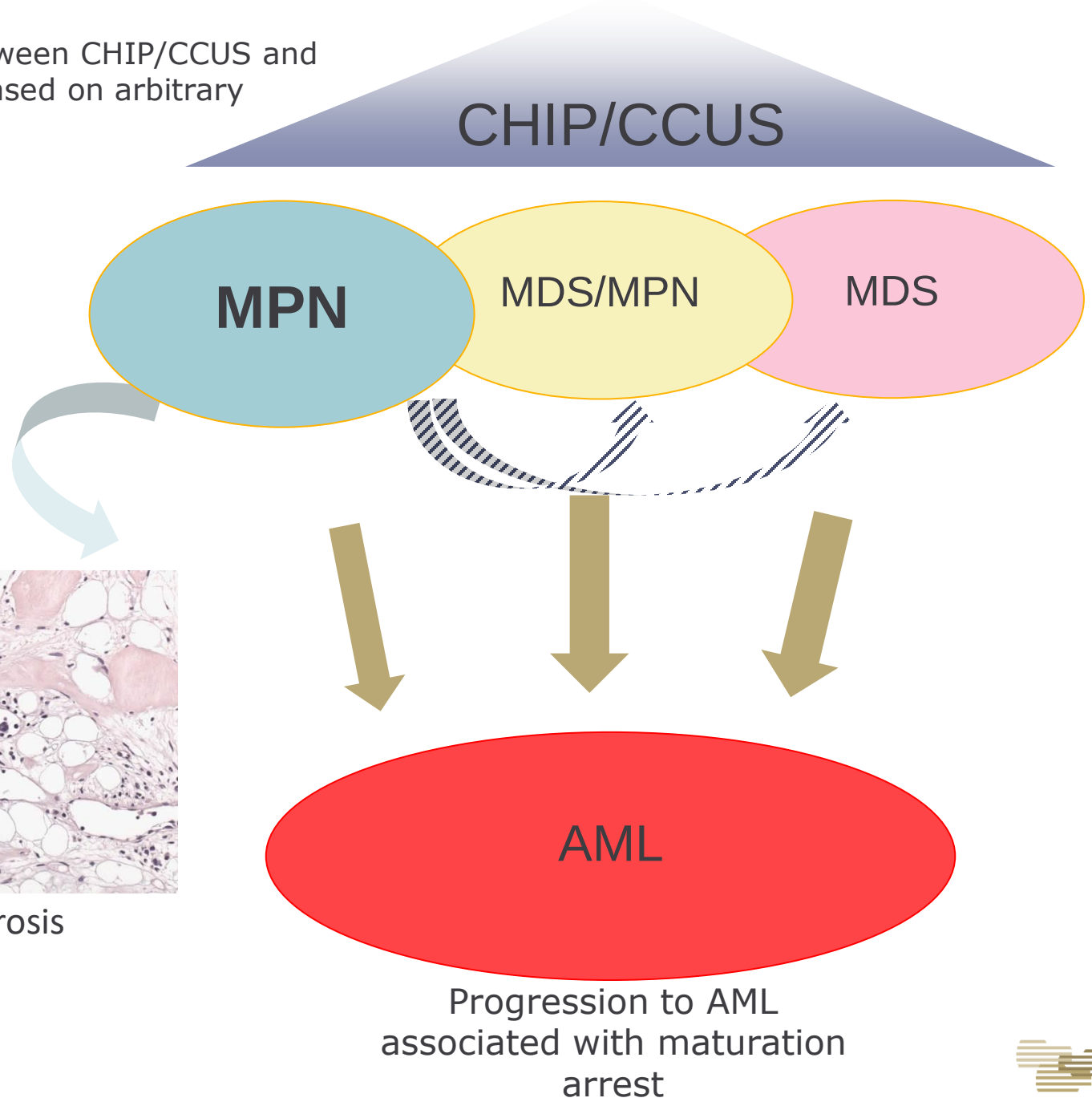
Progression traditionally defined as either myelofibrosis or AML transformation

AML transformation in 20% of PMF and 2.5% of ET and PV after 10 y

Separation between CHIP/CCUS and manifest MN based on arbitrary criteria



Myelofibrosis



Progression to AML associated with maturation arrest

Progression of MPN

Clinical progression

- Increasing hepatosplenomegaly
- Increasing cytopenia
- Change of PB features simulating other myeloproliferative neoplasms – leukocytosis, monocytosis
- Constitutional symptoms

Morphological progression

- Increasing fibrosis +/- osteosclerosis
- Increase in blasts – acute leukemia
- Other changes (dysplasia, CNL/CMML-like features etc.)

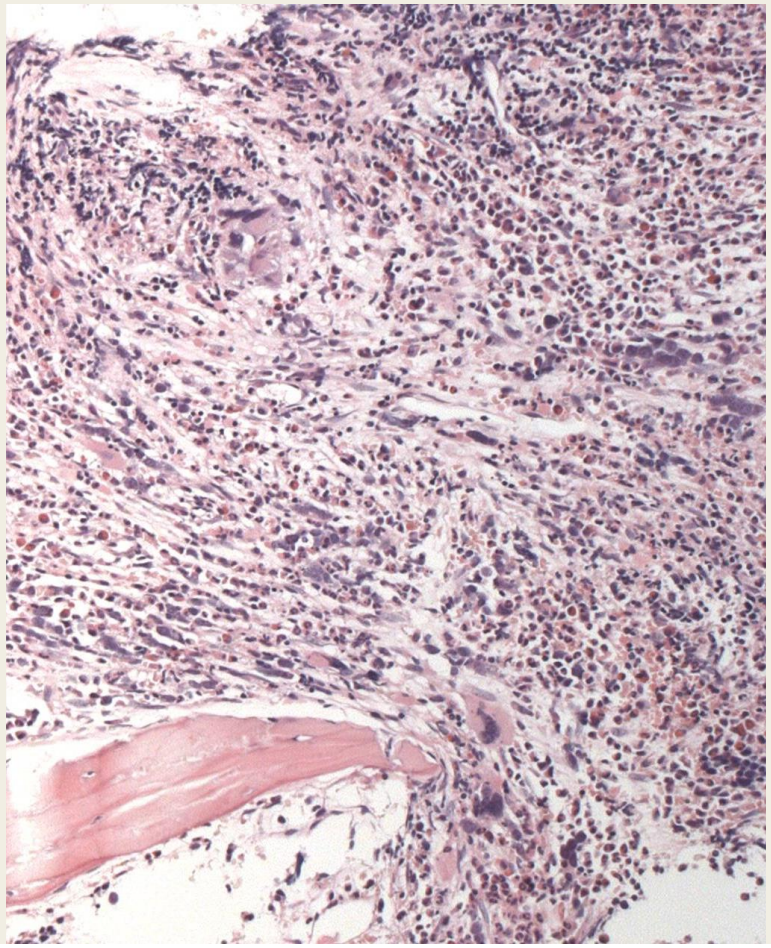
Genetic/molecular progression

- Clonal evolution with acquisition of secondary genetic alterations – clonal outgrowth

Post-PV and Post-ET MF

Late event (>10-15 y) in 10-15% of PV and ET patients after 15y
Parallel increase in cytogenetic alterations (+8 ,+9, del20q, -7,7q del13q, del9p)
Distinct morphological features compared to PMF

- Less atypical megakaryocytes
- Higher cellularity
- More cytogenetic aberrations



Post-PV MF
Required criteria 1. Previous established diagnosis of PV 2. Bone marrow fibrosis of grade 2 or 3 Additional criteria 1. Anemia (ie, below the reference range given age, sex, and altitude considerations) or sustained loss of requirement of either phlebotomy (in the absence of cytoreductive therapy) or cytoreductive treatment for erythrocytosis 2. Leukoerythroblastosis 3. Increase in palpable splenomegaly of >5 cm from baseline or the development of a newly palpable splenomegaly 4. Development of any 2 (or all 3) of the following constitutional symptoms: >10% weight loss in 6 mo, night sweats, unexplained fever (>37.5°C)
The diagnosis of post-PV MF is established by all required criteria and at least 2 additional criteria

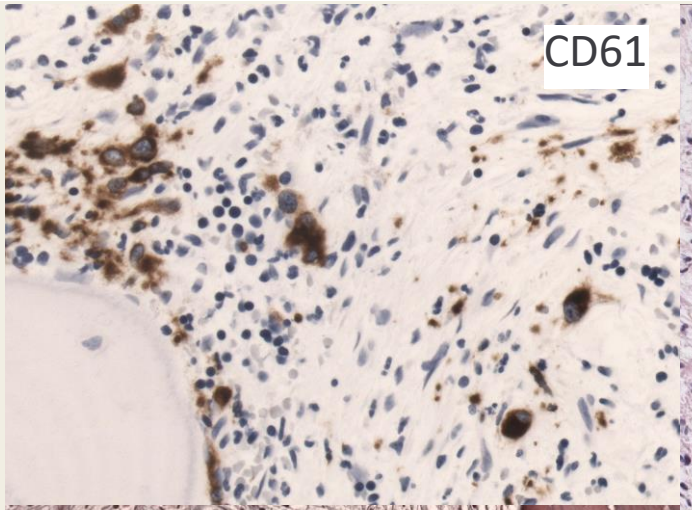
Non-classical forms of MPN progression

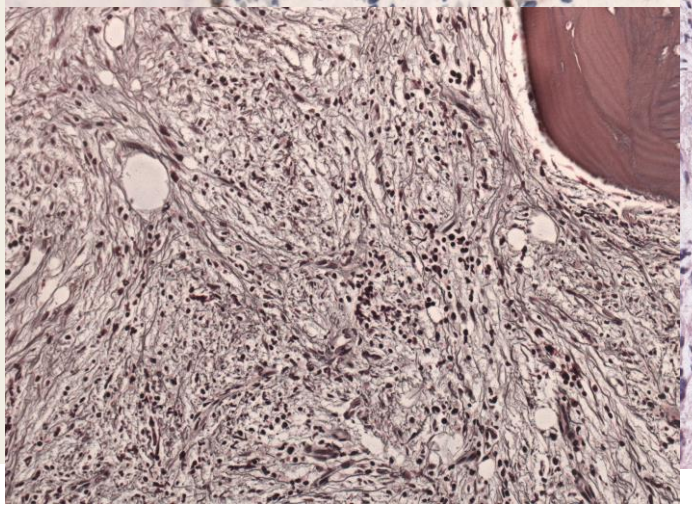
14-year history of ET, new pancytopenia

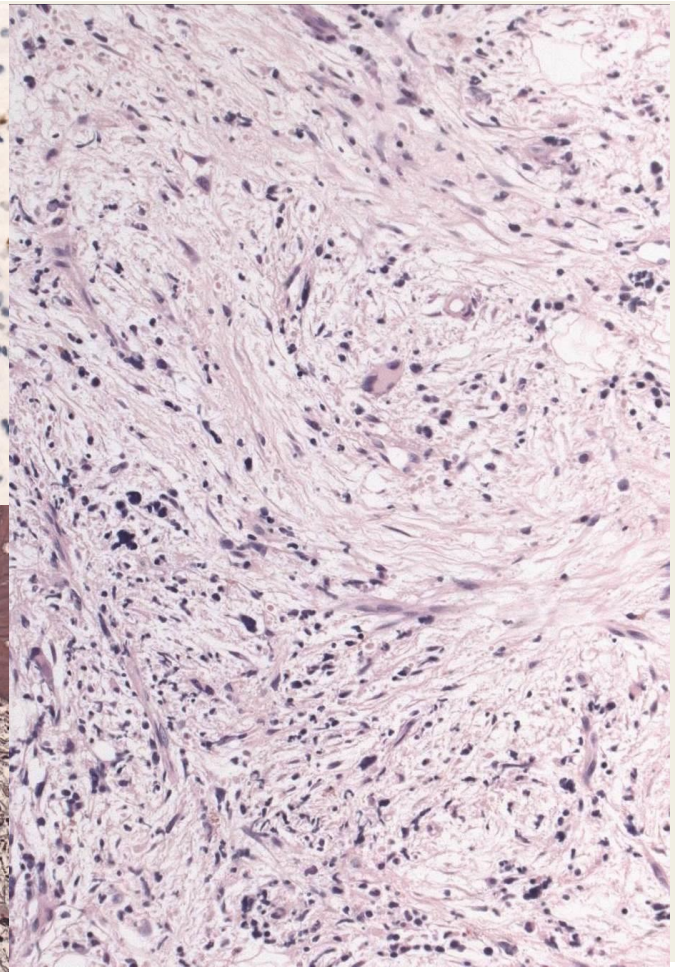
In addition to secondary myelofibrosis and blastic phase, other types of progression can rarely be observed

- MDS-like
  - Dwarf megakaryocytes, increase in blasts
- CNL/aCML/CMML-like
  - massive increase in maturing neutrophils and PB leukocytosis
  - Increase in monocytes

BM histology is an important aspect of monitoring progression and transformation due to frequent dry tap

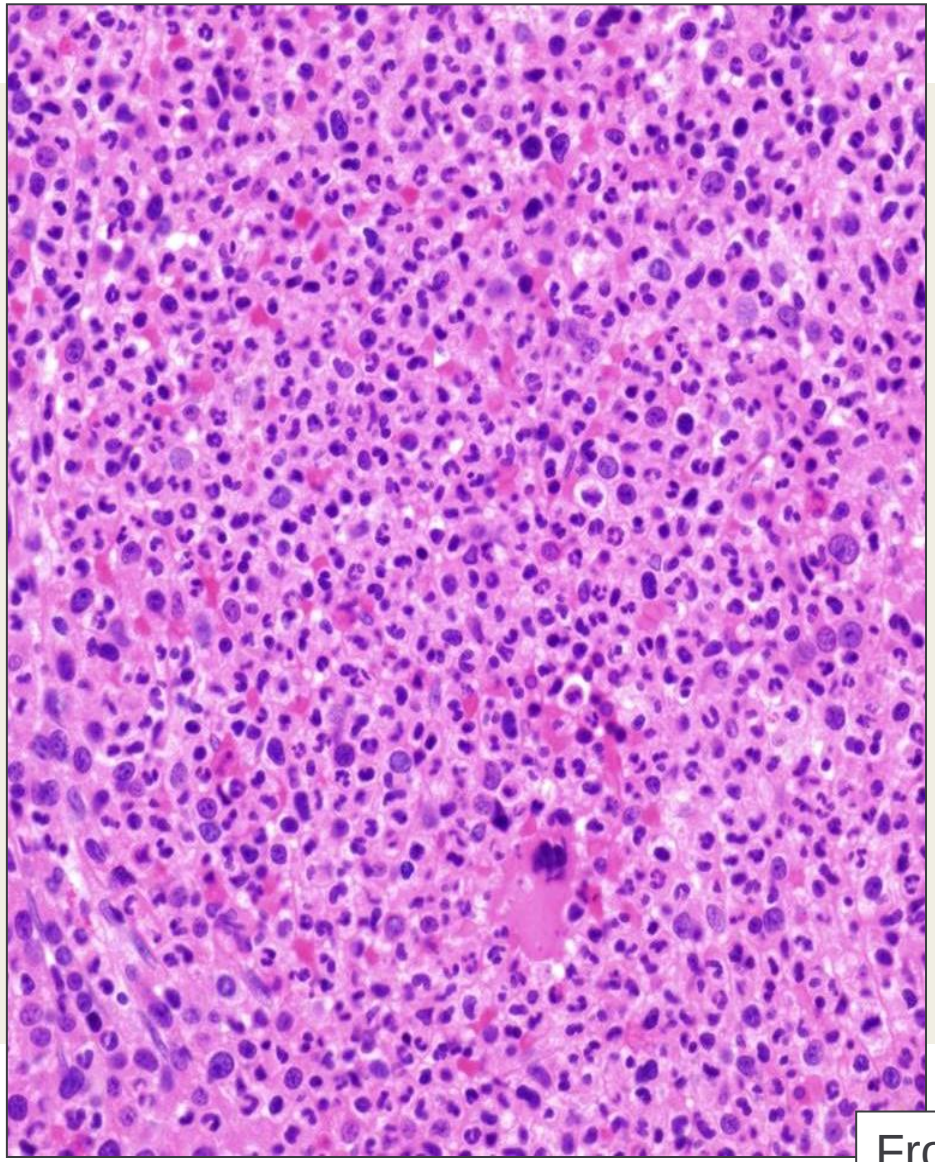


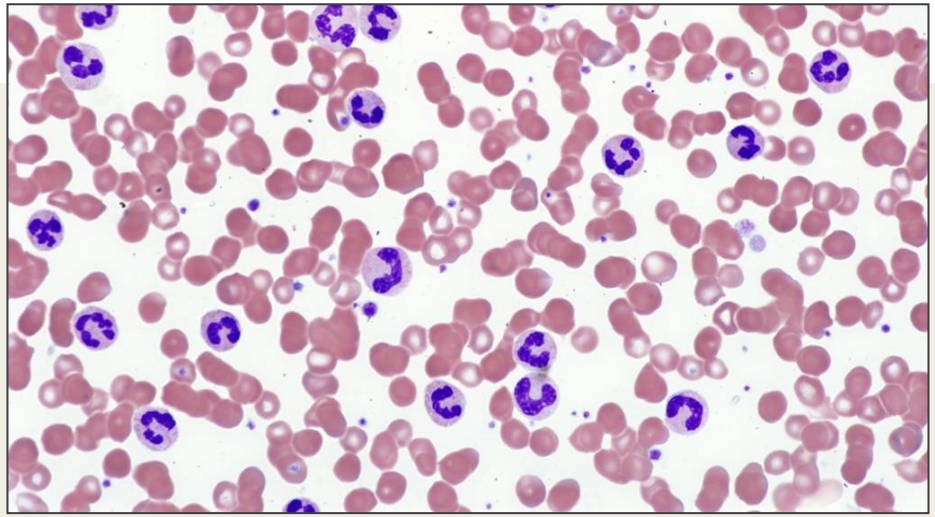


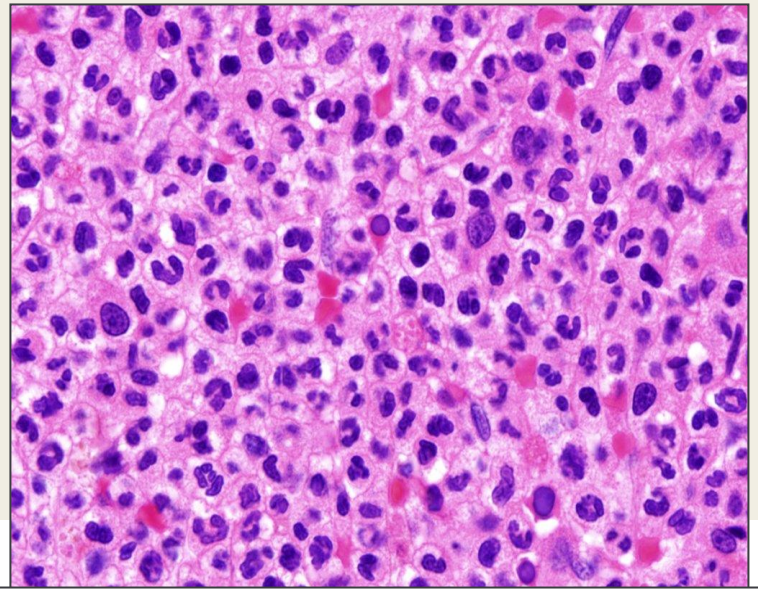


Boiocchi L et al. Mod Pathol. 2013 Federmann B et al, Hum Pathol 2014, Boiocchi L et al. Mod Pathol 2015

Non-classical forms of progression







From Boiocchi et al, Hum Pathol 2017

Post-polycythemic myelofibrosis associated with marked persistent neutrophilic leukocytosis, consistent with progression (neutrophilic type disease progression)

Poor prognosis

No *BCR::ABL1*

The role of genetics in progression of MPN

Types and composition of genetic alterations determine phenotype

Acquisition/outgrowth of additional alterations are responsible for disease progression

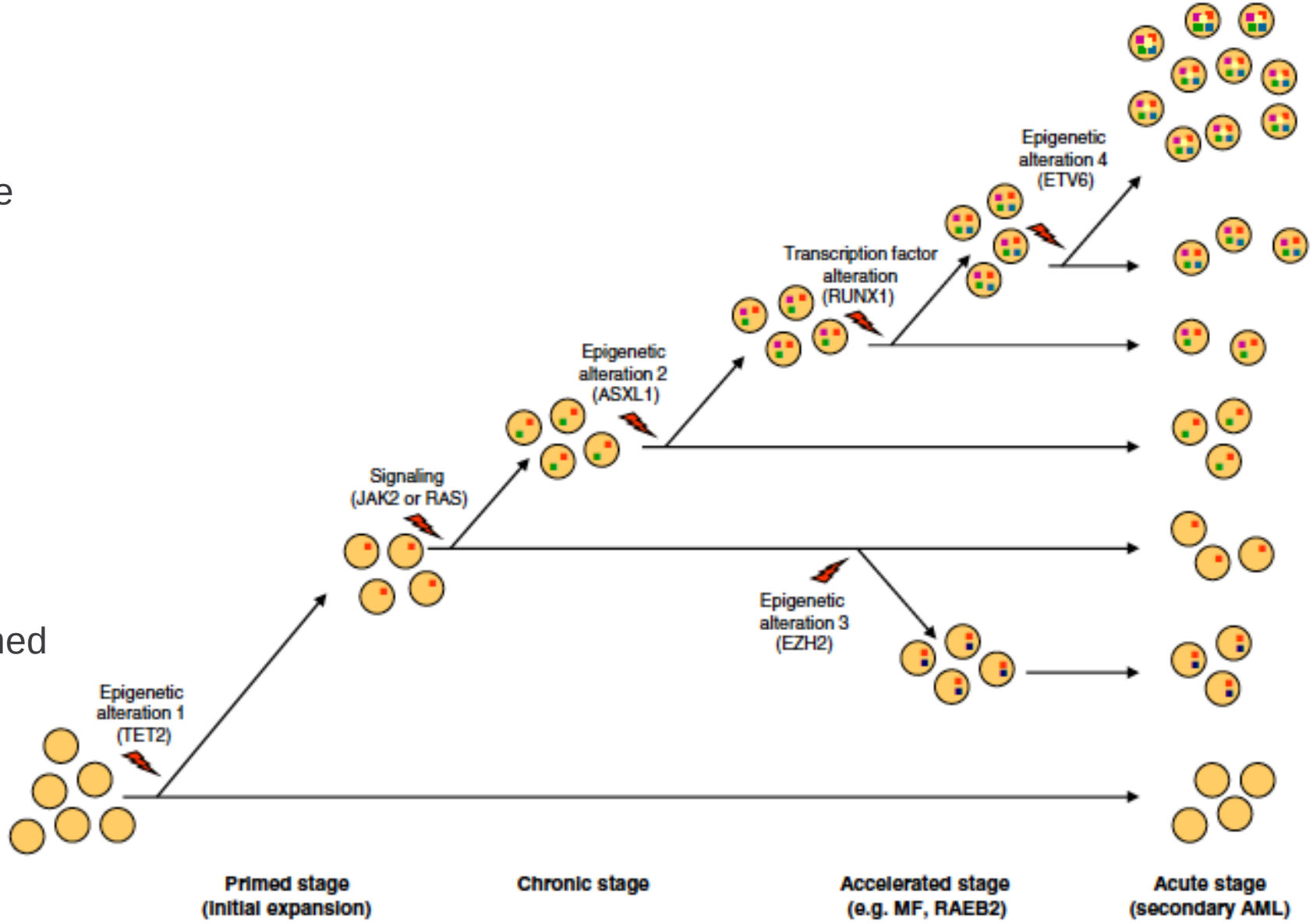
Mixture of strong and weak drivers, modifiers and initiating mutations

Sequence of mutations relevant for disease evolution (*TET2 ->JAK2* vs. *JAK2 -> TET2)*

sAML may be *JAK2-*

TP53 mutations common in transformed MPN

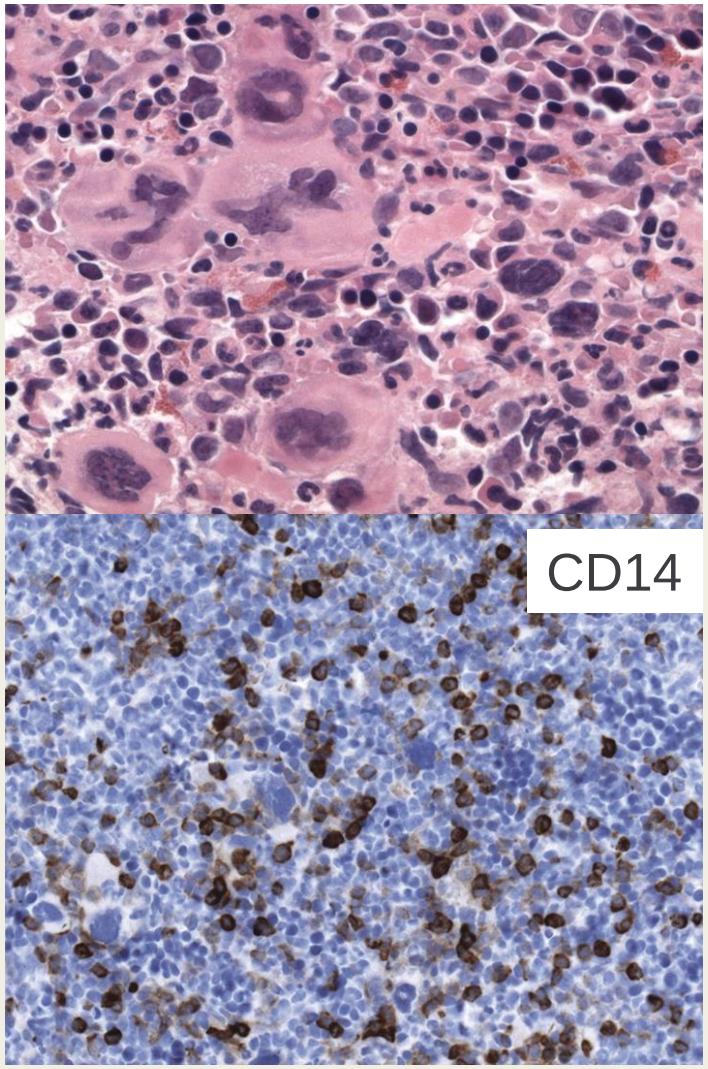
Differences in early vs late transformation



Brune et al, Cancers 2021, ; Luque Paz et al, Blood Adv 2020; Murati et al, 2012

Current molecular diagnostics in MPN

PB: CMML? 12% and 1500 Mono



Identification of driver mutations and disease modifiers

Sensitivity in the 1% range

Stepwise approach feasible for uncomplicated cases with identification of driver mutations first (e.g. *JAK2* V617F)

Look for non-canonical mutations in „triple negative“ cases

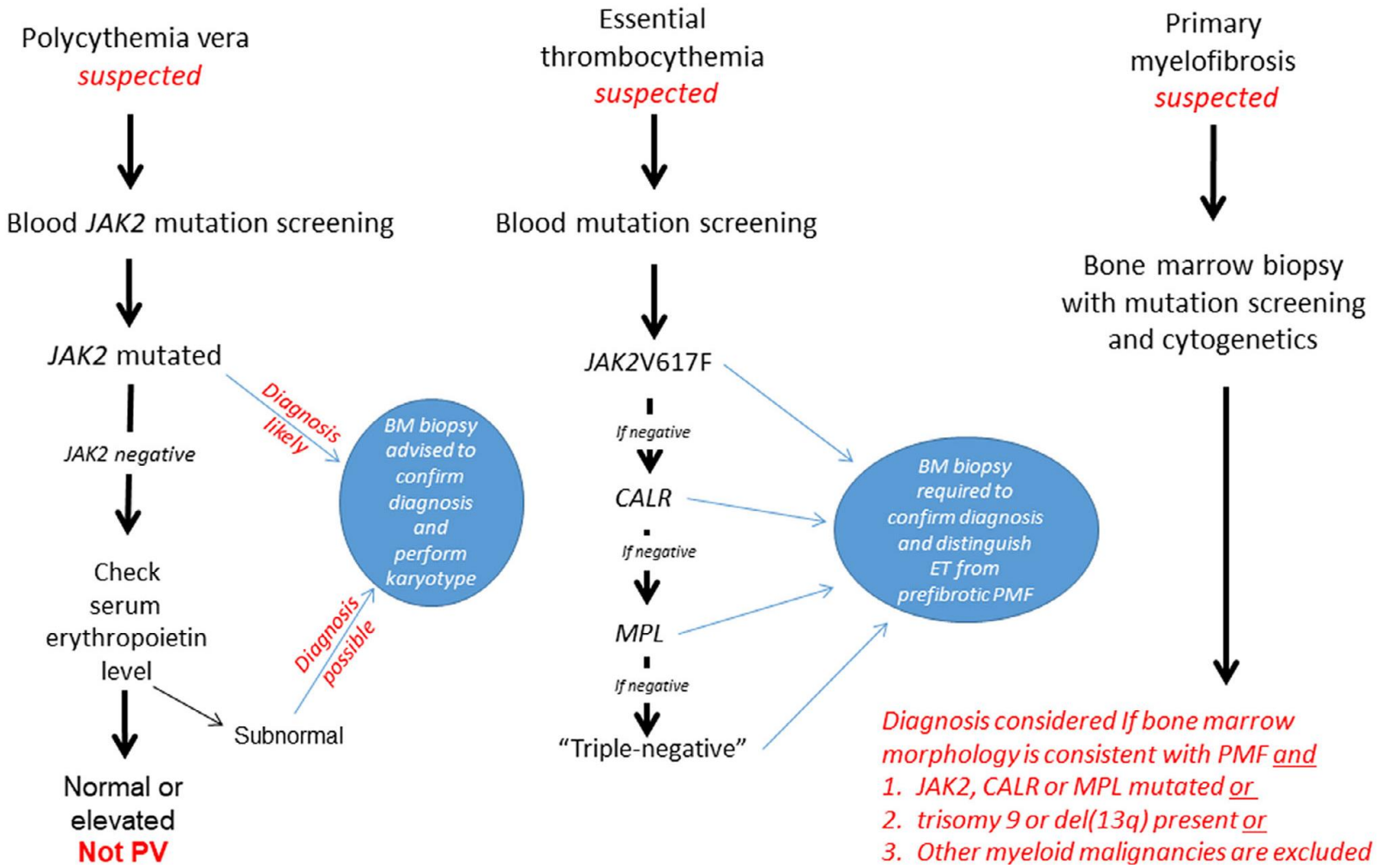
Look for double mutants, if low VAF present

MPN and mastocytosis‡	*ASXL1, CALR, CBL, CSF3R, DNMT3A, EZH2, IDH1, IDH2 JAK2§, KIT, KRAS, MPL, NRAS, PTPN11, RUNX1, SETBP1, SF3B1, SH2B3, SRSF2, TET2, U2AF1, ZRSR2*	*BCR::ABL1§*

Diagnosis of Ph- MPN

Diagnosis of MPN relies on a combination of laboratory and clinical features, detection of driver mutations and BM morphology

BM biopsy remains important for subclassification and assessment of fibrosis



Tefferi & Barbui, AJH 2021

Thank you!