European Association for Haematopathology

Integrative approach for the diagnosis of myeloproliferative neoplasms

Hans Michael Kvasnicka
Wuppertal, Germany

DISCLOSURES

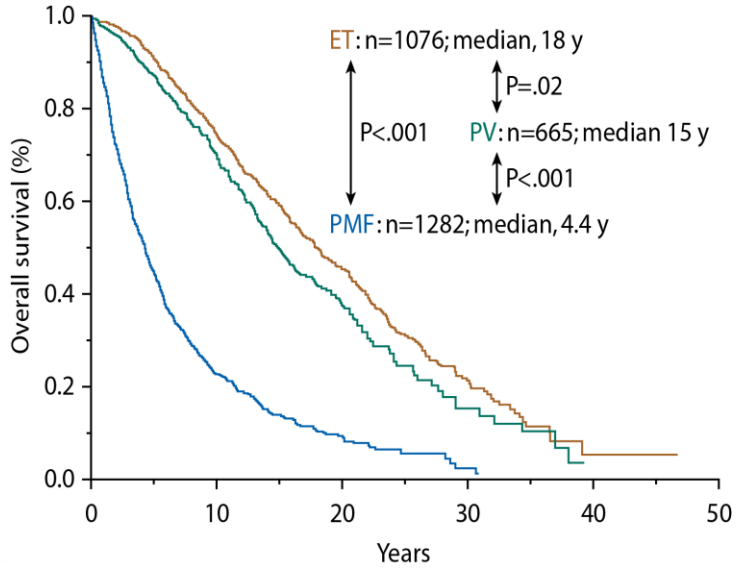
I have no actual or potential conflict of interest in relation to this presentation

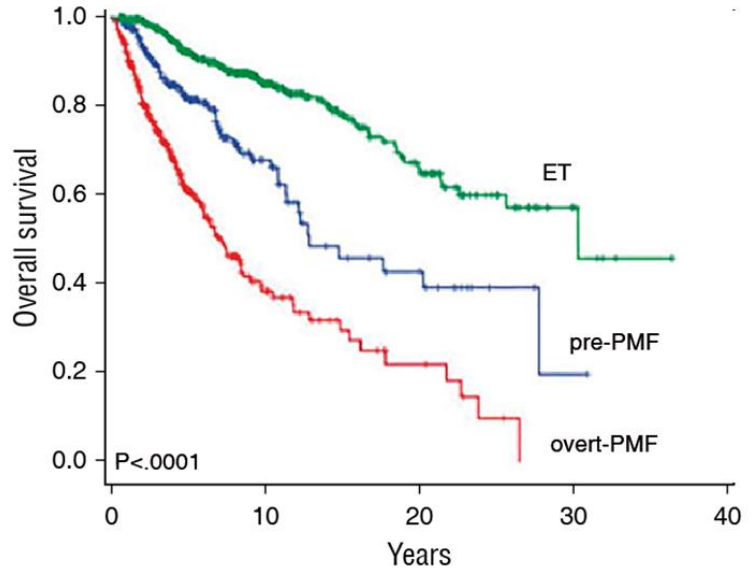
Myeloproliferative neoplasms

- Essential thrombocythemia
- Polycythemia vera
- Primary myelofibrosis
  - Prefibrotic/early primary myelofibrosis
  - Overt primary myelofibrosis
- Myeloproliferative neoplasm, unclassifiable

- Chronic neutrophilic leukemia
- Chronic eosinophilic leukemia, NOS

shifting balance between morphology and genetic features as leading parameter for classification





Barbui et al., J Clin Oncol. 2011;29:761-770
Barbui et al., J Clin Oncol. 2011;29:3179-3184
Guglielmelli et al., Blood 2017 129:3227-3236
Szuber et al., Mayo Clin Proc (2019) 94:599-610

Presentation of MPN subtypes at time of diagnosis

Symptoms

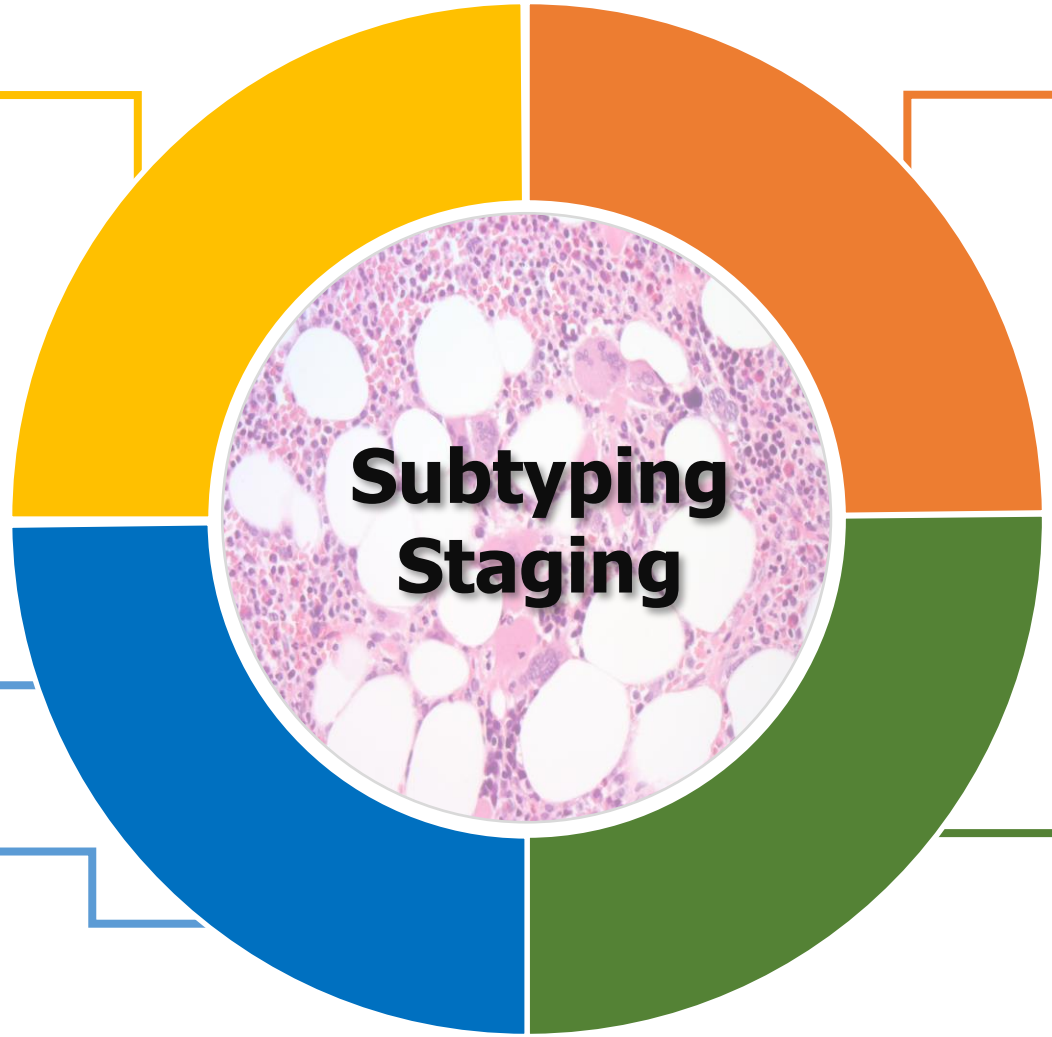
Night sweats
Weight loss
Fever
Fatigue
Itching

Blood counts & PB

Thrombocytosis
Erythrocytosis
Leukocytosis
Monocytosis
Blasts

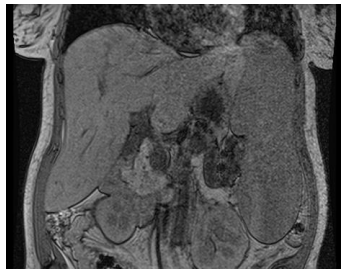
Serum markers

LDH
EPO
Vitamin B12
Tryptase



Organ involvement

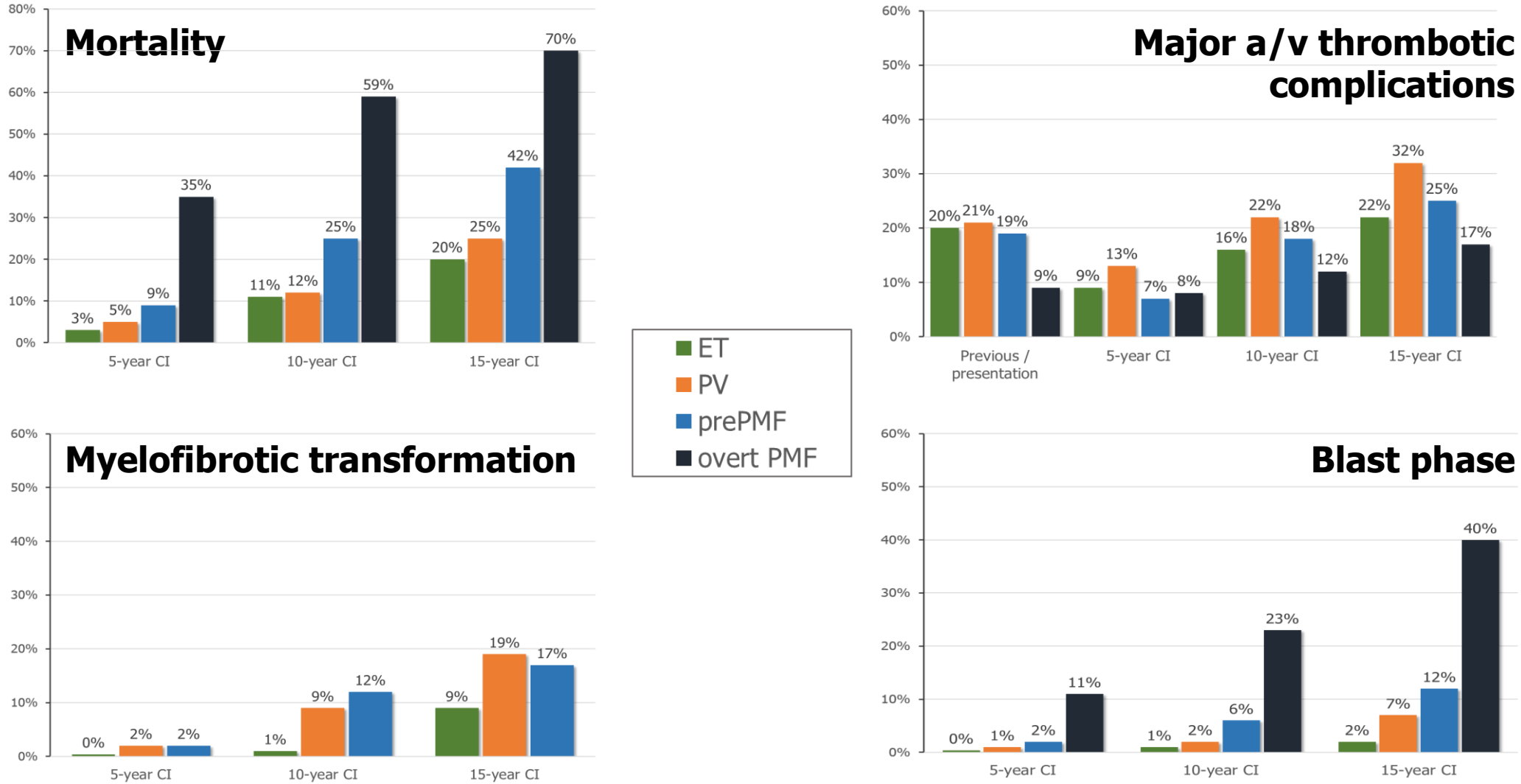
Splenomegaly



Molecular genetics

Driver Mutations
Adverse variants
Cytogenetics
Fusion genes

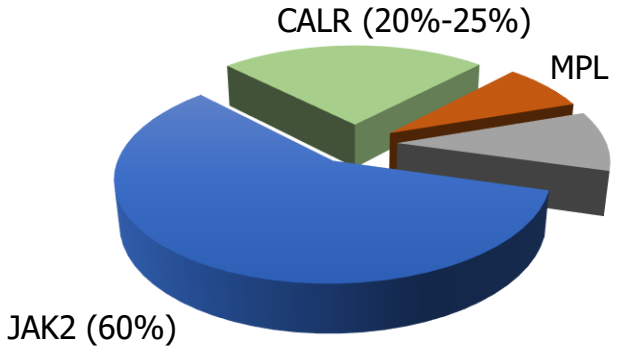
Prevalence of previous events and cumulative incidence during follow-up in ET and other MPN subtypes



Barbui et al. Blood Cancer Journal (2018) 8:15

The current genetic landscape concerning phenotypic driver mutations in MPN

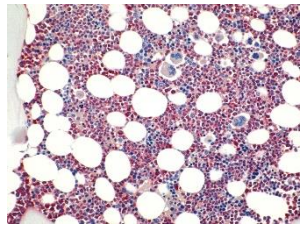
ET



5%-10%

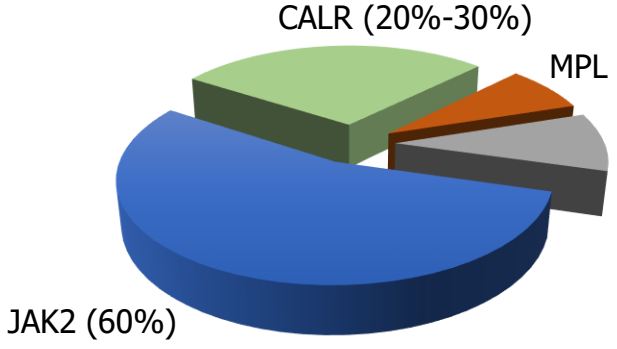
'wild-type'

clonal / unknown



exclude reactive conditions

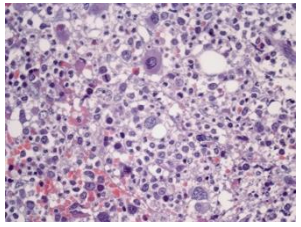
PMF



5%-10%

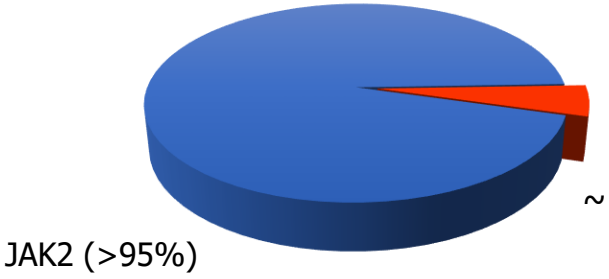
'triple-negative'

clonal / unknown



exclude MDS/MPN or MDS

PV



- be aware of the sensitivity and limitations of the techniques used in the molecular diagnosis of patients with erythrocytosis
- JAK2-negative cases by conventional mutational screening may harbour compound or rare mutations in exon 14 or small exon 12-mutated clones
- investigate for mutations in genes involved in other forms of erythrocytosis (EPOR, VHL, PHD2, HIF2A, etc.)

Vannucchi AM, et al. CA Cancer J Clin. 2009; 59:171-91; Akada H, et al. Blood. 2010;115:3589-97; Harrison C & Vannucchi A, Blood 2016;127:276-278

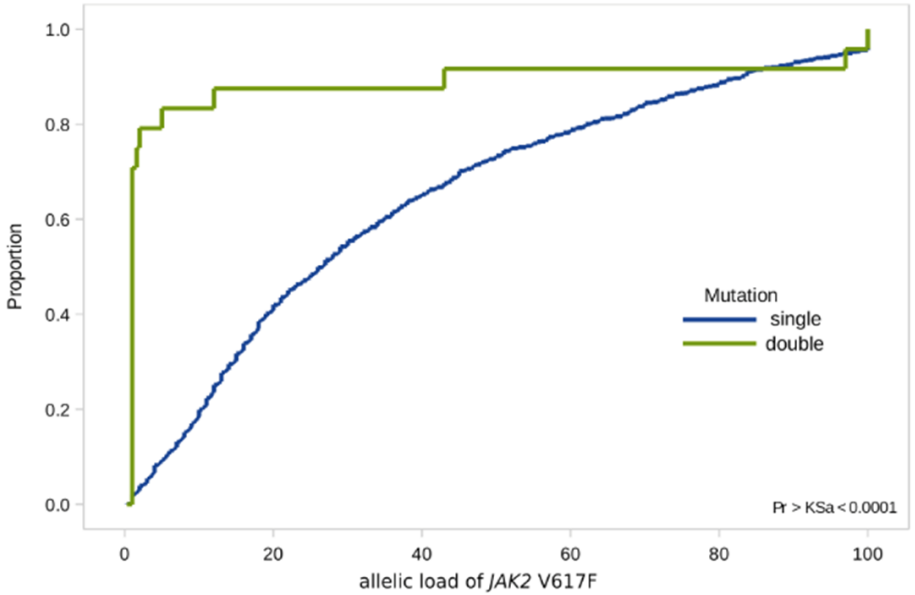
Sources of DNA for driver mutation analysis

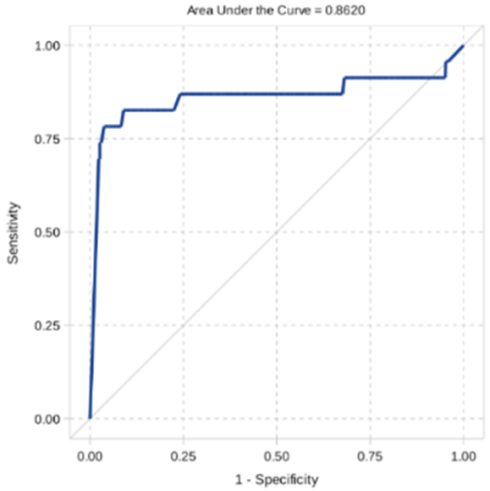
- The optimal source for searching driver mutations in the suspicion of MPN is peripheral blood, purified granulocytes.
- Most routine laboratories use whole bood as the source for DNA preparation. This may lead to under-estimation of the VAF and potentially expose to false negativity results in case analytical methods with a sensitivity of >3% are used.
- This especially applies to JAK2V617F, since most MPL and CALR VAFs are above 5%

Guglielmelli P et al, Leuk Res 2017; 58:63

Type of assay for driver mutation analysis

- a variety of tests used, mostly single target (PCR, ddPCR)
- increasing use of multi-target panel (NGS)
- either approach is acceptable, if laboratory turnaround times and assay sensitivity are satisfactory (e.g., detection of 1-3% VAF or lower for JAK2V617F)





A JAK2V617F VAF <1% should prompt search for CALR (MPL) mutations

Of 34 pts with *JAK2*VF VAF <1% (**4.7% of 787 cases**), **47%** had CALR mutation, compared to **0.9%** of 753 with VAF >1%.

Makarik TV et al, Genes 2021; 12:559

Prevalence and phenotypes of JAK2 V617F and calreticulin mutations in the general population

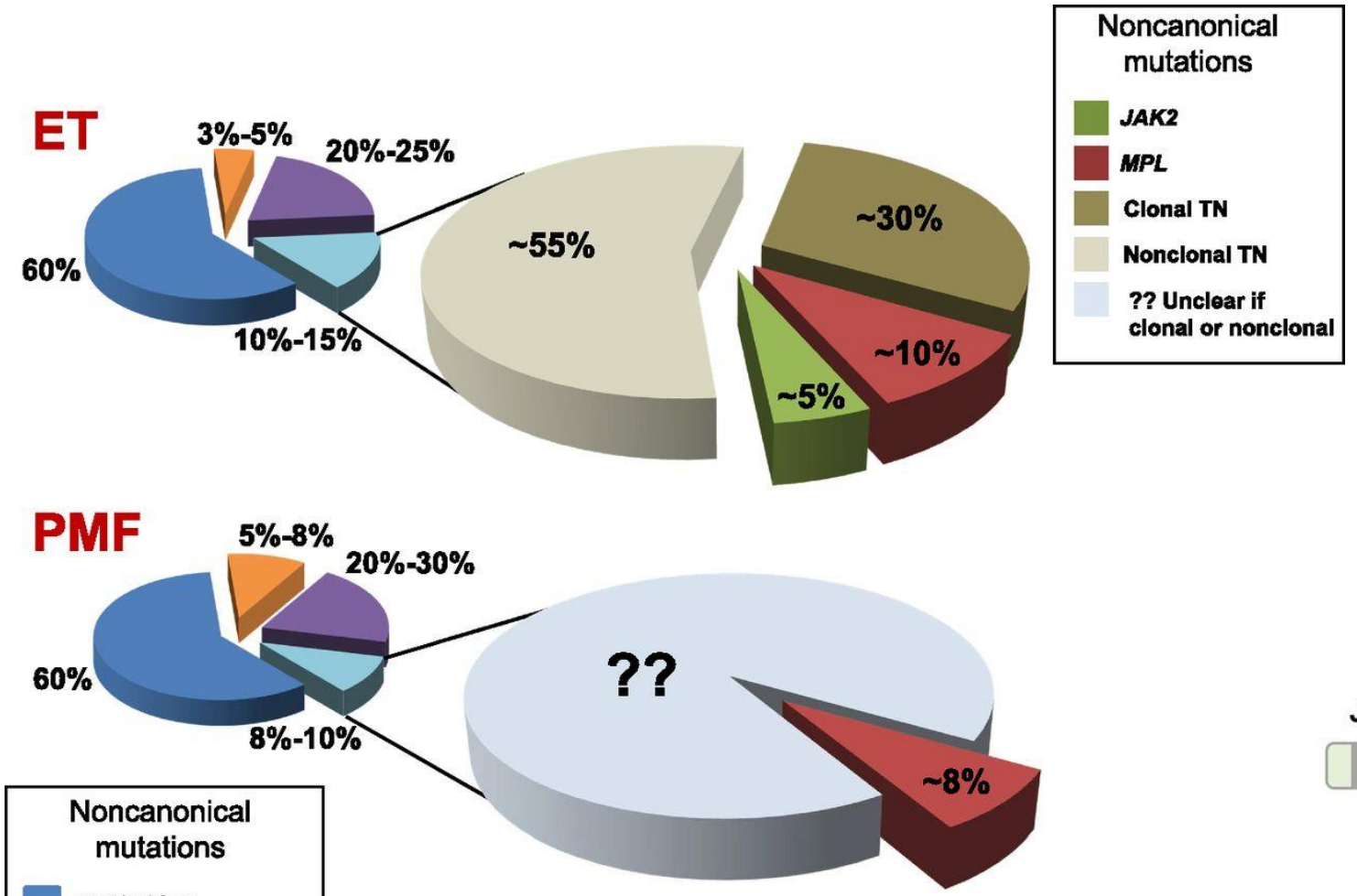
- CALR mutations are prevalent in the general population but are much less frequent compared with the estimated JAK2 V617F prevalence
- JAK2 V617F and CALR mutations in the general population are linked to a distinct blood count profile, also in the absence of MPN diagnosis

Allele burden according to mutation type

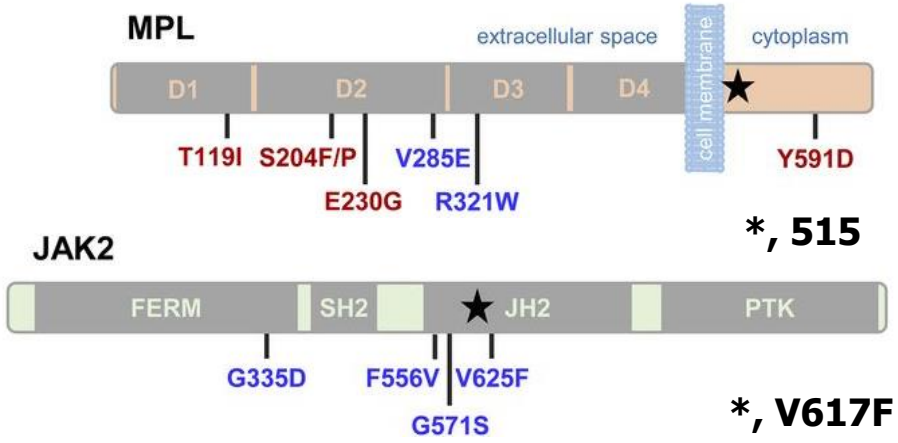
	JAK2 V617F	*CALR*	
		Type 1	Type 2
Number	613	24	8
Allele burden, %			
Mean (SE), range	2.1 (0.34), 0.010-96	6.2 (2.3), 0.020-44	11 (5.9), 0.013-38
<0.1, n (%)	255 (42)	5 (21)	1 (13)
0.1-0.99, n (%)	253 (41)	9 (38)	4 (50)
1-10, n (%)	75 (12)	4 (17)	0
>10, n (%)	30 (5)	6 (25)	3 (38)

Cordua et al., Blood (2019) 134:469-479

Design of mutation assay: beyond the hotspots

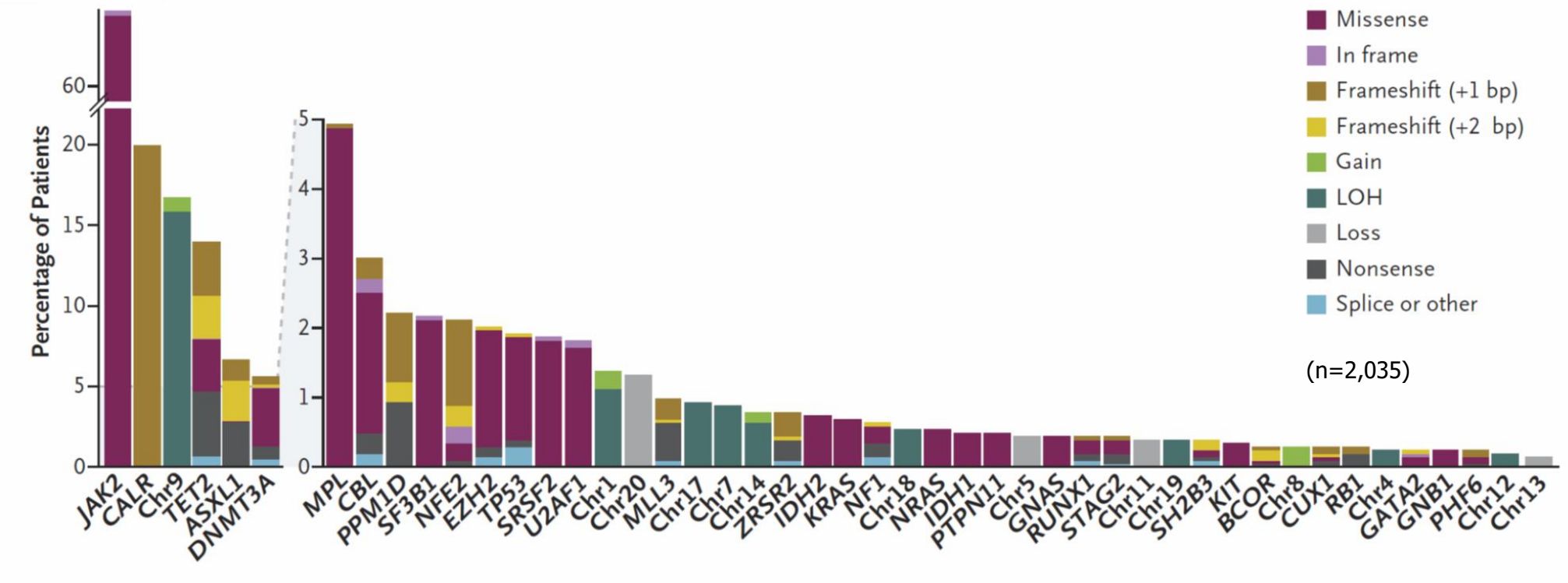


"Non Canonical" JAK2 and MPL Mutation in TN ET and PMF



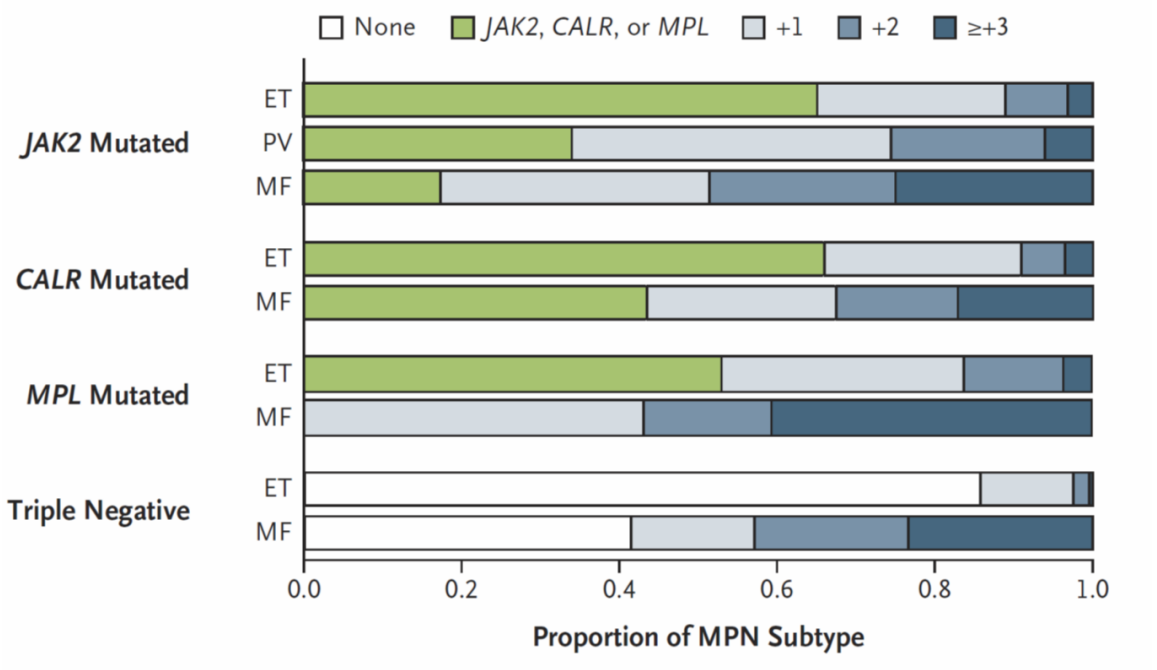
Harrison CN, Vannucchi AM. Blood, 2016, 127:3
Milosevic Feenstra J et al. Blood 2016;127:325-332

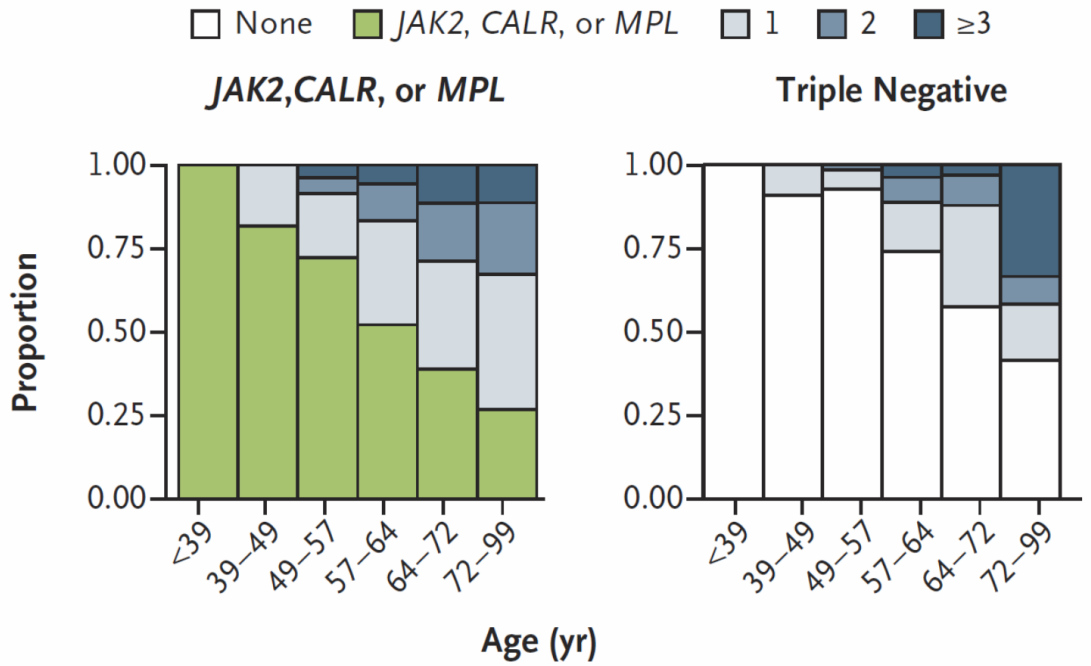
Frequency of recurrently mutated genes and chromosomal abnormalities in MPN



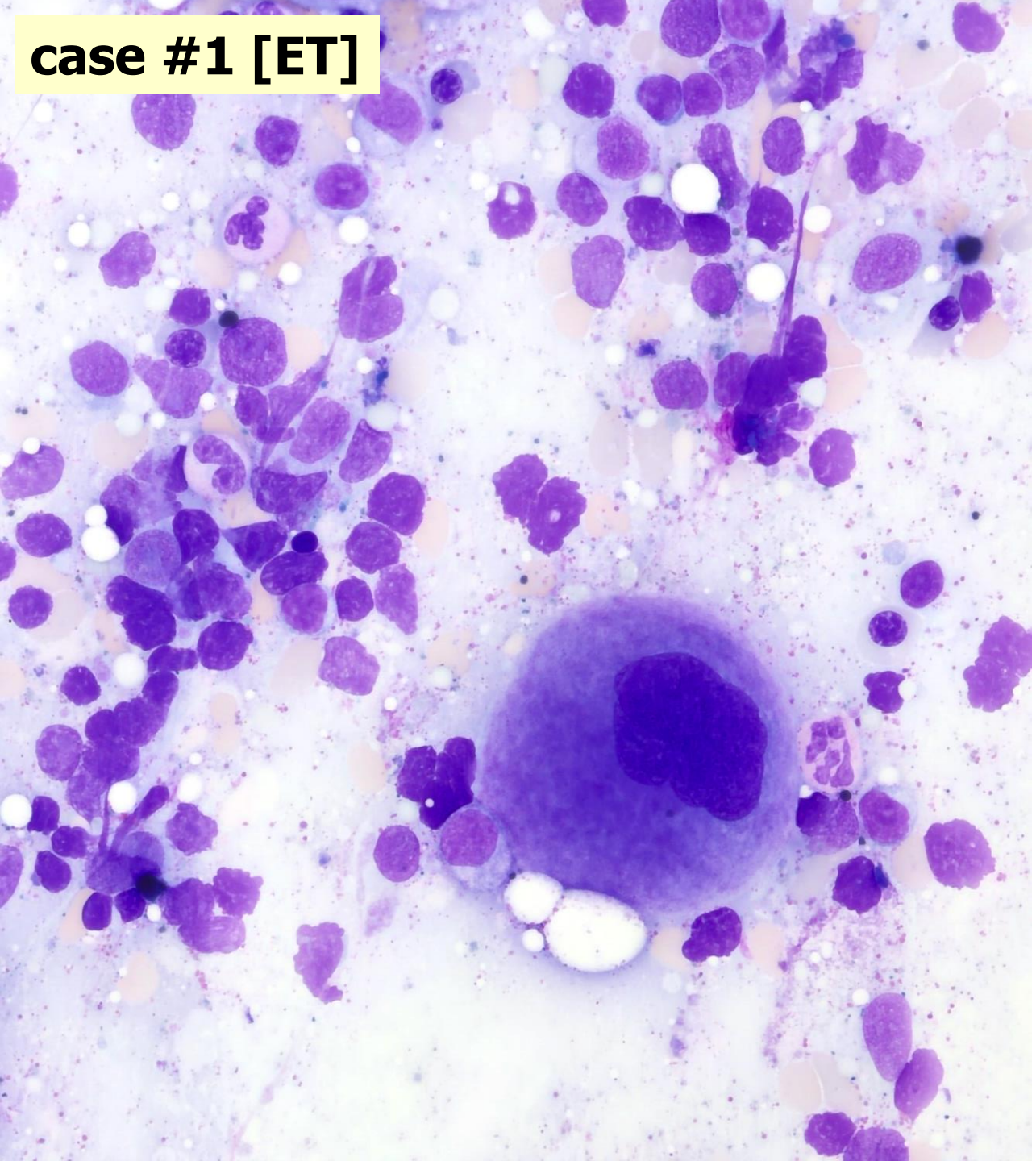
Grinfeld J et al. N Engl J Med 2018;379:1416-1430

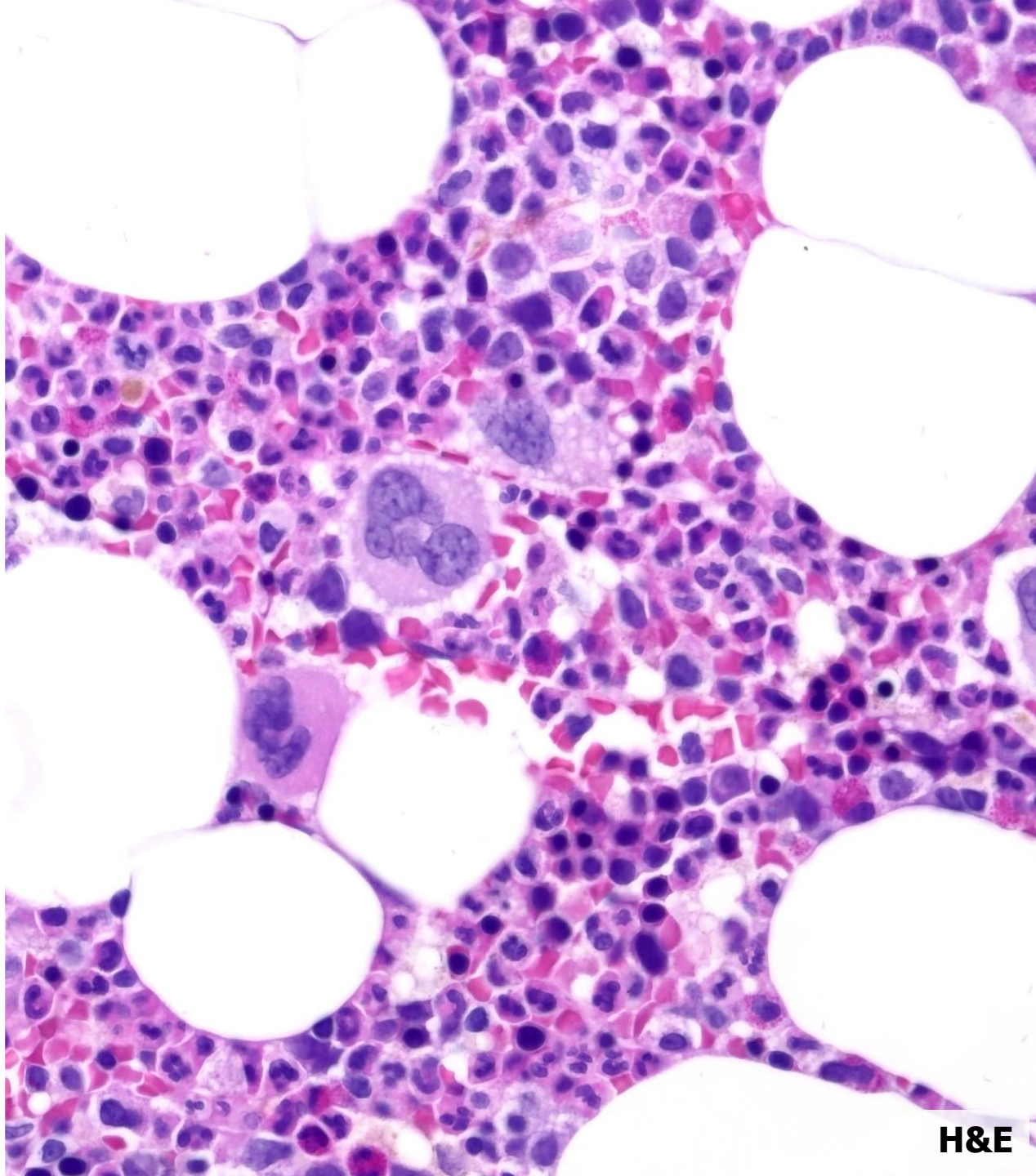
Frequency of driver mutations and additional chromosomal changes in molecular subtypes of MPN

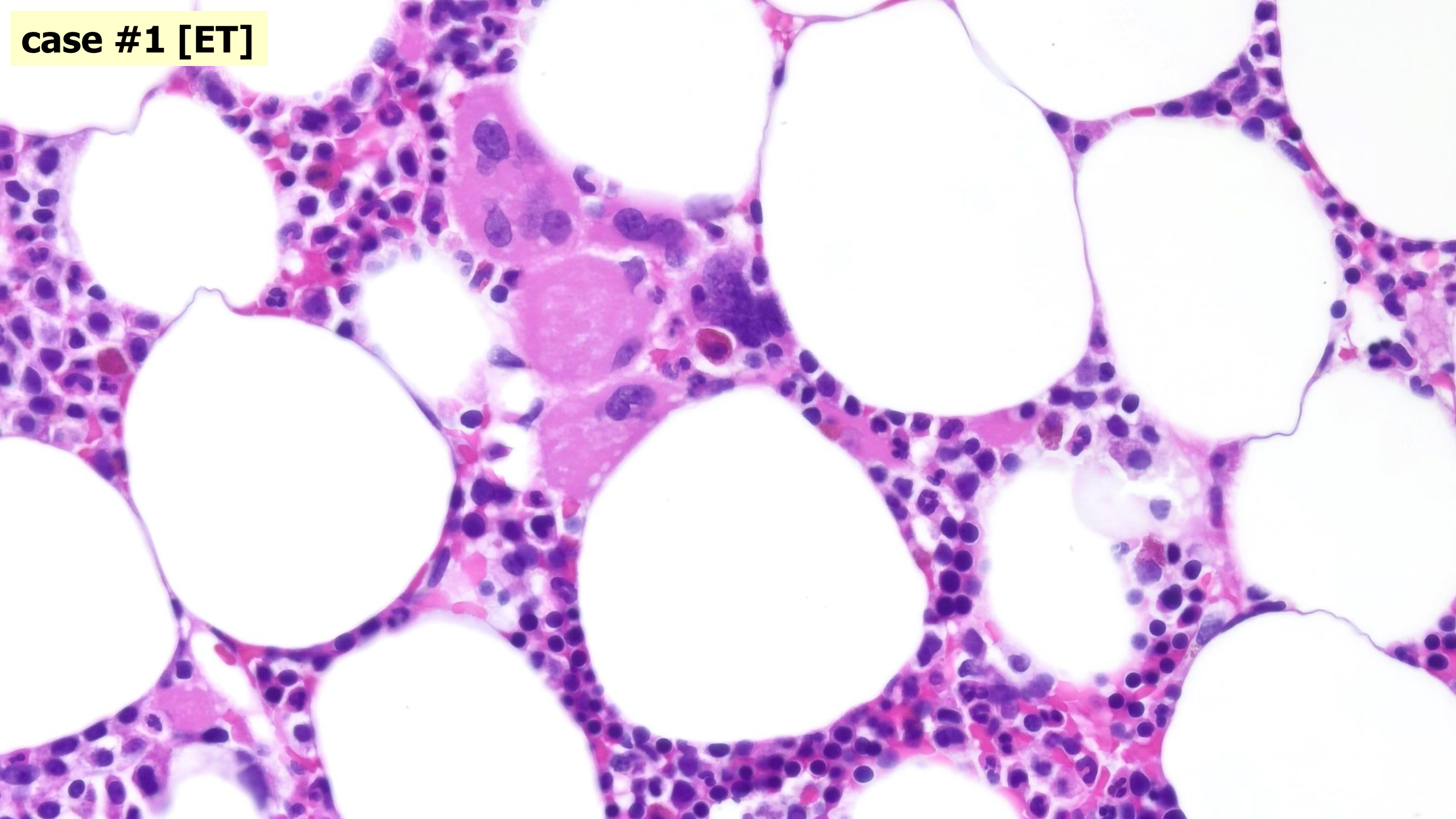




Grinfeld J et al. N Engl J Med 2018;379:1416-1430


case #1 [ET]


H&E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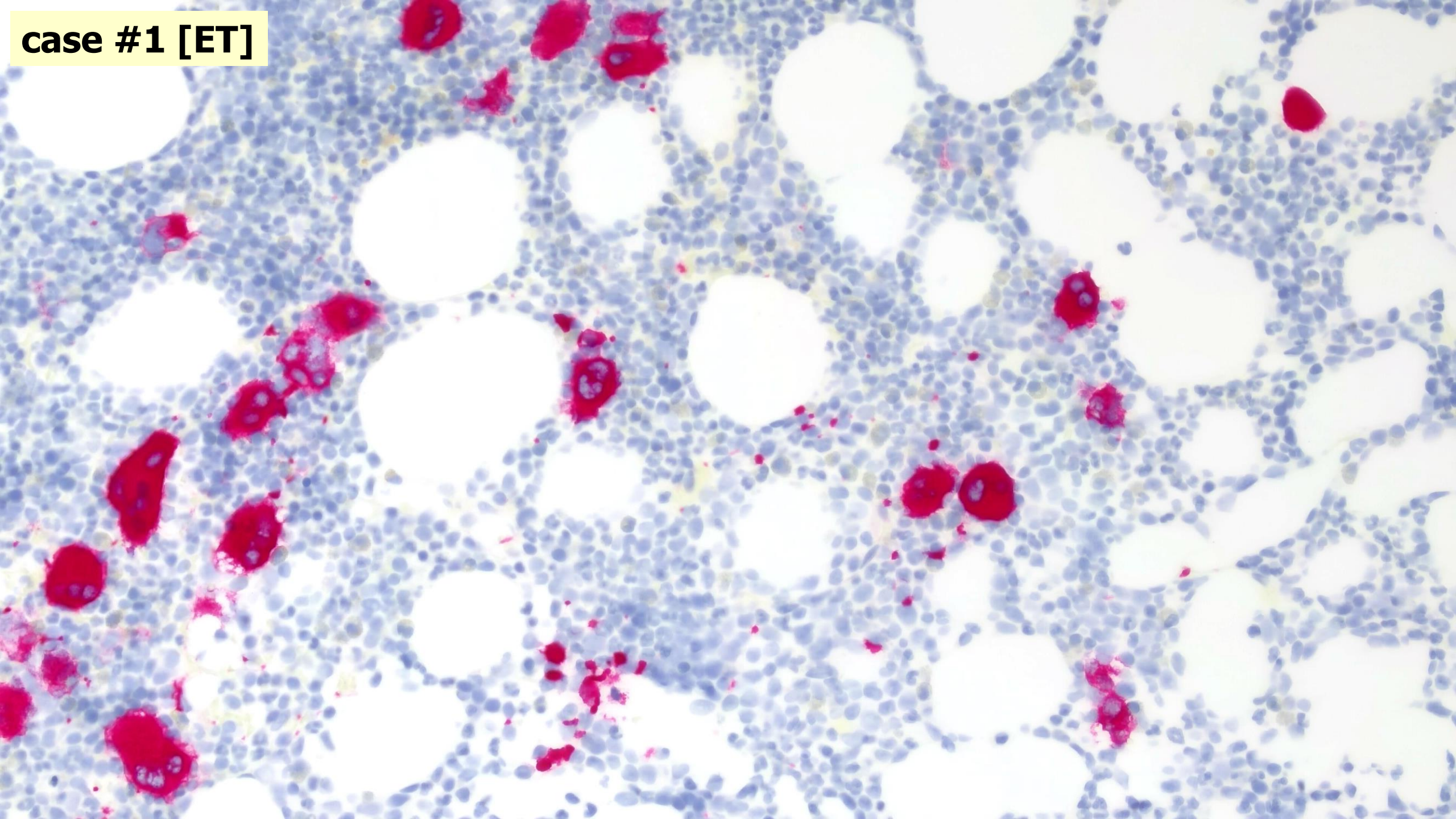

case #1 [ET]

ICC diagnostic criteria for ET and Post-ET MF

Essential thrombocythemia (ET)	Post-ET MF
Major criteria 1. Platelet count ≥ 450 x 10^9/L 2. JAK2, CALR, or MPL mutation[a)] 3. 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[b)]; no significant increase or left shift in neutrophil granulopoiesis or erythropoiesis; no relevant BM fibrosis[c)] 4. Diagnostic criteria for BCR::ABL1-positive chronic myeloid leukemia, polycythemia vera, primary myelofibrosis, or other myeloid neoplasms are not met	**Required criteria** 1. Previous established diagnosis of ET 2. Bone marrow fibrosis of grade 2 or 3
Minor criteria 1. Presence of a clonal marker[d)] or Absence of evidence of reactive thrombocytosis	**Additional criteria** 1. Anemia (i.e., below the reference range given age, sex, and altitude considerations) and a >2 g/dL decrease from baseline hemoglobin concentration 2. Leukoerythroblastosis 3. Increase in palpable splenomegaly of >5 cm from baseline or the development of a newly palpable splenomegaly 4. Elevated lactate dehydrogenase (LDH) level above the reference range 5. Development of any 2 (or all 3) of the following constitutional symptoms: >10% weight loss in 6 months, night sweats, unexplained fever (>37.5 °C)
The diagnosis of ET requires either all major criteria or the first 3 major criteria plus the minor criteria	**The diagnosis of Post-ET MF is established by all required criteria and at least two additional criteria**

(a) It is recommended to use highly sensitive assays for *JAK2* V617F (sensitivity level <1%) and *CALR* and *MPL* (sensitivity level 1-3%) - in negative cases, consider to search for non-canonical *JAK2* and *MPL* mutations (b) **three or more megakaryocytes lying adjacent without other BM cells in between; in most of these rare clusters ≤ 6 megakaryocytes may be observed, increase in huge clusters (> 6 cells) accompanied by granulocytic proliferation is a morphological hallmark of prePMF** (c) very rarely a minor increase in reticulin fibers may occur at initial diagnosis (grade 1) (d) assessed by cytogenetics or sensitive NGS techniques

Clinical features and outcomes of CALR-mutant ET and MF

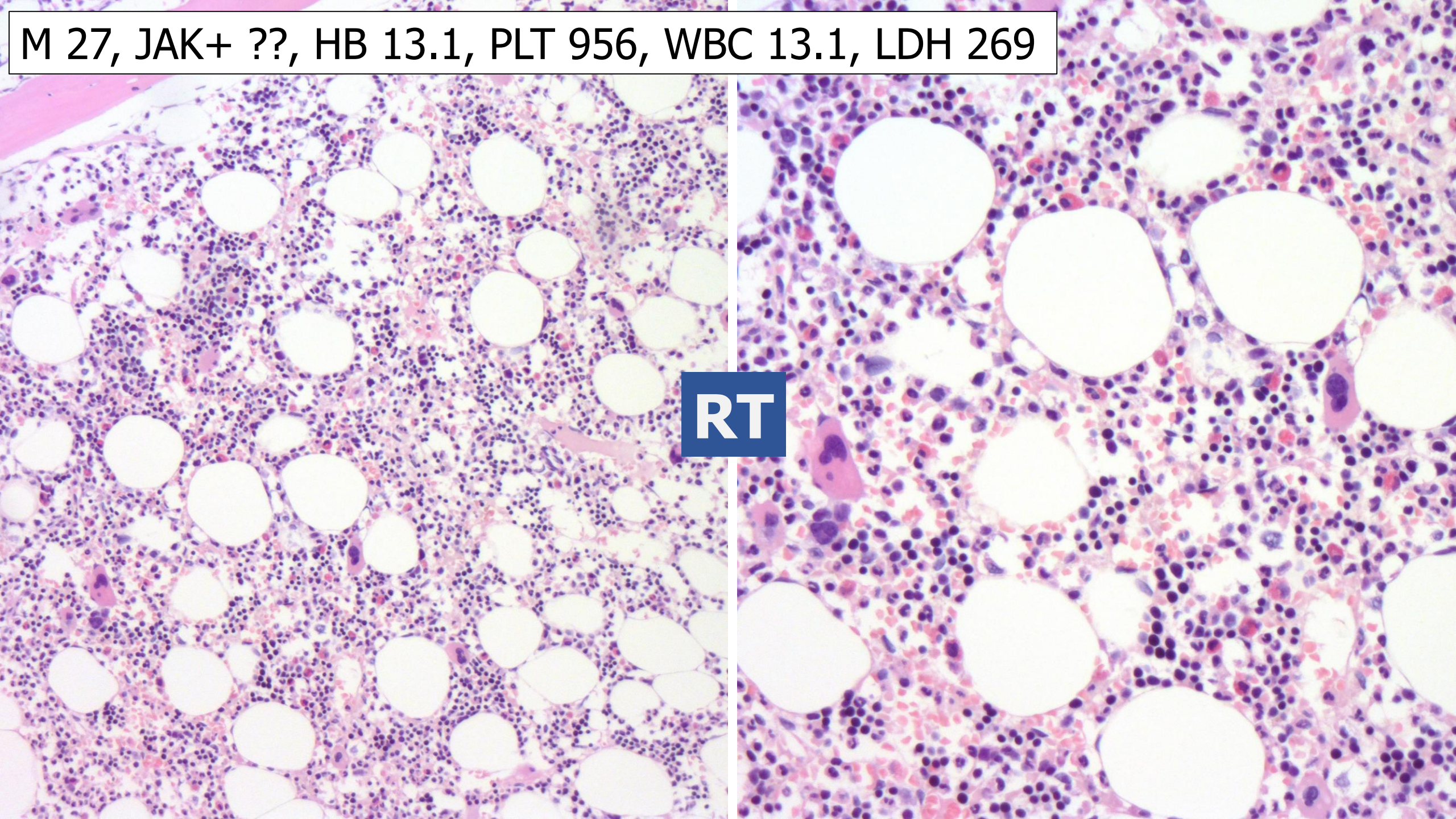
	CALR vs *JAK2*	*CALR* vs *MPL*	*CALR* vs triple negative
ET			
Clinical	Younger, male predominance, lower WBC count, lower Hg/Hct, higher platelets	Male predominance, otherwise similar	Male predominance, higher platelets
Thrombosis	Decreased risk	Decreased risk	Similar
Post-ET MF	Similar to increased	Similar	Similar
Overall prognosis	Similar	Similar	Similar
PMF			
Clinical	Younger, lower WBC count, higher Hg/Hct, higher platelets	Younger, higher Hg/Hct, higher platelets	Younger, higher Hg/Hct, higher platelets
Thrombosis	Similar, possibly decreased risk	Similar	Similar
Leukemic transformation	Similar	Similar	Improved
Overall prognosis	Improved*	Similar	Improved

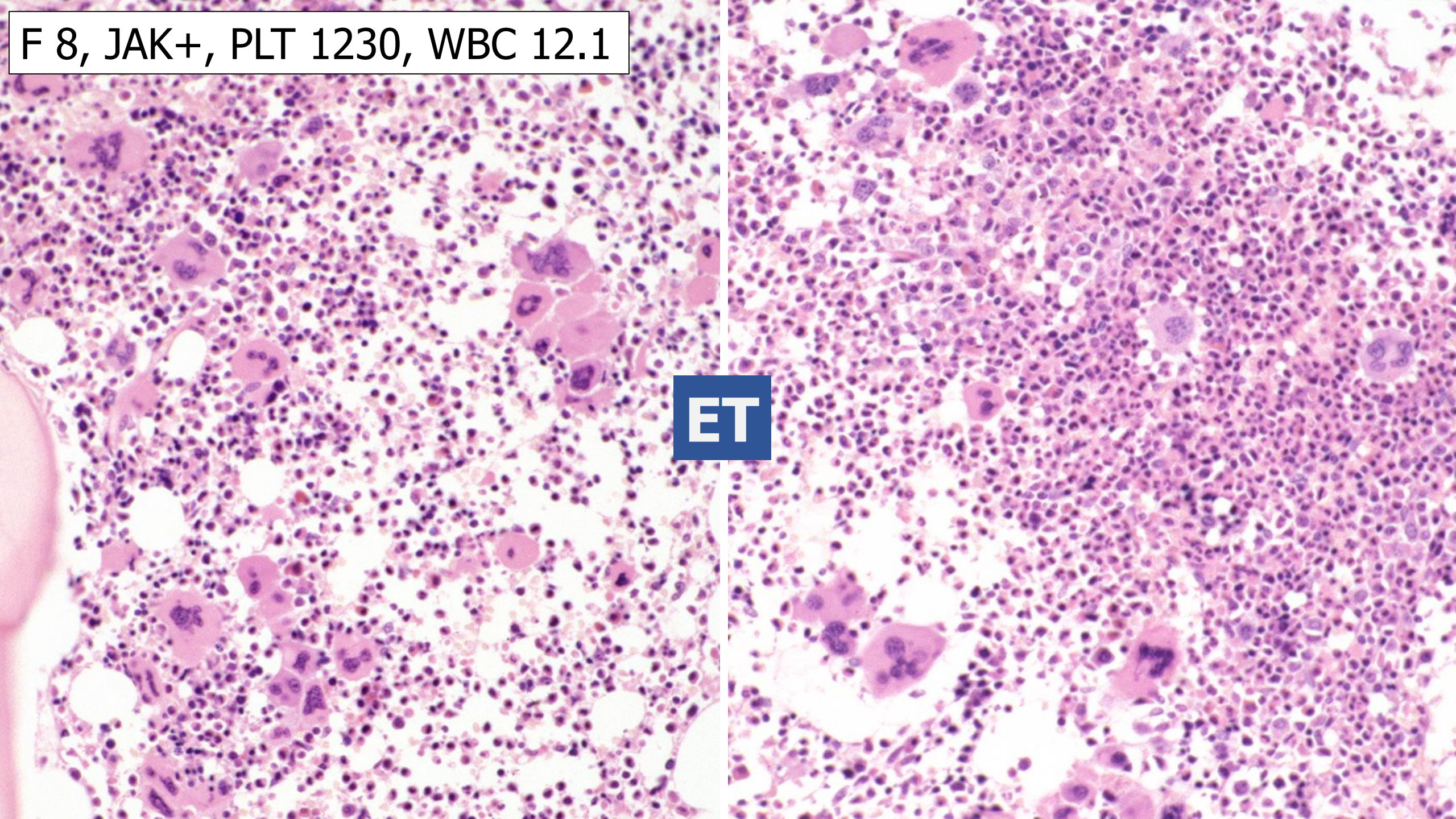
	Type 1–like *CALR*	Type 2–like *CALR*
Most common mutation	52-bp deletion (L367fs*46)	5-bp insertion (K385fs*47)
Prevalence	More common in MF	More common in ET
Clinical (ET)	Similar; lower platelet counts vs type 2 like	Similar; higher platelet counts vs type 1 like
Clinical (MF)	Less splenomegaly, leukocytosis, anemia, and circulating blasts; lower DIPSS score; higher platelets (all vs type 2 like)	More splenomegaly, leukocytosis, anemia, and circulating blasts; higher DIPSS score; lower platelets (all vs type 1 like). More similar to *JAK2 V617F*
Post-ET MF	Similar to/increased vs type 2 like	Similar to/decreased vs type 1 like
Overall prognosis (ET)	Similar to type 2 like	Similar to type 1 like
Overall prognosis (MF)	Improved vs type 2 like and *JAK2 V617F*	Worsened vs type 1 like; more similar to *JAK2 V617F*

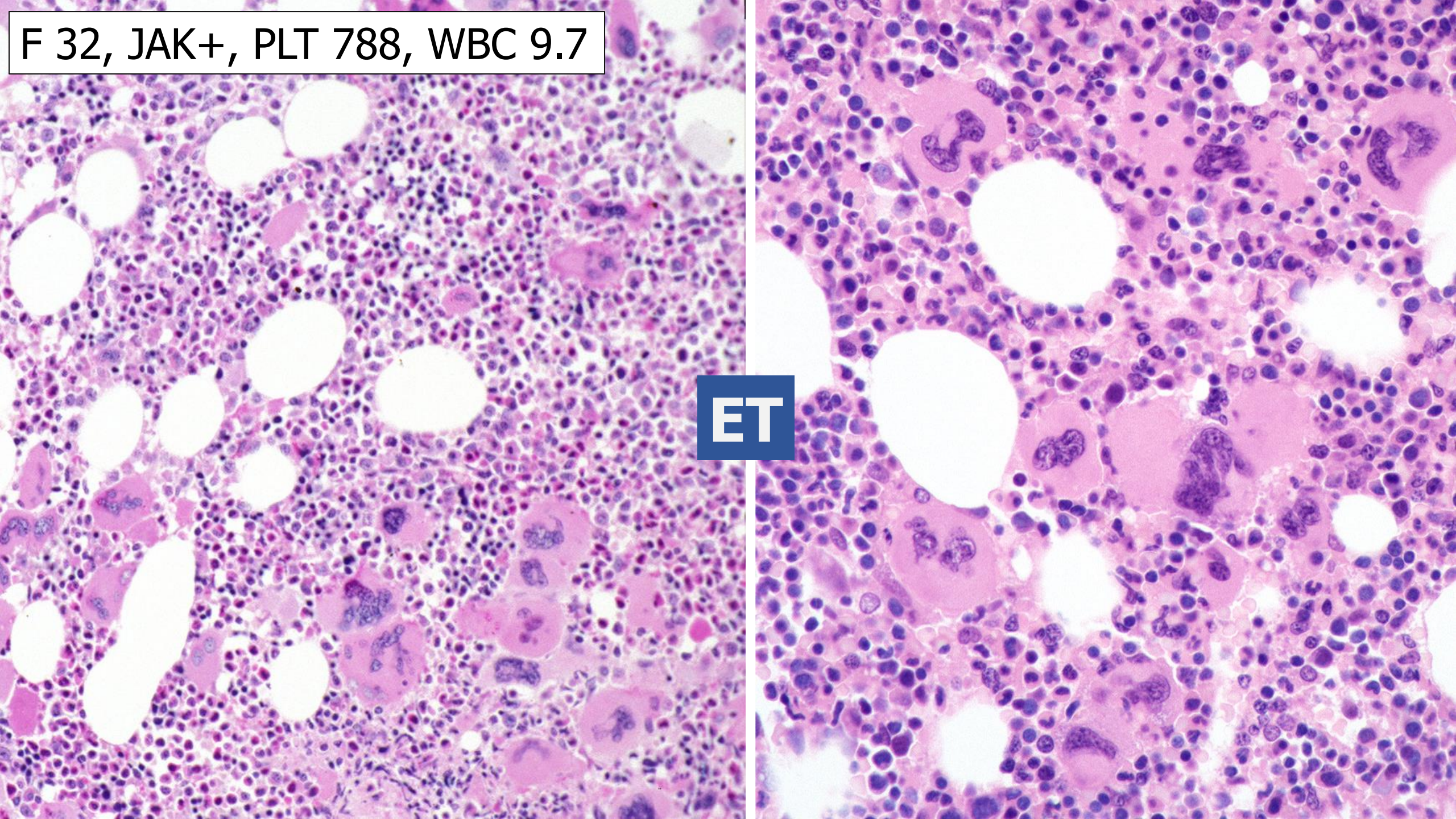
- CALR mutated and 'wild-type' patients may be at a very low risk of thrombosis, and the effect of the CALR mutation may be particularly evident in younger patients
- Type 2-like CALR is more common in ET and is correlated with higher PLT counts
- Type 1-like CALR has no impact on overall prognosis

How et al., Blood (2019) 134:2242-2248

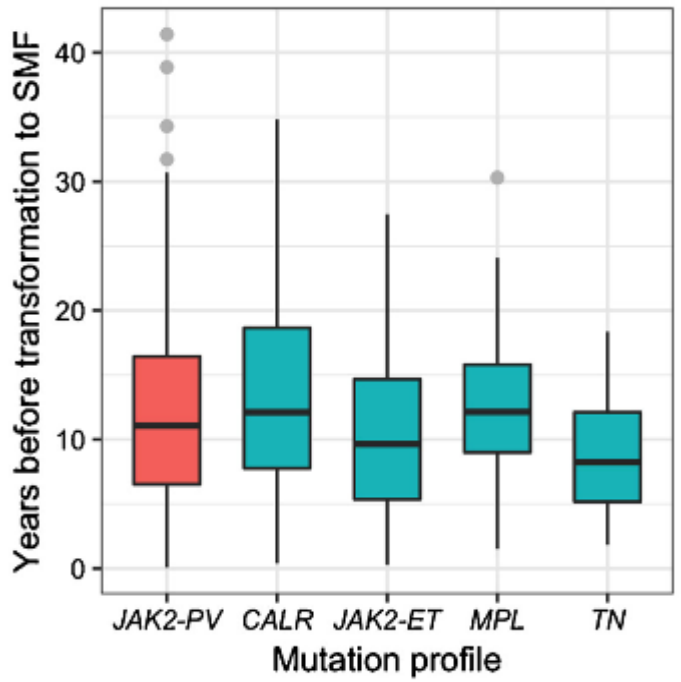
M 27, JAK+ ??, HB 13.1, PLT 956, WBC 13.1, LDH 269




F 8, JAK+, PLT 1230, WBC 12.1
ET


F 32, JAK+, PLT 788, WBC 9.7
ET

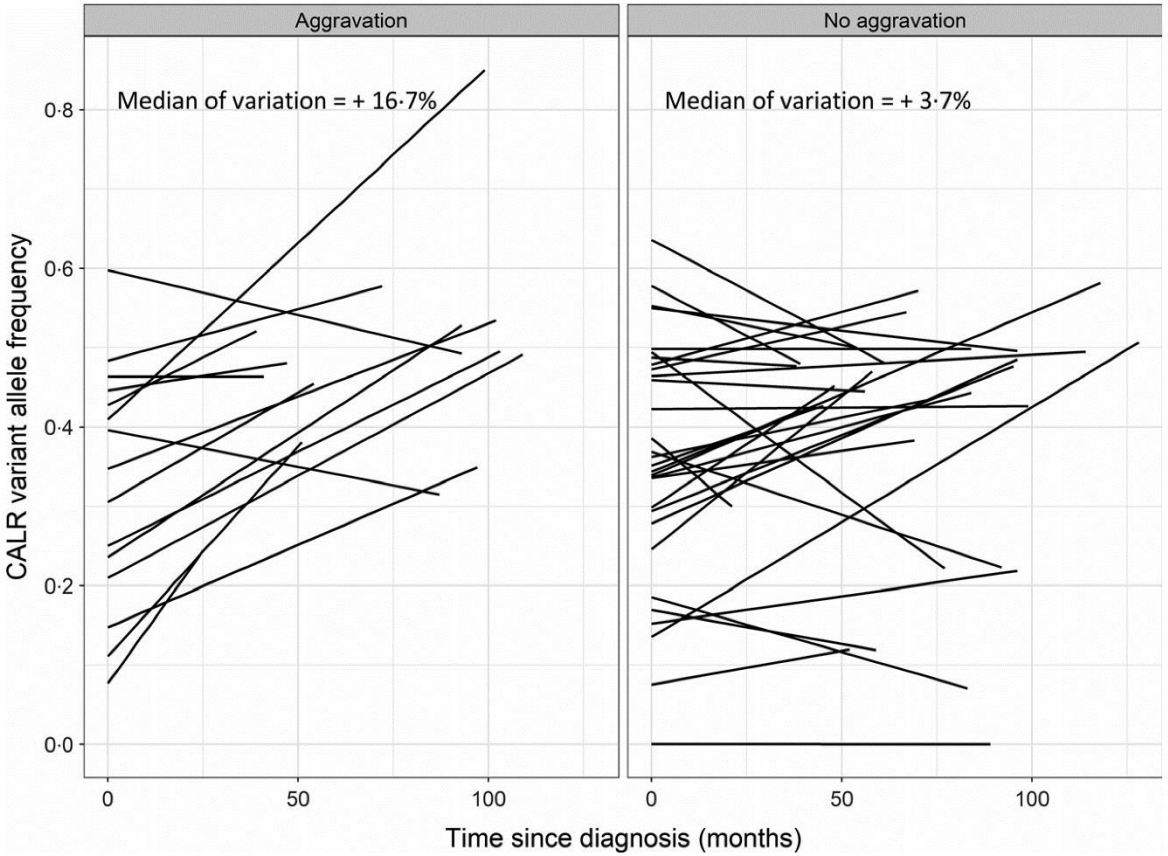
Prediction of post-ET MF



Time to Progression

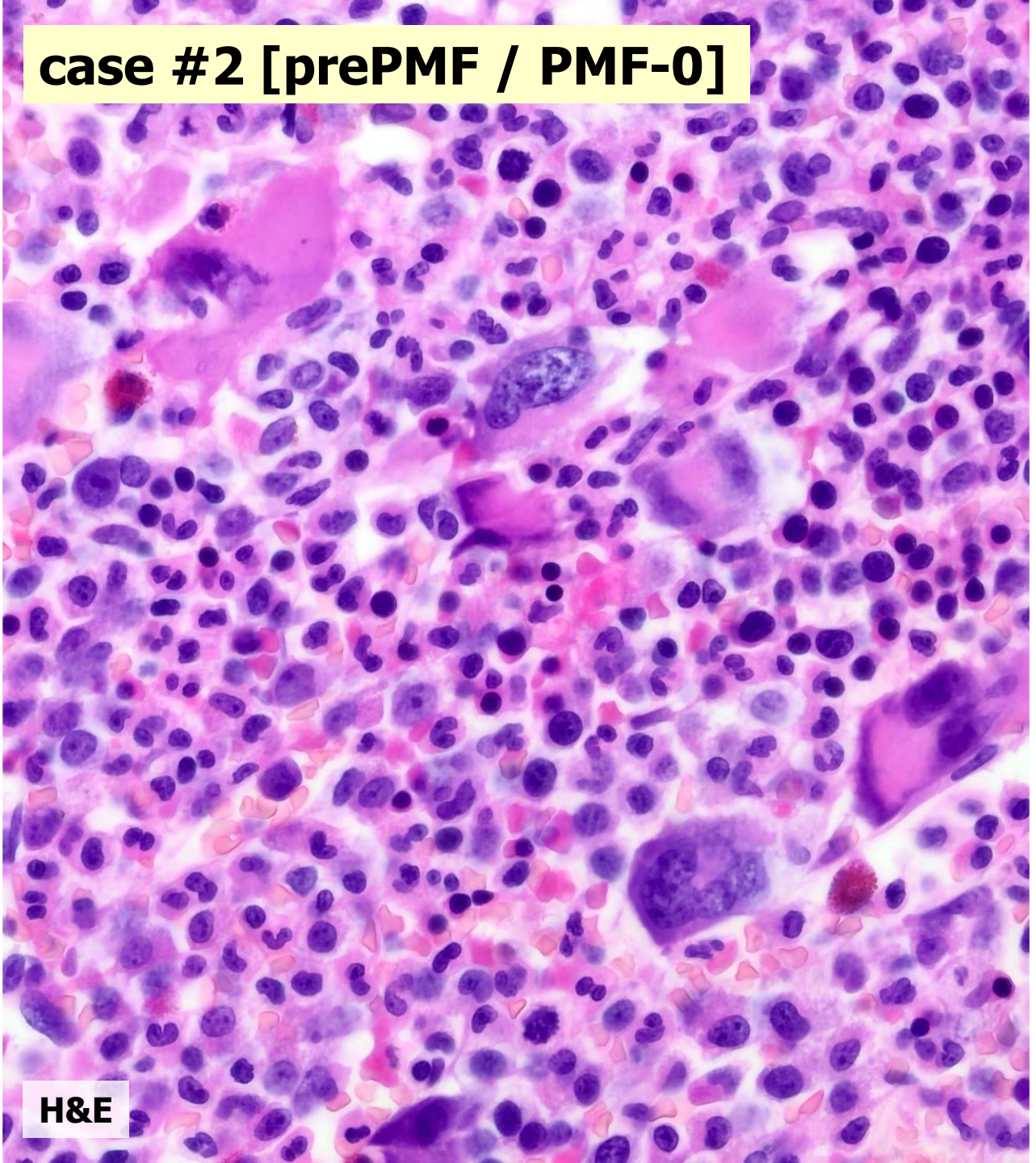
- JAK2+ 9.6 yrs (range, 0.3–27.4)
- CALR+ 12.1 yrs (range,0.4–34.8)
- MPL 12.2 (range, 1.5–30.3)
- wild type 8.2 (range, 1.8–18.4)

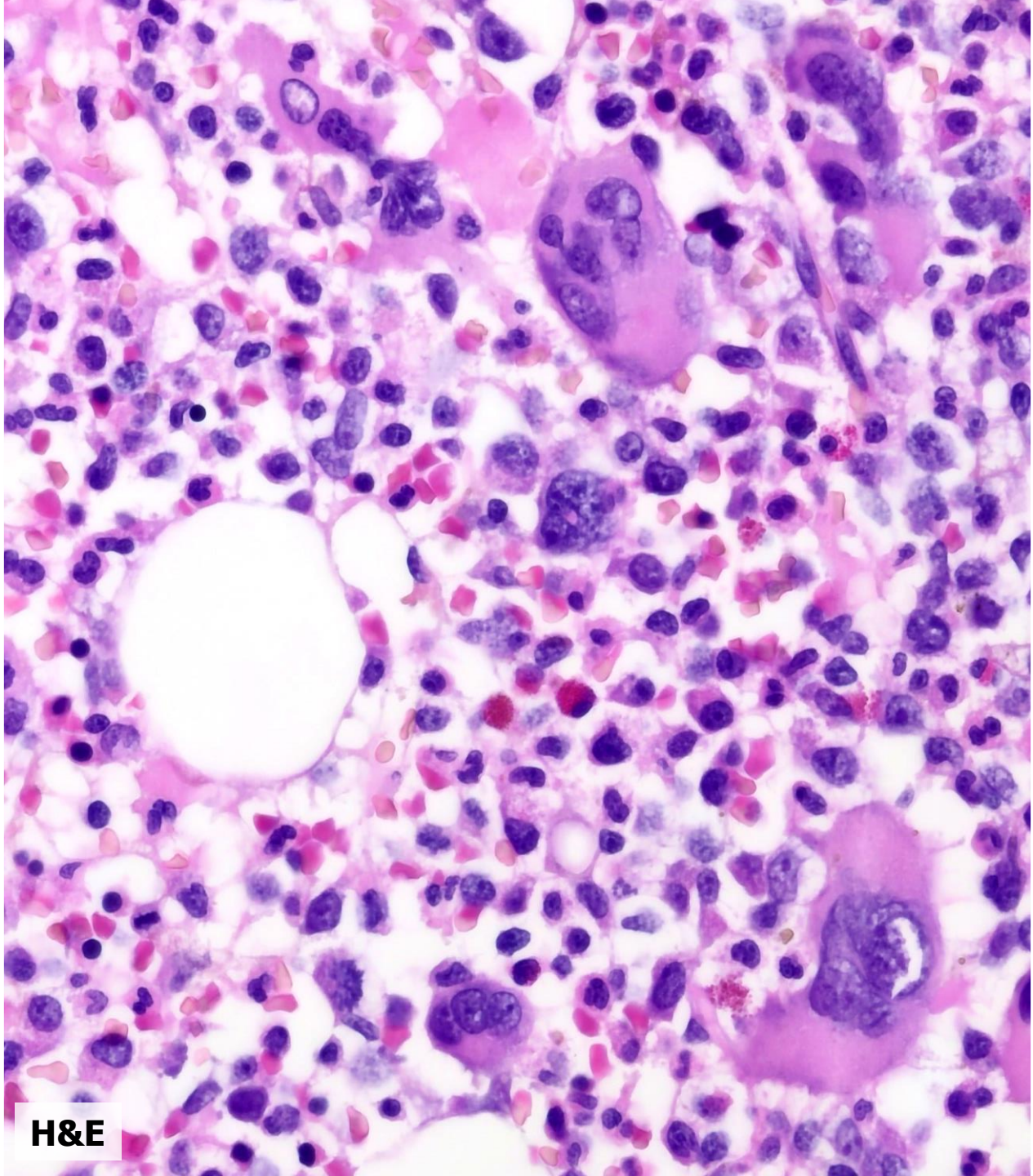
No differences between CALR type1/type1 like and type2/type2 like (p=0.36)



Evolution of CALR allele burden is associated with disease progression

Mora B et al. Leukemia 2018; Pietra D et al Leukemia. 2016 Feb;30(2):431-8
Cottin et al., Br J Haematol (2020) 188:935-944


case #2 [prePMF / PMF-0]
H&E


H&E

ICC diagnostic criteria for PMF

Primary myelofibrosis, early/prefibrotic stage (prePMF)	Primary myelofibrosis, overt fibrotic stage
Major criteria 1. Megakaryocytic proliferation and atypia[a)], BM fibrosis grade <2, increased age-adjusted BM cellularity, granulocytic proliferation, and (often) decreased erythropoiesis 2. JAK2, CALR, or MPL mutation[b)] or Presence of another clonal marker[c)] or Absence of reactive bone marrow reticulin fibrosis[d)] 3. Diagnostic criteria for BCR::ABL1-positive chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, myelodysplastic syndromes, or other myeloid neoplasms are not met	Major criteria 1. Megakaryocytic proliferation and atypia[a)], accompanied by reticulin and/or collagen fibrosis grades 2 or 3 2. JAK2, CALR, or MPL mutation[b)] or Presence of another clonal marker[c,f)] or Absence of reactive myelofibrosis[d)] 3. Diagnostic criteria for essential thrombocythemia, polycythaemia vera, BCR::ABL1-positive chronic myeloid leukemia, myelodysplastic syndrome, or other myeloid neoplasms[e)] are not met
Minor criteria ▪ Anemia not attributed to a comorbid condition ▪ Leukocytosis ≥11 x 10^9/L ▪ Palpable splenomegaly ▪ Lactate dehydrogenase level above the above the reference range	Minor criteria ▪ Anemia not attributed to a comorbid condition ▪ Leukocytosis ≥11 x 10^9/L ▪ Palpable splenomegaly ▪ Lactate dehydrogenase level above the above the reference range ▪ Leukoerythroblastosis
The diagnosis of prePMF or overt PMF requires all 3 major criteria and at least 1 minor criterion confirmed in 2 consecutive determinations	

ICC diagnostic criteria for PMF

a) Morphology of megakaryocytes in prePMF and overt PMF usually demonstrates a higher degree of megakaryocytic atypia than in any other MPN-subtype; distinctive features of megakaryocytes include small to giant megakaryocytes with a prevalence of severe maturation defects (cloud-like, hypolobulated and hyperchromatic nuclei) and presence of abnormal large dense clusters (mostly >6 megakaryocytes lying strictly adjacent)

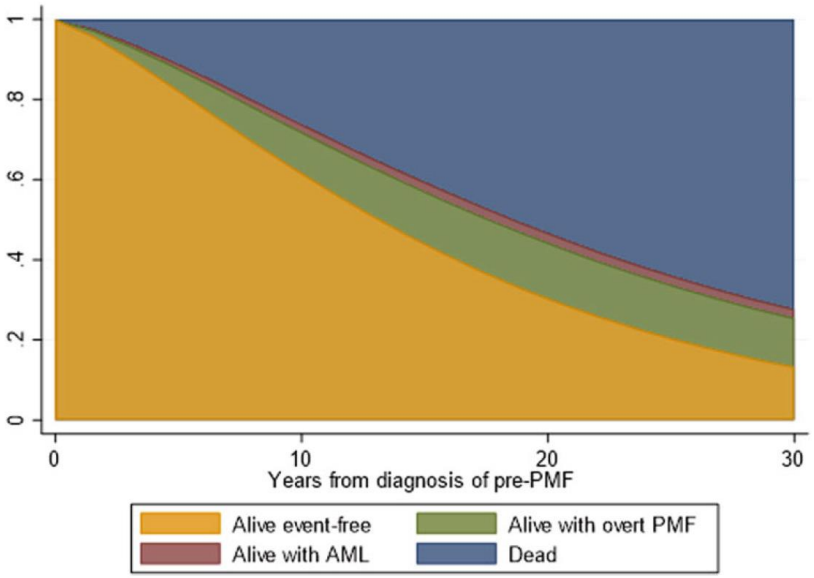
b) It is recommended to use highly sensitive assays for *JAK2* V617F (sensitivity level <1%) and *CALR* and *MPL* (sensitivity level 1-3%) - in negative cases, consider to search for non-canonical *JAK2* and *MPL* mutations

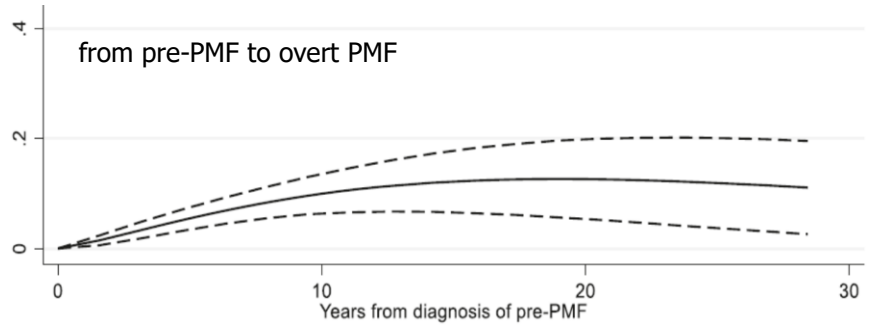
c) Assessed by cytogenetics or sensitive NGS techniques; detection of mutations associated with myeloid neoplasms (e.g. *ASXL1*, *EZH2*, *IDH1*, *IDH2*, *SF3B1, SRSF2*, *and TET2* mutations) supports the clonal nature of the disease

d) Minimal reticulin fibrosis (grade 1) secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or another lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies

e) Monocytosis can be present at diagnosis or develop during the course of PMF; in these cases, a history of MPN excludes CMML, whereas a higher variant allelic frequency for MPN-associated driver mutations is supporting the diagnosis of PMF with monocytosis rather than CMML

Transition probabilities over time from pre- to overt PMF, blast phase, and death

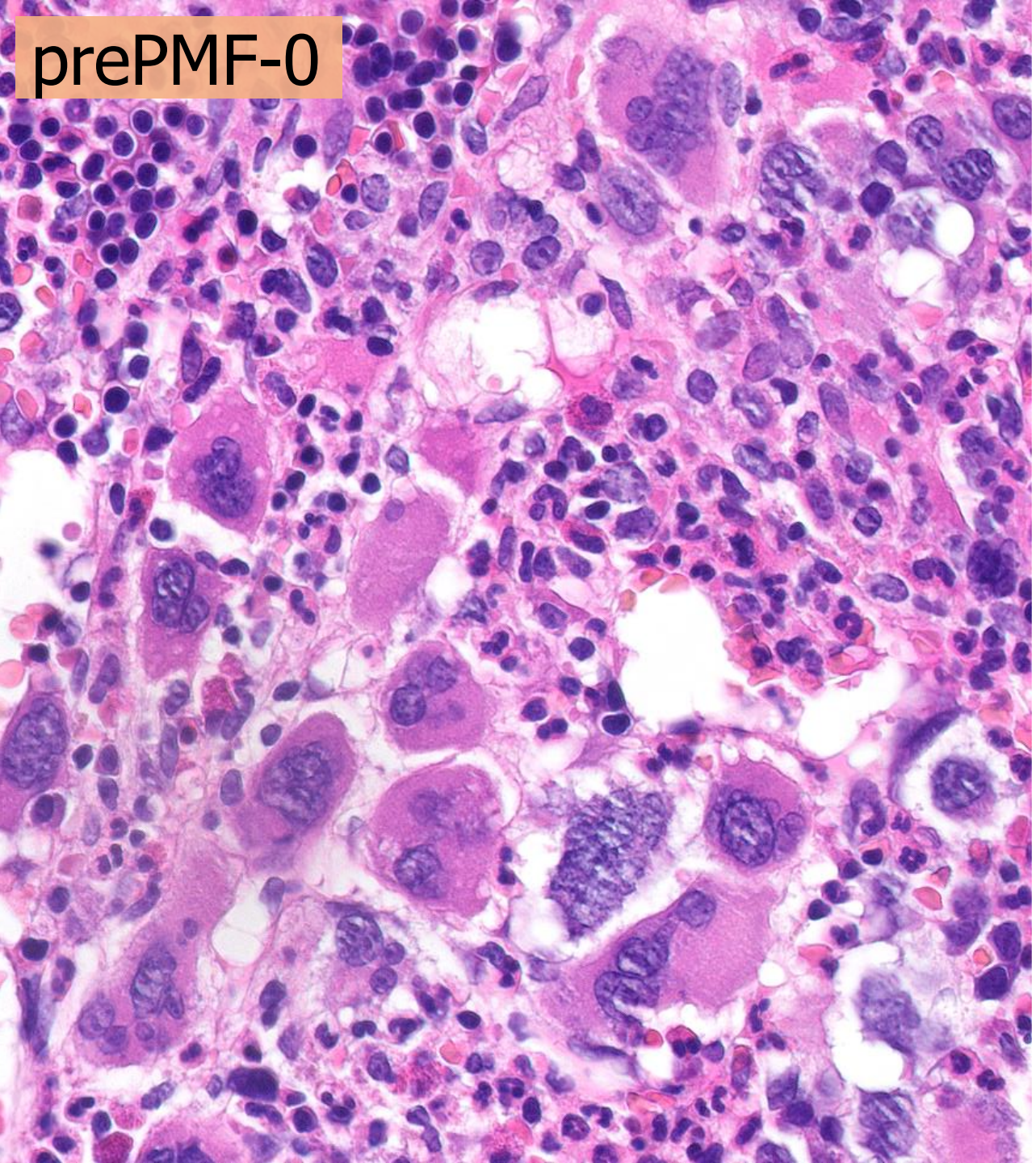


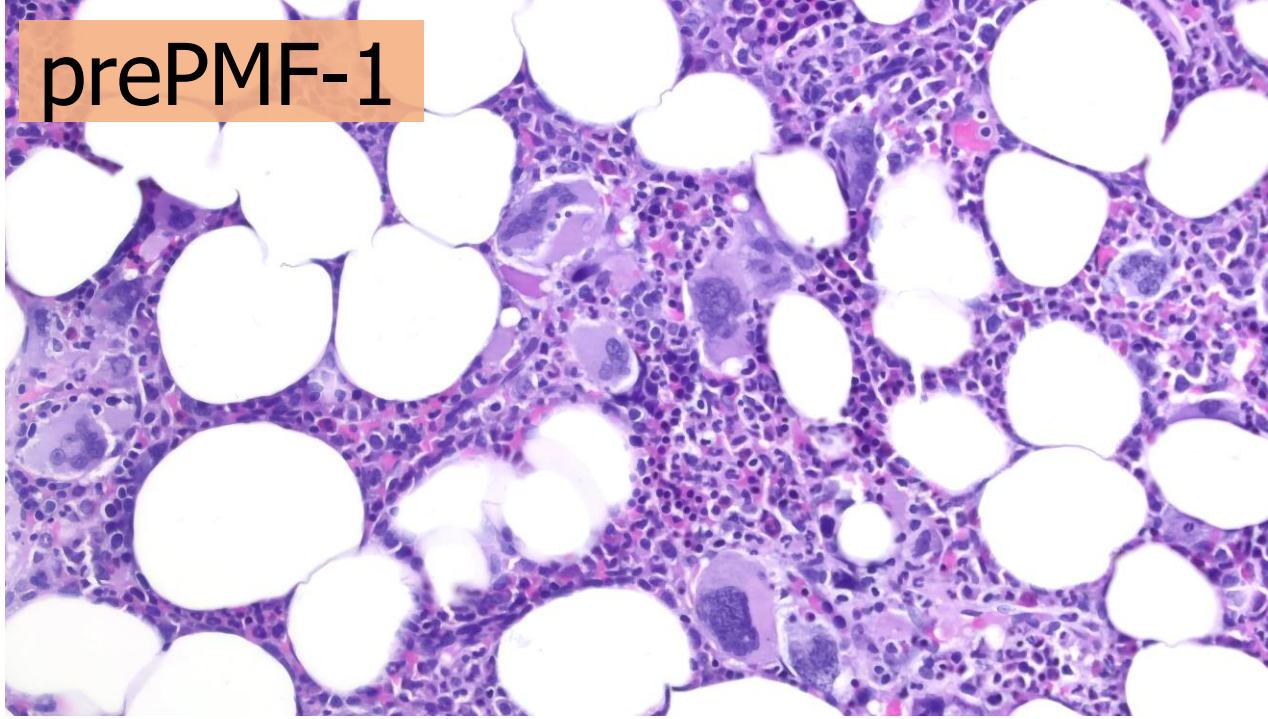


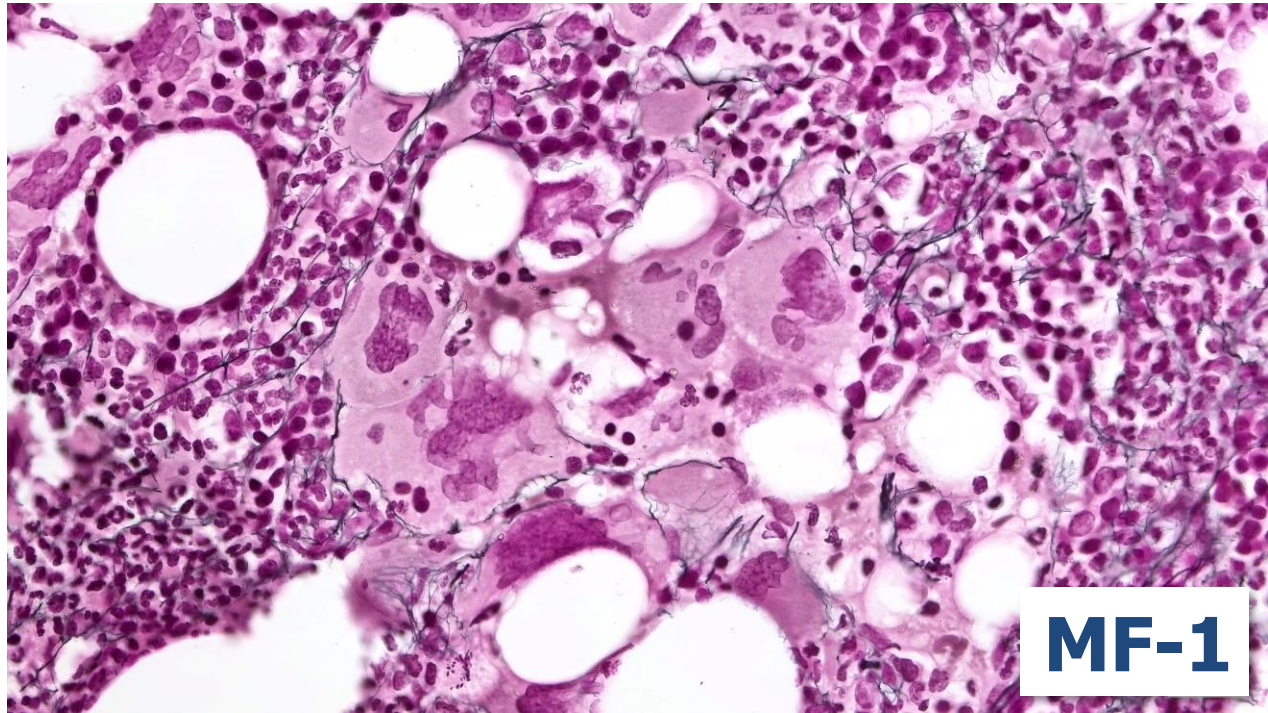
Covariate effect in each transition-specific model

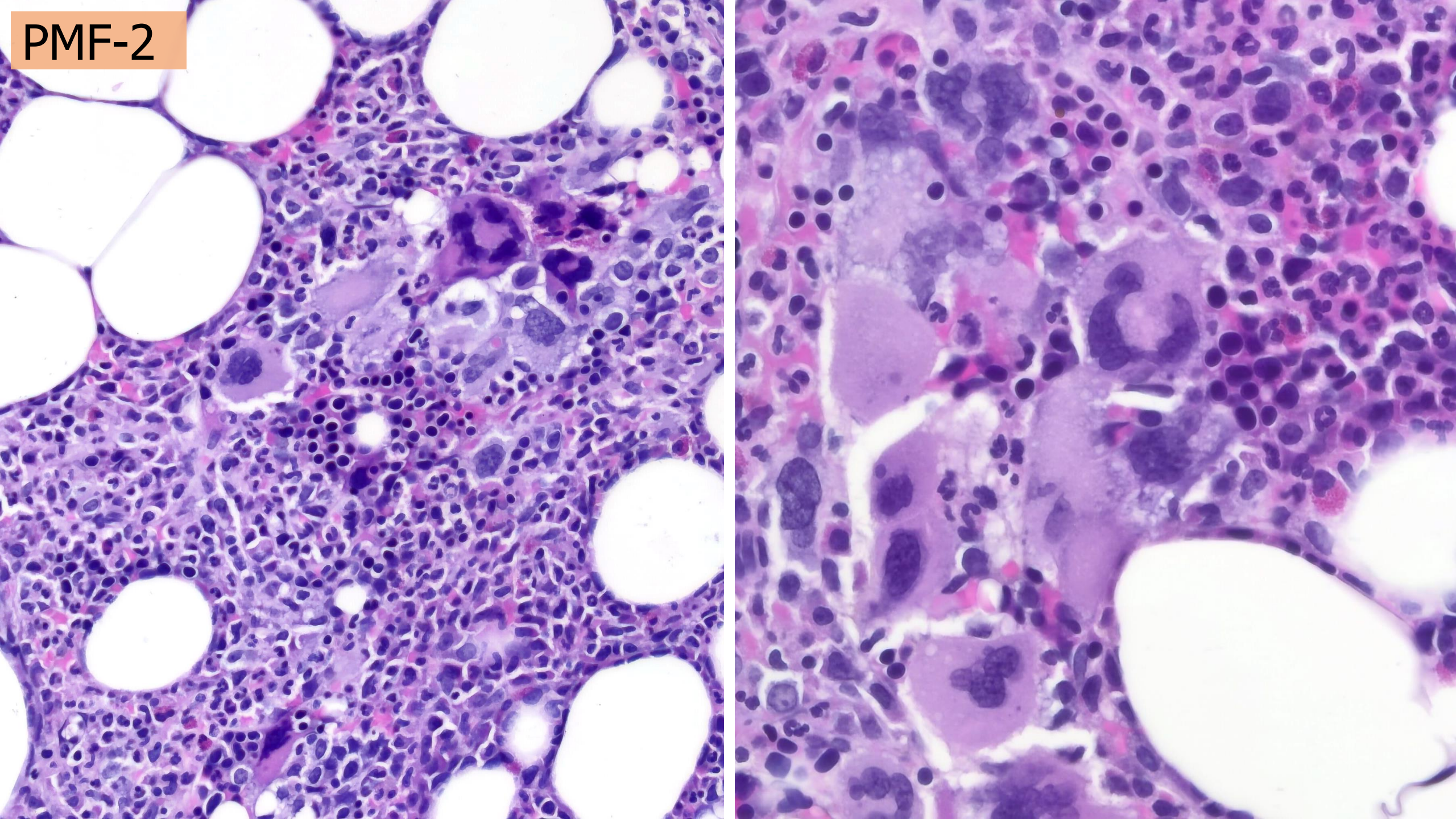
Covariate	Transition 1: evolution in overt PMF		Transition 2: evolution in AML		Transition 3: death	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
Clinical						
Male sex	0.91 (0.44–1.87)	0.790	0.34 (0.08–1.46)	0.147	1.66 (0.64–4.26)	0.296
Age >65 years	0.56 (0.25–1.30)	0.176	**10.3 (2.33–45.6)**	**0.002**	**6.53 (2.38–17.9)**	**<0.0001**
Anemia	**2.18 (1.01–4.72)**	**0.047**	1.34 (0.31–5.92)	0.695	2.30 (0.88–5.37)	0.153
WBC > 15×10^9/L	0.91 (0.29–2.84)	0.870	**4.80 (1.22–18.9)**	**0.025**	**3.49 (1.35–9.01)**	**0.010**
Plt > 1000×10^9/L	1.96 (0.64–5.97)	0.236	0.47 (0.07–3.16)	0.438	1.21 (0.30–4.84)	0.786
Fibrosis grade 1	**3.20 (1.08–9.41)**	**0.035**	0.34 (0.09–1.29)	0.112	2.96 (0.82–10.7)	0.100
Spleen size > 5 cm	0.59 (0.17–2.05)	0.404	2.59 (0.66–10.2)	0.175	0.79 (0.25–2.53)	0.694
LDH > 1.5	1.58 (0.76–3.30)	0.221	**8.13 (1.46–45.2)**	**0.017**	0.84 (0.36–1.98)	0.687
Abnormal cytogenetics	1.60 (0.64–4.00)	0.310	**6.92 (1.61–29.8)**	**0.009**	1.84 (0.71–4.76)	0.209
Mutational						
Driver						
*JAK2*V617F	0.87 (0.04–17.4)	0.925	0.12 (0.02–6.00)	0.286	0.82 (0.07–10.2)	0.875
*MPL*W515L/K	0.93 (0.06–14.4)	0.955	0.52 (0.01–24.7)	0.740	1.91 (0.21–17.8)	0.570
CALR	0.91 (0.06–14.4)	0.955	0.13 (0.02–7.27)	0.319	0.36 (0.04–3.68)	0.390
Triple negatives	2.70 (0.10–74.3)	0.557	0.13 (0.00–10.6)	0.368	3.31 (0.23–46.6)	0.376
Nondriver						
HMR	**3.15 (1.08–9.21)**	**0.036**	–	–	**4.62 (2.09–10.2)**	**<0.0001**
HMR 2	–	–	**6.62 (1.11–39.3)**	**0.038**	–	–

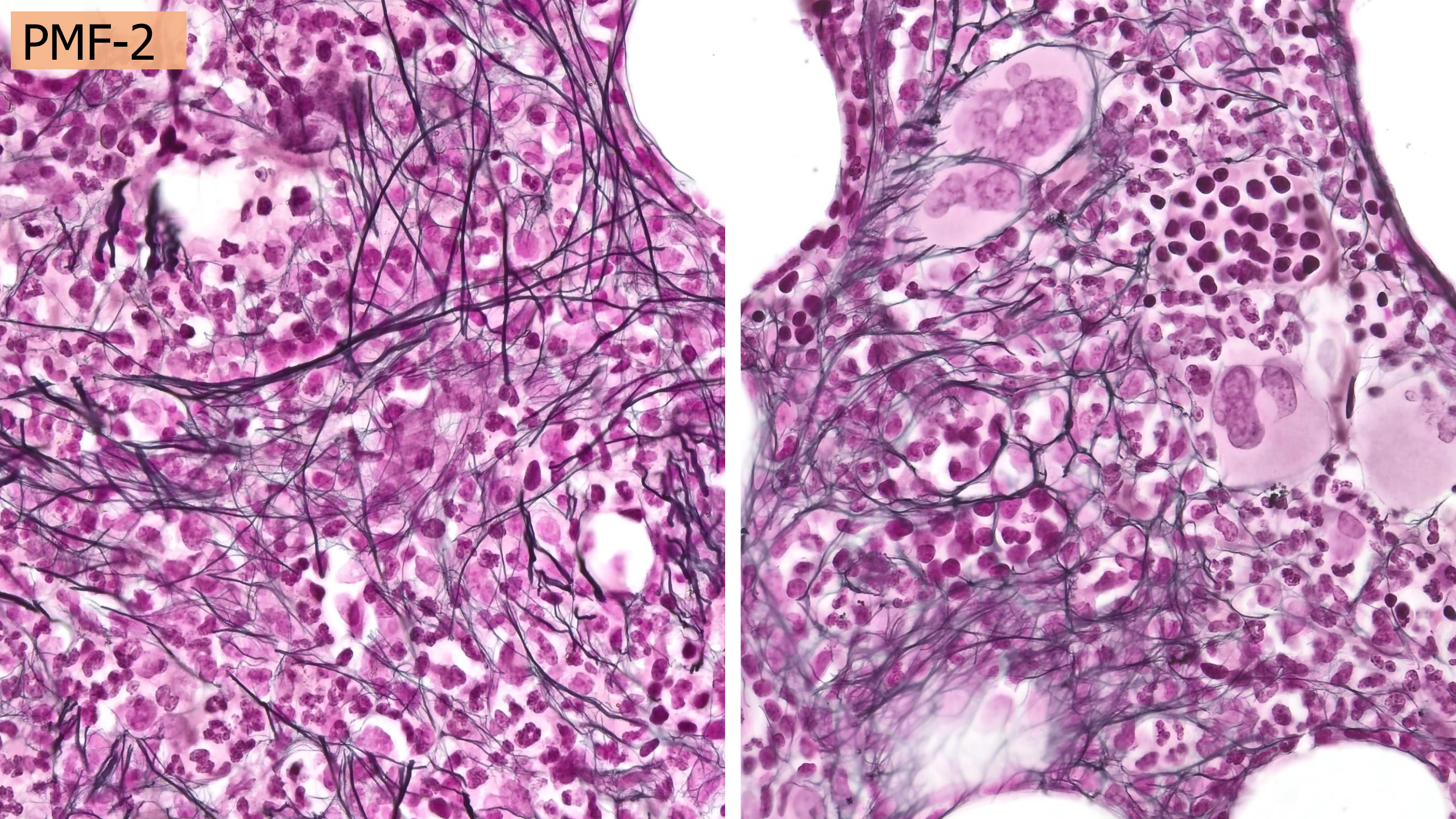
Carobbio et al., Blood Cancer J (2020) 10:100


prePMF-0

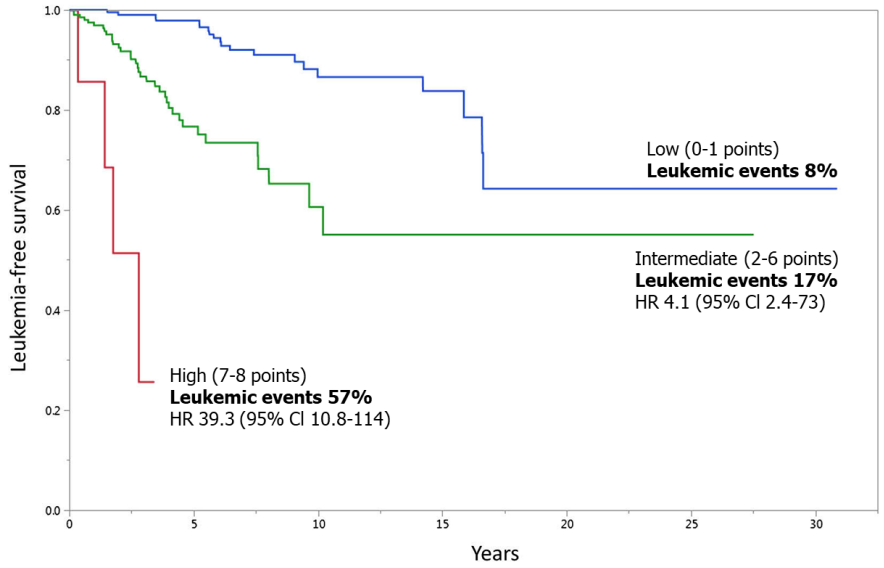

prePMF-1


MF-1


PMF-2


PMF-2

Prediction Model for Leukemic Transformation in PMF



Covariates	points
IDH1 mut	3
Circulating blast cells >3%	2
SRSF2 mut	2
Age >70 yrs	1
ASXL1 mut	1
Moderate/severe anemia*	1

Vallapureddy RR. et al, BCJ 2019 Feb; 9(2): 12

Clinical and cytogenetic/molecular risk factors for blast phase (post-MPN AML)

Risk factors
Clinical
Age >60-65 y
Red blood cell transfusion dependency
Prior treatment exposure (pipobroman, ^{32}P, chlorambucil, busulfan)
Prior thrombosis*
Myelofibrosis or prefibrotic ET/PV
Laboratory
Leukocytosis (>15 × 10^9/L to 30 × 10^9/L)
Anemia (Hgb <10 g/dL)
Thrombocytopenia (<50 × 10^9/L to 100 × 10^9/L)
Peripheral blast count (>1% to 10%)
Extreme thrombocytosis (>1000 × 10^9/L)*
Elevated serum IL-8†
Elevated serum C-reactive peptide†
Cytogenetics
Monosomal karyotype‡
Complex karyotype or sole or 2 abnormalities that include +8, −7/7q, i(17q), −5/5q−, 12p−, inv(3), 11q23 rearrangement§
Chromosome 17p deletion
Molecular
TP53, TET2, ASXL1, EZH2, SRSF2, IDH1/2, RUNX1, U2AF1Q157

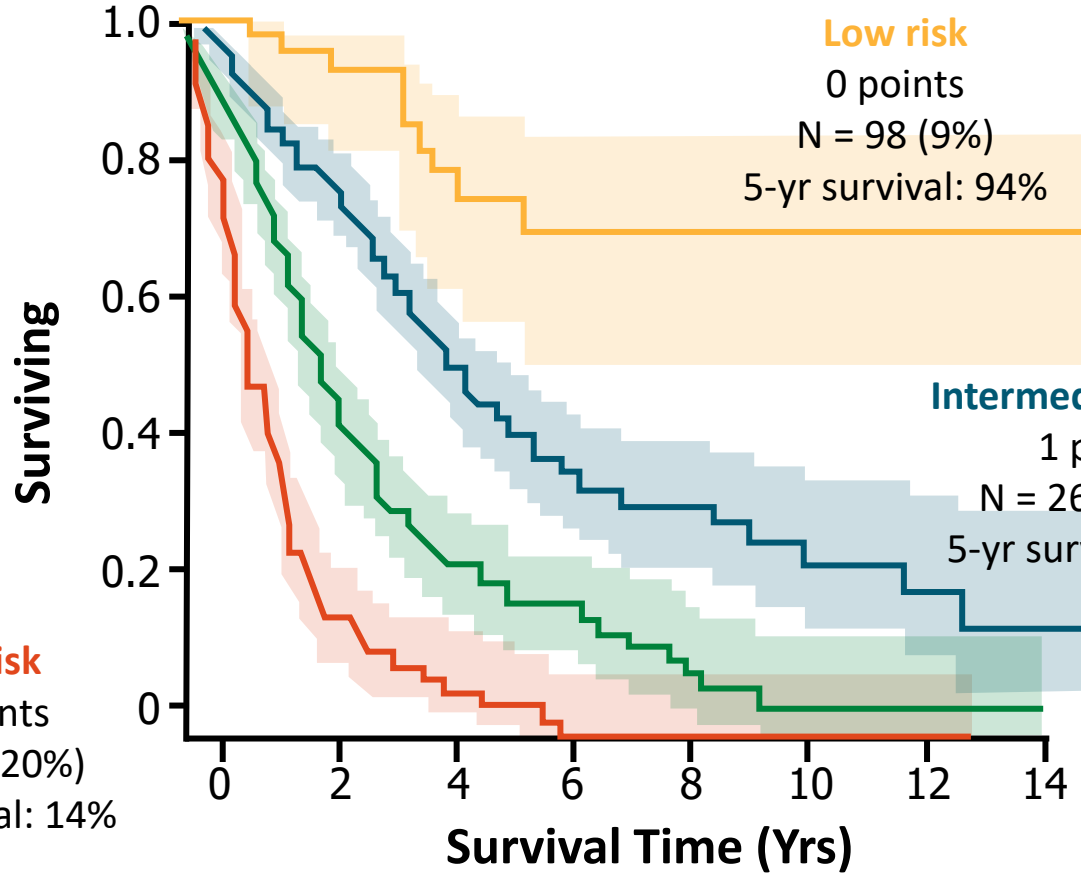
Dunbar et al., Blood (2020) 136:61-70

Impact of mutations on phenotype

- JAK2 exon 12/14, CALR exon 9, MPL exon 10
  - Thrombotic events (JAK2 > CALR/MPL)
  - Progression of fibrosis (mutation burden)

- Additional somatic mutations (e.g. ASXL1, SRSF2, EZH2)
  - Progression of fibrosis
  - Progression to blast phase

Genetically Inspired Prognostic Scoring (GIPSS) for PMF



Low risk
0 points
N = 98 (9%)
5-yr survival: 94%

Intermediate-1 risk
1 point
N = 260 (41%)
5-yr survival: 73%

Intermediate-2 risk
2 points
N = 192 (30%)
5-yr survival: 40%

High risk
≥ 3 points
N = 131 (20%)
5-yr survival: 14%

Karyotype:
Very high = 2 points
Unfavorable = 1 point

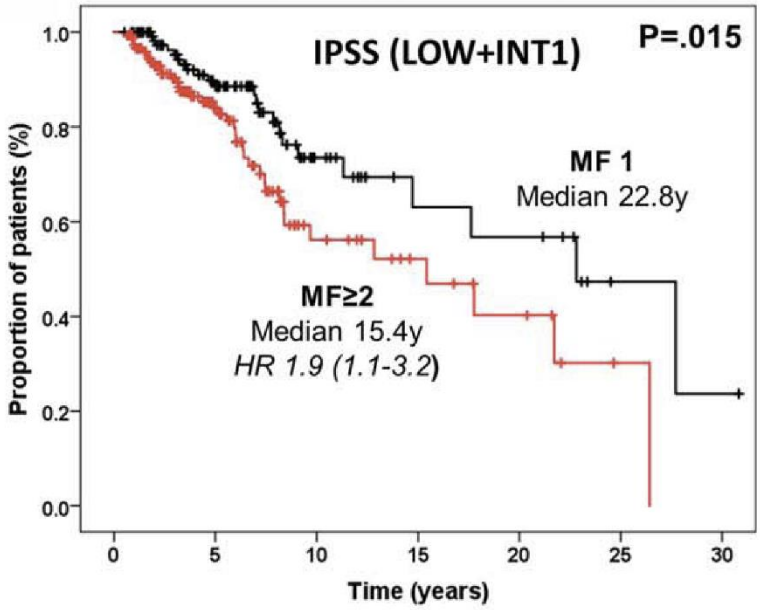
Driver mutations:
Type 1/like *CALR* absent = 1 point

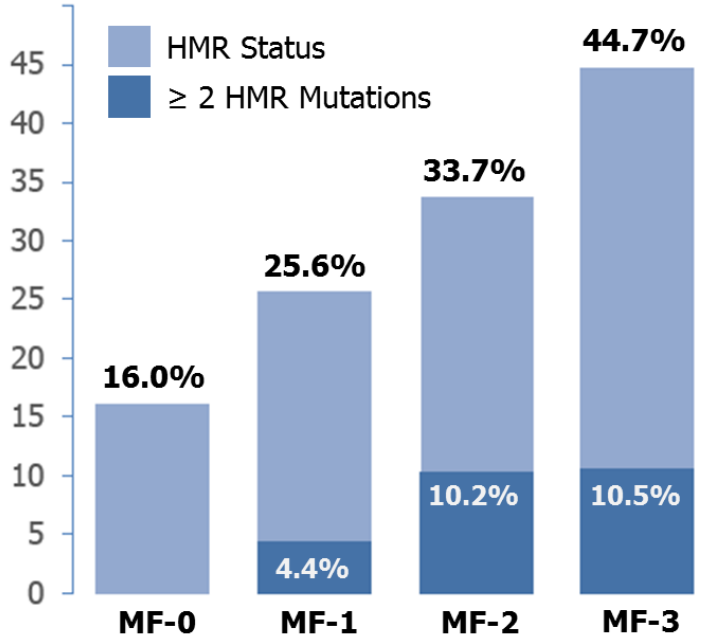
High risk mutations:
ASXL1 mut = 1 point
SRSF2 mut = 1 point
U2AF1 Q157mut = 1 point

Tefferi. Leukemia. 2018;32:1631.

Clinical impact of BM fibrosis in PMF

- grading of MF is not only important to diagnose MPN, but also to guide treatment decisions and stratify patients in clinical trials
- recent data suggest that degree of BM fibrosis is an independent prognostic factor in PMF in multivariate analysis
- impact of BM fibrosis is significant in low and int-1 risk categories
- no correlation between fibrosis grade and phenotypic driver mutations
- frequency of HMR is increasing progressively with grade of fibrosis
- significant association between grade of fibrosis and ASXL1 and EZH2

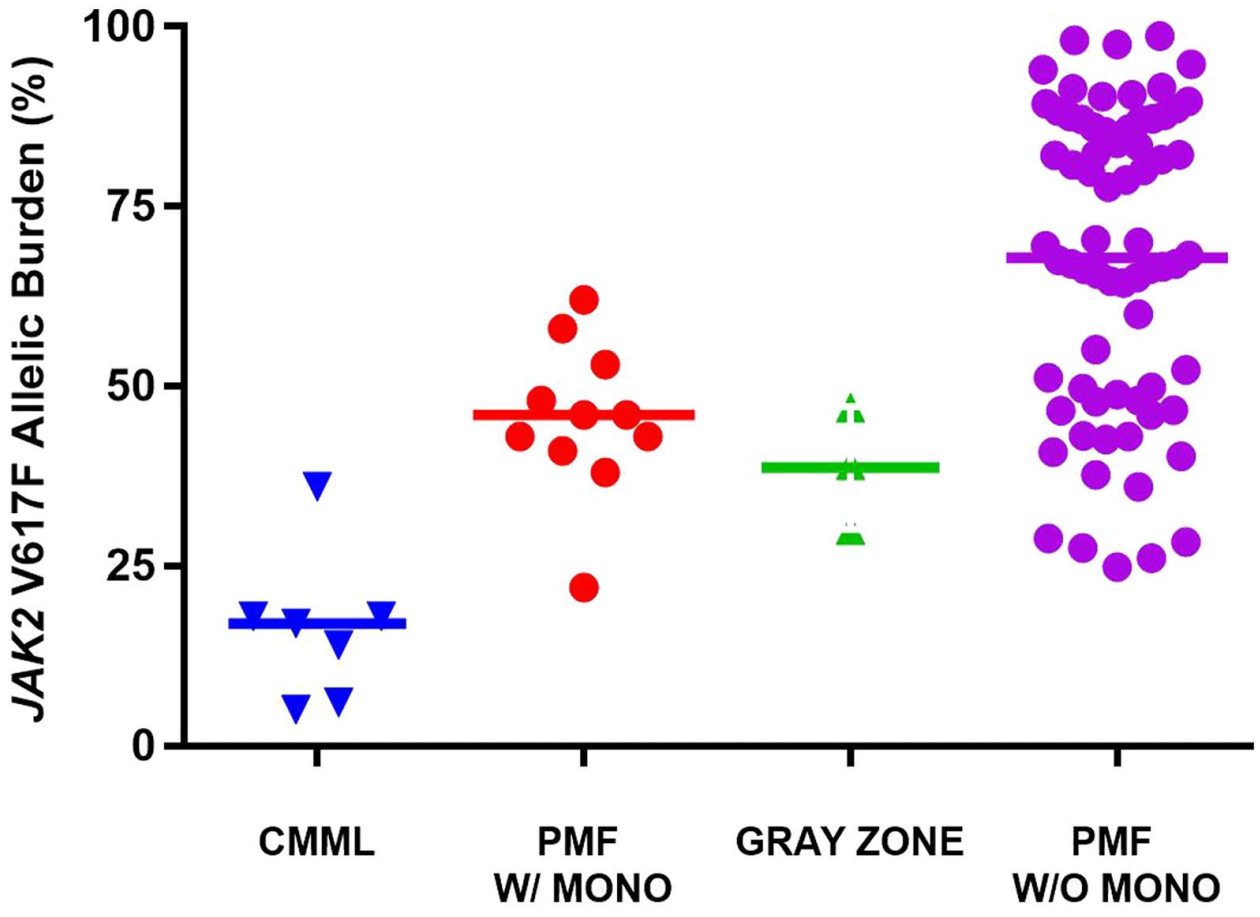




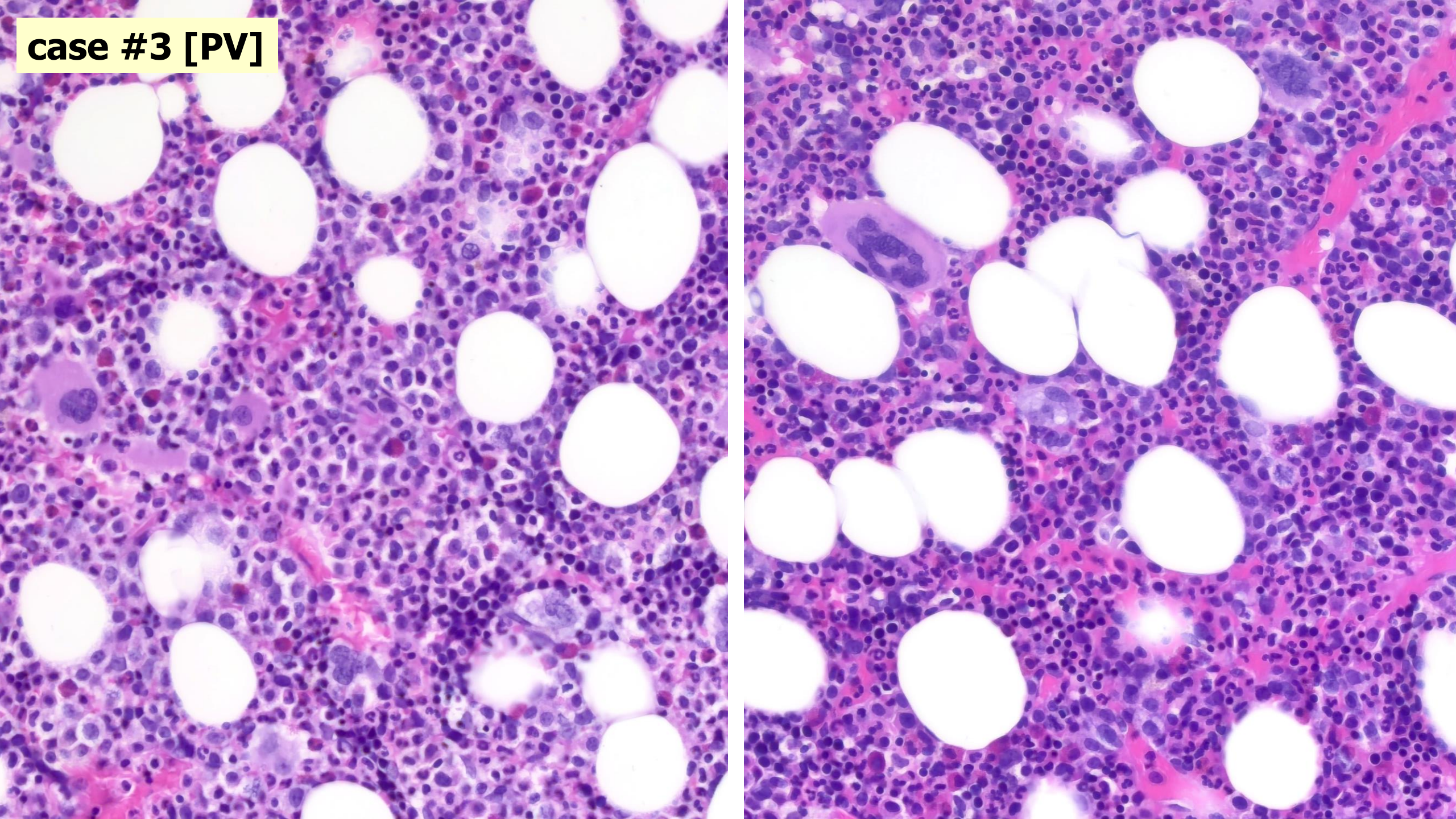
Kvasnicka & Thiele, Semin Thromb Hemost, 2006, 32, 219-230
Thiele J, Kvasnicka HM, et al. Histopathology. 2003;43:470-479
Guglielmelli, et al. Am J Hematol. 2016;91:918-922

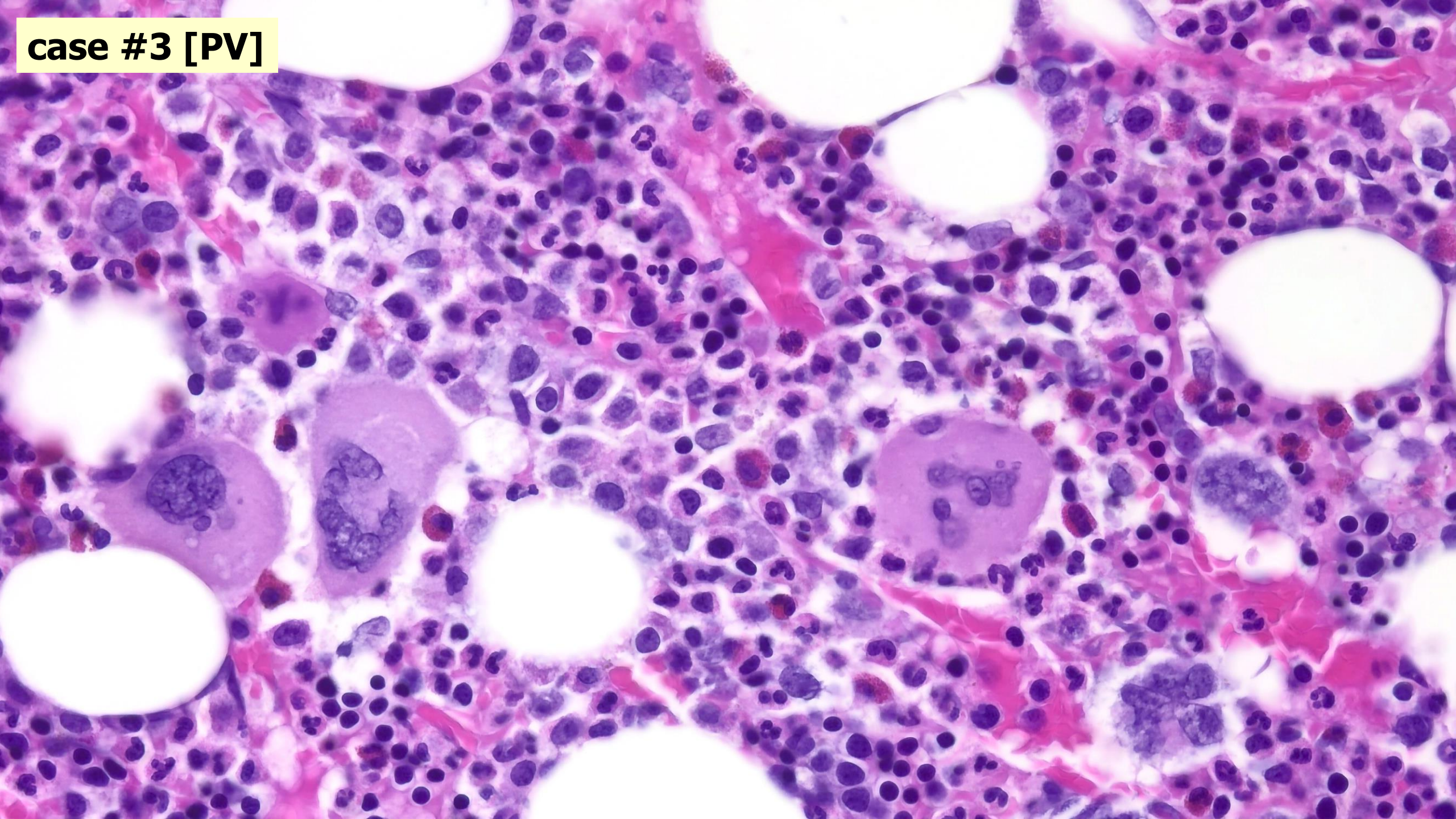
Monocytosis in PMF and MDS/MPN - CMML

- **JAK2 V617F, monocytosis and BM fibrosis can be seen in both PMF and CMML**
- JAK2 V617F allelic burden is significantly higher in PMF compared to CMML
- megakaryocyte morphology is key to distinguish PMF from CMML
- gray zone cases between CMML and PMF exist with borderline features



Hu et al., Hum Pathol (2019) 85:290-298


case #3 [PV]

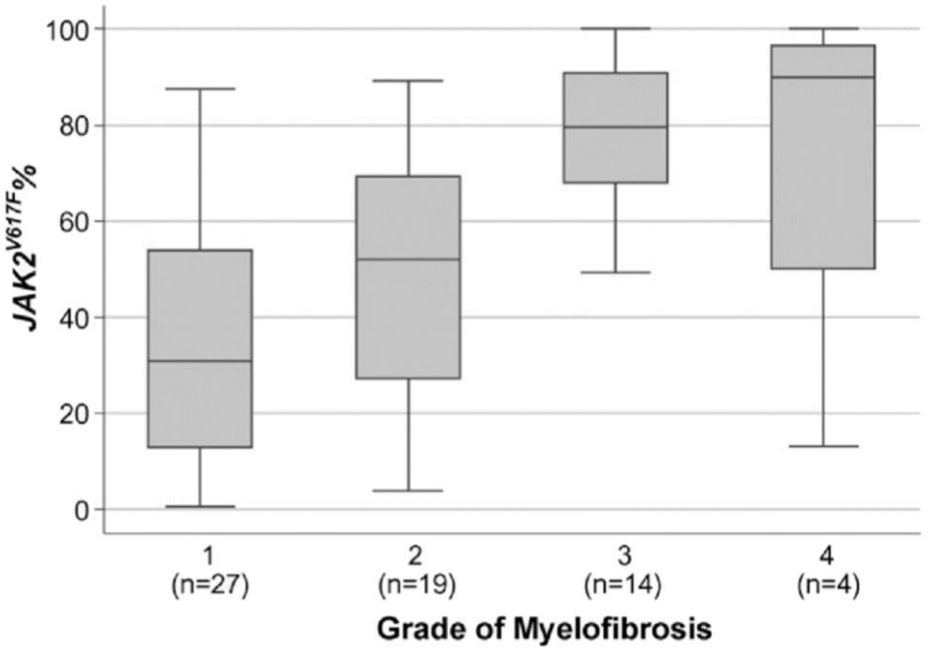

case #3 [PV]

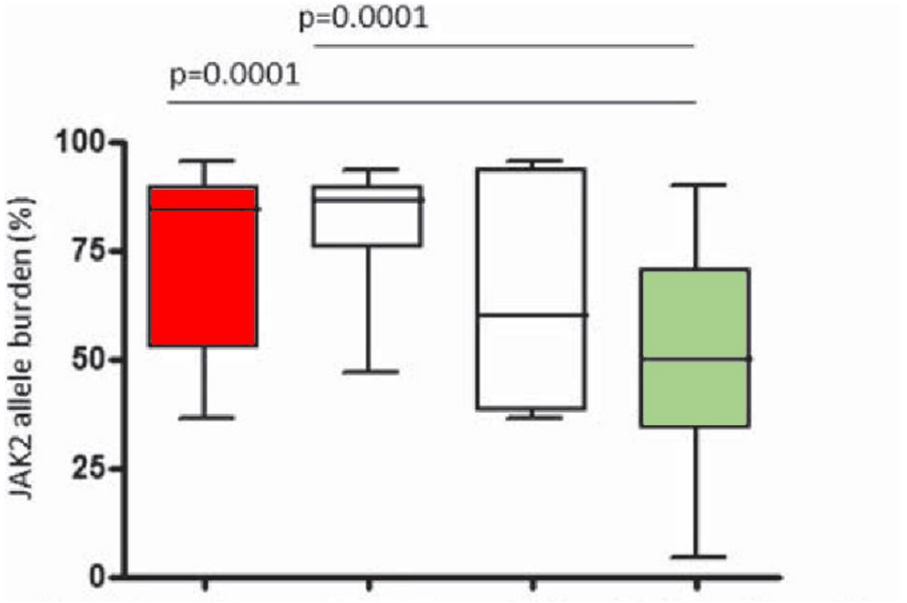
ICC diagnostic criteria for PV and Post-PV MF

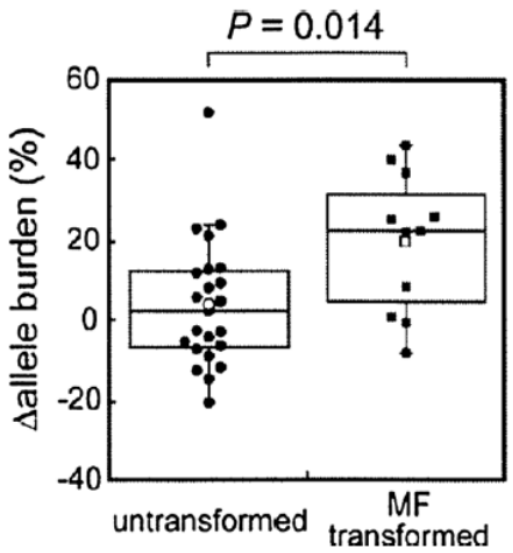
Polycythemia vera (PV)	Post-PV MF
Major criteria 1. Elevated hemoglobin concentration or Elevated hematocrit or Increased red blood cell mass[a)] 1. Presence of JAK2 V617F or JAK2 exon 12 mutation[b)] 2. Bone marrow biopsy showing age-adjusted hypercellularity with trilineage proliferation (panmyelosis), including prominent erythroid, granulocytic, and increase in pleomorphic, mature megakaryocytes without atypia	**Required criteria** 1. Previous established diagnosis of PV 2. Bone marrow fibrosis of grade 2 or 3
Minor criterion ▪ Subnormal serum erythropoietin level	**Additional criteria** 1. Anemia (i.e. 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 months, night sweats, unexplained fever (> 37.5 °C
The diagnosis of PV requires either all 3 major criteria or the first 2 major criteria plus the minor criterion[c)]	**The diagnosis of Post-PV MF is established by all required citeria and at least two additional criteria**

(a) Diagnostic thresholds: hemoglobin: > 16.5 g/dL in men and > 16.0 g/dL in women; hematocrit: > 49% in men and > 48% in women; red blood cell mass: > 25% above mean normal predicted value (b) It is recommended to use highly sensitive assays for *JAK2* V617F (sensitivity level <1%) and *CALR* and *MPL* (sensitivity level 1-3%) - in negative cases, consider searching for non-canonical *JAK2* mutations (c) A BM trephine biopsy may not be required in patients with sustained absolute erythrocytosis (hemoglobin concentrations of >18.5 g/dL in men or >16.5 g/dL in women and hematocrit values of >55.5% in men or >49.5% in women) and the presence of a *JAK2* V617F or *JAK2* exon 12 mutation

JAK2 allele burden in PV correlates with grade of myelofibrosis and transformation to post-PV MF/BP





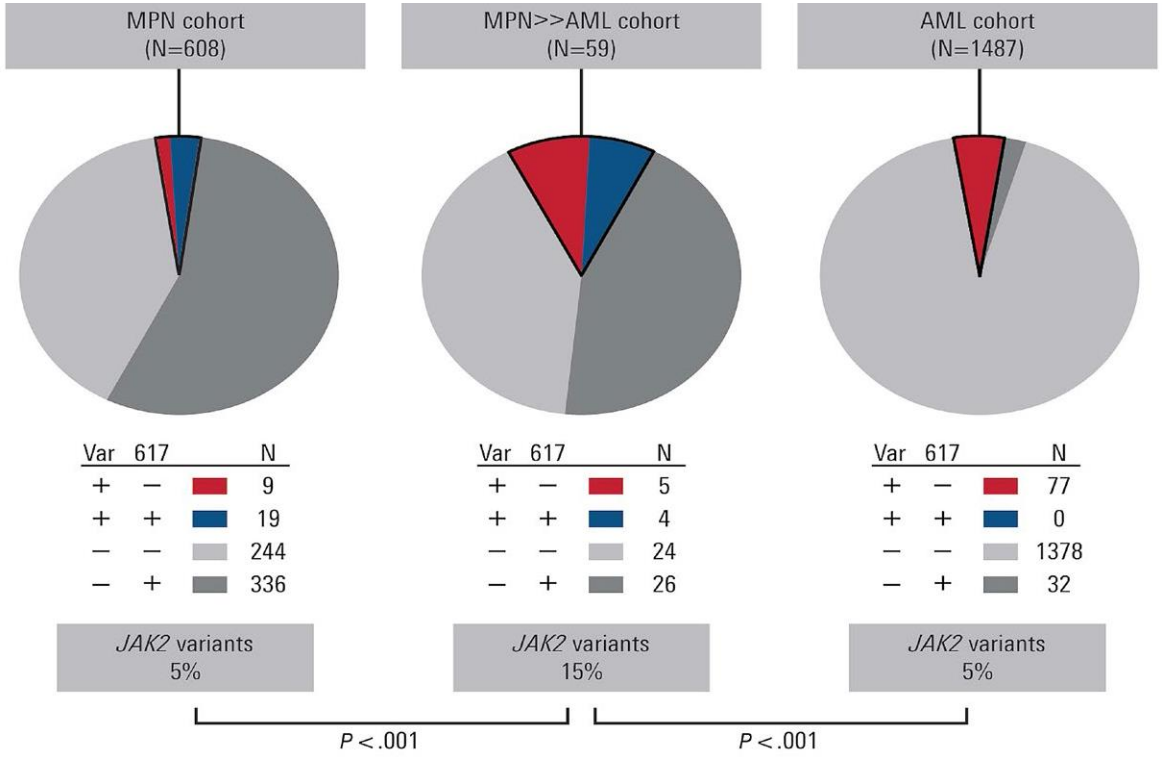


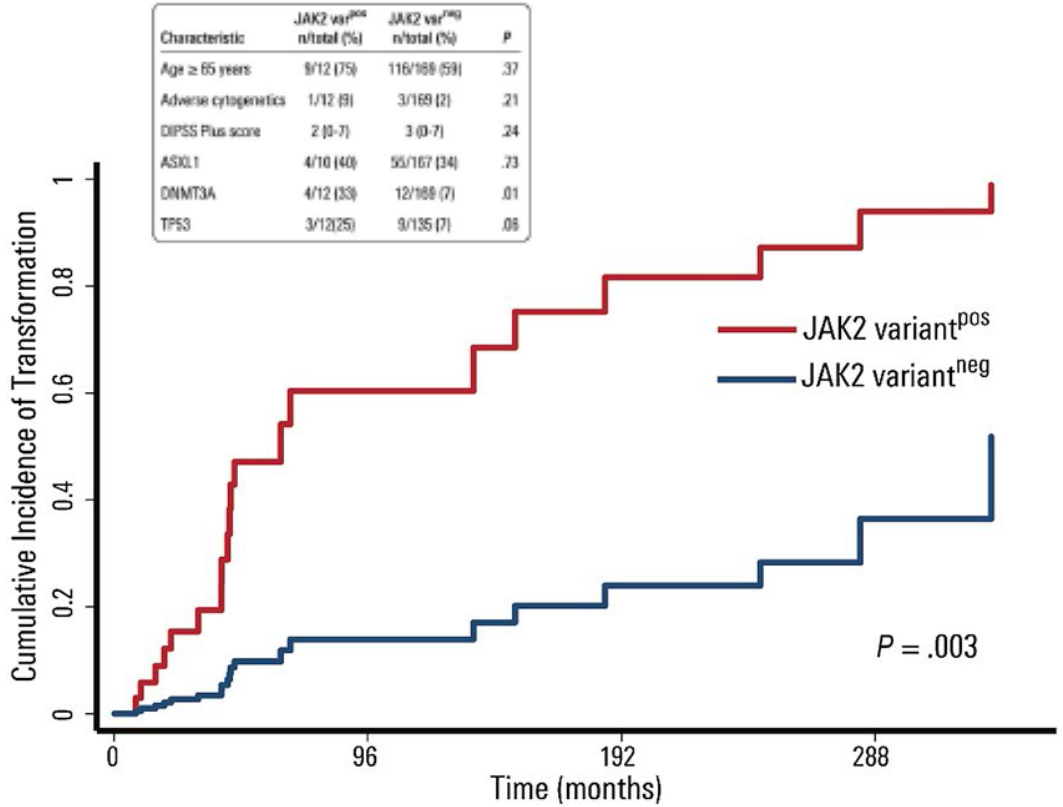
chronological changes in JAK2 allele burden

Silver et al., Leuk Res (2011) 35:177-182
Koren-Michowitz et al., Leuk Lymphoma (2012) 53:2210-2213
Passamonti et al., Leukemia (2010) 24:1574-1579
Shirane S et al., Int J Hematol (2015) 101:148–153

JAK2 variants are associated with transformation into MPN-BP

- besides *JAK2* exon 12 and 14 mutations point mutations across the entire pseudokinase domain, including exons 13 and 15, can be obsered in patients with MPN–related phenotypes
- other important domains of the *JAK2* gene include the four-point-one ezrin, radixin, moesin (FERM; JH4-JH7) and src homology 2 (SH2; JH4) domains

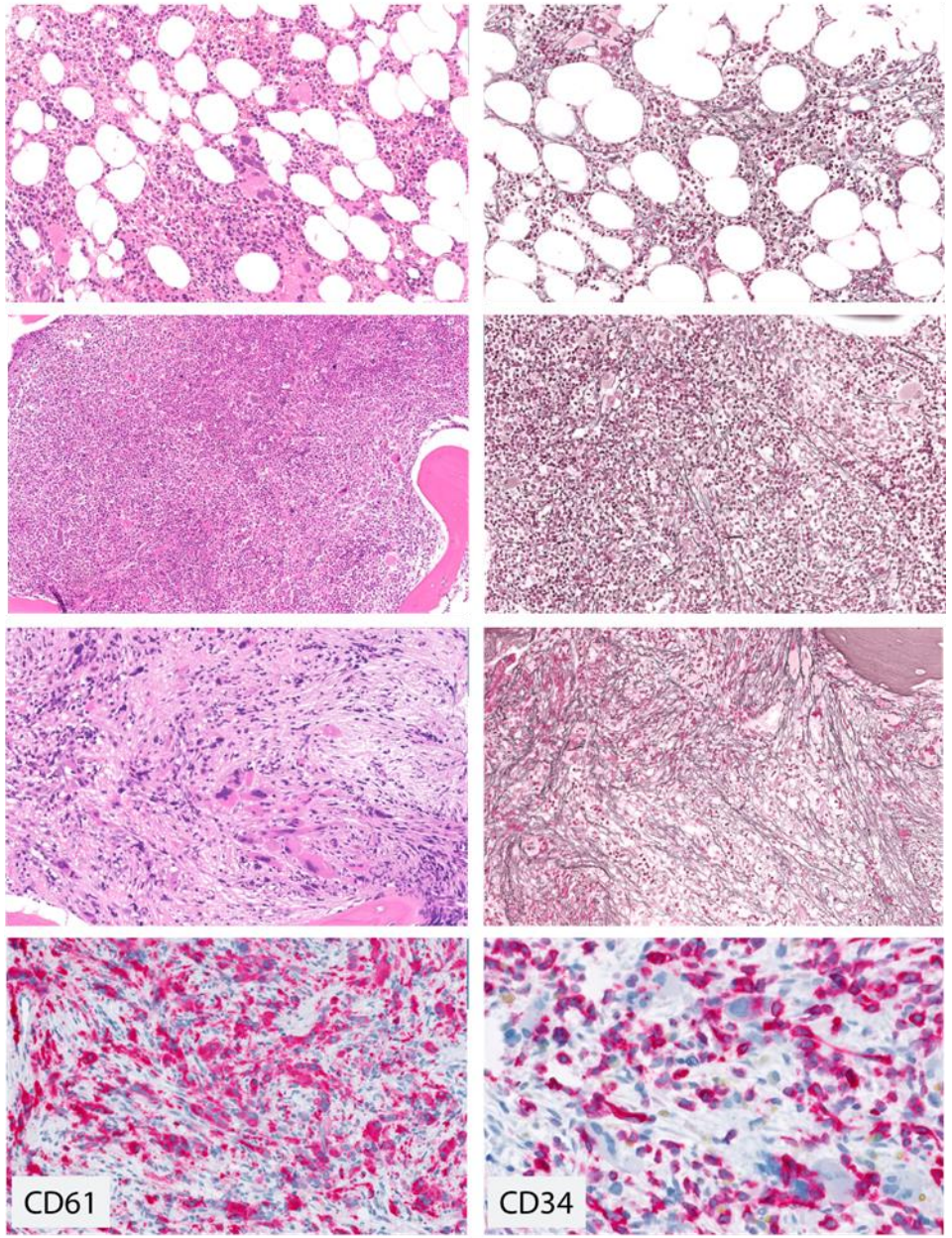




Benton et al., Cancer (2019) 125:1855-1866

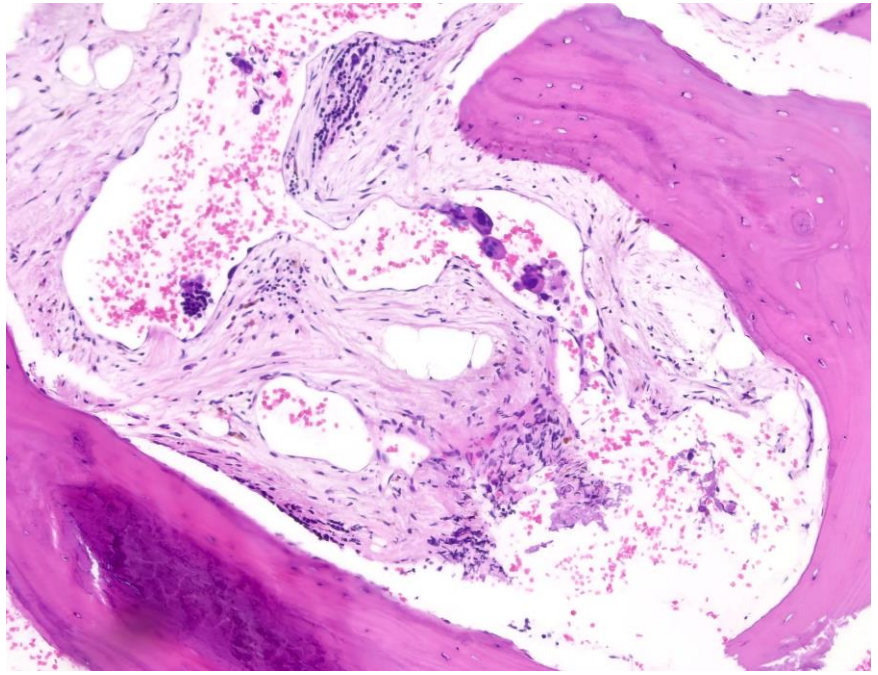
Key features of Post-PV MF

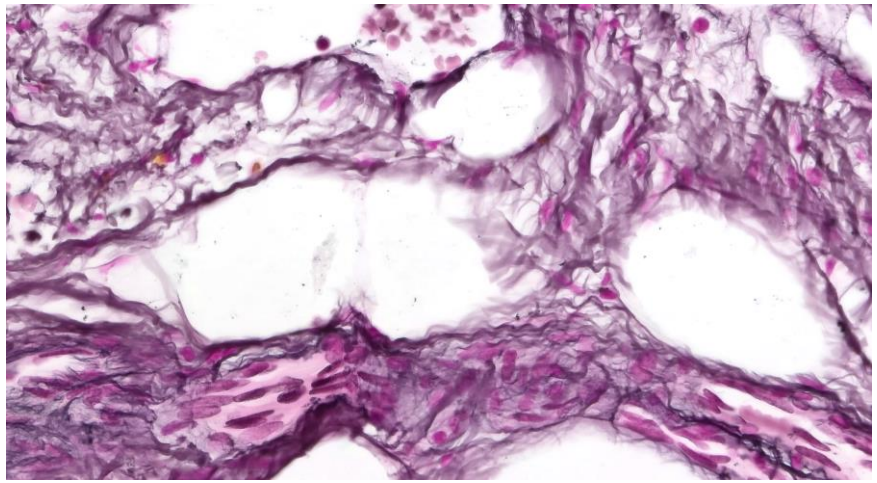
- Post-PV MF is not a uniform stage
- Post-PV MF represents the most common type of disease progression in patients with PV
- develops late in the disease, usually >10-15 years from the initial diagnosis in 20-40% of PV patients
- manifest bone marrow fibrosis (collagen, osteosclerosis), MF-2 or MF-3
- splenomegaly, anemia, L/E, and development of constitutional symptoms



Myeloproliferative neoplasm (MPN), unclassifiable

- Most cases of MPN-U fall into one of three groups:
  - early MPN with overlapping features and/or not yet fully developed diagnostic thresholds (ET-like, PMF-like, PV-like) - cases presenting with portal or splanchnic vein thrombosis
  - progressed advanced-stage MPN with myelodysplastic changes
  - cases with molecular evidence of MPN, but coexisting neoplastic or inflammatory disorder that obscures some of the usual diagnostic features





ICC diagnostic criteria for MPN-U

Myeloproliferative neoplasm, unclassifiable (MPN-U)

1. Clinical and hematological features of an MPN are present[a)]
2. JAK2, CALR, or MPL mutation[b)]
   or
   Presence of another clonal marker[c)]
3. Diagnostic criteria for any other MPN, myelodysplastic syndrome, myelodysplastic/ myeloproliferative neoplasm[d)], or BCR::ABL1-positive chronic myeloid leukemia are not met

The diagnosis of MPN-U requires all 3 criteria

a) In cases presenting with BM fibrosis reactive causes must be excluded, in particular BM fibrosis secondary to infection, autoimmune disorder or another chronic inflammatory condition, hairy cell leukemia or another lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathy

b) It is recommended to use highly sensitive assays for *JAK2* V617F (sensitivity level <1%) and *CALR* and *MPL* (sensitivity level 1-3%) - in negative cases, consider searching for non-canonical *JAK2* and *MPL* mutations

c) Assessed by cytogenetics or sensitive NGS techniques; detection of mutations associated with myeloid neoplasms (e.g., *ASXL1*, *EZH2*, *IDH1*, *IDH2*, *SF3B1, SRSF2, and TET2* mutations) supports the clonal nature of the disease

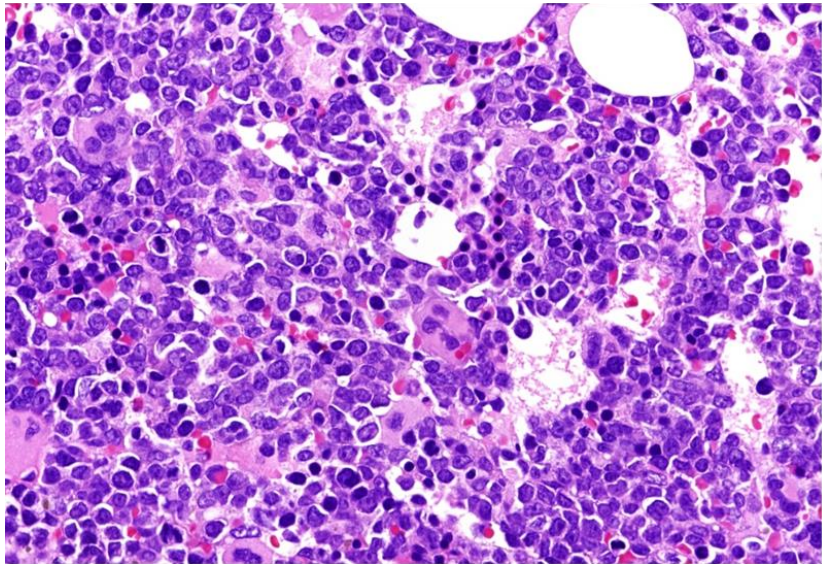
d) In cases presenting with myelodysplastic features effects of any previous treatment, severe comorbidity, and changes during the natural progression of the disease process must be carefully excluded

MPN-BP is not synonymous of AML

- **Distinct differences at the morphologic, molecular and clinical level:**
  - a higher frequency of erythroid and megakaryoblastic morphology in MPN-BP compared to de novo AML
  - Favorable karyotype is much rarer in MPN-BP compared to de novo AML
  - Mutations of TP53, SRSF2, IDH1/2, ASXL-1 and LNK are more common in MPN-BP
  - Mutations in N/KRAS, DNMT3a, NPM1, and FLT3 are more frequently observed in de novo AML
  - Median survival of LT is < 6 months, and induction chemotherapy response rates and overall outcome remain dismal

Morphology of MPN-BP

- Myeloblastic (AML-MRC like features in most cases)
- Megakaryoblastic
- Erythroblastic (pure erythroid leukemia)
- Myeloid sarcoma (type of leukemic presentation)



Mesa R et al. Blood 2005; Mascarenhas J. Clin Lymphoma Myeloma Leuk. 2016;16 Suppl:S124-9; Tefferi A et al, Mayo-AGIMM study. Leukemia 2018

Take home message

- in the era of genetic and genomic tests, diagnosis of MPN still relies on close clinicopathological correlation, with evaluation of both peripheral blood and bone marrow samples being essential in this sense
- non-clonal ET (wild-type) cases rarely have thrombotic and hemorrhagic events and can be considered as low-risk patients
- differentiation of ET from prePMF is essential regarding clinical risk (thrombosis, bleeding) and progression
- prognostically relevant mutations in PMF
- the diagnostic ICC criteria for MPN can be used in children as in adults