# Myelofibrotic Ph Negative Myeloproliferative Neoplasms Marrow Morphology: Key take aways from WHO 2016 diagnostic update

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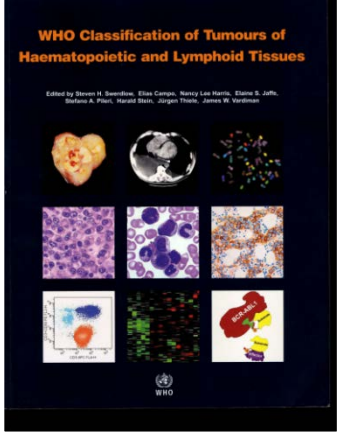
# Disclosures

Consultant or Advisory Role: Incyte

# Outline

- WHO Classification of Myeloproliferative Neoplasms (MPN) 2008, 2016 revision
- Clinical and Morphological Progression in MPN
- Novel Definition of Histomorphological response to targeted therapies (ELN-MDACC)
- Summary

# WHO Classification of Myeloid Neoplasms in Adults 2008 Revision




**Myeloproliferative Neoplasms (MPN)**

**Classic**
- CML, BCR-ABL1+
- Polycythemia vera (PV)
- Essential Thrombocythemia (ET)
- Primary myelofibrosis (PMF)

- CNL
- CEL (NOS)/HES
- Mastocytosis
- MPN-U

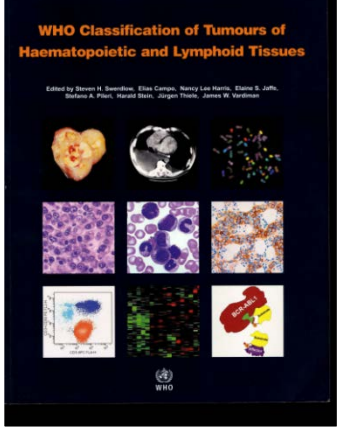
**MDS/ MPN**

**CMML**
**JMML**
**aCML, BCR-ABL1-**
**MDS/MPN-U**
**i.e. RARS-T**

**Myeloid & lymphoid neoplasms w/**
- Eosinophilia and
- Abnormalities of PDGFRA/B
- Abnormalities of FGFR1

**Rearranged PDGFRA**
- PDGFRB
- FGFR1

# WHO Classification of Myeloid Neoplasms in Adults - 2016




**Myeloproliferative Neoplasms (MPN)**

**Mastocytosis**

**MDS/ MPN**

**Myeloid & lymphoid neoplasms w/**
- **Eosinophilia and**
- **Rearrangement of PDGFRA/B or**
- **FGFR1 or PCM1-JAK2**

**Classic**
- **CML, BCR-ABL1+**
- **Polycythemia vera (PV)**
- **Essential Thrombocythemia (ET)**
- **Primary myelofibrosis (PMF)**

- **CNL**
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**CMML**
**JMML**
**aCML, BCR-ABL1-**
**MDS/MPN-U**
**MDS/MPN-RS-T**

**Rearranged PDGFRA**
- **PDGFRB**
- **FGFR1**
- **PCM1-JAK2**

D.A. Arber et al. *Blood* 2016:2391

# 2016 Revision World Health Organization (WHO) Diagnostic Criteria for *BCR-ABL1*-Negative MPN

| | *Polycythemia vera (PV)*[a] | *Essential thrombocythemia (ET)*[b] | *Primary myelofibrosis (PMF)*[c] |
|---|---|---|---|
| *Major criteria* | | | |
| 1 | Hemoglobin >16.5 g/dl (men) >16 g/dl (women) or hematocrit >49% (men) >48% (women) | Platelet count $\geq 450 \times 10^9$/l | Megakaryocyte proliferation and atypia[d] accompanied by hypercellularty, increased Grans, either reticulin and/or collagen fibrosis or[e] |
| 2 | BM hypercellular trilineage myeloproliferation with pleomorphic megakaryocytes | Megakaryocyte proliferation with large and mature morphology | Not meeting WHO criteria for CML, PV, ET, MDS or other myeloid neoplasm |
| 3 | Presence of *JAK2* V617F or exon 12 mutation | Not meeting WHO criteria for CML, PV, PMF, MDS or other myeloid neoplasm | Presence of *JAK2*, *CALR* or *MPL* mutation or presence of a clonal marker |
| 4 | | Presence of *JAK2*, *CALR* or *MPL* W515K/L mutation | Absence of reactive myelofibrosis |
| *Minor criteria* | | | |
| 1 | Subnormal serum erythropoietin level | Presence of a clonal marker (e.g. abnormal karyotype) or absence of evidence for reactive thrombocytosis | Leukocytosis ≥11K/uL |
| 2 | | | Presence of anemia or palpable splenomegaly |
| 3 | | | Presence of leukoerythroblastosis[f] or increased lactate dehydrogenase[f] |

[a] PV diagnosis requires meeting either all three major criteria or the first two major criteria and one minor criterion.

[b] ET diagnosis requires meeting all four major criteria or first three major criteria and one minor criterion.

[c] PMF diagnosis requires meeting all three major criteria or the first two major criteria and alt least 1 minor criteria. Confirmed x2

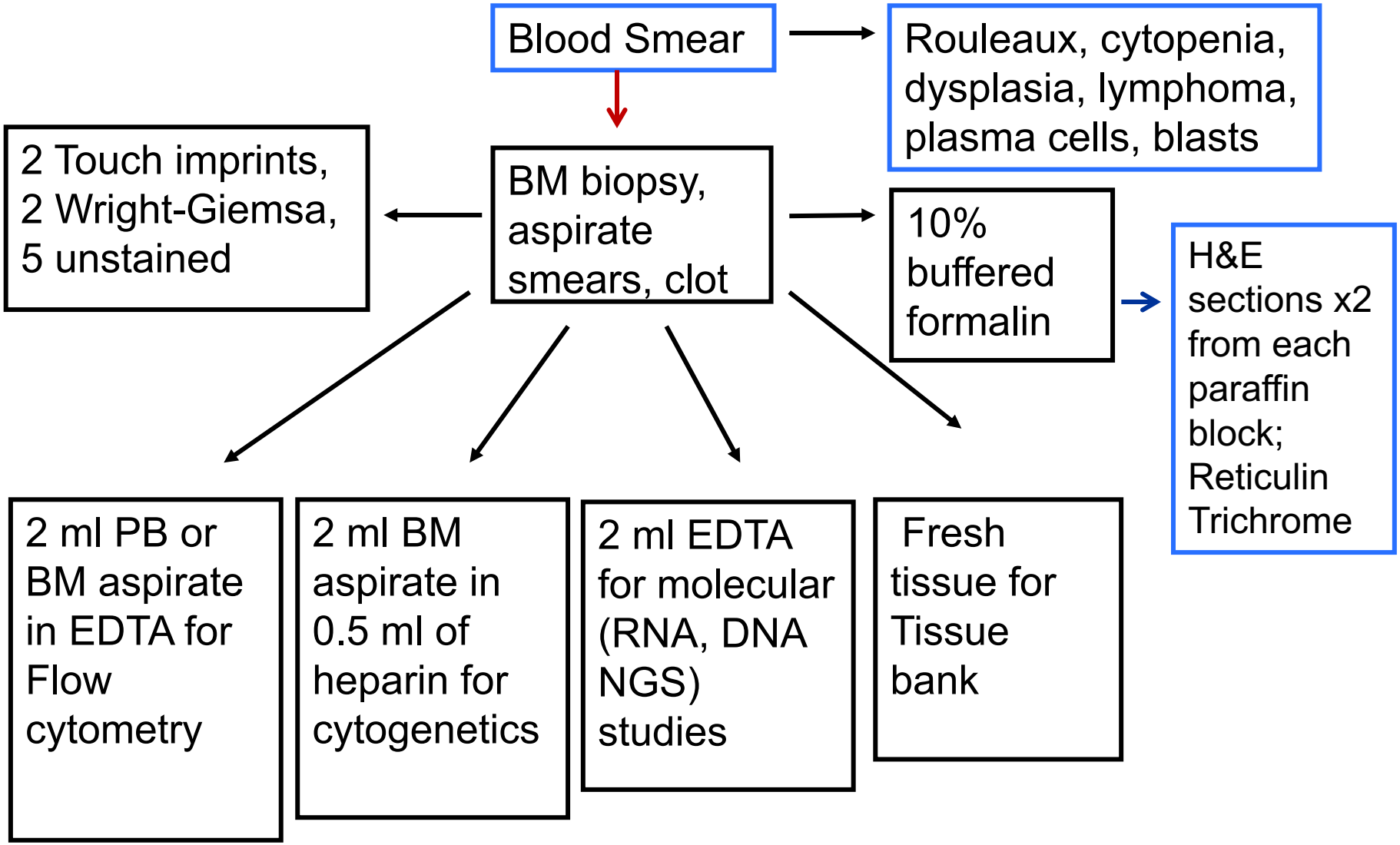
[d] Small-to-large megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering.

[e] In the absence of reticulin fibrosis, the megakaryocyte changes must be accompanied by increased marrow cellularity, granulocytic proliferation and often decreased erythropoiesis (that is, prefibrotic PMF).

[f] Degree of abnormality can be borderline or marked and institutional reference range should be used for lactate dehydrogenase level.

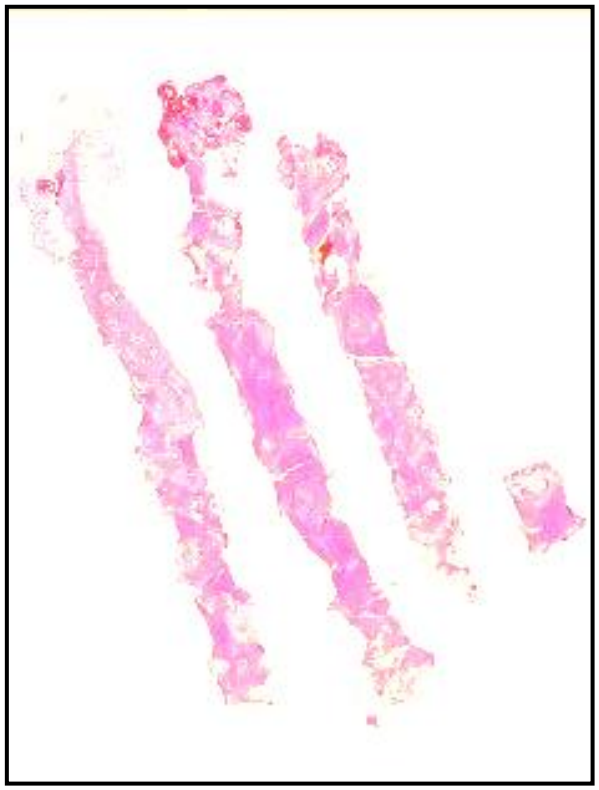
***Blood (2016) 2391 - 2405***

# BM specimen workflow of suspected Hematolymphoid Malignancy



# Adequacy

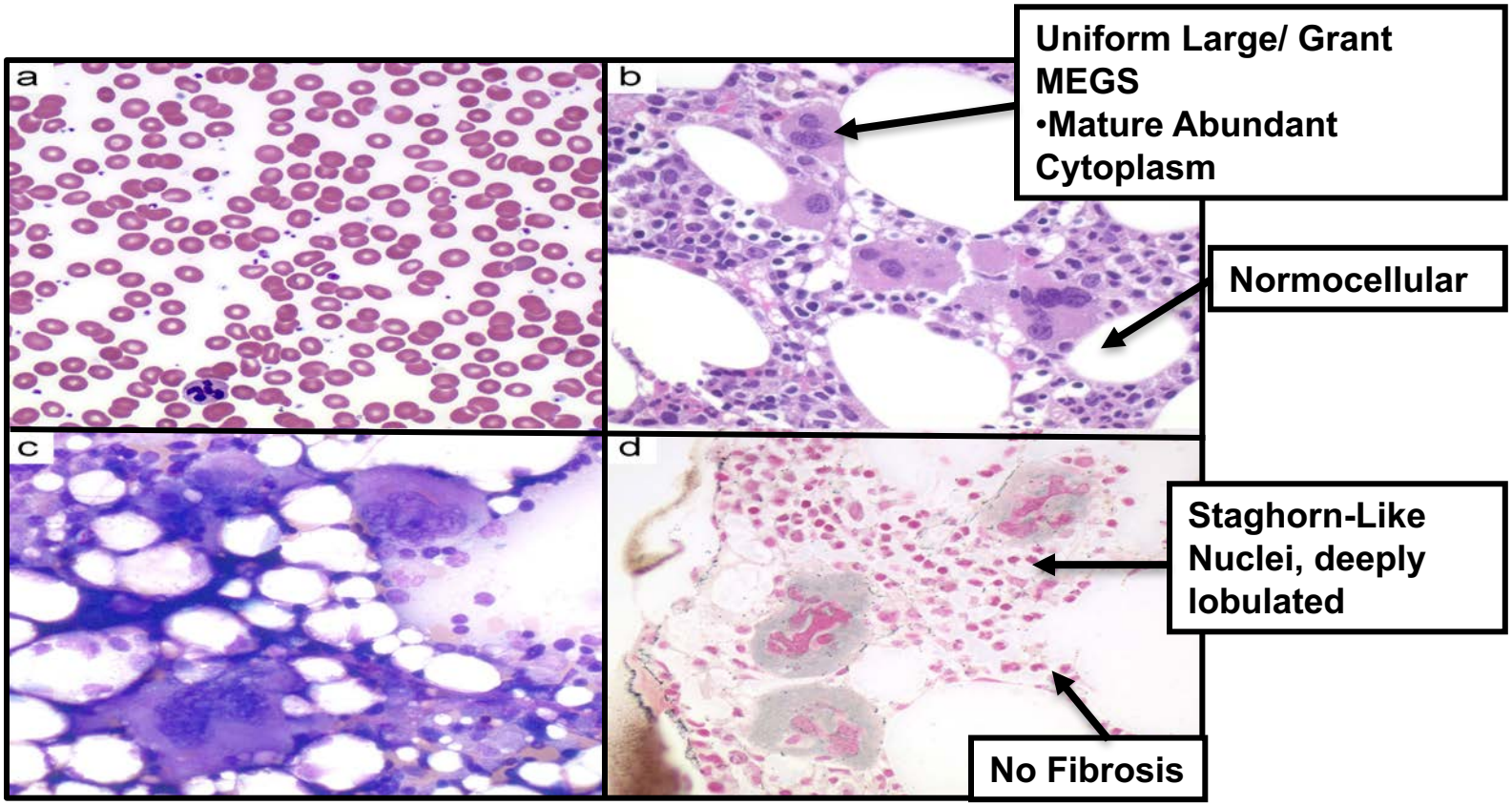
- Bilateral iliac crest trephine biopsies
- Biopsy 1.5 cm
- Fix 3 hrs. in 10% buffered formalin
- Decal Stat 1 hr.; or 5% formic acid 12 hrs
- B5 fix/EDTA decal
- Embedded in paraffin, cut sections 3 microns thick



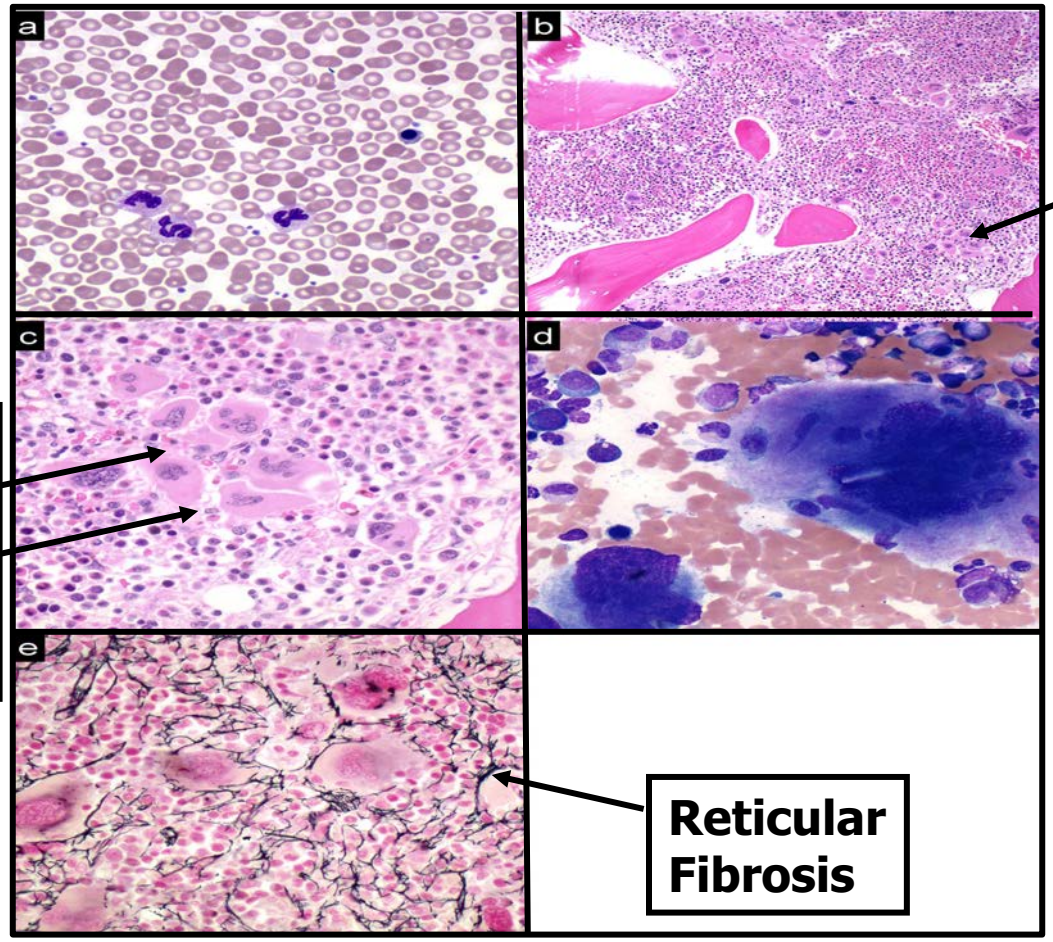
Adequate

Inadequate

# Essential Thrombocythemia in a 74 year-old female



# Polycythemia Vera with Myelofibrosis

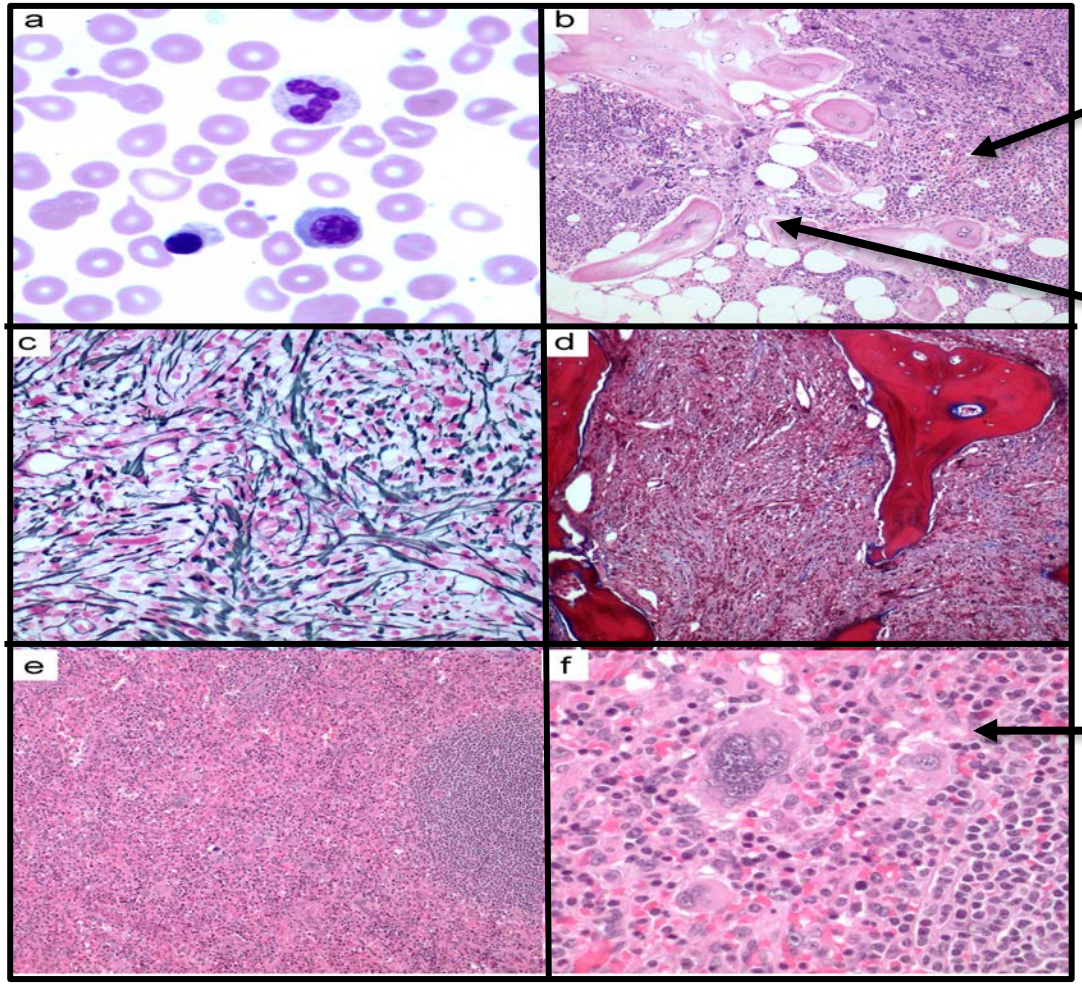



**Hypercellular Increase in all cell lineages**

**Loose Clusters of MEGS**
- **Small to Giant**
- **Normal N/C ratio**

**Reticular Fibrosis**

# Primary Myelofibrosis
# Fibrotic Stage

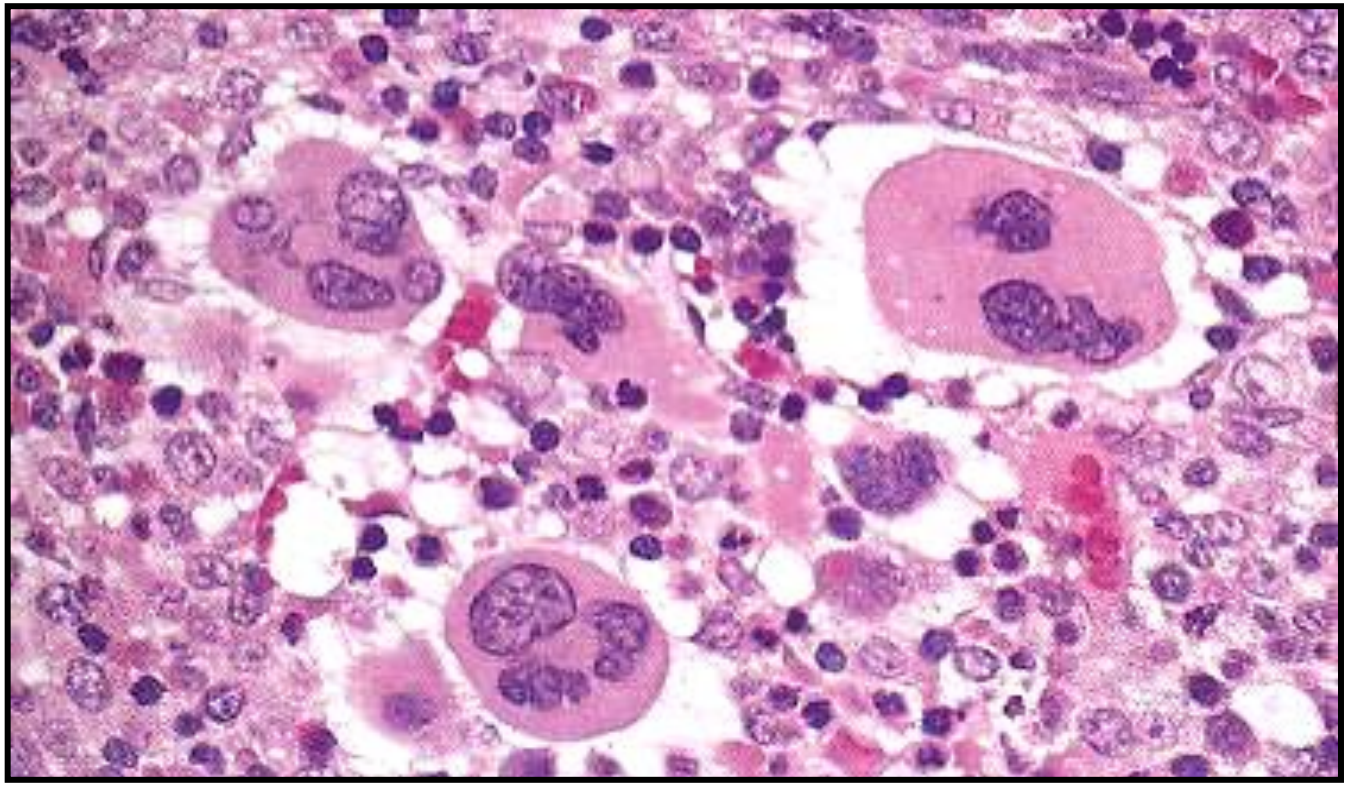



**Dense Atypical MEG Clusters**

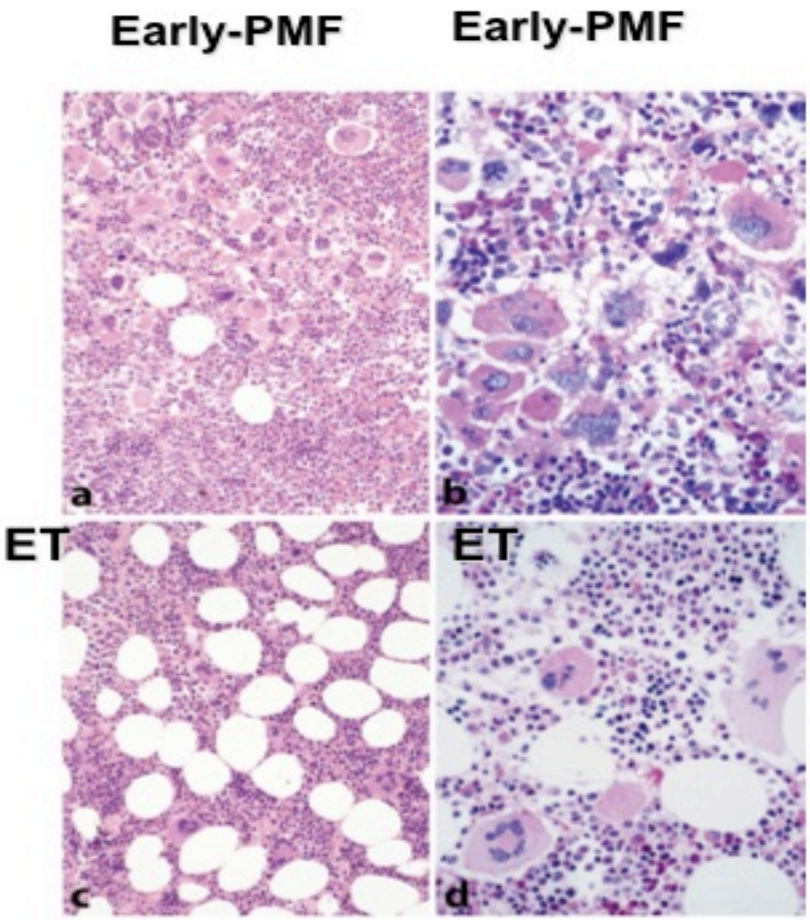
**Translocate close to Bone**

**Trilineage Hematopoiesis Spleen**

# Prefibrotic/early PMF: Disorganized "balloon-like" Nuclear Lobules



# Comparative histopathology in early stage PMF and ET




## *Early Primary Myelofibrosis*

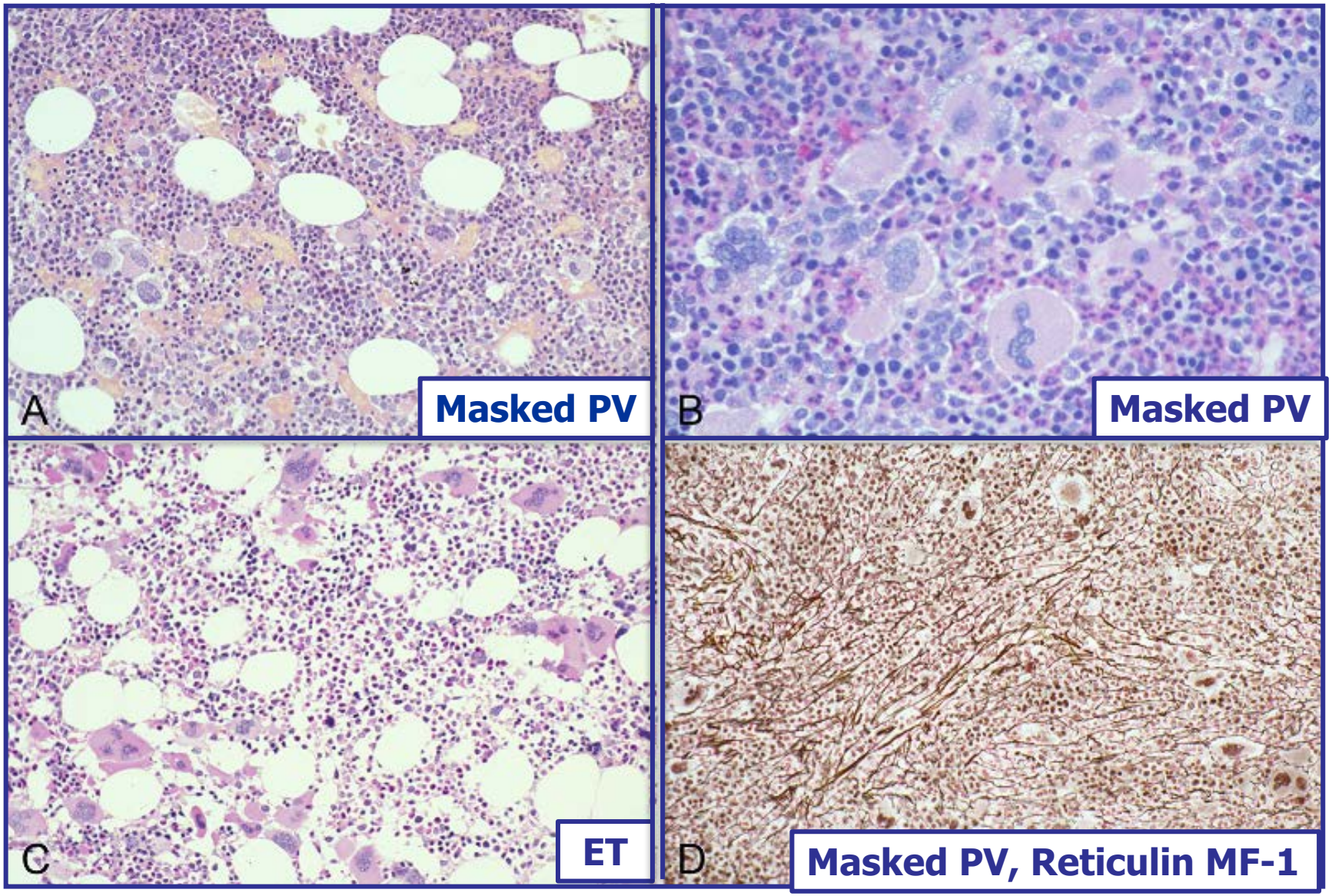
- ✓ hypercellular
- ✓ prominent clustering of abnormal megakaryocytes
- ✓ hypolobulated / hyperchromatic nuclei
- ✓ Granulocytic proliferation

## *Essential Thrombocythemia*

- ✓ normocellular
- ✓ Dispersed large to giant megakaryocytes

**Barbui et al. *Blood* 2010**

# EL Net




Kvasnicka H-M *et al. Am J Hematol*. 2017 Jul 7 [Epub ahead of print]

# Key Points – ET / PV

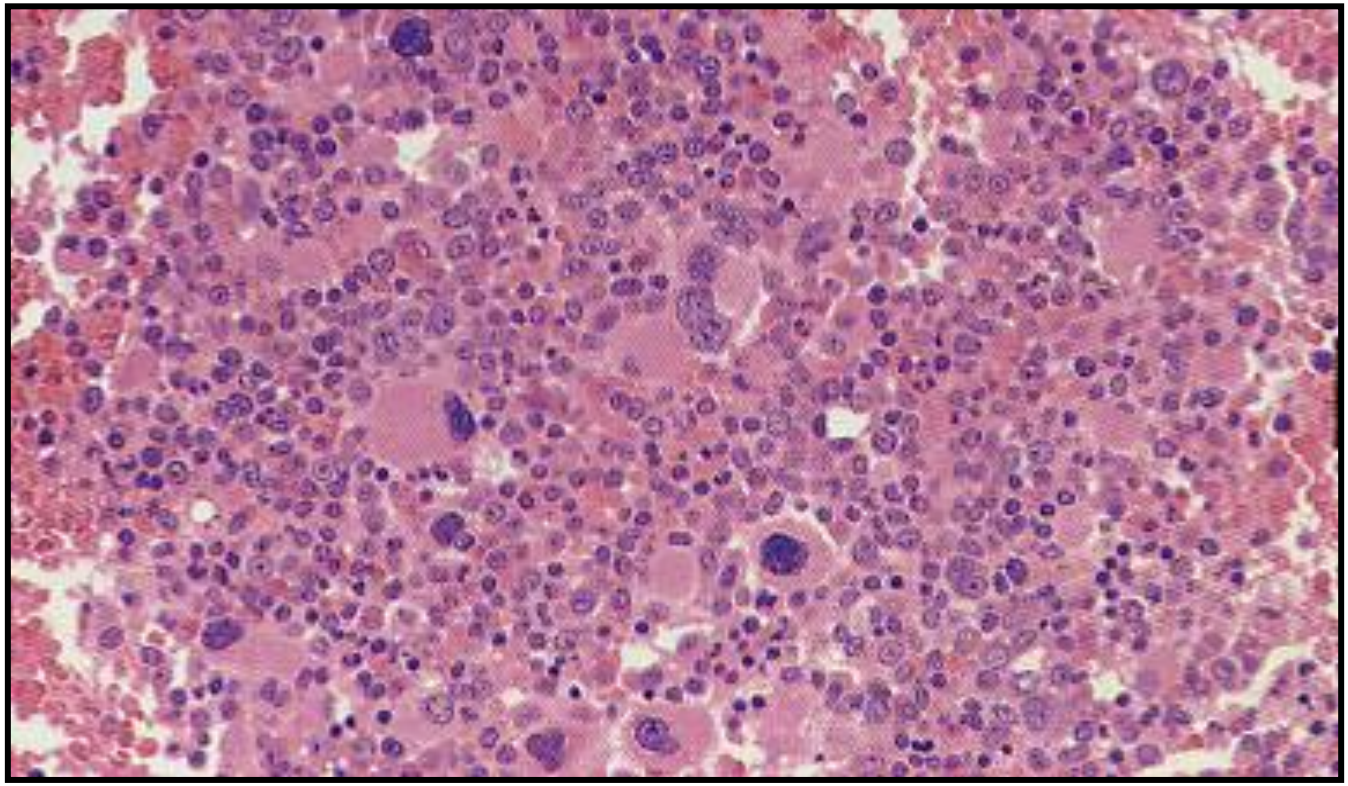
| ET | PV |
|---|---|
| Cellularity (age-matched) | |
| • Normocellular (<20% over expected cellularity for age) | • Hypercellular (>20% over expected cellularity for age, usually nearly 100%) |
| Increased lineage(s) | |
| • Megakaryocytes only<br>• No left shift in erythro-/granulopoiesis | • Erythro-/megakaryo-/granulopoiesis (panmyelosis)<br>• Left shift in erythro- and granulopoiesis |
| • Morphological characteristics | |
| • Large/giant, mature megakaryocytes with hyperlobulated nuclei | • mature megakaryocytes with significant variability in size (pleomorphism) |
| • Bone marrow stroma | |
| • usually no increase in reticulin fibers (<5%)<br>• normal sinuses<br>• lymphoid nodules rare or absent | • mild increase in reticulin fibers (<20%)<br>• dilated sinuses, some with intraluminal erythrocytes<br>• lymphoid nodules up to 20% |
| • Molecular features* data from Rotunno et al. [33] | |
| • JAK2: 64%<br>• CALR: 15%<br>• MPL: 4%<br>• Triple-negative: 16% | • 100% JAK2 / Exon12<br><br>**Kvasnicka H-M *et al. Am J Hematol* . 2017 Jul 7 [Epub ahead of print]** |

# Hematological data in patients with mPV vs overt PV (mean ± 95 confidence interval) *cm below left costal margin

| | mPV | PV |
|---|---|---|
| **No. of Patients** | 48 | 31 |
| **HB (g/dL)** | 16.2 [16.0 – 16.6] | 19.2 [18.6 – 19.6] |
| **HCT (%)** | 49.6 [48.0 – 51.6] | 59.4 [59.7 – 62.2] |
| **WBC (x $10^9$/L)** | 12.1 [10.7 – 13.5] | 18.3 [6.9 – 29.6] |
| **Platelets (x $10^9$/L)** | 687 [568 – 806] | 483 [377 – 590] |
| **Spleen size (cm)*** | 0.9 [0.3 – 1.5] | 0.9 [0.4 – 1.3] |
| **EPO-subnormal (%)** | 84 | 100 |
| **LDH (U/L)** | 268 [237 – 298] | 318 [221 – 414] |

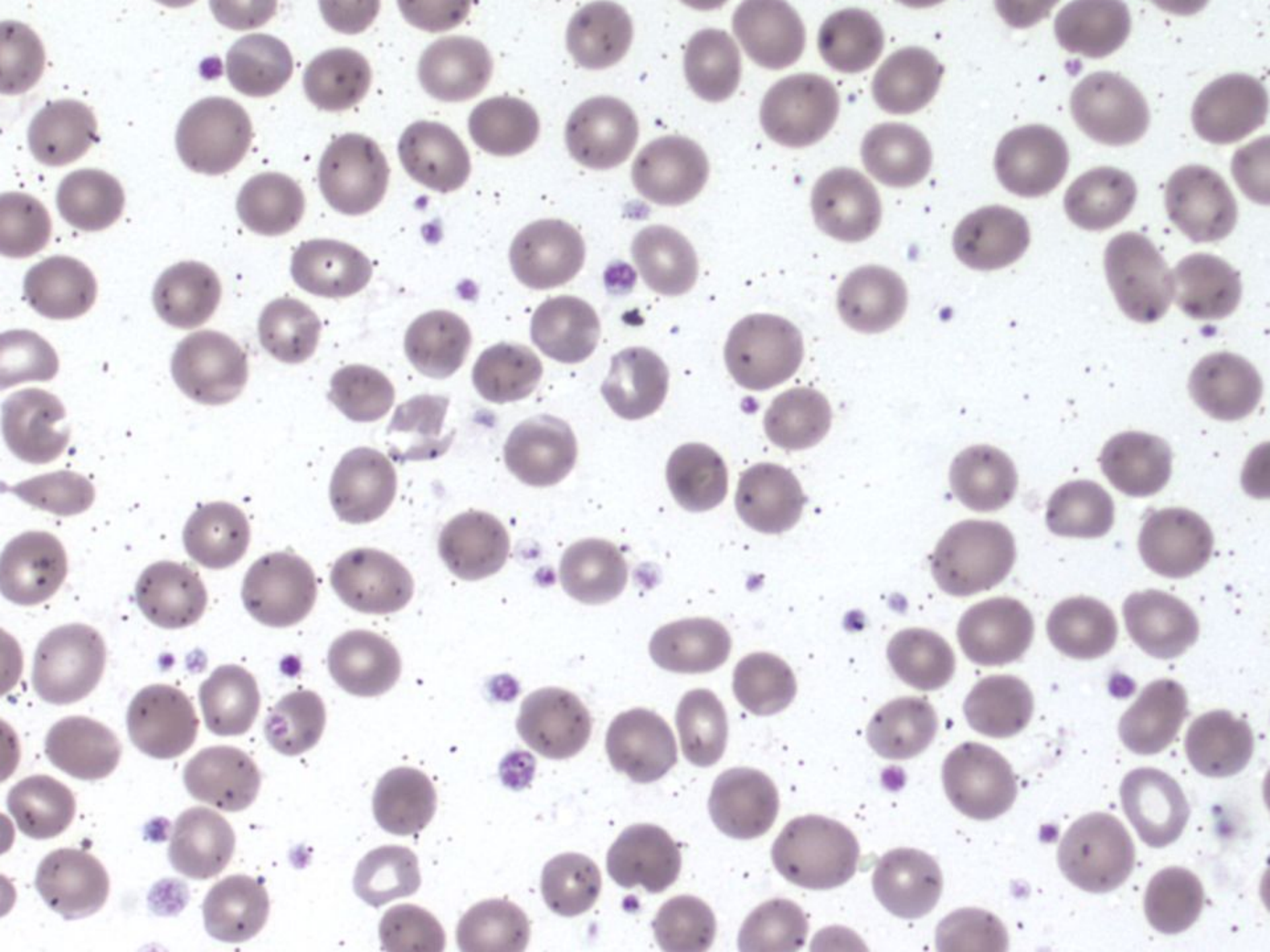
**Kvasnicka H-M *et al*. *Am J Hematol*. 2017 Jul 7 [Epub ahead of print]**

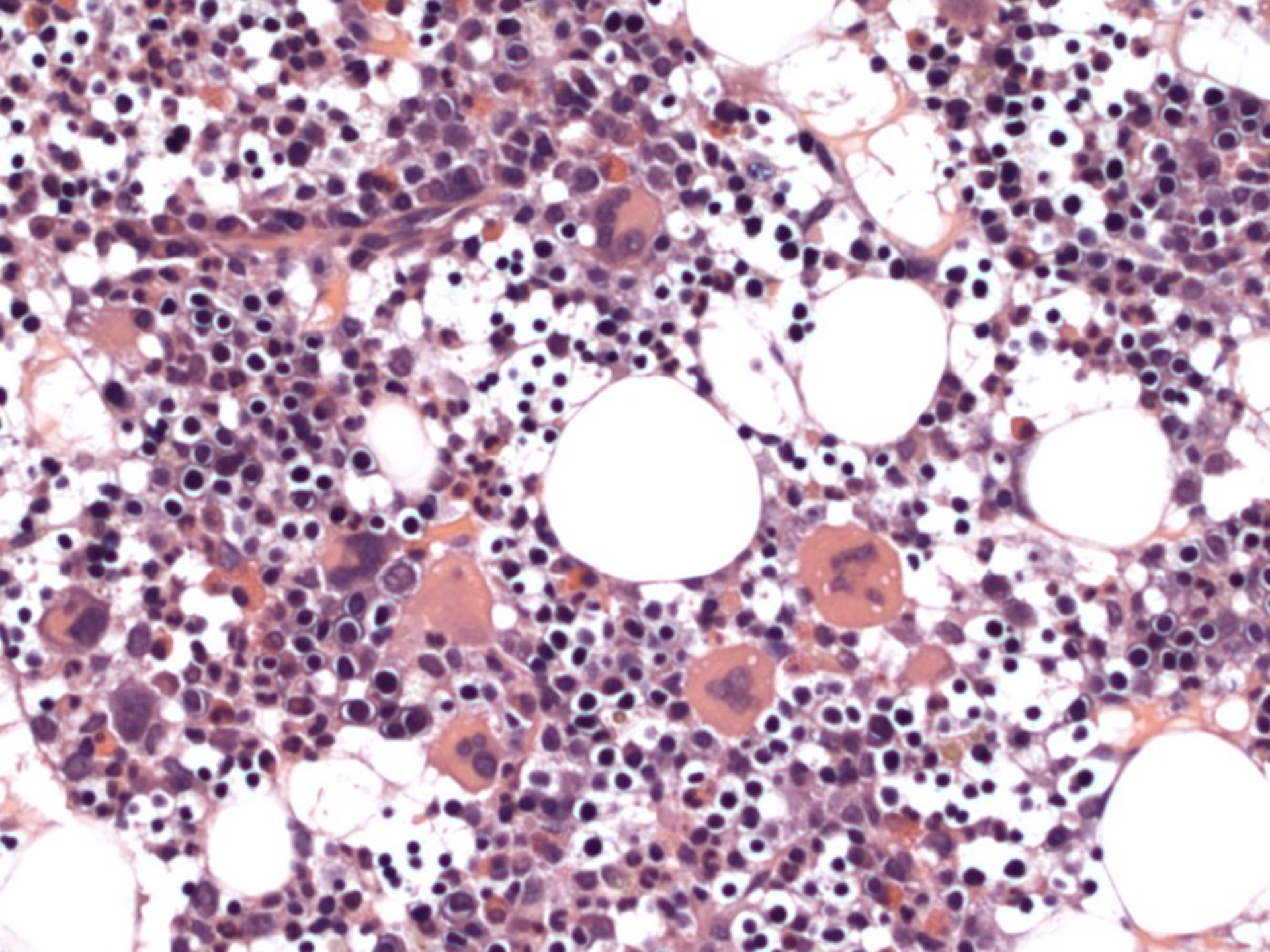
# CML with p230: Marked Thrombocytosis, Minimal Leukocytosis

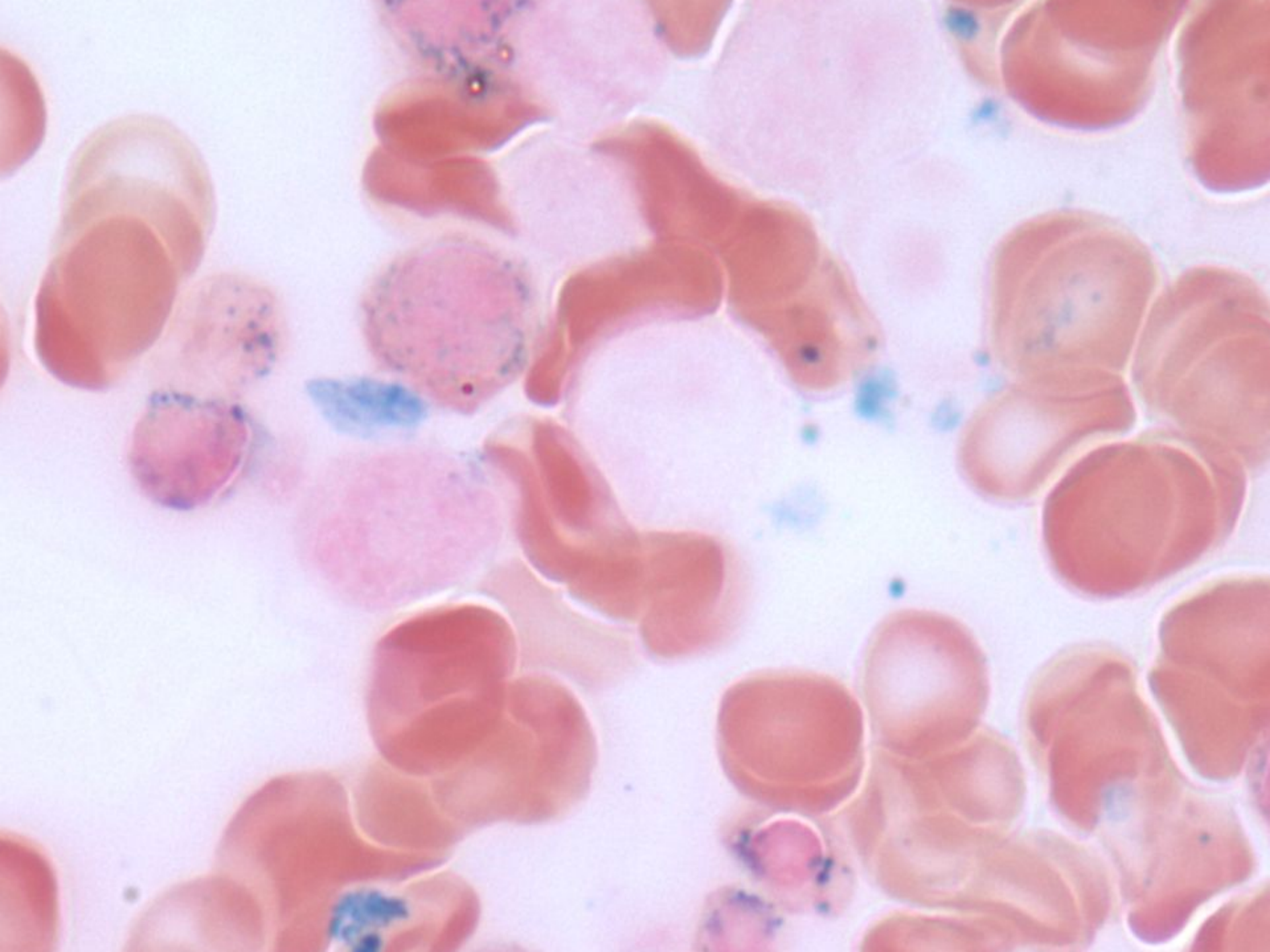


# Case

- 62 year-old woman presents with anemia and thrombocytosis at the time of a general medical exam
- Labs:
  - Hemoglobin 9.7 g/dL
  - Leukocytes 7.2 x 10(9)/L
  - Platelets 600 x 10(9)/L
- Exam:
  - Spleen tip palpable







# Case

- Bone marrow
  - Dysplasia in erythroid lineage
  - Ringed sideroblasts are noted

- What if she had the JAK2-V617F mutation?
- What if 1+ fibrosis

How do we separate MDS/MPN-RS-T from PMF or ET?

# ARS Polling Question

According to 2016 revision to the WHO of Myeloid Neoplasms, which of the following statement about the diagnostic criteria for MDS/MPN with ring sideroblasts and thrombocytosis is correct?

a. Anemia is uncommon; <15% ring sideroblasts
b. Preceding history of MPN, MDS or other MDS/MPN
c. PCM1-JAK2 is detected in most of the cases
d. Presence of mutated *SF3B1,* anemia, ≥15% ring sideroblasts, <5% blasts in the BM, persistent thrombocytosis ≥450K
e. Presence of mutated *SF3B1*, anemia, 5% ring sideroblasts, <5% blasts in the BM, persistent thrombocytosis ≥450K

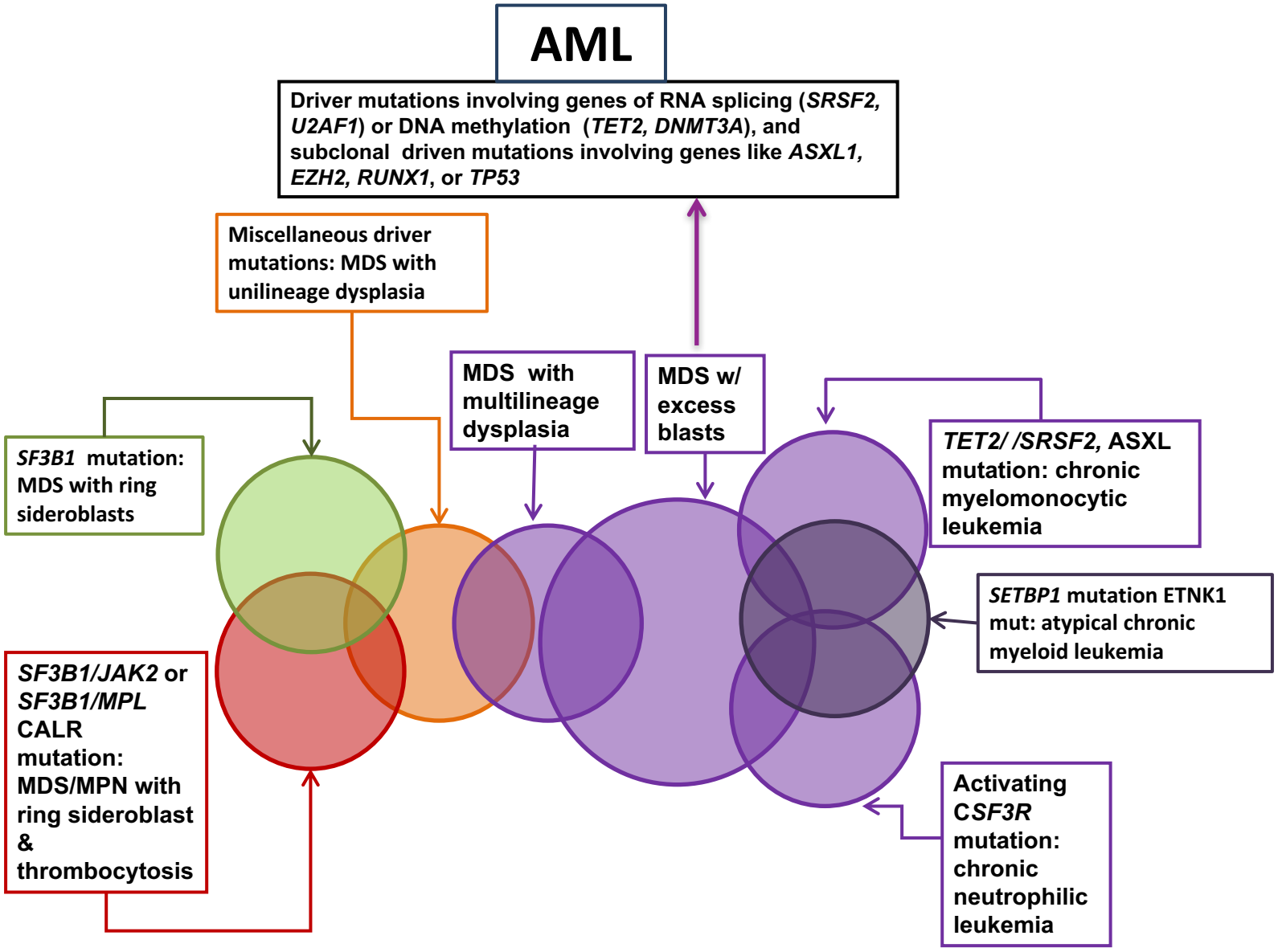
# Diagnostic criteria for MDS/MPN with ring sideroblasts and thrombocytosis

| |
|---|
| • Anemia associated with erythroid lineage dysplasia with or without multilineage dysplasia, ≥15% ring sideroblasts,* <1% blasts in PB and <5% blasts in the BM |
| • Persistent thrombocytosis with platelet count ≥450 × $10^9$/L |
| • Presence of a SF3B1 mutation or, in the absence of SF3B1 mutation, no history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myeloproliferative features† |
| • No BCR-ABL1 fusion gene, no rearrangement of PDGFRA, PDGFRB, or FGFR1; or PCM1-JAK2; no (3;3)(q21;q26), inv(3)(q21q26) or del(5q)‡ |
| • No preceding history of MPN, MDS (except MDS-RS), or other type of MDS/MPN |

**Diagnostic criteria for MDS/MPN with ring sideroblasts and thrombocytosis**

- *** At least 15% ring sideroblasts required even if *SF3B1* mutation is detected.**
- **† A diagnosis of MDS/MPN-RS-T is strongly supported by the presence of *SF3B1* mutation together with a mutation in *JAK2* V617F, *CALR*, or *MPL* genes.**
- **‡ In a case which otherwise fulfills the diagnostic criteria for MDS with isolated del(5q)-no or minimal absolute basophilia; basophils usually <2% of leukocytes.**

Arber et al. Blood 2016:2391




**Modified from *Blood* 2013;122(25):4021-4034**

Clinical Lymphoma Myeloma and Leukemia

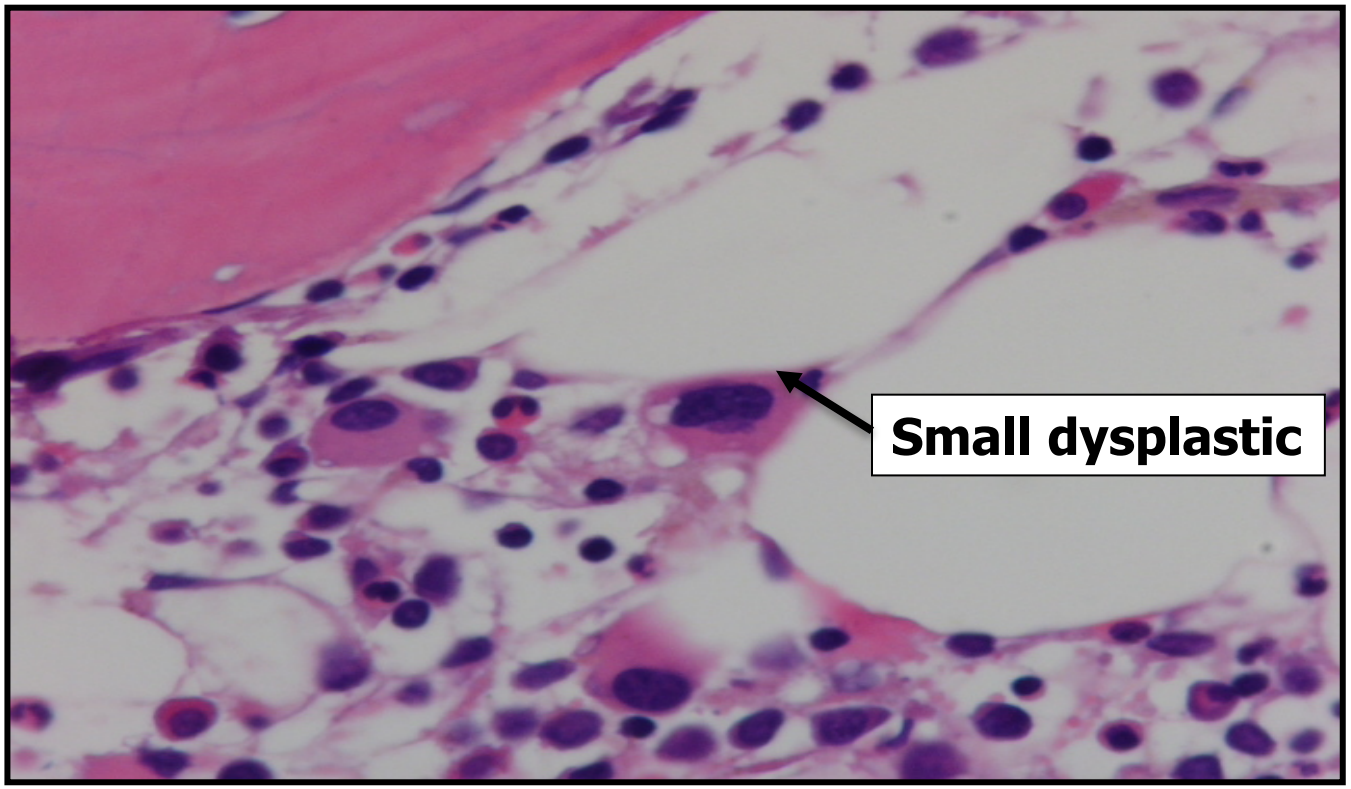
Volume 13, Issue 5, October 2013, Pages 629–633

Case report

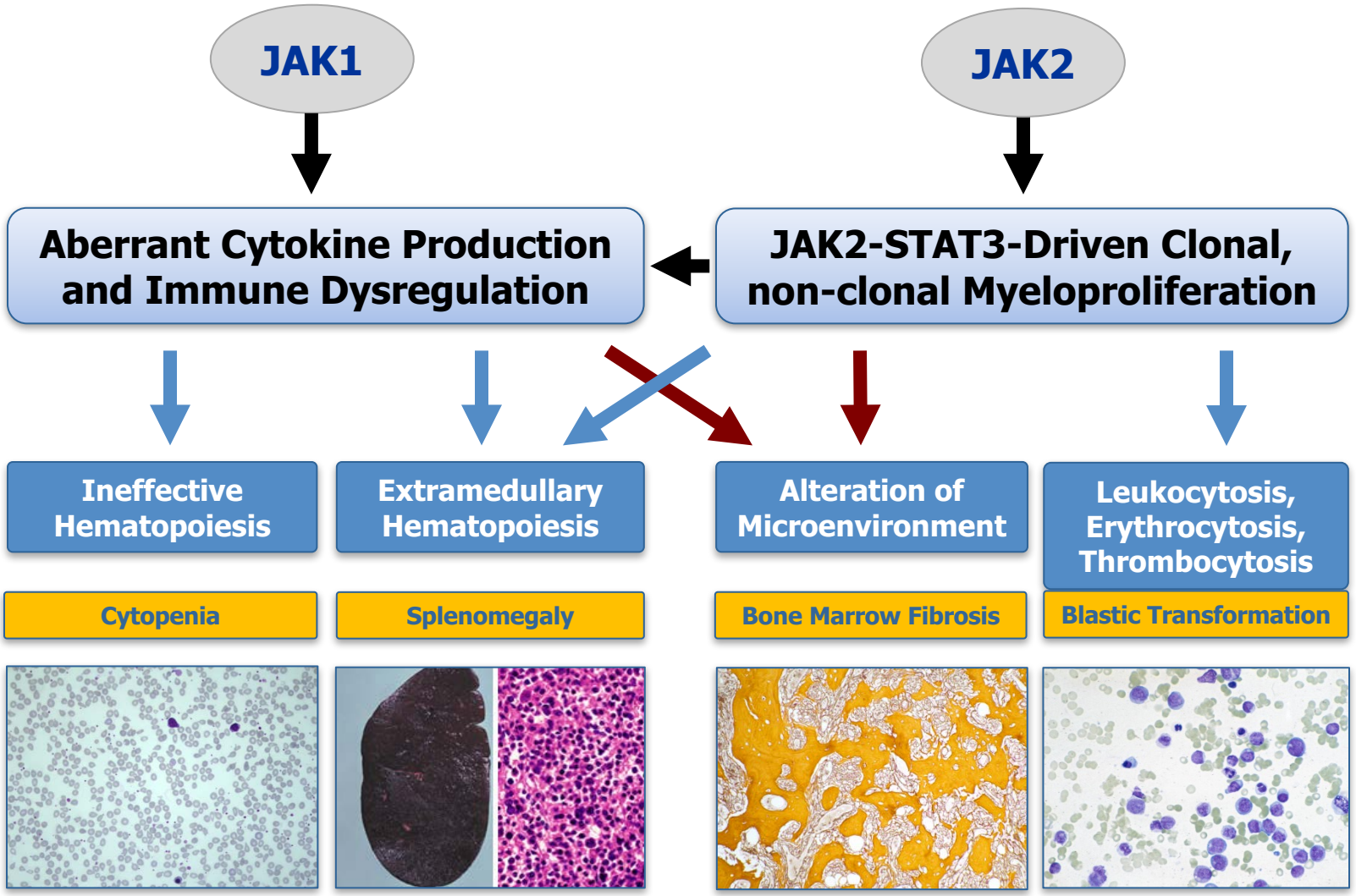
# Metastatic Splenic Angiosarcoma Presenting With Thrombocytopenia and Bone Marrow Fibrosis Mimicking Idiopathic Thrombocytopenic Purpura and Primary Myelofibrosis: A Diagnostic Challenge

Shimin Hu, Carlos E. Bueso-Ramos, Srdan Verstovsek, Roberto N. Miranda, C. Cameron Yin, Timothy McDonnell, L. Jeffrey Medeiros, Pei Lin

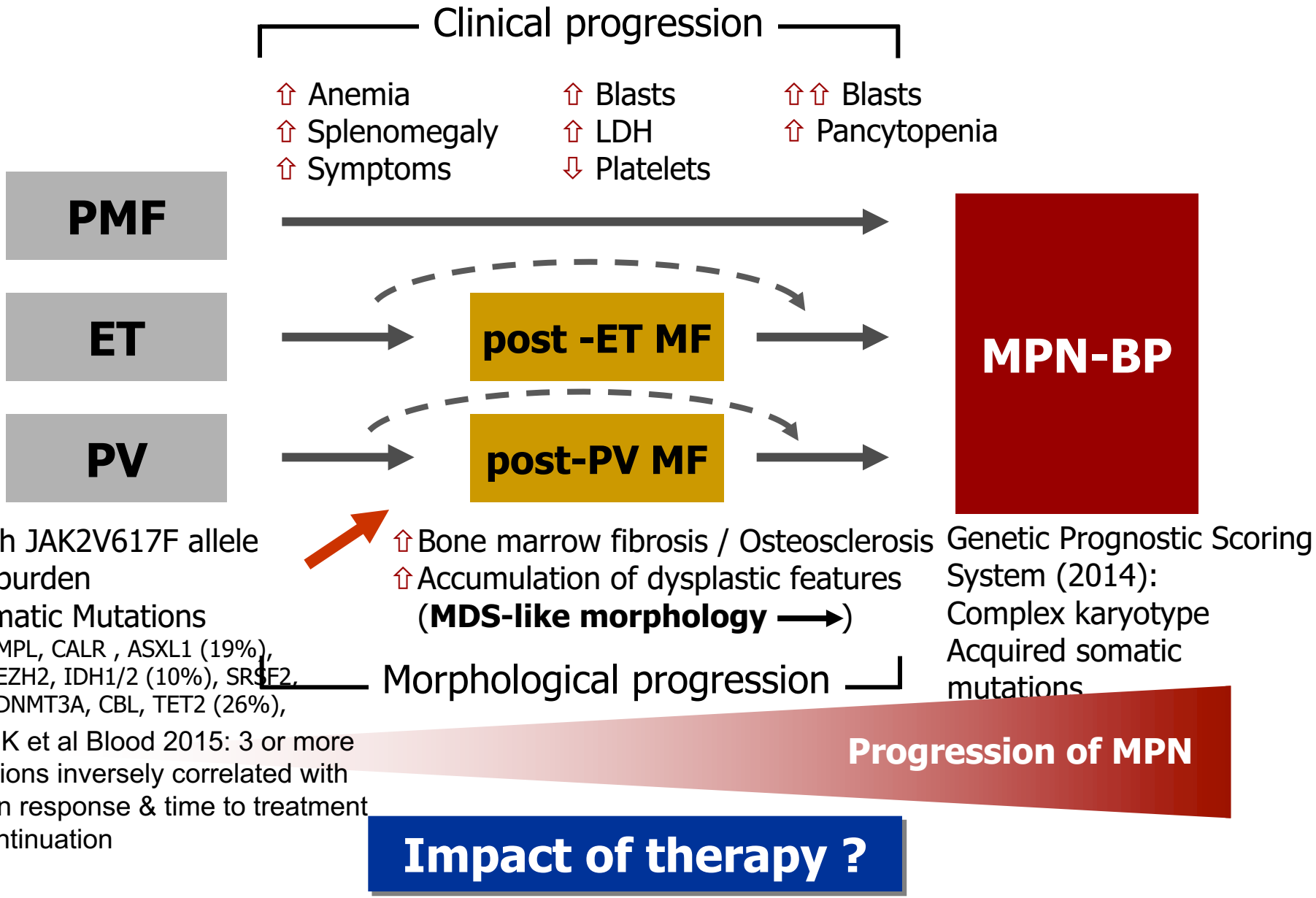
# MDS with myelofibrosis (MDS-F)



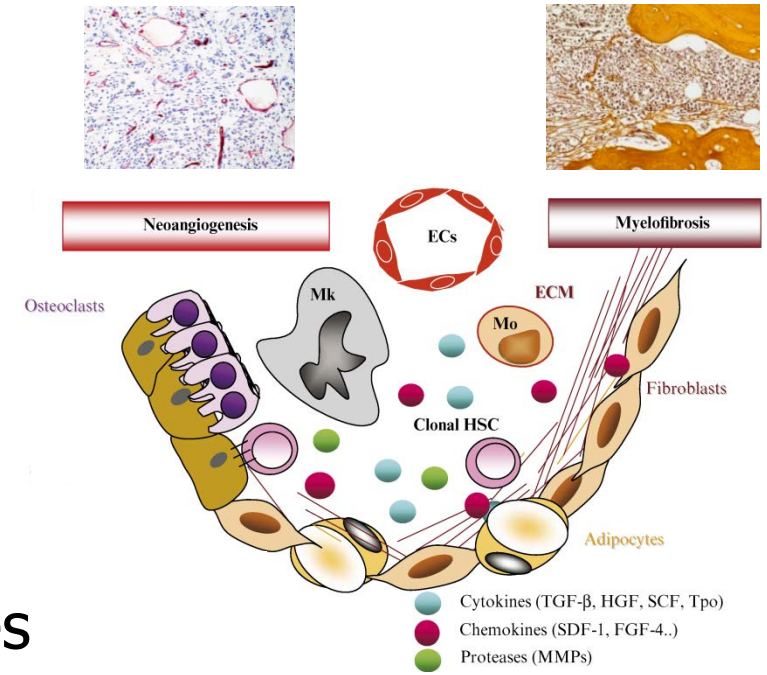
# Mechanisms of Disease in Ph-MPN



# Clinical and Morphological Progression in MPN



# Myelofibrosis

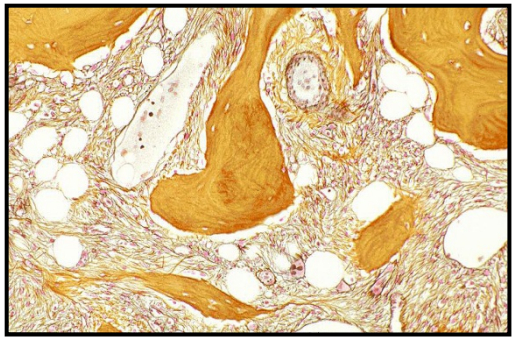



- **Accumulation of reticulin and/or collagen bone marrow fibers**
- Reactive and neoplastic etiologies
- In MPN, myelofibrosis is mediated by clonal and non-clonal cell–derived cytokines and autoimmune reactions to the altered bone marrow stroma
- Grading of MF is important to diagnose MPN and to guide treatment decisions and stratify patients in clinical trials

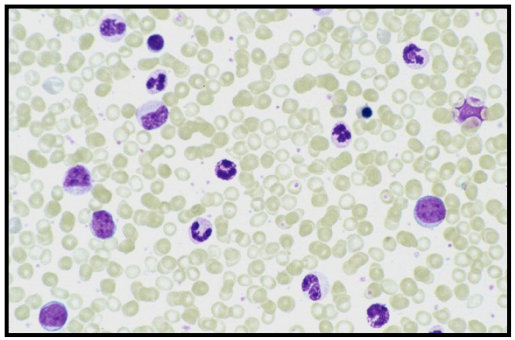
**Quintas-Cardama A, Bueso-Ramos C, Verstovsek S. *J Clin Oncol*. 2009**
**Thiele J, et al. *Histol Histopathol*. 2005**
**Kleppe M, et al. *Cancer Discov*. 2015**

# Histopathological vs clinical features in progressive myelofibrosis

- **Morphology:** increase in BM fibers



- **Clinic:** myelofibrosis associated with extramedullary hematopoiesis (MMM)
  - Progressive anemia
  - Splenomegaly
  - Tear drop poikilocytosis
  - Leukoerythroblastosis
  - BM & MMM exhibit similar miRNA profiles



Nazha A, Estrov Z, Bueso-Ramos C , Verstovsek S, et al. *Leuk Lymphoma* 2013; Loghavi S et al USCAP 2016

# Response and progression criteria for MPN

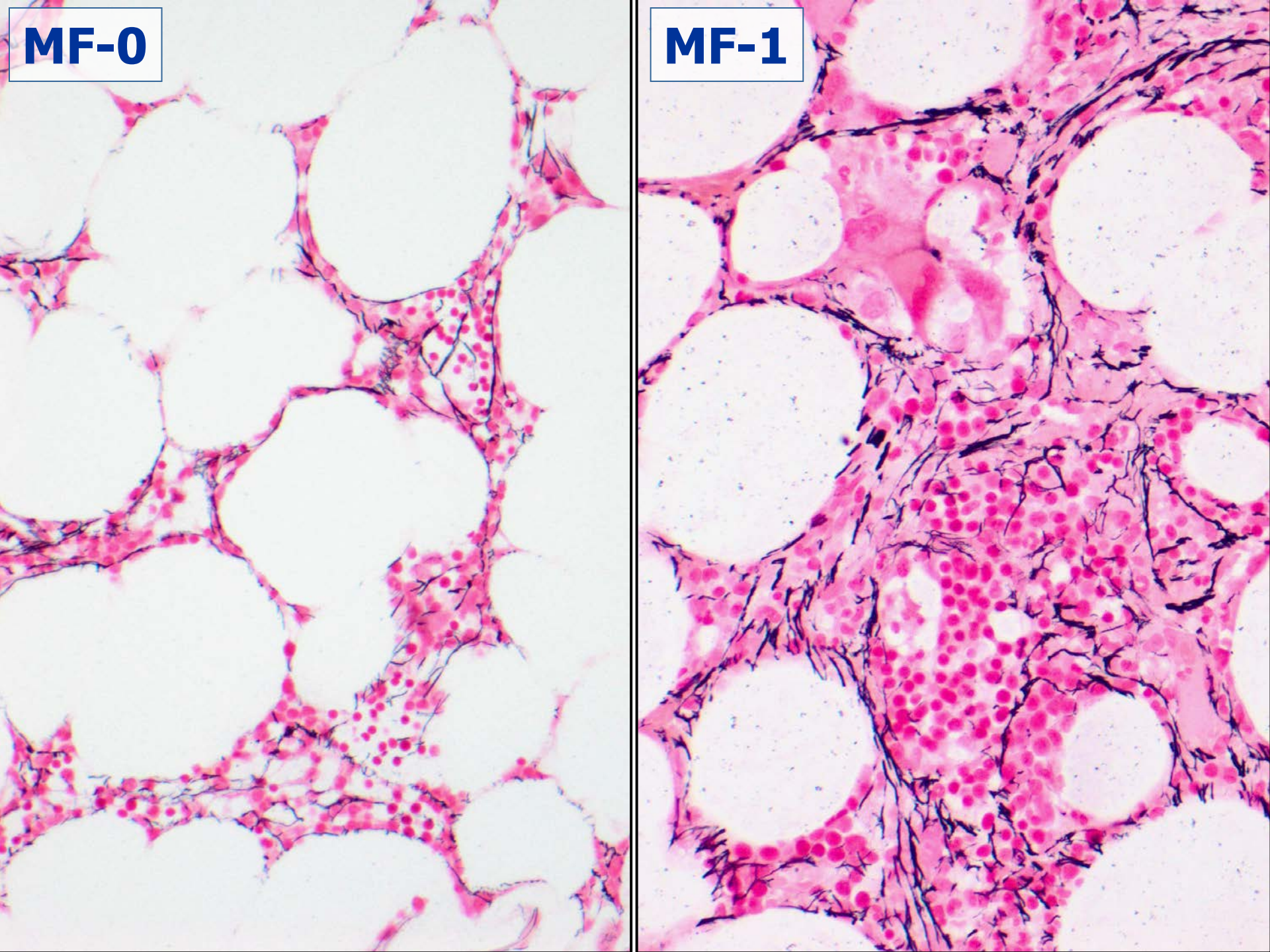
- **IWG-MRT criteria 2006**
  - Clinical based definition
  - Minor impact of BM morphology (only for CR)

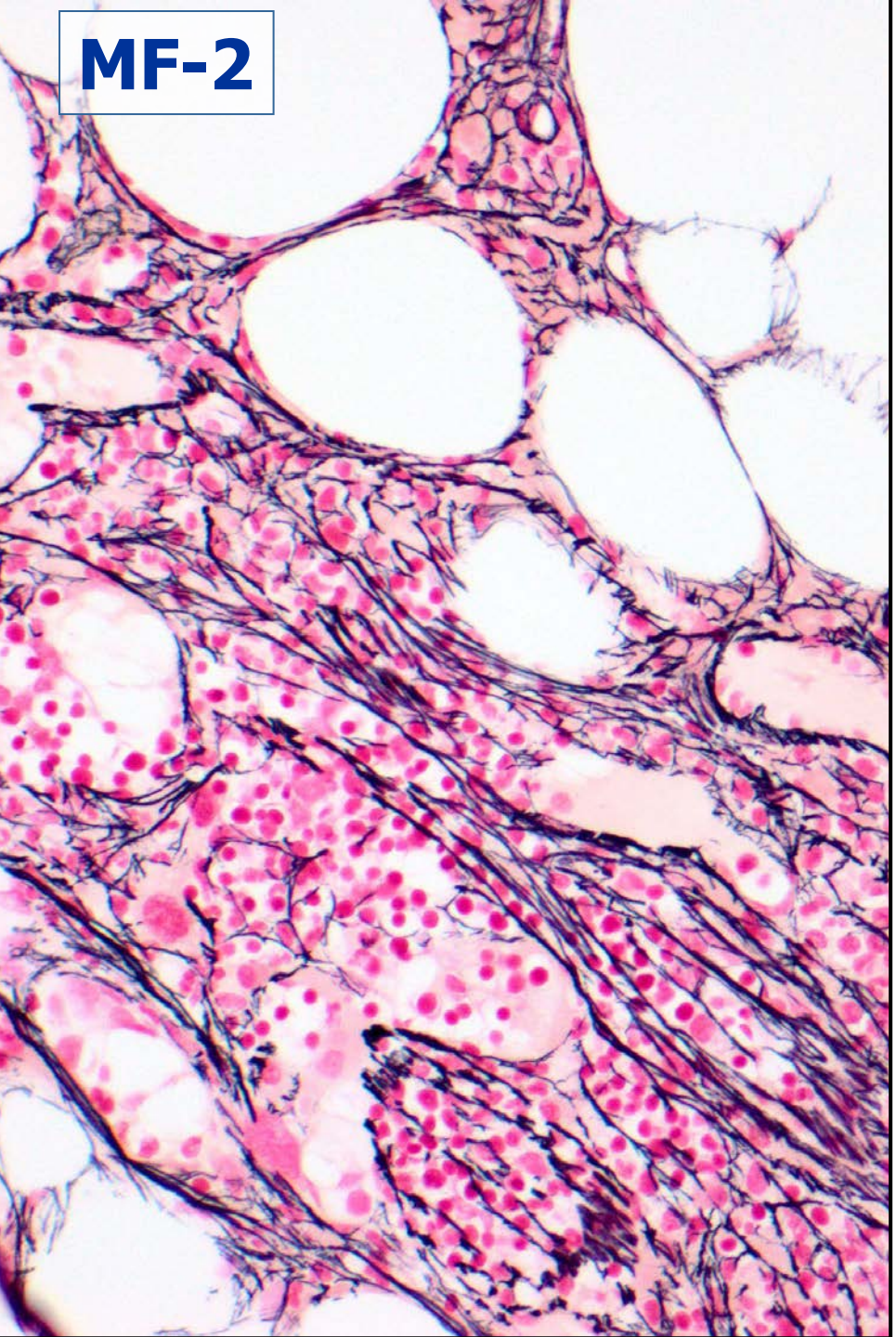
- **Current WHO 2008 guidelines**
  - No definition of response
  - Progression according to blast count (AP: 10% - 19%; BP: ≥20%)

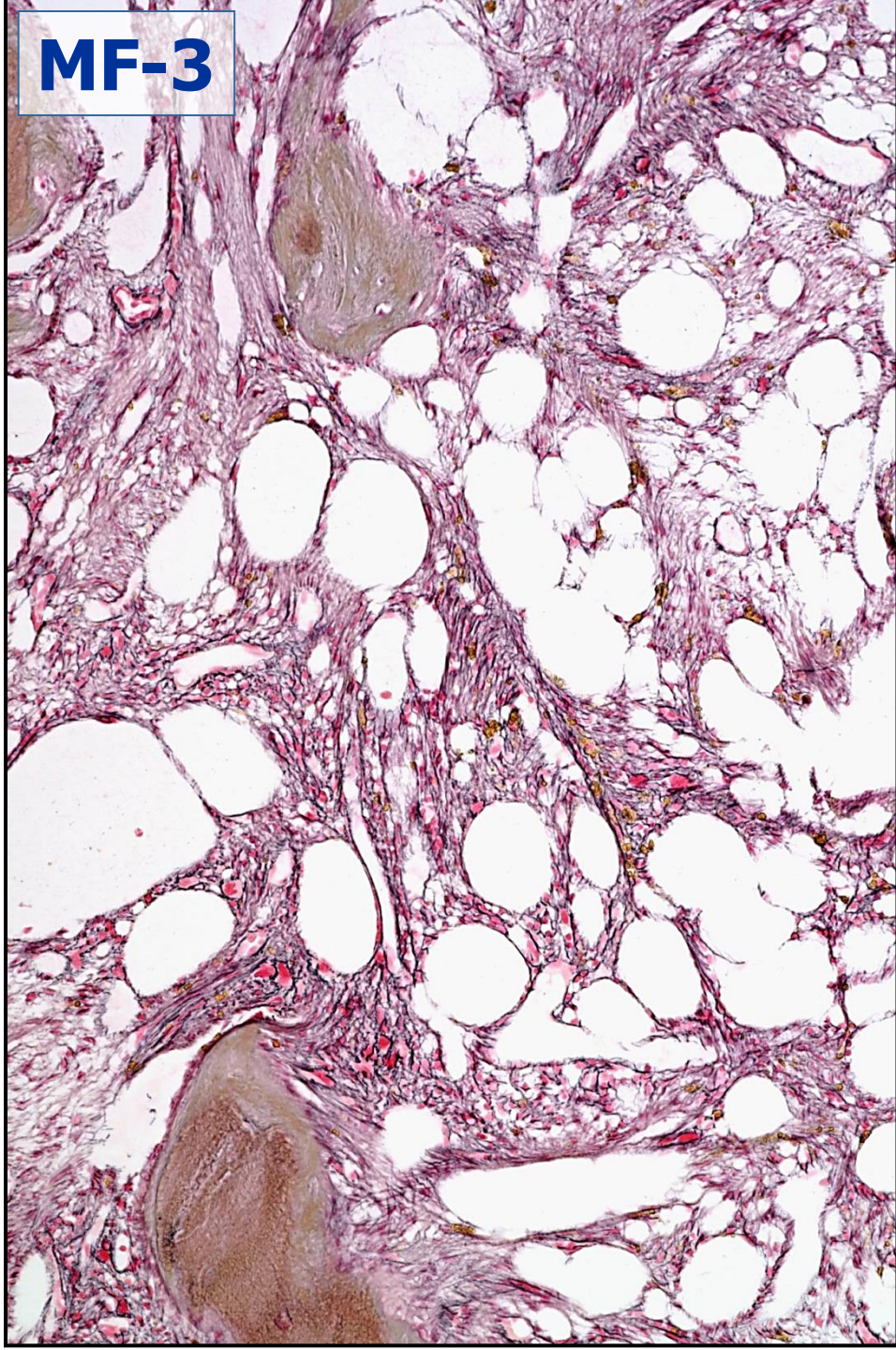
- **ELN / IWG-MRT consensus criteria 2013**
  - Clinical definition of response and progression
  - **Definition of histomorphological response**

# Key Points

- BM core bx taken at 90 degree from cortical bone, sufficient length without fragmentation for grading osteosclerosis
- Gomori or Gordon and Sweets' for reticulin fibers with positive internal perivascular control
- In MF-2 or MF-3 trichrome stain is required
- Fiber density should be assessed in hematopoietic areas (fatty areas are excluded)
- If the pattern is heterogeneous, the score is determined by the highest grade present in at least 30% of BM area


MF-0
MF-1

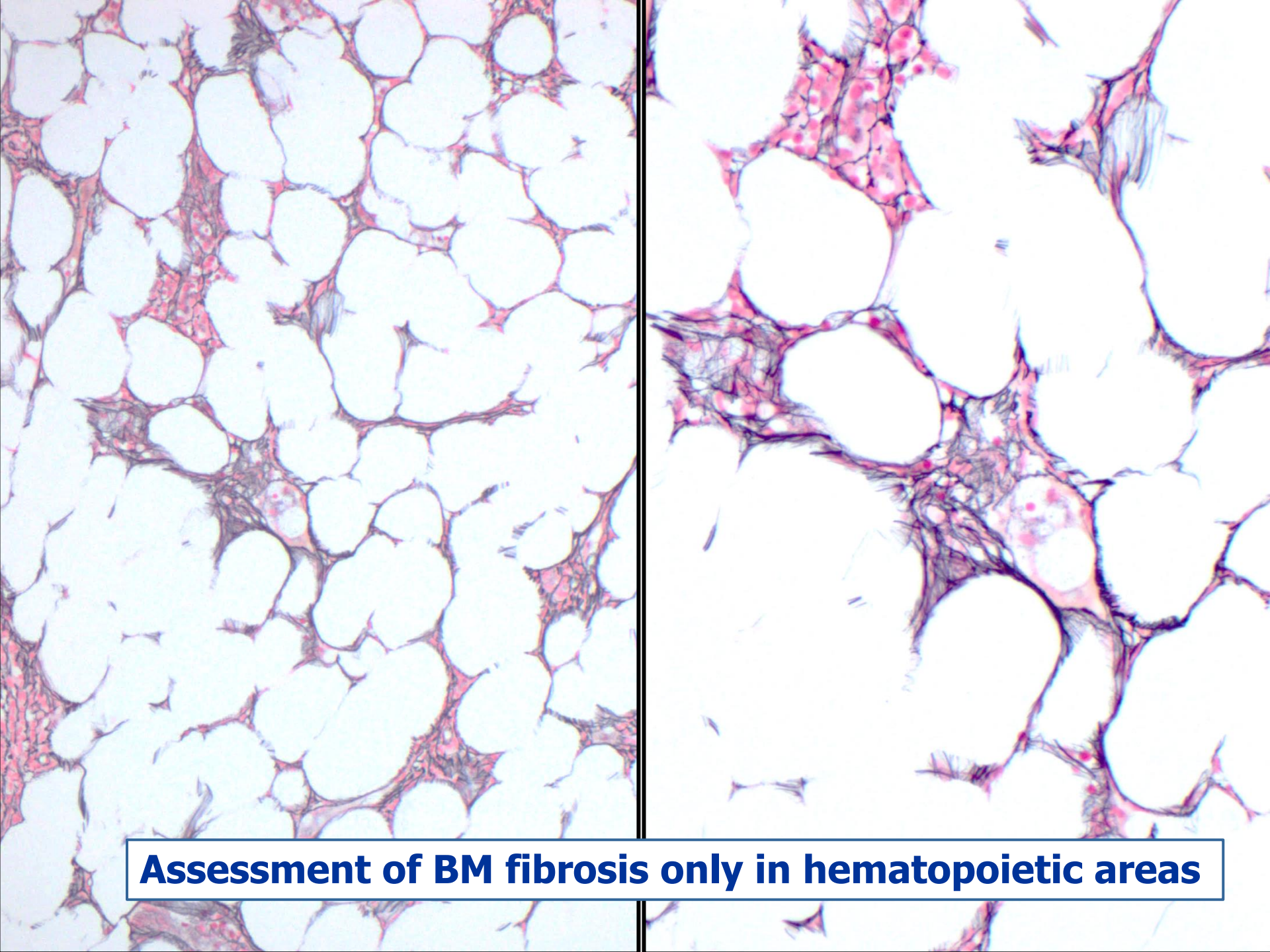

MF-2


MF-3

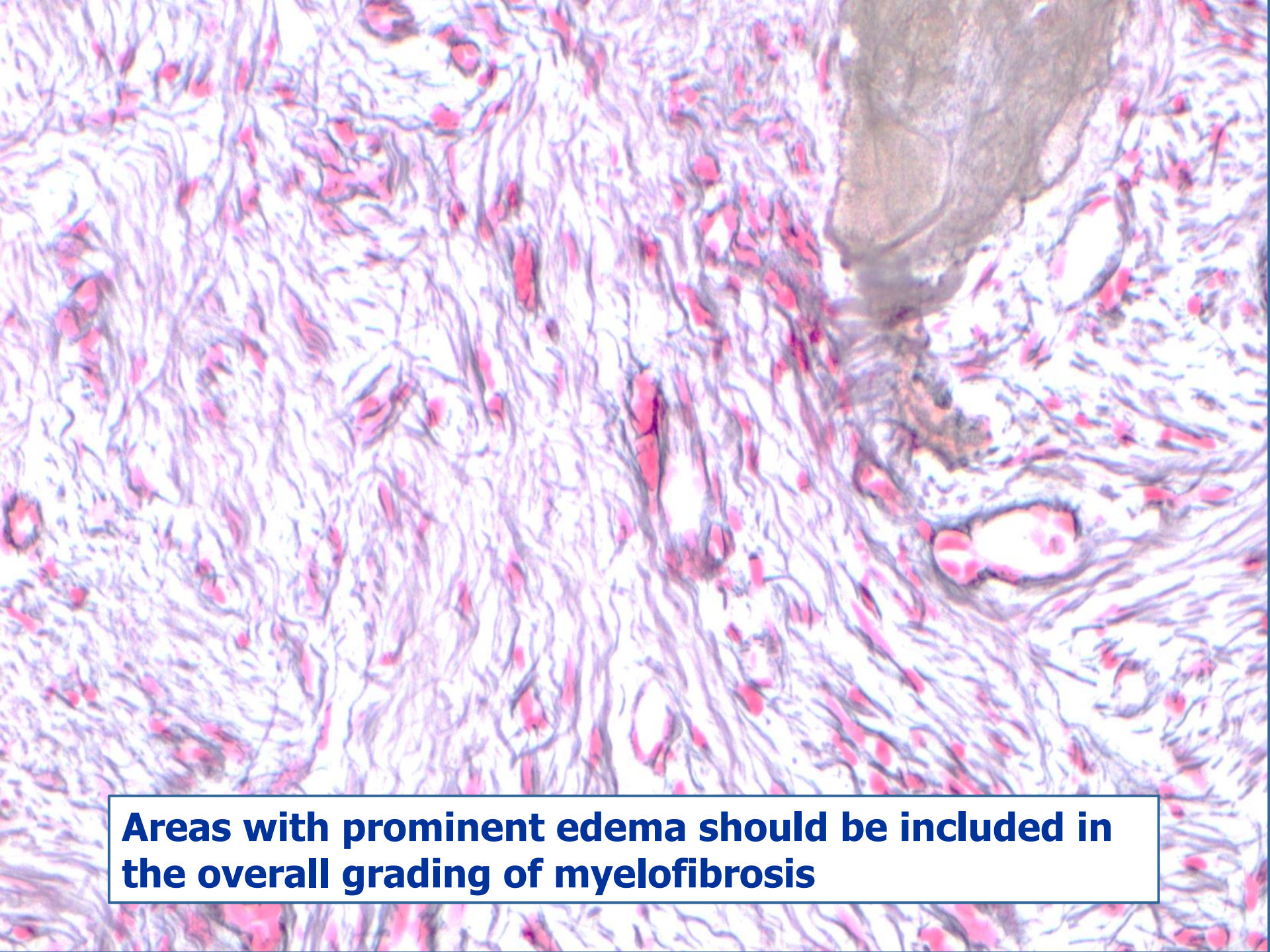
# Grading of BM Fibrosis (updated WHO 2016 grading system)*

| | |
|---|---|
| MF-0 | Scattered linear reticulin with no intersections (cross-overs) normal BM |
| MF-1 | Loose network of reticulin with many intersections, especially in perivascular areas |
| MF-2 | Diffuse dense increase in reticulin, extensive intersections, **occasionally** focal bundles of **thick fibers mostly consistent with collagen, and/or focal osteosclerosis** |
| MF-3 | Diffuse dense increase reticulin with extensive intersections, **coarse bundles** of **thick fibers mostly consistent with collagen, usually** with osteosclerosis |

*Note: Fiber density should be assessed only in hematopoietic areas. In grades MF-2 or MF-3 additional trichrome stain is recommended. 2016 changes are marked in bold.



**Assessment of BM fibrosis only in hematopoietic areas**


Areas with prominent edema should be included in the overall grading of myelofibrosis

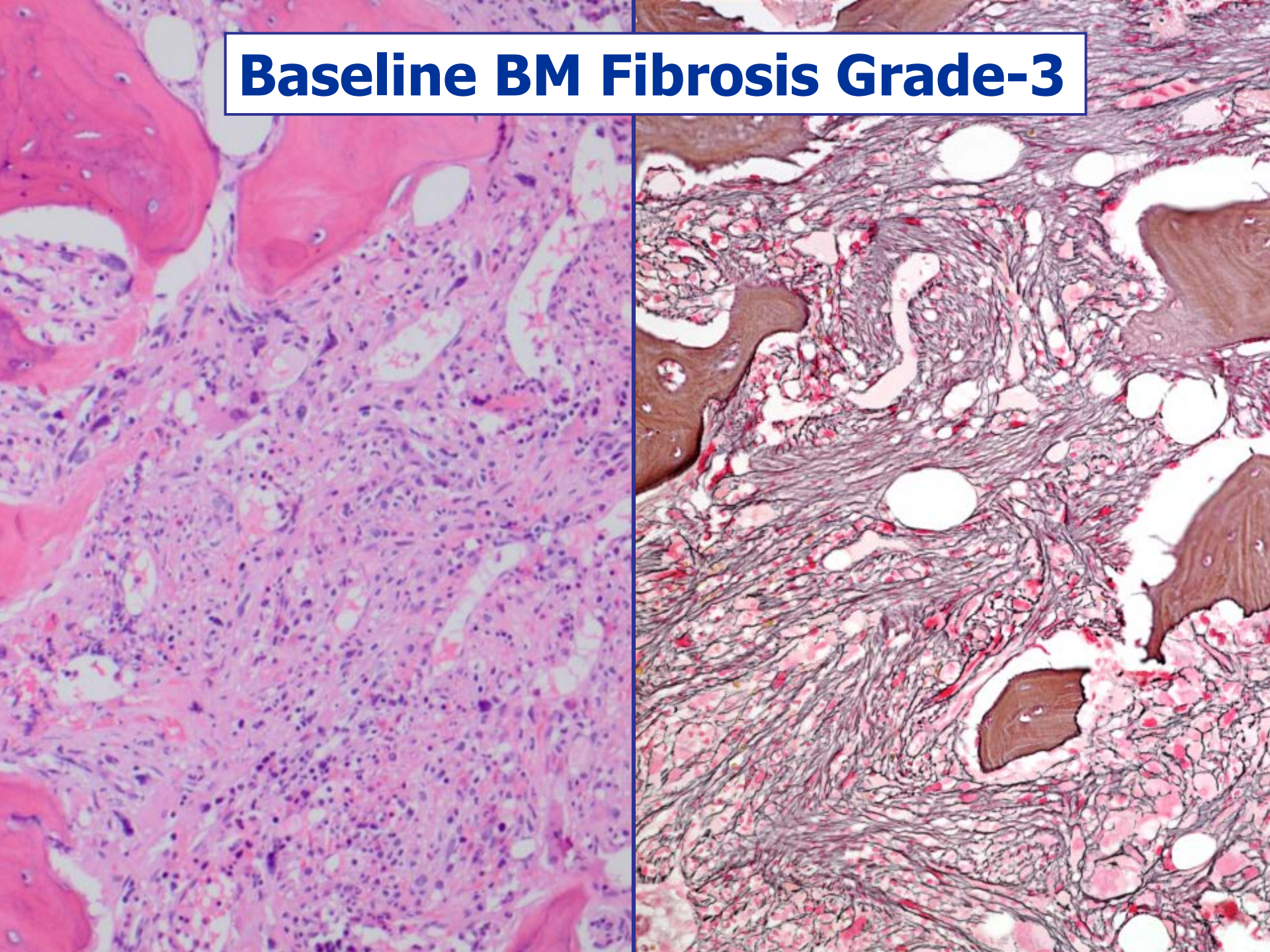
# ELN consensus on assessment of therapy response in patients with myelofibrosis

- **Definition of <u>type of response</u>**
  - Histopathological response can be characterized as:
  - Complete response
  - Major response
  - Minor response
  - No histomorphological response

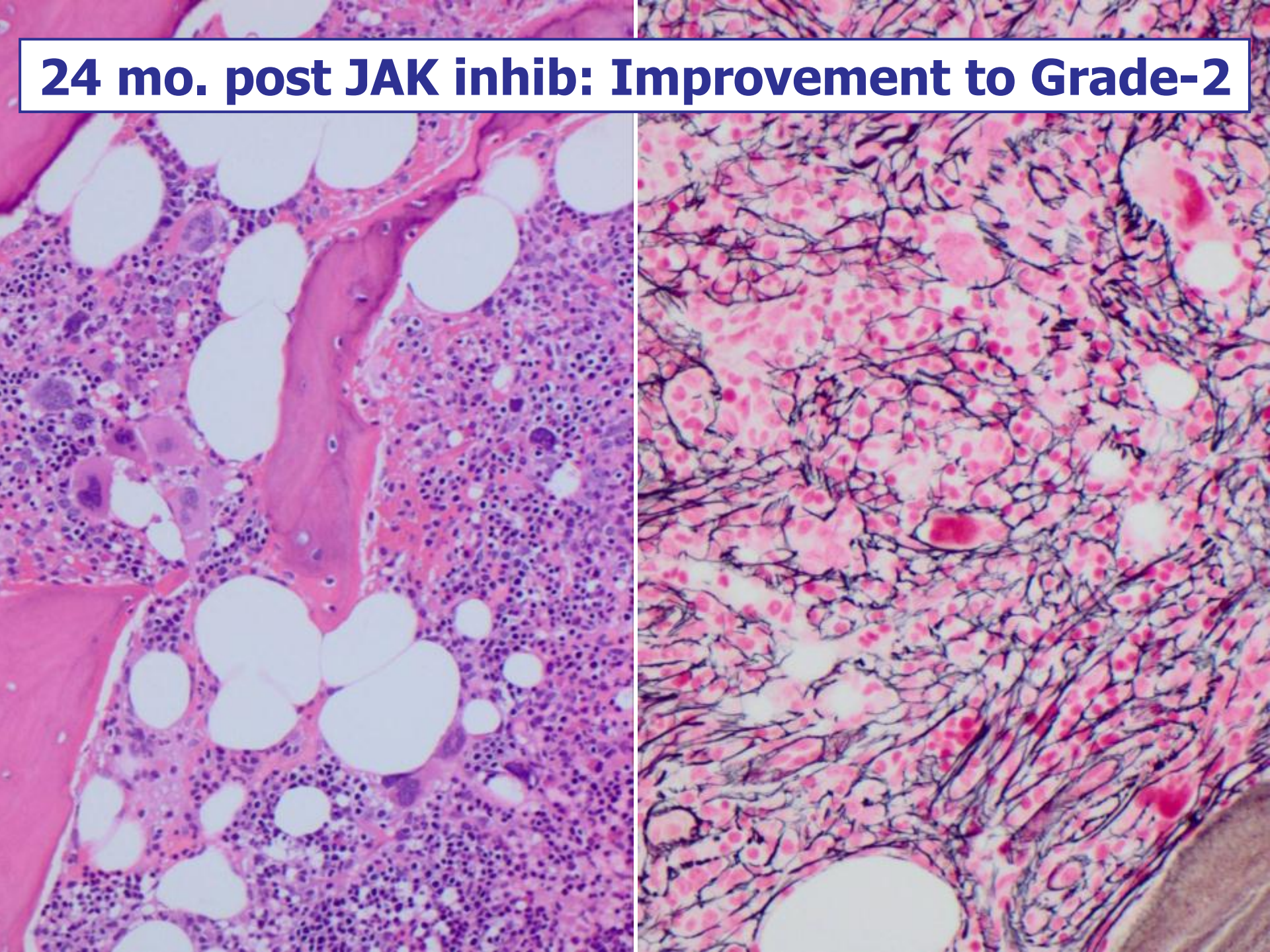
- **Definition of criteria to assess morphological response**

| Major criteria | Minor criteria |
|---|---|
| • Reticulin fibers<br>• Deposition of collagen<br>• Osteosclerosis | • Megakaryopoiesis<br>• BM blast count<br>• Granulopoiesis<br>• Erythropoiesis |

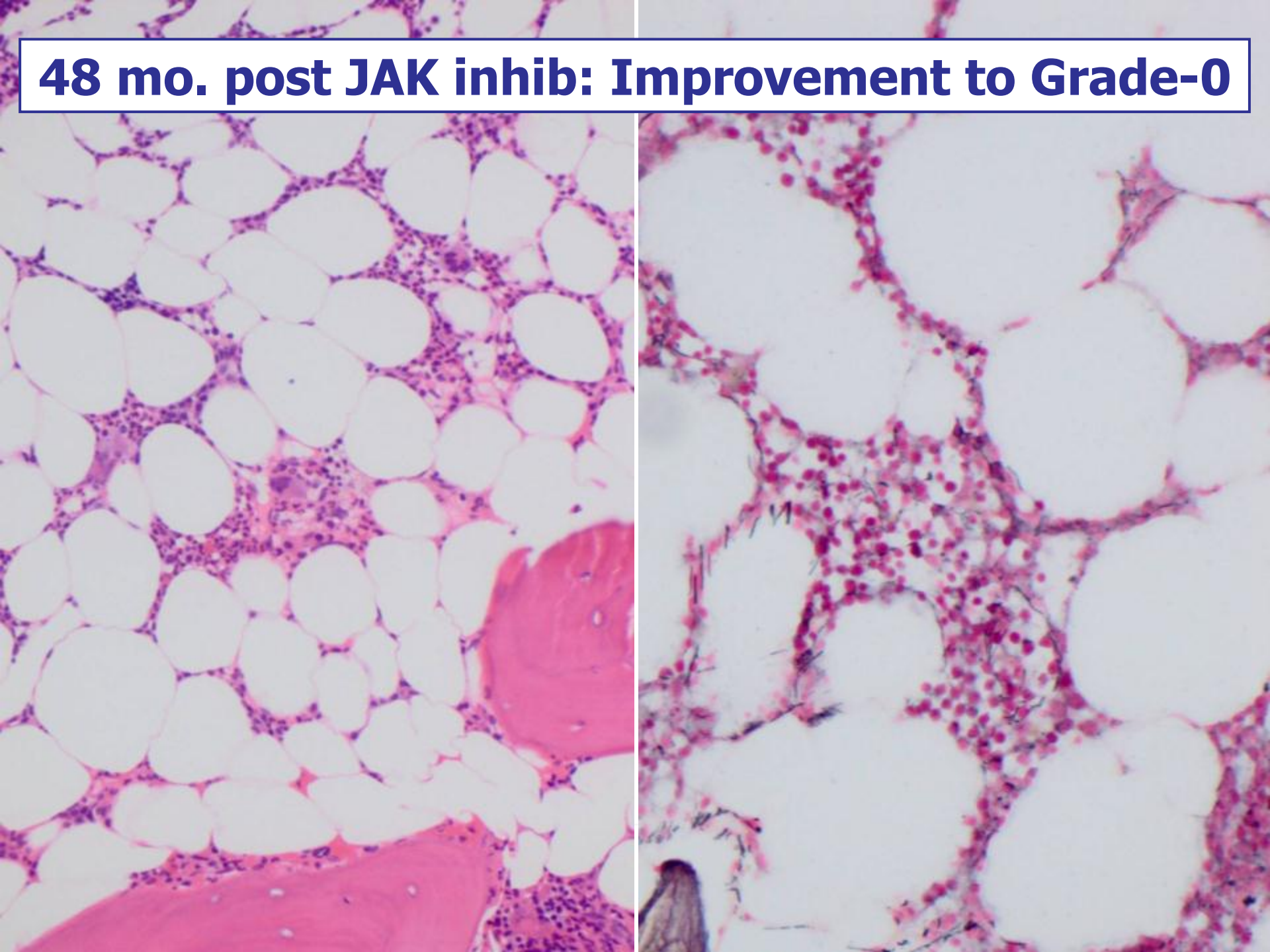
# Baseline BM Fibrosis Grade-3



# 24 mo. post JAK inhib: Improvement to Grade-2



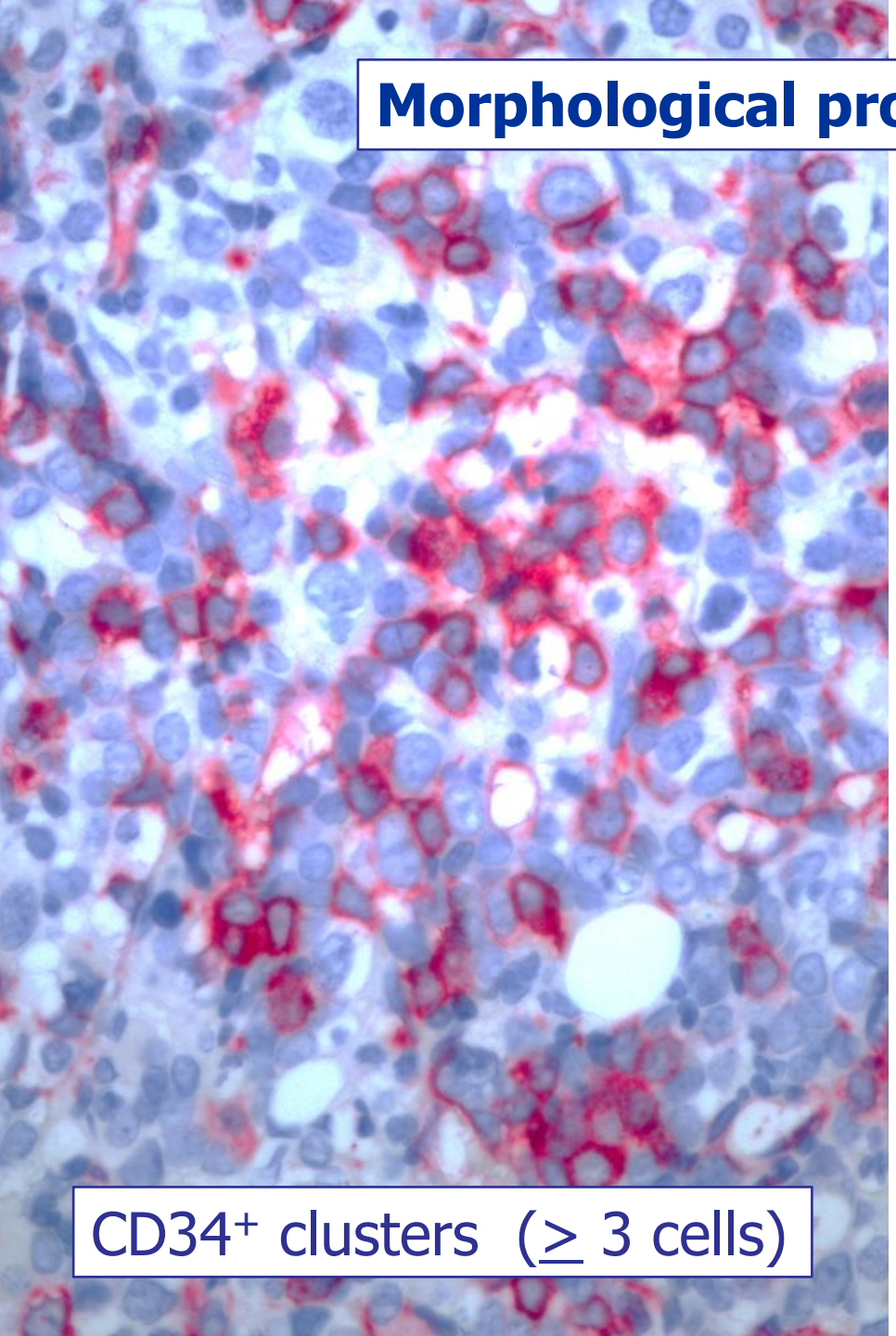
# 48 mo. post JAK inhib: Improvement to Grade-0



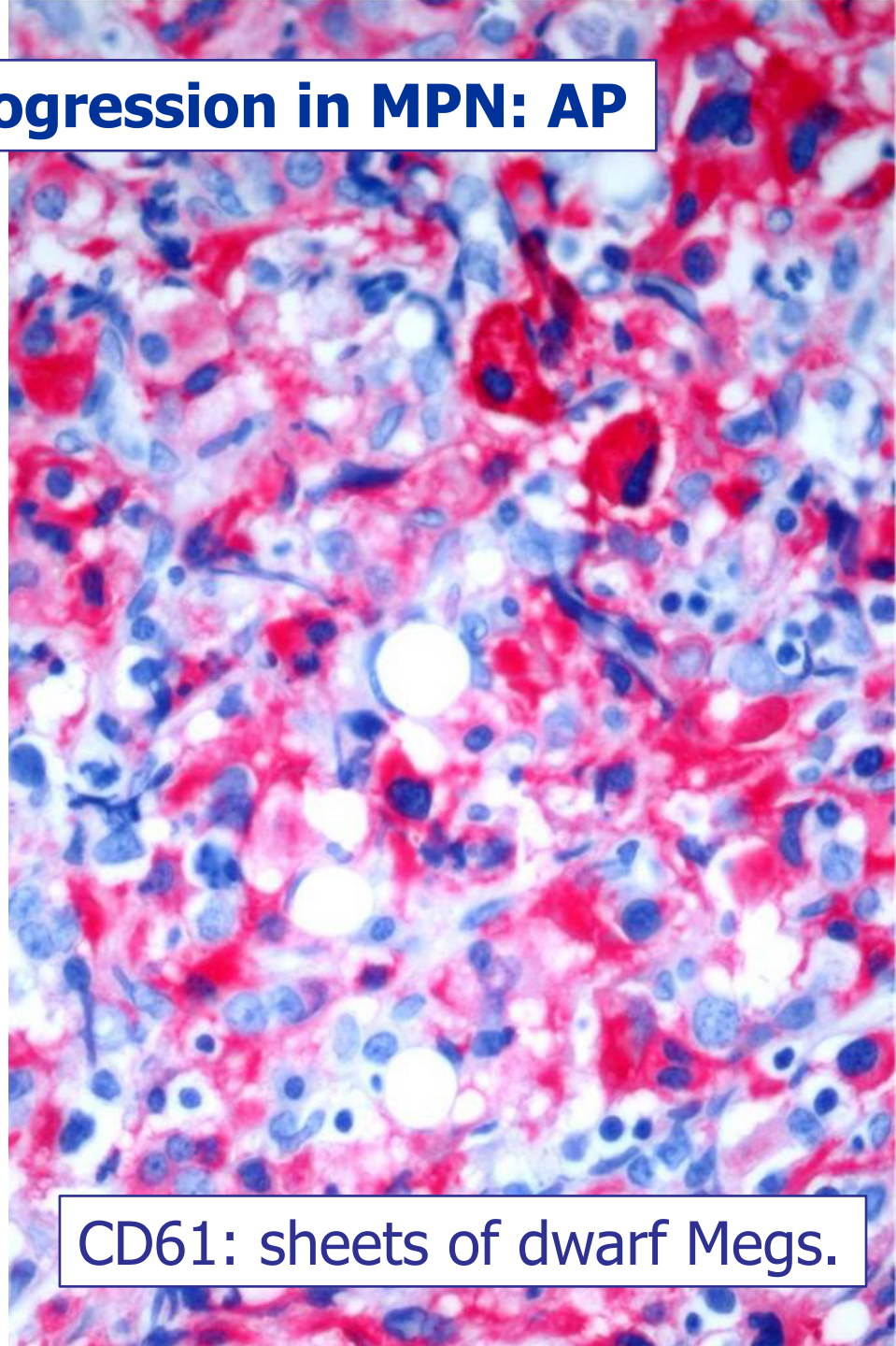
# Disease Progression in MPN

- Accelerated (AP) and blast phase (BP)
- Post-PV (-ET) myelofibrosis (MF)
  - Classical and in accelerated phase
- Other types of disease progression:
  - MDS-type
  - PV: neutrophilic, CNL-like or with dysplasia aCML-like
  - PMF: monocytic, CMML-like

# Morphological progression in MPN: AP



CD34$^{+}$ clusters ($\geq$ 3 cells)



CD61: sheets of dwarf Megs.

# WHO Criteria for Post-PV (-ET) MF

**Thiele J, Kvasnicka H-M, Orazi A, et al. WHO Classification. IARC. Lyon 2008**

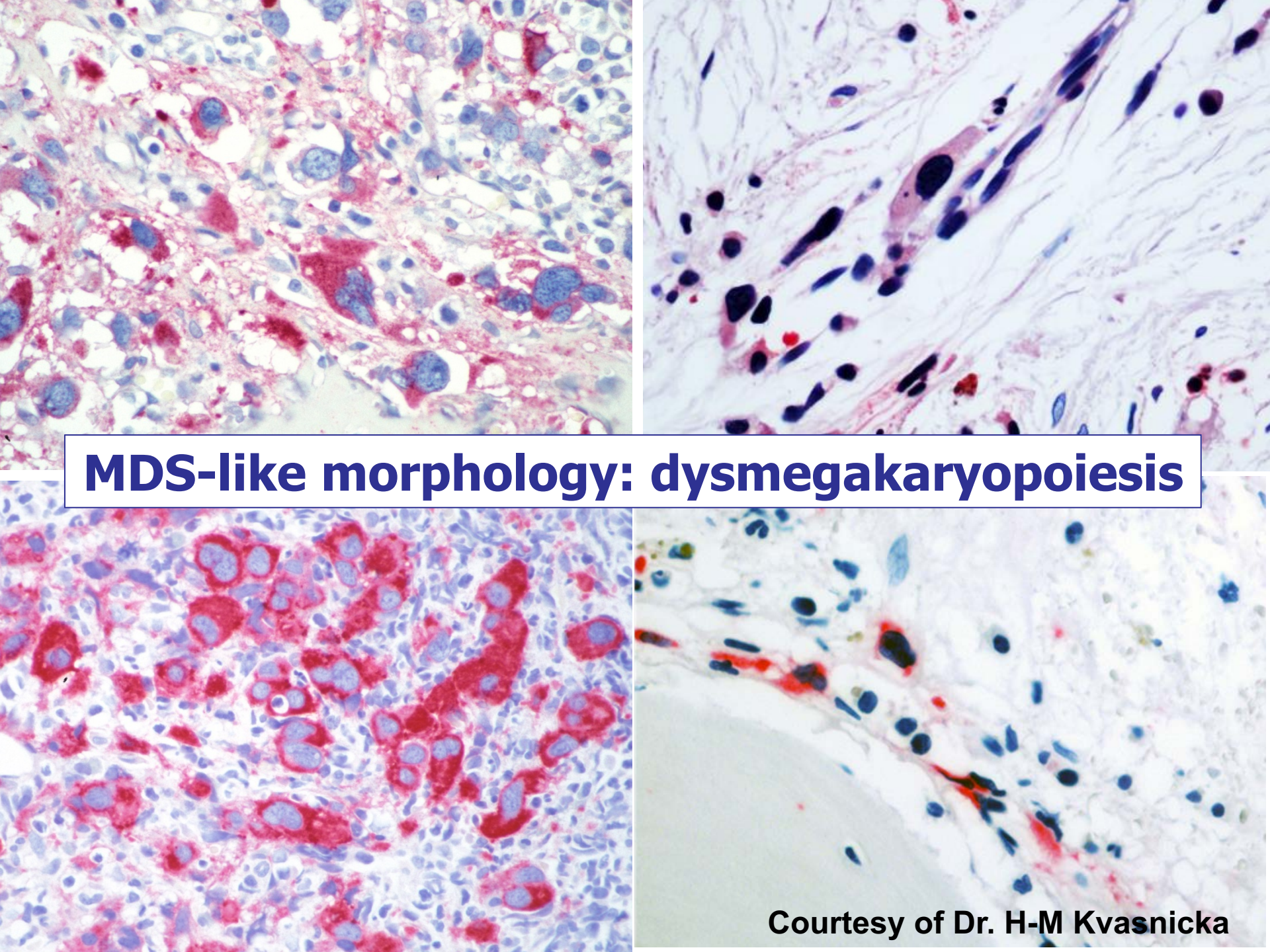
## Required criteria

1. Documentation of a previous diagnosis of WHO-defined PV or ET
2. BM fibrosis grade 2-3 (on 0-3 scale) or grade 3-4 (on 0-4 scale)

## Additional criteria (2 are required)

1. Anemia* sustained loss of either phlebotomy in the absence of cytoreductive therapy or cytoreductive treatment requirement for erythrocytosis
2. Leucoerythroblastic PB
3. Increasing splenomegaly increase in palpable spleen of >5 cm from baseline or the appearance of a newly palpable splenomegaly
4. Development of >1 of 3 constitutional symptoms: >10% weight loss in 6 mo., night sweats, unexplained fever >37.5°C

*Below the reference range for appropriate age, sex, gender, and altitude considerations


MDS-like morphology: dysmegakaryopoiesis
Courtesy of Dr. H-M Kvasnicka

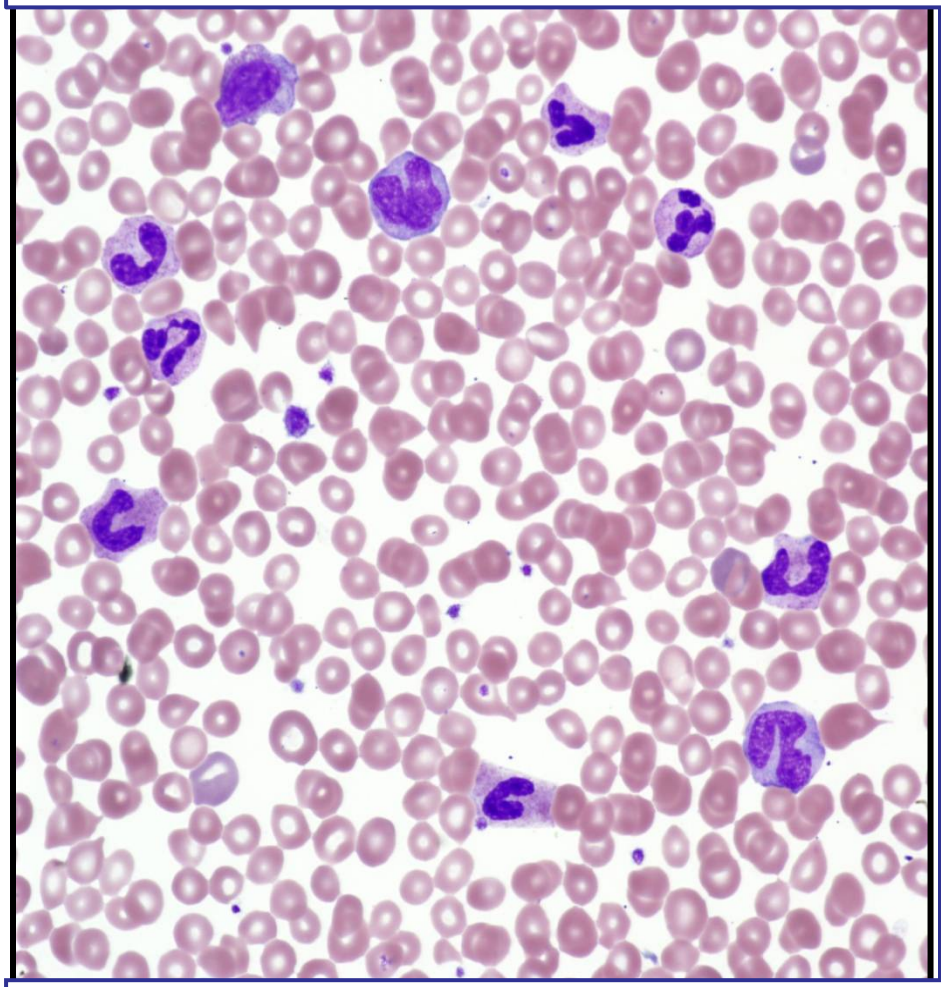
# Less common types of progression:

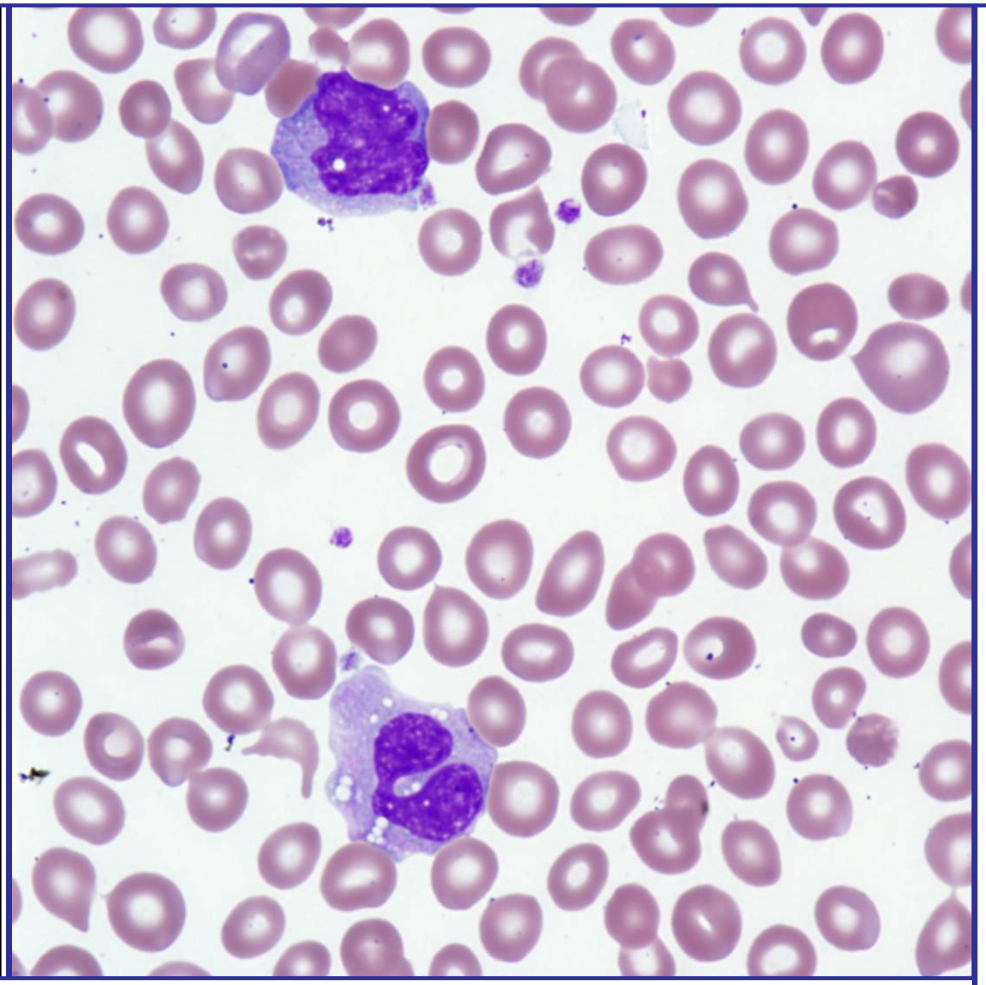
- Development of persistent neutrophilia or monocytosis
  - WBC $\geq 25 \times 10^9$/L, or
  - WBC $\geq 13 \times 10^9$/L with dysgranulopoiesis and neutrophil precursors ≥10% of WBC
  - Monocytosis $>1.0 \times 10^9$/L and >10% monocytes

Boiocchi L. et al. Development of monocytosis in course of PMF. *Mod Pathol*. Mod Pathol. 2013 Feb;26(2):204-12.

Boiocchi L et al. Neutrophilic progression during the fibrotic stage of PV. Submitted to 103rd USCAP Annual Meeting. March 1-7, 2014 San Diego Convention Center, San Diego, CA

# PMF (MF-2) associated with marked persistent monocytosis and BM showing CMML-like features. Findings c/w disease progression





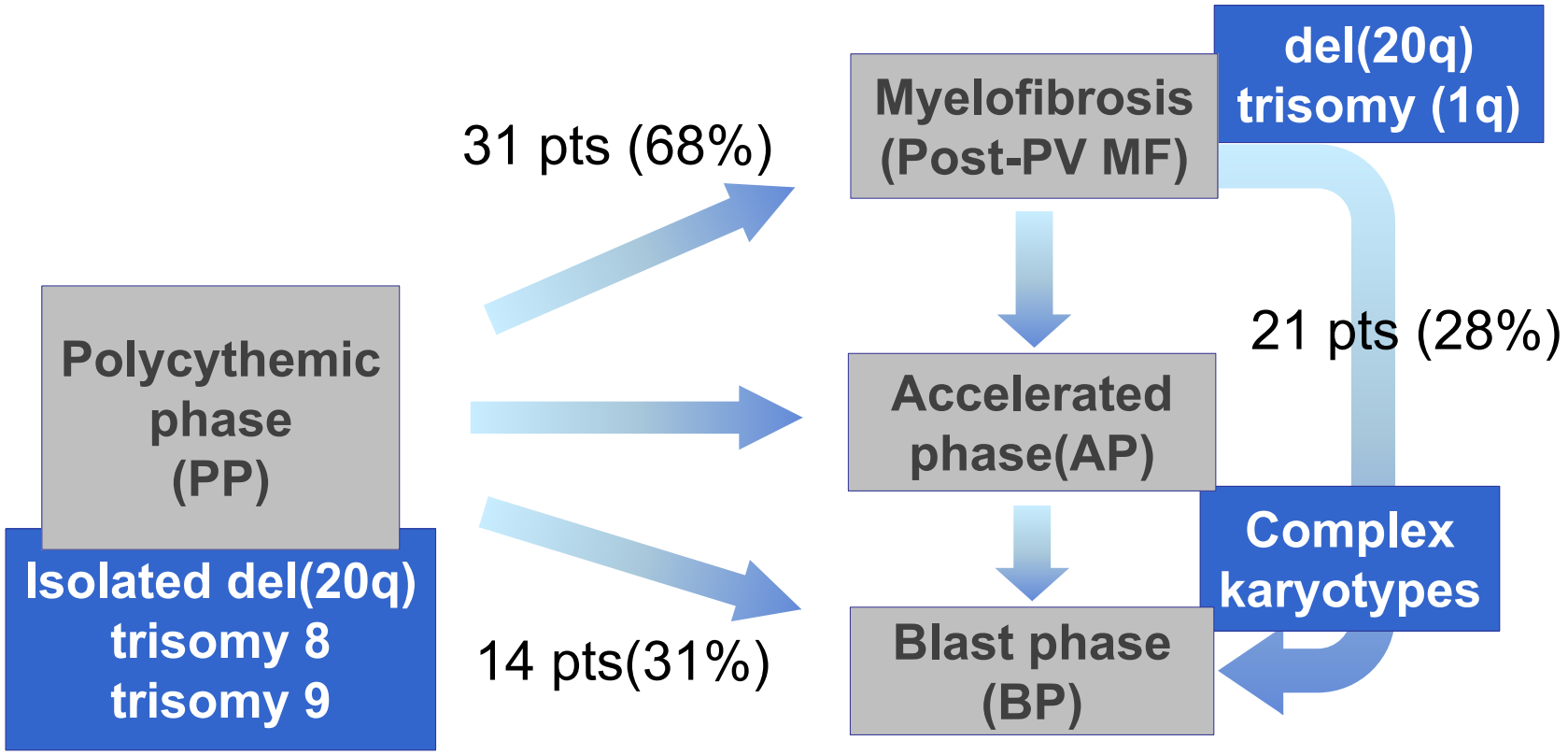
Hb: 8.8; MCV: 93.4; WBC: 120.5 (N 39, E: 1, BA 5, BL 2, L: 20, M: 33). NRBC: 1/100. PLT: [36]

Courtesy of Dr. A. Orazi

# Take Home Messages

- Initial BM biopsy evaluation is crucial for accurate diagnosis
- Grading of collagen (trichrome staining) and osteosclerosis are important to evaluate therapy-related effects on BM stroma (e.g., decrease of fibrosis)
- Hematopoiesis, in particular megakaryopoiesis may show partial resolution of maturation defects in different degree. These changes should always be recorded
- Always assess for disease progression

# Cytogenetic vary in different phases of PV




*Tang et al, Hematologica 2017*

# Proposed MDACC model of Cytogenetic risk in PV

Overall survival

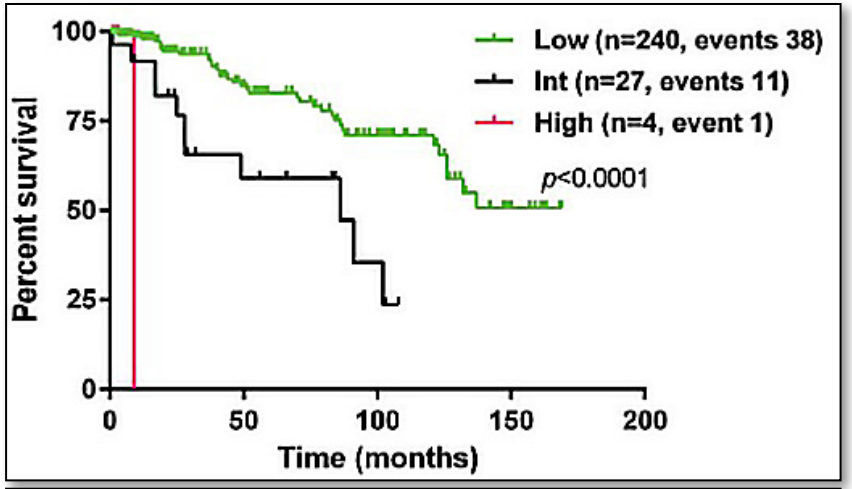
**Low**
normal karyotype, sole +8, +9 and other single abnormality

**Intermediate**
sole del20q, +1q and other two abnormalities

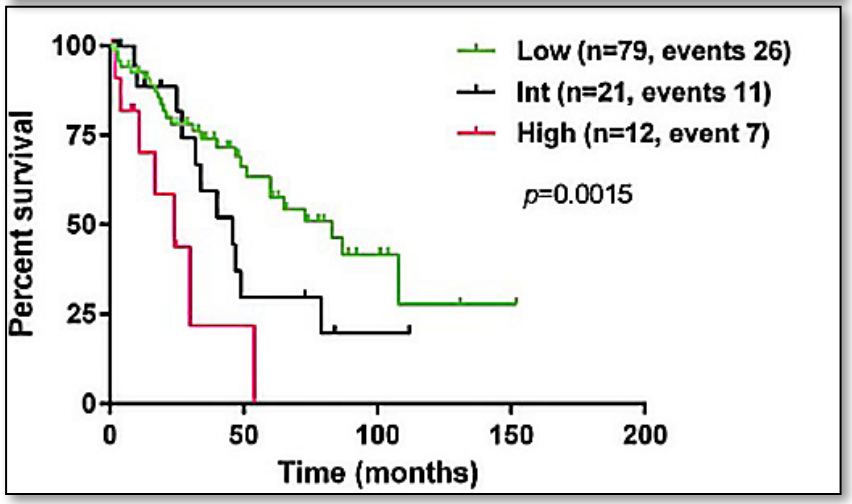
**High-risk**
complex karyotype

Overall Survival

PP

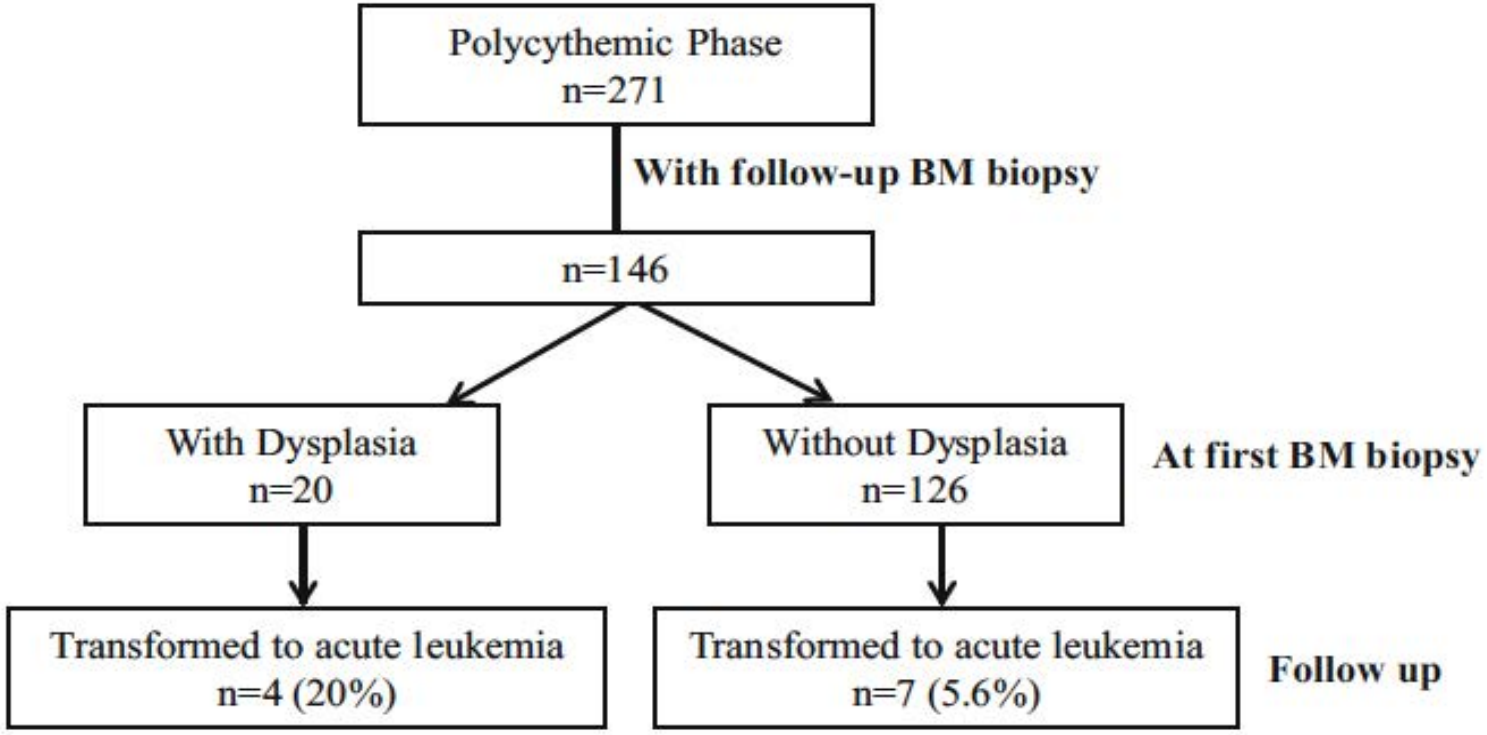



Post-PV MF




*Tang et al, Hematologica 2017*

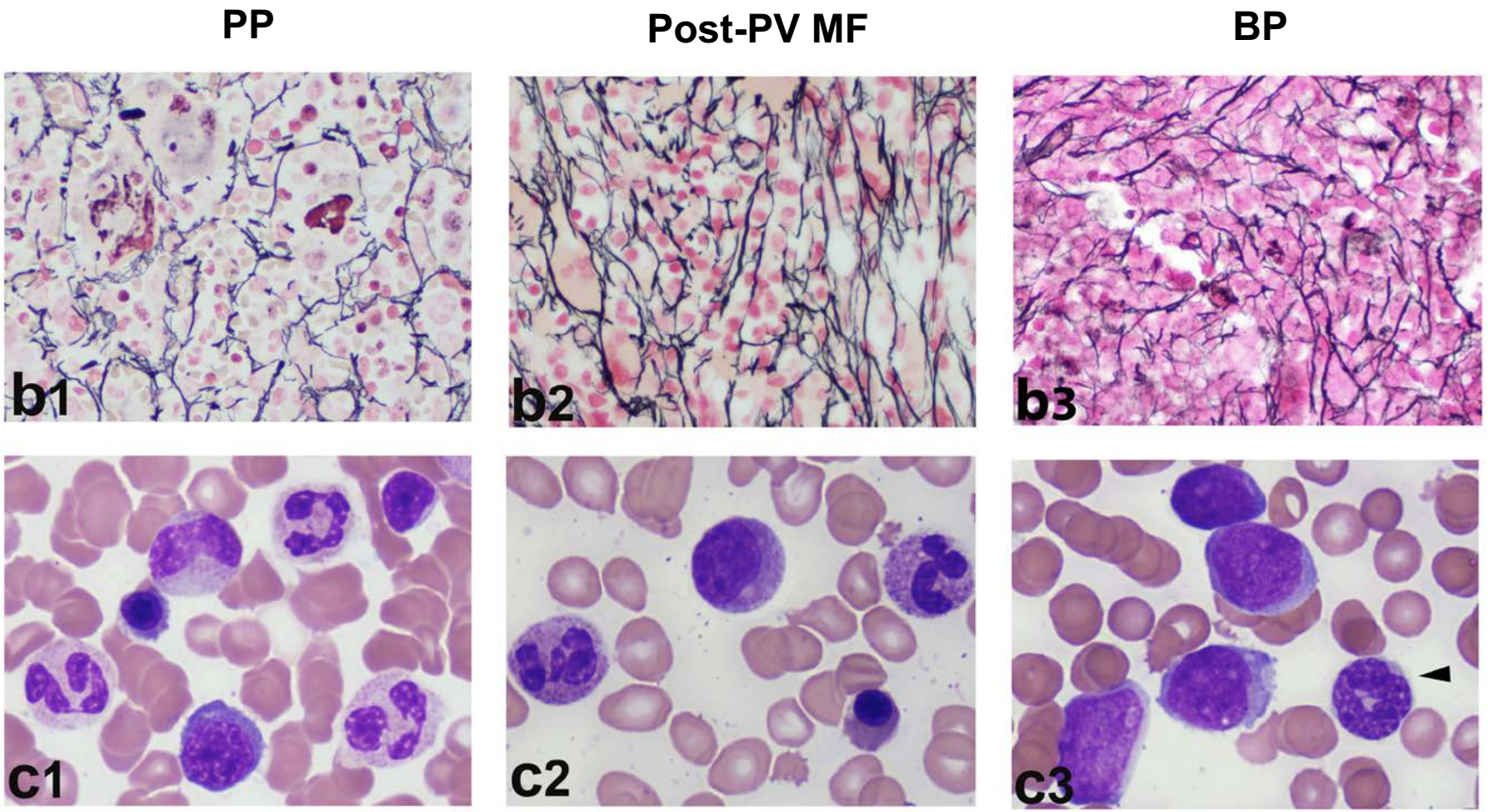
# Myelodysplasia detected at PP had higher risk for BP transformation




P=0.0453

*Hidalgo-Lopez et al, Ann Hematol J 2018*

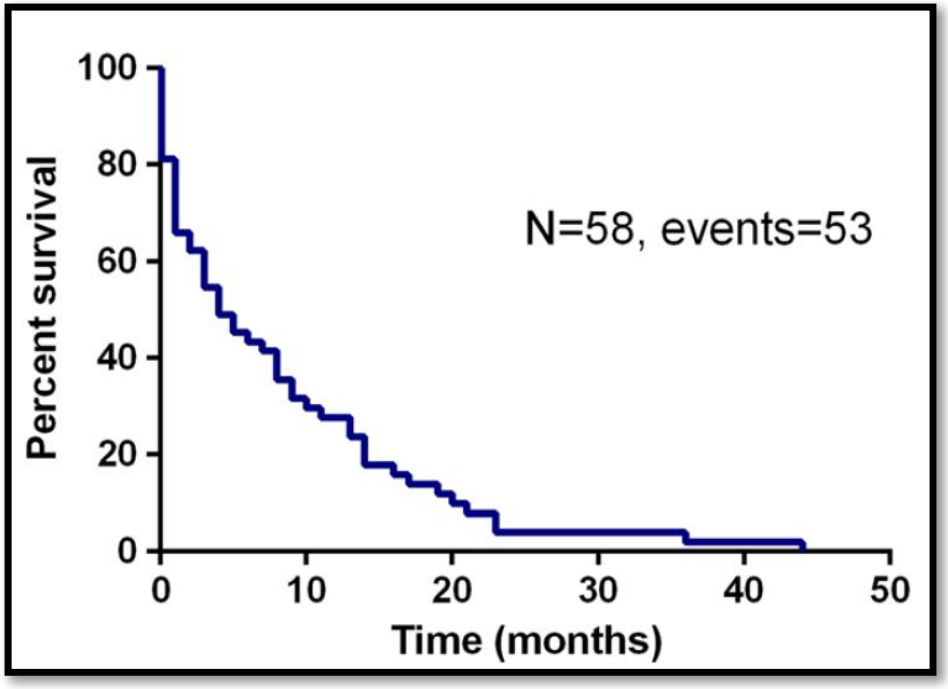
# BP PV presents with myelodysplasia and MF-2/MF-3




*Hidalgo-Lopez et al, Ann Hematol J 2018*

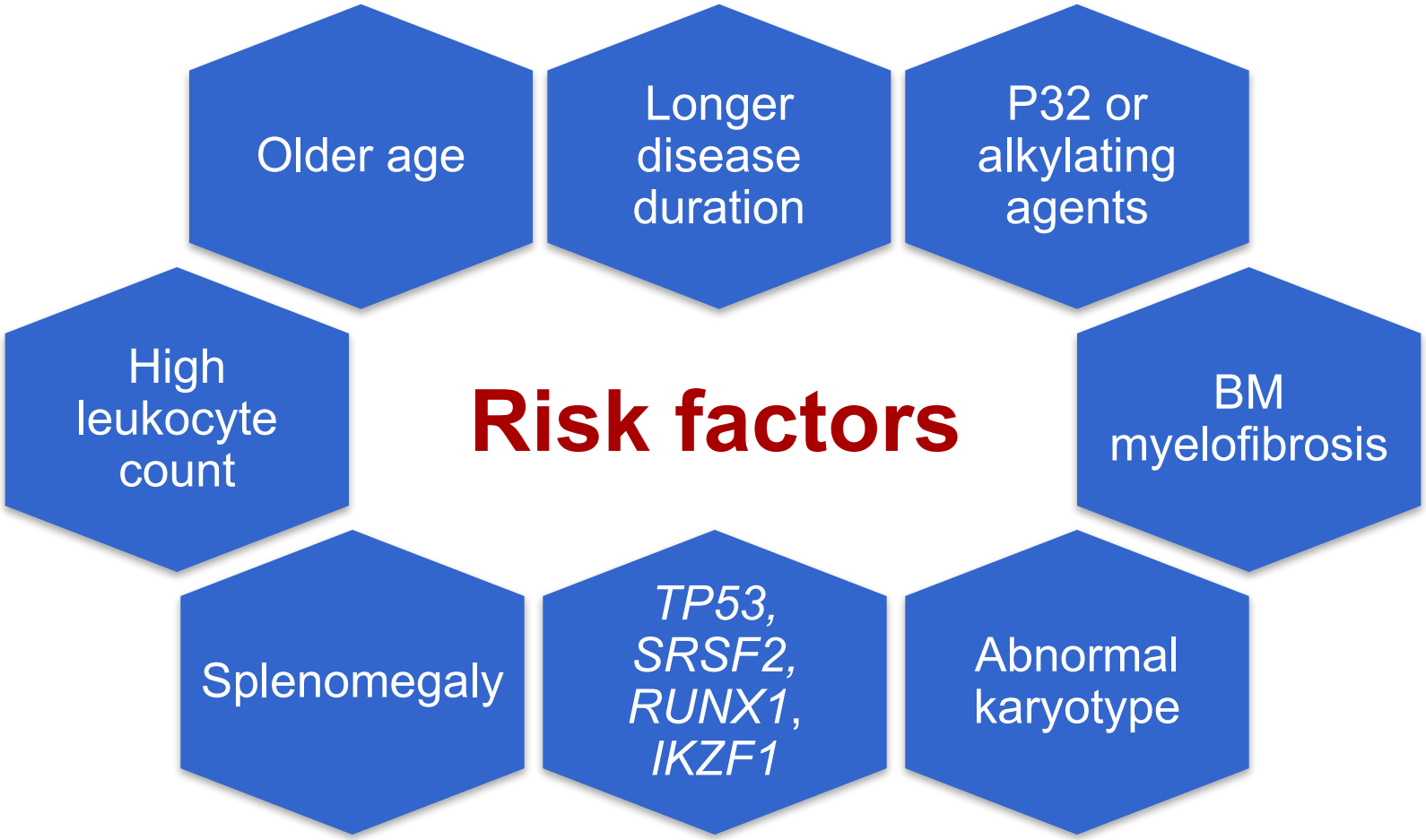
# BP of PV presents with high risk cytogenetics and molecular

- Abnormal karyotype:
  - Complex karyotype (72%)
- Mutations at BP
  - *TP53*: 55%
  - *TET2*: 27%
  - *DNMT3A*: 25%




*Hidalgo-Lopez et al, Ann Hematol J 2018*

# Blastic phase of PV




*Cerquozzi and Tefferi, Blood Cancer Journal 2015*
*Hidalgo-Lopez et al, Ann Hematol J 2018*

# Summary

- Development of myeloid neoplasms involve multiple genetic lesions that complement each other
- Aberrant signal transduction enhances survival and proliferation of Leukemic Cells
- Functional dependencies of Leukemic Cells result in activation of limited effector pathways
- Mutational and non-mutational mechanisms to small molecule inhibitors have emerged
- Early identification of secondary mutations and small molecular combinations may overcome resistances.

# THANK YOU

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