Myeloproliferative Neoplasms

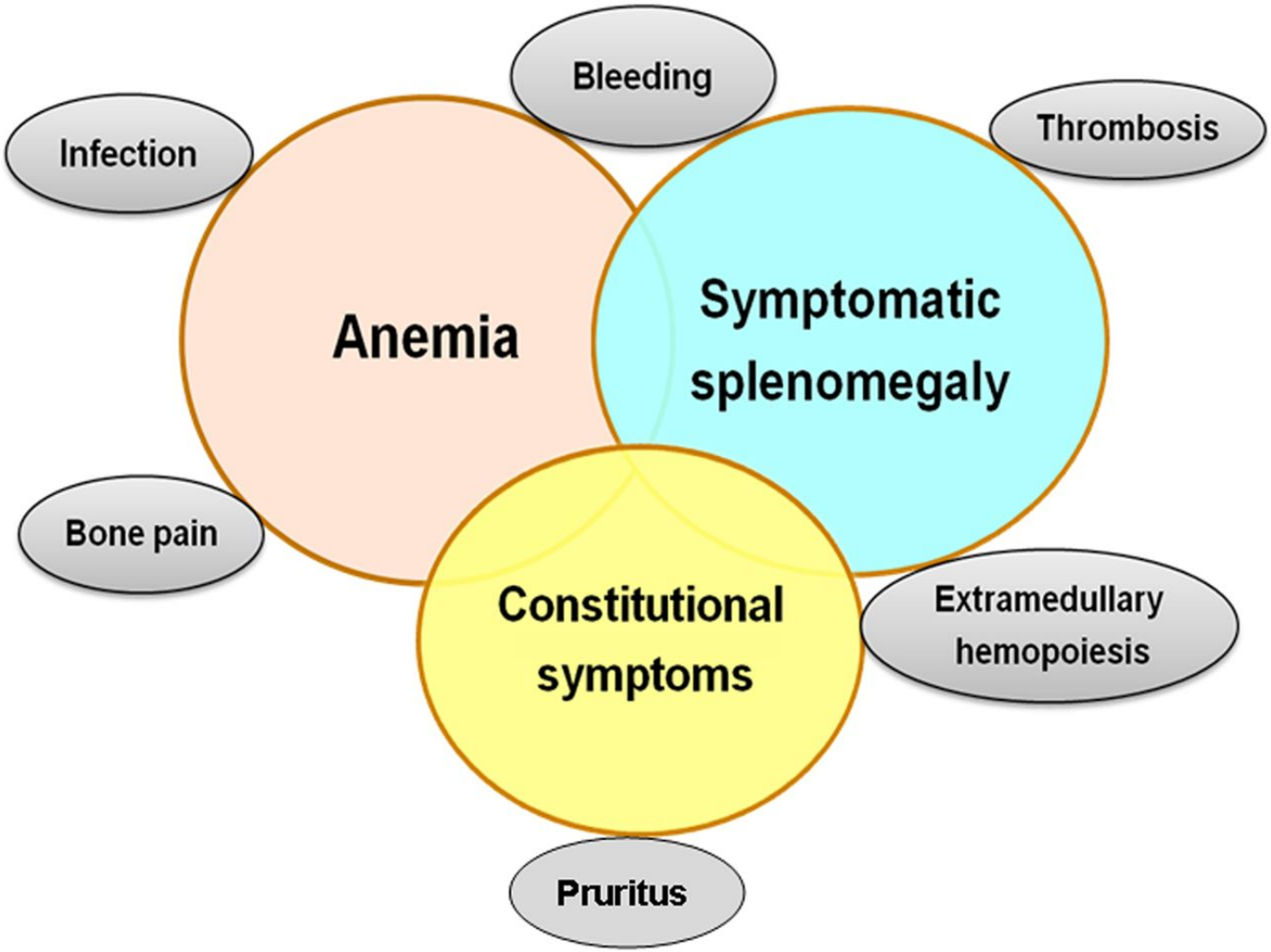
Lymphoma Tumor Board

March 17, 2017

Myelofibrosis (MF)

- Relatively rare bone marrow disorder
- Can be primary or secondary
- Characteristic of Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs)
- Proliferation of an abnormal clone of hematopoietic stem cells results in fibrosis
- Symptoms include:
  - Abdominal fullness – usually caused by splenomegaly or hepatomegaly
  - Bone pain
  - Bruising and easy bleeding – caused by thrombocytopenia
  - Cachexia
  - Fatigue
  - High uric acid levels and gout
  - Pallor and shortness of breath
  - Increased susceptibility to infection

Clinical manifestations of Myelofibrosis



Francisco Cervantes Blood 2014;124:2635-2642

Philadelphia Chromosome-Negative Neoplasms

- Three types of Philadelphia chromosome-negative myeloproliferative neoplasms:
  - Essential thrombocythemia (ET)
  - Polycythemia vera (PV)
  - [Primary] Myelofibrosis (MF)
- Most Philadelphia chromosome-negative cases have an activating *JAK2* (*e.g.*, V617F) or *MPL* mutation
- Mutations in *CALR* (calreticulin) are found in a majority of *JAK2* and *MPL*-negative essential thrombocythemia and myelofibrosis cases
- ET is associated with *JAK2* V617F mutation in up to 55% of cases, and with a *MPL* mutation in up to 5% of cases
  - **Cellular** phase - increased large megakaryocytes with fibrosis and little increase in other bone marrow elements
  - **Fibrotic** phase – collagenous fibrosis with lack of marrow elements
- PV is associated most often with the *JAK2* V617F mutation (greater than 95% of cases), whereas the remainder have a *JAK2* exon 12 mutation
  - **Cellular phase** - increased megakaryocytes which cluster, reticulin fibrosis, later trichrome fibrosis and increased myeloid and erythroid precursors
  - **Fibrotic phase** - collagenous fibrosis with lack of marrow elements

Philadelphia Chromosome-Negative Neoplasms

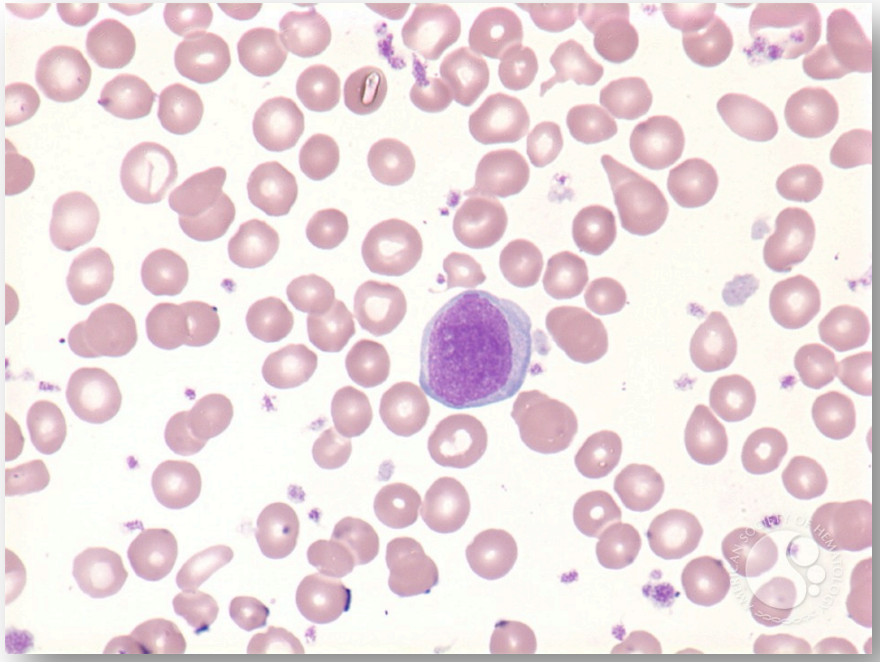
Table 1. Philadelphia-negative classical MPNs: clinical, morphological, and molecular features

Nosologic entity	Clinical and morphological features	Driver genes	Relationships between genotype, phenotype, and clinical outcome
PV	Erythrocytosis frequently combined with thrombocytosis and/or leukocytosis (that is, polycythemia) and typically associated with suppressed endogenous erythropoietin production. Bone marrow hypercellularity for age with trilineage growth (that is, panmyelosis)	• *JAK2* (V617F) in about 96% of patients • *JAK2* exon 12 mutations in about 4% of patients (isolated erythrocytosis in most of these patients) • Patients with wild-type *JAK2* extremely rare, if any	• PV patients are at increased risk of thrombosis • PV may progress to myelofibrosis and less commonly to a blast phase similar to AML, sometimes preceded by a myelodysplastic phase
ET	Thrombocytosis. Normocellular bone marrow with proliferation of enlarged megakaryocytes	• *JAK2* (V617F) in 60%-65% of patients • *CALR* exon 9 indels in 20%-25% of patients • *MPL* exon 10 mutations* in about 4%-5% of patients • Noncanonical *MPL* mutations* in <1% of patients • About 10% of patients do not carry any of the above somatic mutations (the so-called triple-negative cases)	• ET involves increased risk of thrombosis and bleeding, and may progress to more aggressive myeloid neoplasms • *JAK2* (V617F)-mutant ET involves a high risk of thrombosis, and may progress to PV or myelofibrosis • *CALR*-mutant ET involves lower risk of thrombosis and higher risk of progression to myelofibrosis • Triple-negative ET is an indolent disease with low incidence of vascular events
PMF	Prefibrotic PMF • Various abnormalities of peripheral blood • Granulocytic and megakaryocytic proliferation in the bone marrow with lack of reticulin fibrosis Overt PMF • Various abnormalities of peripheral blood. Bone marrow megakaryocytic proliferation with atypia, accompanied by either reticulin and/or collagen fibrosis grades 2/3. Abnormal stem cell trafficking with myeloid metaplasia (extramedullary hematopoiesis in the liver and/or the spleen)	• *JAK2* (V617F) in 60%-65% of patients • *CALR* exon 9 indels in 25%-30% of patients • *MPL* exon 10 mutations* in about 4%-5% of patients • Noncanonical *MPL* mutations* in <1% of patients • About 5%-10% of patients do not carry any of the above somatic mutations (the so-called triple-negative cases)	• PMF is associated with the greatest symptom burden and the worst prognosis within MPNs, with a variable risk of progression to AML • *CALR*-mutant PMF is associated with longer survival compared with other genotypes • *JAK2* (V617F)- and *MPL*-mutant PMF have worse prognosis than *CALR*-mutant PMF • Triple-negative PMF is an aggressive myeloid neoplasm characterized by prominent myelodysplastic features and high risk of leukemic evolution

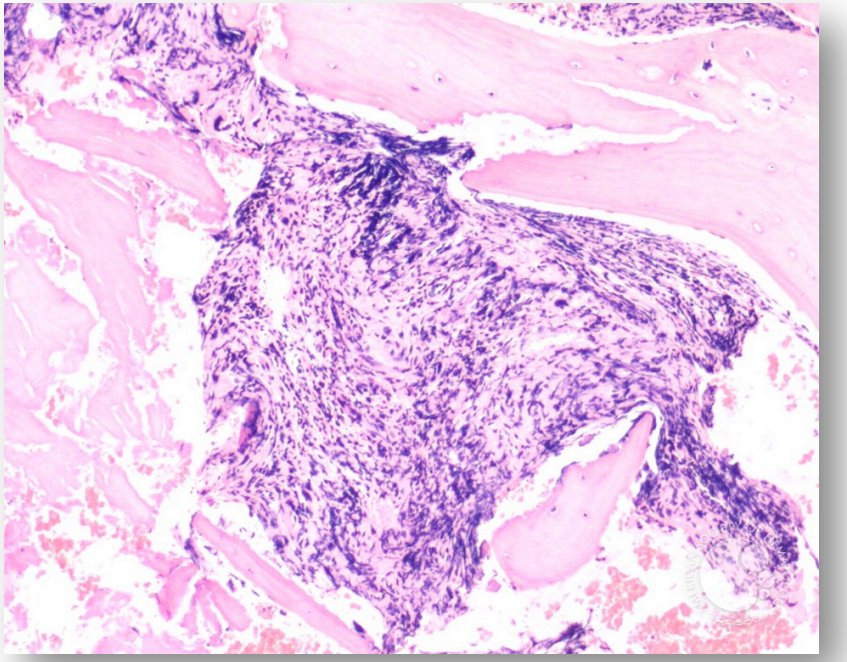
AML, acute myeloid leukemia.

*Canonical *MPL* exon 10 mutations include W515L/K/A/R, S505N/C, and V501A (transmembrane domain of MPL); noncanonical *MPL* mutations (outside exon 10) include T119I, S204F/P, E230G, Y252H (extracellular domain) and Y591D/N (intracellular domain).[12]

Pathology of Myelofibrosis

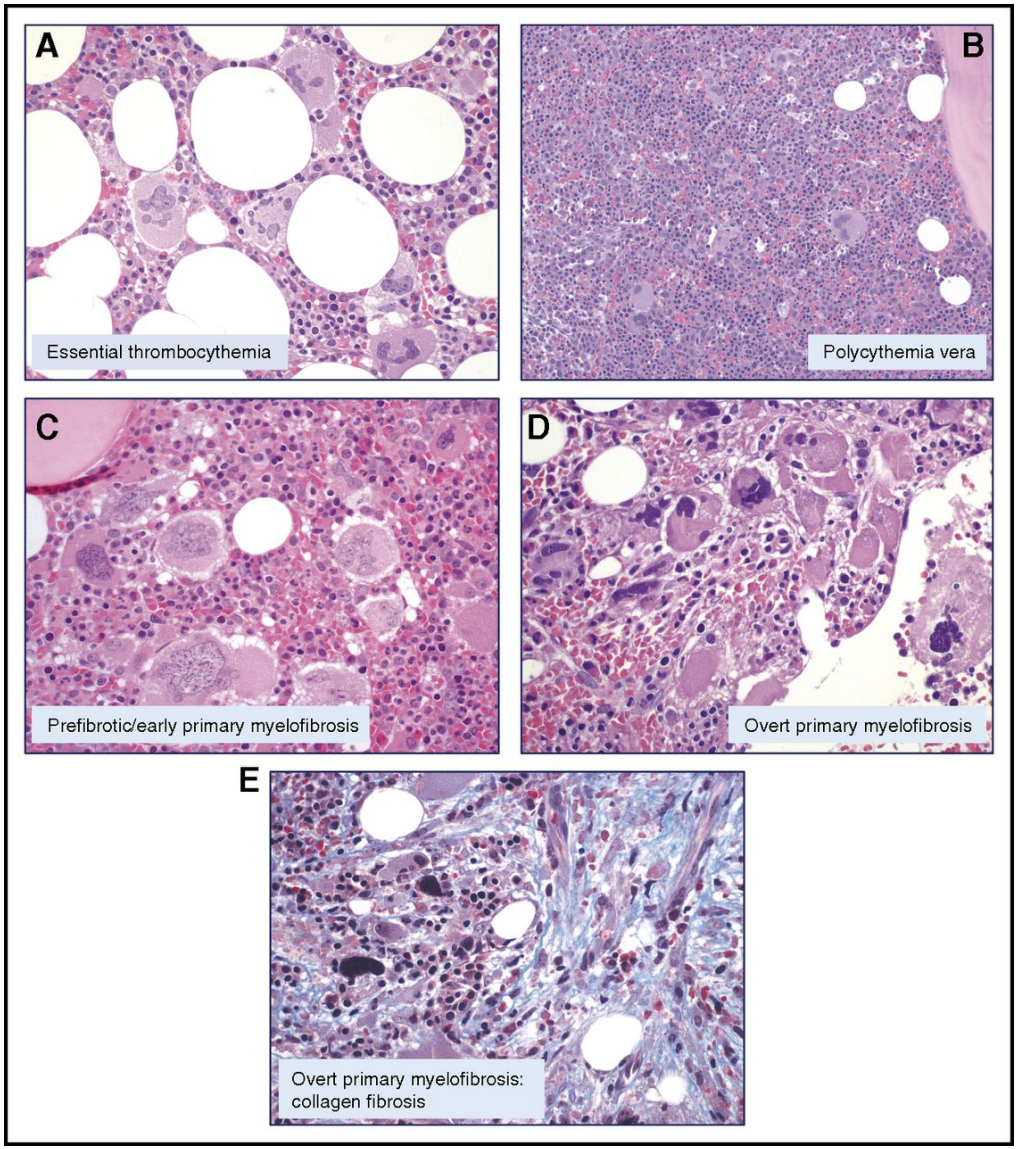


Blasts circulating in the peripheral blood can be found.



Bone marrow biopsy from a patient with primary (also called idiopathic) myelofibrosis (IMF) shows extensive fibrosis with clustered megakaryocytes noted in the center of the specimen. Other hematopoietic elements are not noted.

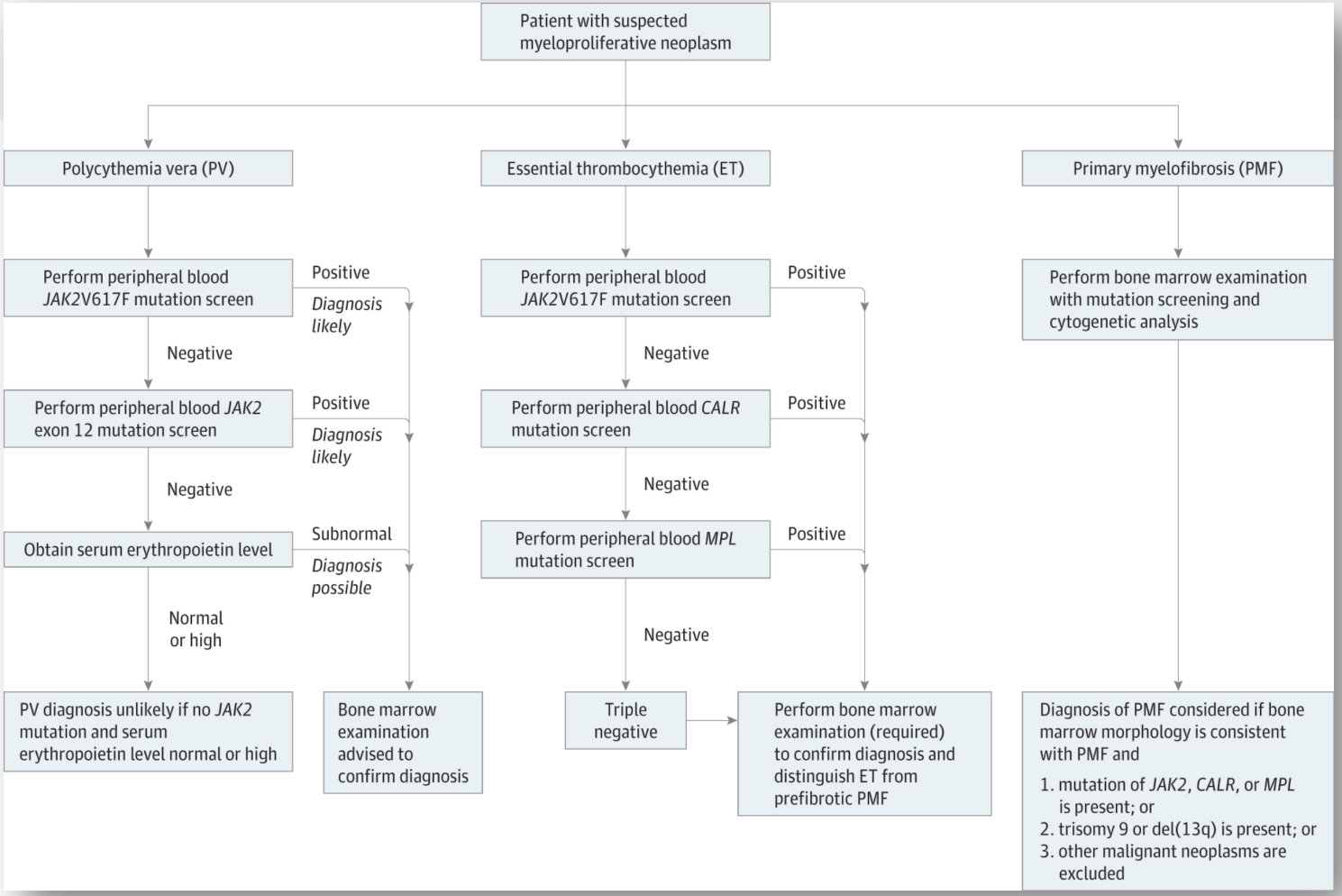
Representative bone marrow biopsies from patients with MPNs



Elisa Rumi, and Mario Cazzola Blood 2017;129:680-692

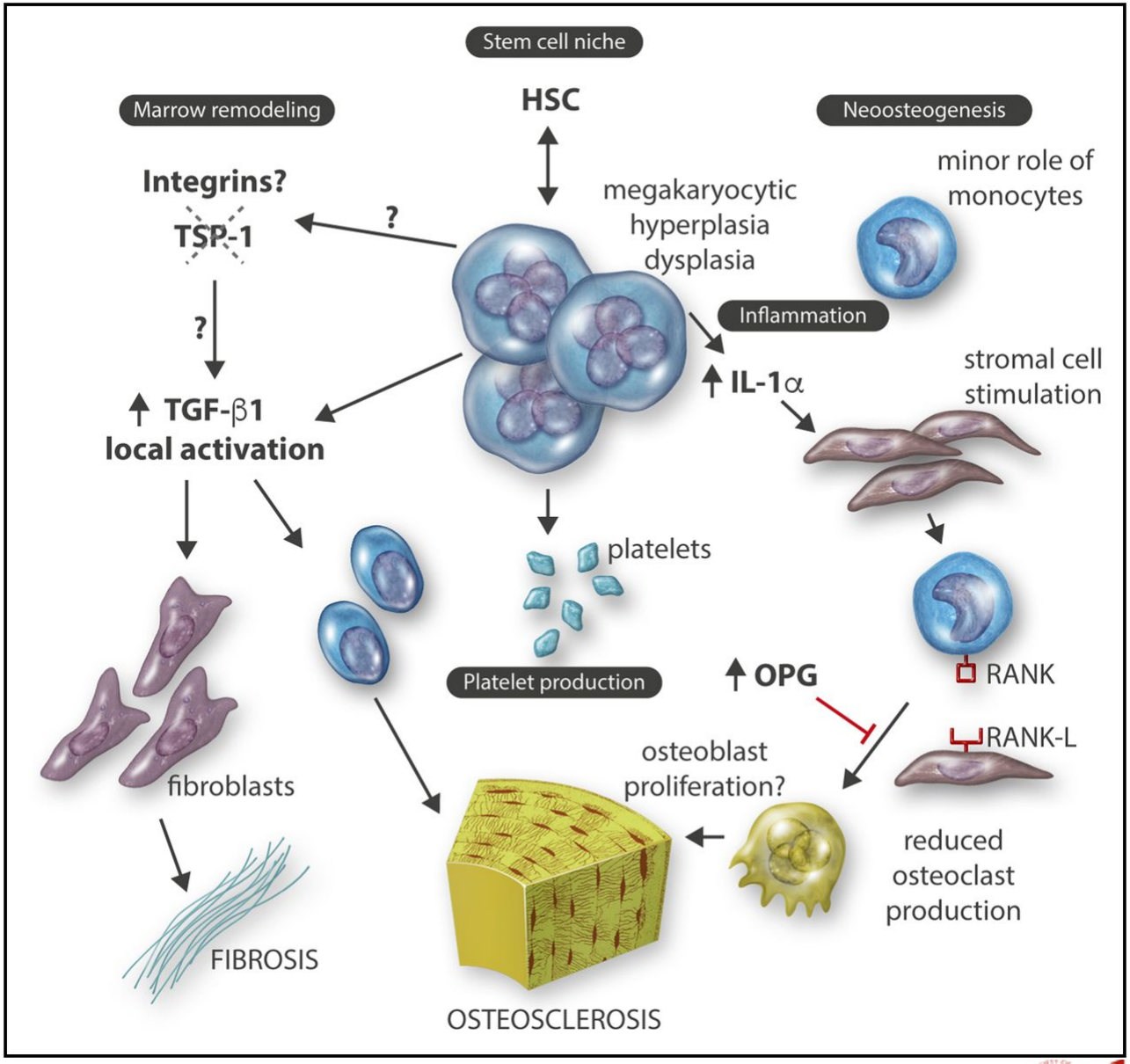
blood

JAMA Oncol. 2015;1(1):97-105. doi:10.1001/jamaoncol.2015.89



Practical Algorithm for Diagnosis of Polycythemia Vera (PV), Essential Thrombocythemia (ET), and Primary Myelofibrosis (PMF)

Megakaryocytes play a central role in MPN pathogenesis



William Vainchenker, and Robert Kralovics Blood
2017;129:667-679

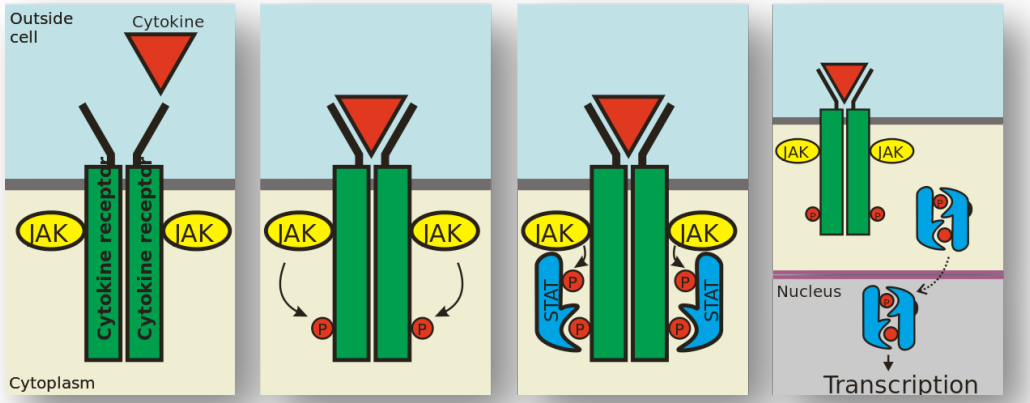
JAK-STAT signaling pathway

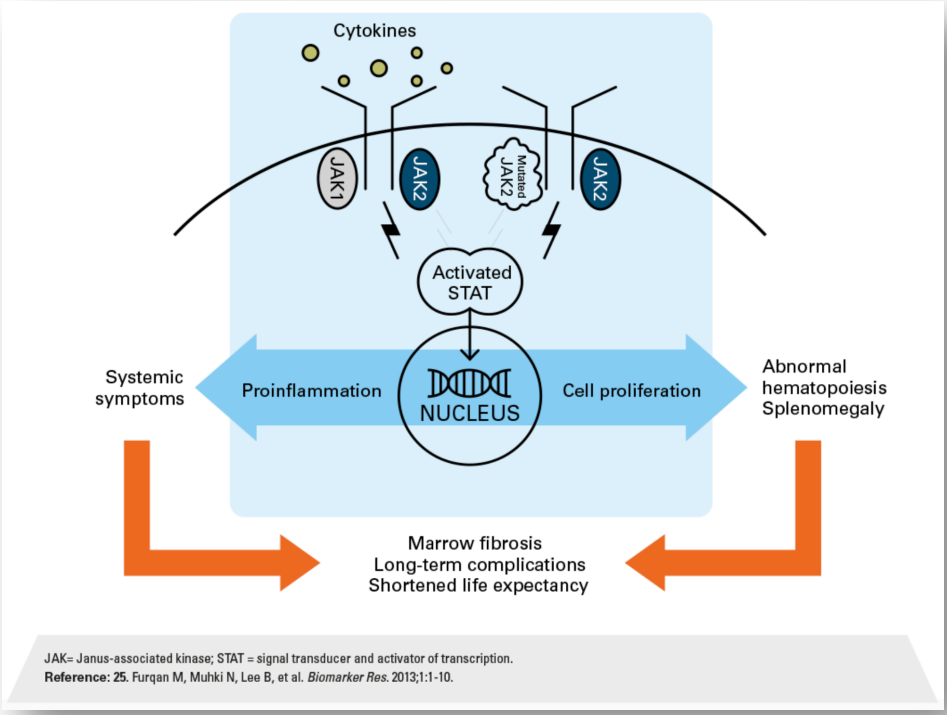
- JAK-STAT signaling pathway transmits information to the nucleus resulting in DNA transcription and expression of genes.

- Signaling cascade consists of three main components:
  - Cell surface receptor
  - Janus kinase (JAK)
  - Two Signal Transducer and Activator of Transcription (STAT) proteins

- Disruption of the JAK-STAT functionality can result in immune deficiency syndromes and cancers

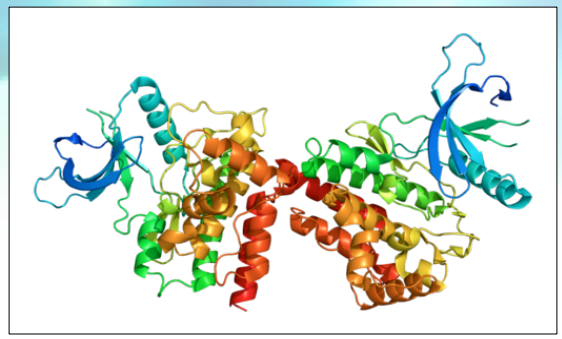
- Overactive signaling of the JAK pathway is key to the pathogenesis of myelofibrosis.





JAK2

- Janus kinase 2 (JAK2) – non-receptor tyrosine kinase
- Member of the Janus kinase family
- Implicated in signaling by members of the following families:
  - Type II cytokine receptor family
  - GM-CSF receptor family (IL-3R, IL-5R and GM-CSF-R)
  - GP130 receptor family (IL-6)
  - Single chain receptors (Epo-R, Tpo-R, GH-R, PRL-R
- Signaling is activated downstream from the prolactin receptor
- JAK2 lacks the Src homology binding domains (SH2/SH3) & up to 7 JAK homology domains (JH1-JH7)



Common Somatic Mutations in MPNs

Table 1.

Somatic mutations in MPN

Gene function and symbol	Location	Type of mutations	Protein function	Frequency	Consequences	Reference
Signaling MPN driver						
JAK2	9p24	JAK2V617F	Tyrosine kinase associated with cytokine receptors	95% PV, 50%–60% PMF and ET	Increased RBC, platelet, and granulocyte production	4–7
		JAK2 exon 12		3% PV	Increased RBC	17
MPL	1p34	MPL515L/K/A/R MPLS505N	TPOR	2%–3% ET	Increased platelet production	19, 22, 23
		Other missense mutations		3–5% PMF		25
CALR	19p13	Indel exon 9	Mutant: activator of MPL	20%–25% ET, 25%–30% PMF	Increased platelet production	28, 29

Less Common Somatic Mutations in MPNs (1)

DNA methylation						
TET2	4q24	Missense, nonsense deletion	α-Ketoglutarate-dependent dioxygenase Oxidation of 5mC into 5hmC and active 5mC demethylation	10%–20% MPN (ET, PV, and PMF)	Initiation, Mutations on 2 alleles associated with progression	70
DNMT3A	2p23	Missense, hotspot	DNA methylase, de novo methylation	5%–10% MPN (ET, PV, and PMF)	Initiation	74
IDH1	2q33.3	Missense, hotspot	Neomorphic enzyme, generation of 2-hydroxyglutarate blocking α-ketoglutarate-dependent enzymes	1%–3% PMF	Initiation, Disease progression	111
IDH2	15q26.1	Missense, hotspot	Neomorphic enzyme, generation of 2-hydroxyglutarate blocking α-ketoglutarate-dependent enzymes	1%–3% PMF	Initiation, Disease progression	111
Histone modifications						
EZH2	7q35–36	Missense, indel	H3K27 methyltransferase, loss of function	5%–10% PMF	Initiation Disease progression	82, 83
ASXL1	20q11	Nonsense/ indel	Chromatin-binding protein associated with PRC1 and 2	25% PMF 1%–3% ET/PV	Initiation Rapid progression	69

Less Common Somatic Mutations in MPNs (2)

Transcription factors						
TP53	17p13.1	Missense/ indel	Transcription factor regulating cell cycle, DNA repair and apoptosis	<5% (20% of sAML)	Progression to leukemia (mutations on both alleles) complex karyotype	98
CUX1	7q22	Deletion 7p	Transcription factor regulating TP53 and ATM	<3%	Progression to leukemia	112
IKZF1	7p12.2	Deletion 7p, indel	Master transcription factor in lymphopoiesis	<3%	Progression to leukemia	112
ETV6	12p13	Missense/ indel	Transcription factor of the ETs family	<3%	Progression to leukemia	112
RUNX1	21q22.3	Nonsense/ missense/ indel	Master transcription factor in hematopoiesis	<3% (10% of sAML)	Progression to leukemia	112
RNA splicing						
SRSF2	17q25.1	Missense, hotspot	Serine/arginine-rich pre- RNA splicing factor	<2% ET 10%–15% PMF (association with IDH mutations)	Initiation? Progression	94
SF3B1	2q33.1	Missense	RNA-splicing factor 3b subunit 1, part of U2	<3% ET	Phenotypic change (anemia)	113
U2AF1	21q22.3	Missense	U2 small nuclear RNA-splicing factor	10%–15% PMF	Phenotypic change (anemia)	94

Less Common/Rare Somatic Mutations in MPNs

Other signaling						
LNK	12q24	Missense (loss of function) deletion	Negative regulator of JAK2	1% ET, 2% PMF	Synergy with JAK2V617F–Disease progression	35
CBL	11q23;3	Missense (loss of function)	Cytokine receptor internalization	4% PMF	Disease progression (progression to AML)	110
NRAS	1p13.2	Missense (activation)	ERK/MAPK signaling	Rare PMF	Progression to leukemia (5%–10% in secondary AML)	65
NF1	17q11	Missense deletion	ERK/MAPK signaling	Rare PMF	Progression to leukemia (5%–10% in secondary AML)	66
FLT3	13q12	FLT3–ITD	Cytokine receptor (FLT3–L)	MPN (<3%)	Progression to leukemia (10%–15% in secondary AML)	100

Co-occurrence of somatic mutations in MPNs

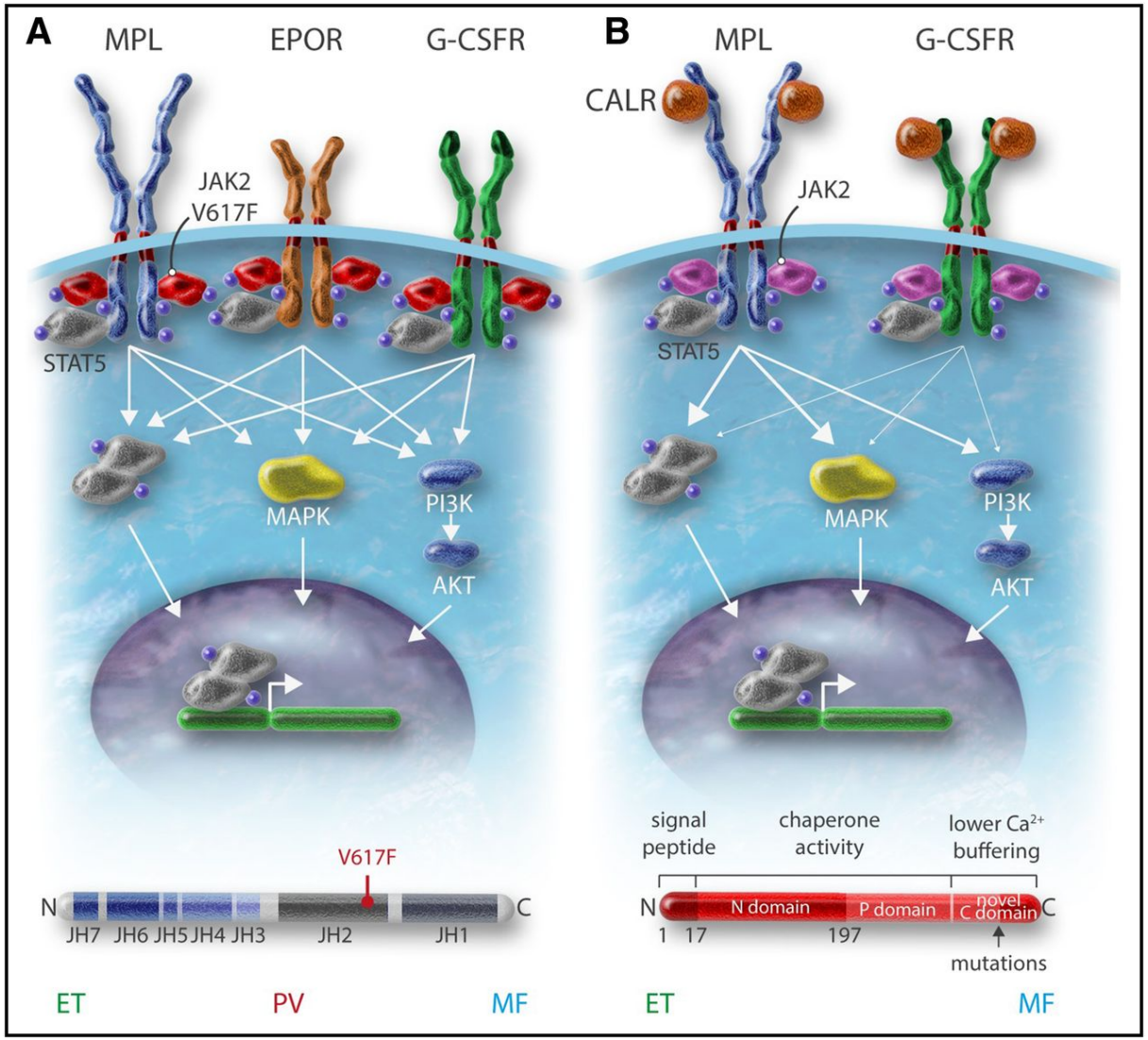
Table 3.

Co-occurrence of mutations

	PV JAK2V617F	ET JAK2V617F	ET CALRmut	ET MPLm	PMF JAK2V617F	PMF CALRmut	PMF MPLm
	% (No. of patients)						
TET2	8.33 (4)	5.26 (3)	0 (0)	0 (0)	28.95 (11)	2.63 (1)	2.63 (1)
DNMT3A	6.25 (3)	7.02 (4)	0 (0)	3.51 (2)	7.89 (3)	0 (0)	0 (0)
ASXL1	0 (0)	3.51 (2)	0 (0)	1.75 (1)	15.79 (6)	7.89 (3)	0 (0)
IDH1/IDH2	2.08 (1)	1.75 (1)	0 (0)	0 (0)	2.63 (1)	0 (0)	0 (0)
EZH2	2.08 (1)	0 (0)	1.75 (1)	0 (0)	2.63 (1)	2.63 (1)	0 (0)
SF3B1	4.17 (2)	0 (0)	0 (0)	0 (0)	2.63 (1)	0 (0)	0 (0)
U2AF1	0 (0)	0 (0)	0 (0)	0 (0)	7.89 (3)	0 (0)	0 (0)

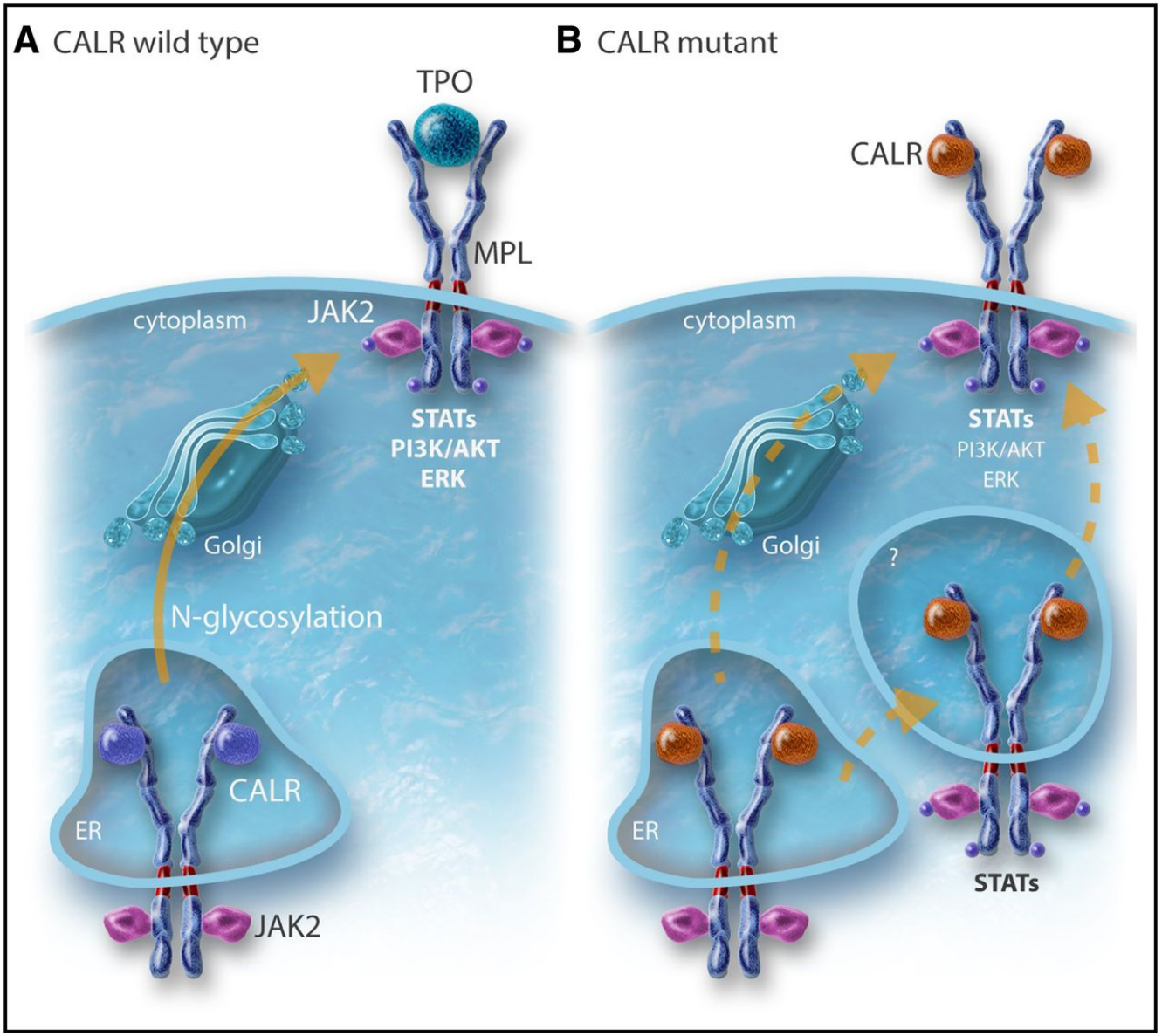
Results are extracted from Nangalia et al[29] because it is one of the rare studies in which all types of MPNs are available. Overall, 143 patients: PV, 43; ET, 57; and PMF, 38. Percentages are expressed for each MPN, irrespective of the MPN drive mutation. Thus, percentages are calculated on a very low number of patients due to the heterogeneity of the disorder, explaining differences with other studies, more particularly for the mutations in splicing genes.

Role of cytokine receptors in the oncogenic properties of JAK2 V617F and CALR mutants



William Vainchenker, and Robert Kralovics Blood
2017;129:667-679

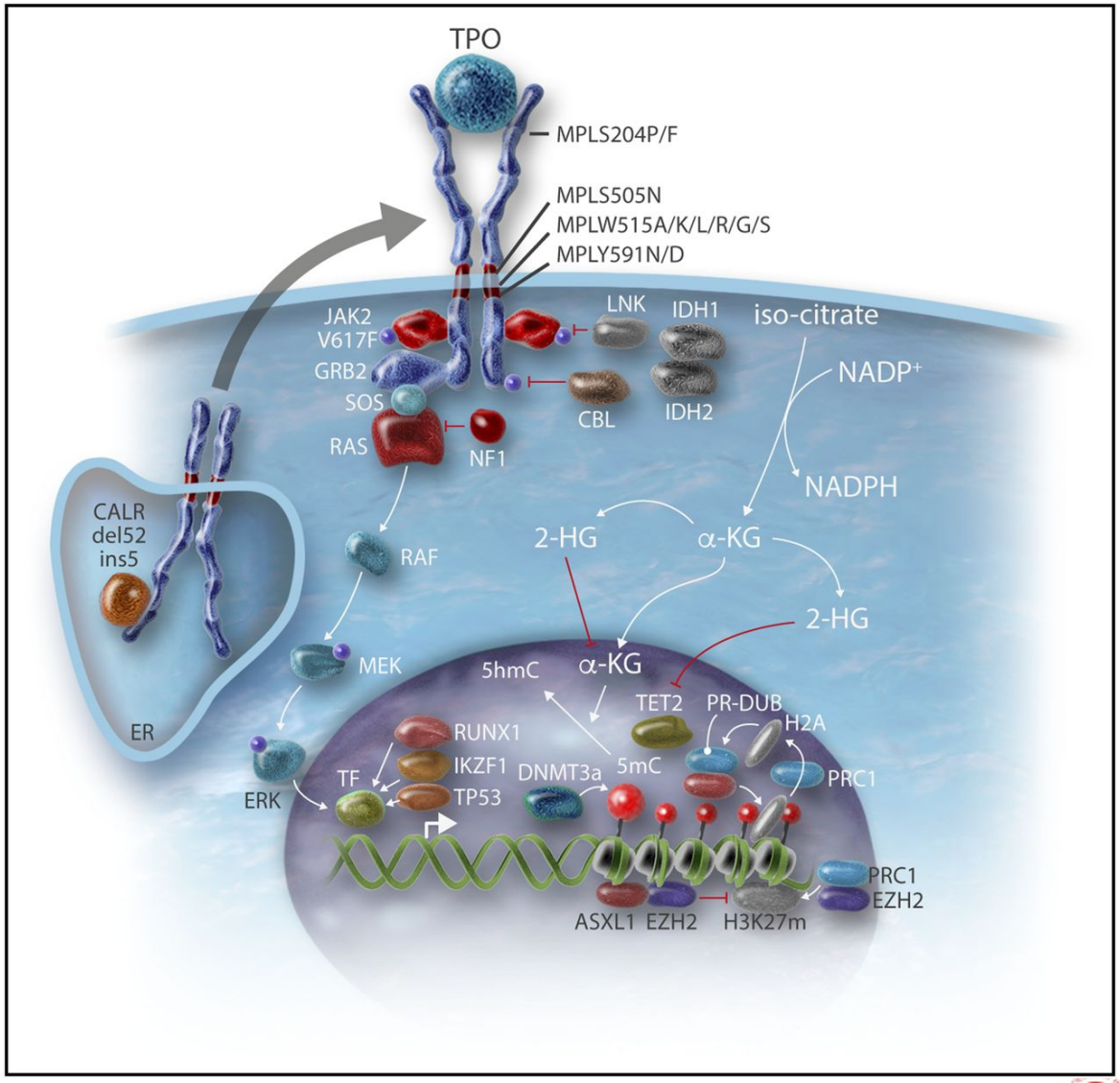
CALR mutants bind and activate MPL



William Vainchenker, and Robert Kralovics Blood
2017;129:667-679

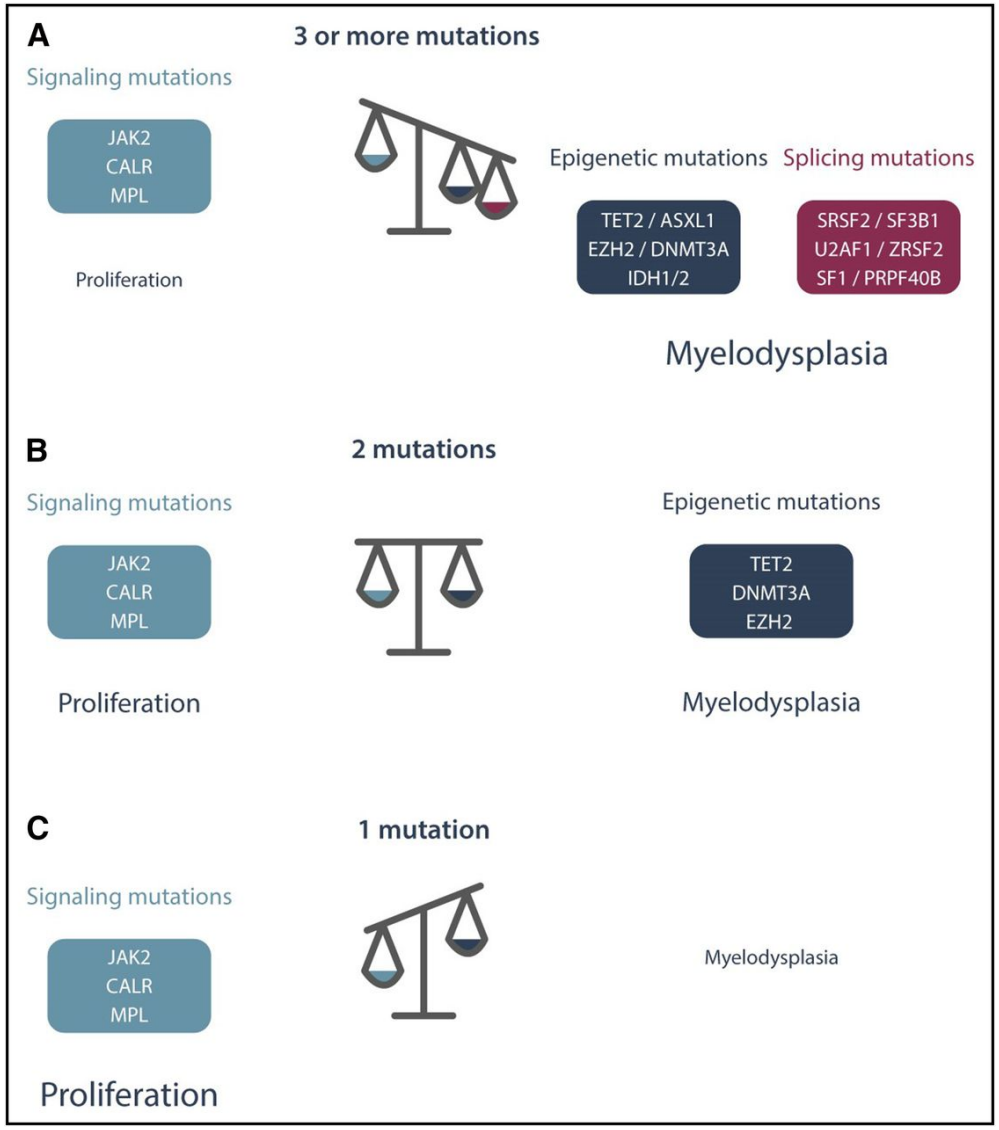
blood

Genes involved in epigenetic regulation and leukemic transformation



William Vainchenker, and Robert Kralovics Blood
2017;129:667-679

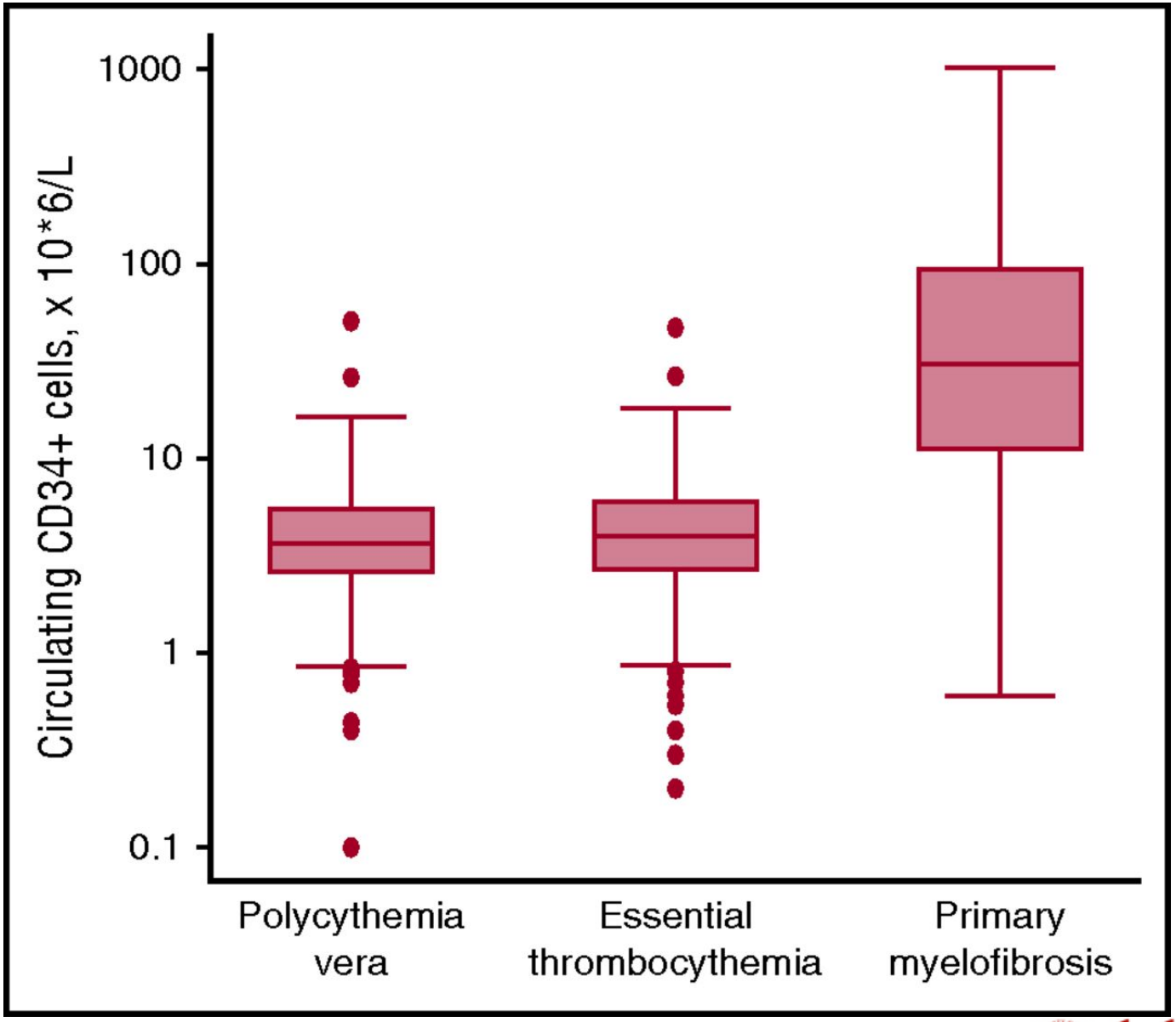
Presence of myeloproliferative and myelodysplastic features in MPN



William Vainchenker, and Robert Kralovics Blood 2017;129:667-679

blood

Circulating CD34+ cells in patients with MPNs

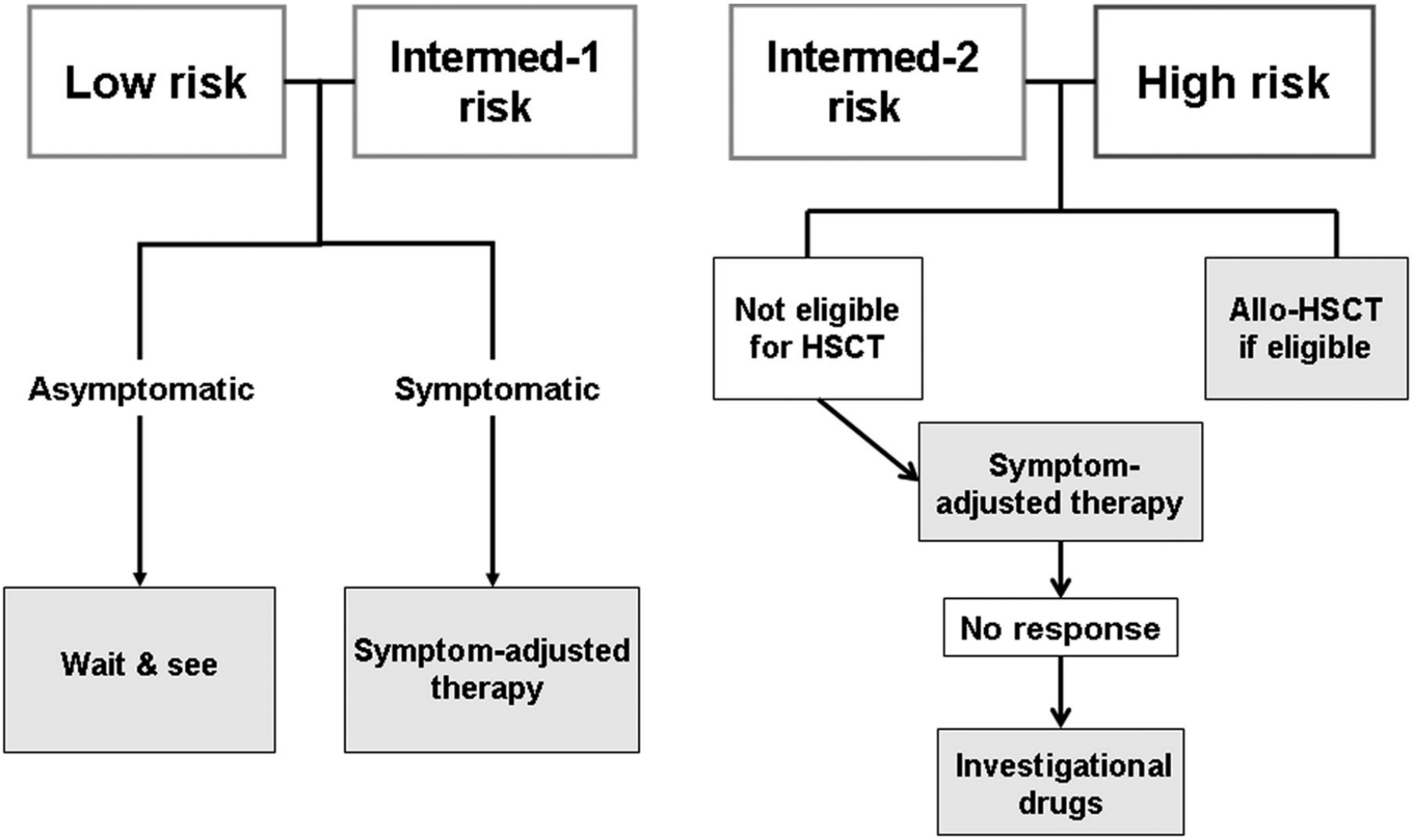


Elisa Rumi, and Mario Cazzola Blood 2017;129:680-692

Treatment of MPNs

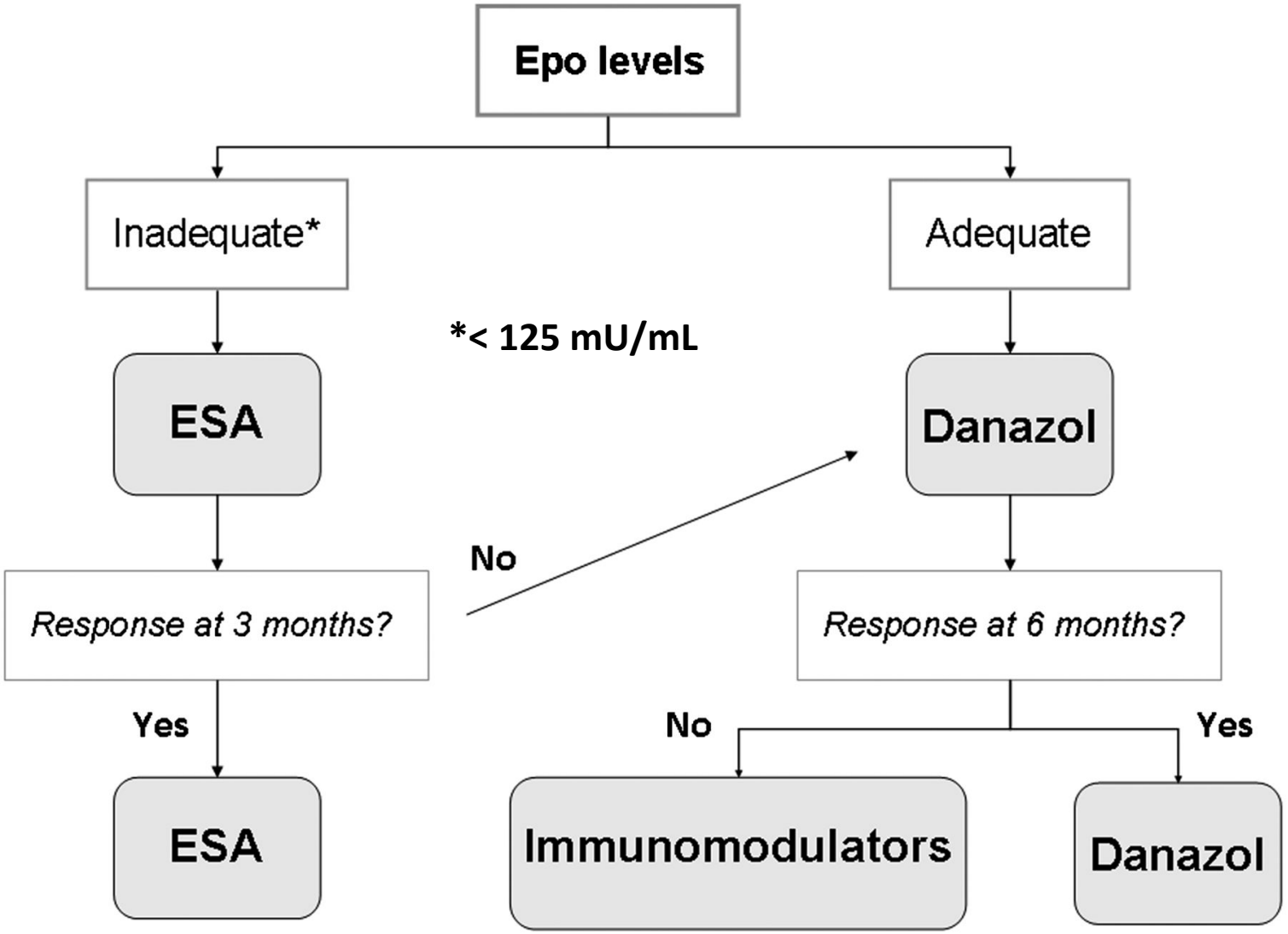
- There are currently no curative treatments for MPNs other than allogeneic stem cell transplantation, which involves significant risks
- For ET and PV, the goal is prevention of thrombohemorrhagic complications
- For MF, the goal is amelioration of anemia, splenomegaly, and other symptoms
- Treatment options include:
  - Low-dose aspirin in the setting of PV and ET
  - Tyrosine kinase inhibitors (imatinib)
  - Ruxolitinib, which is a JAK2 inhibitor and approved for primary myelofibrosis
- Frequent blood transfusions may be needed
- In the setting of primary myelofibrosis, lenalidomide and thalidomide may be used
  - Peripheral neuropathy will be a common side-effect
- If the patient is diabetic and taking sulfonylurea, this should be stopped periodically to rule out drug-induced thrombocytopenia

Treatment algorithm for patients with MF



Francisco Cervantes Blood 2014;124:2635-2642

Treatment algorithm for anemia in MF



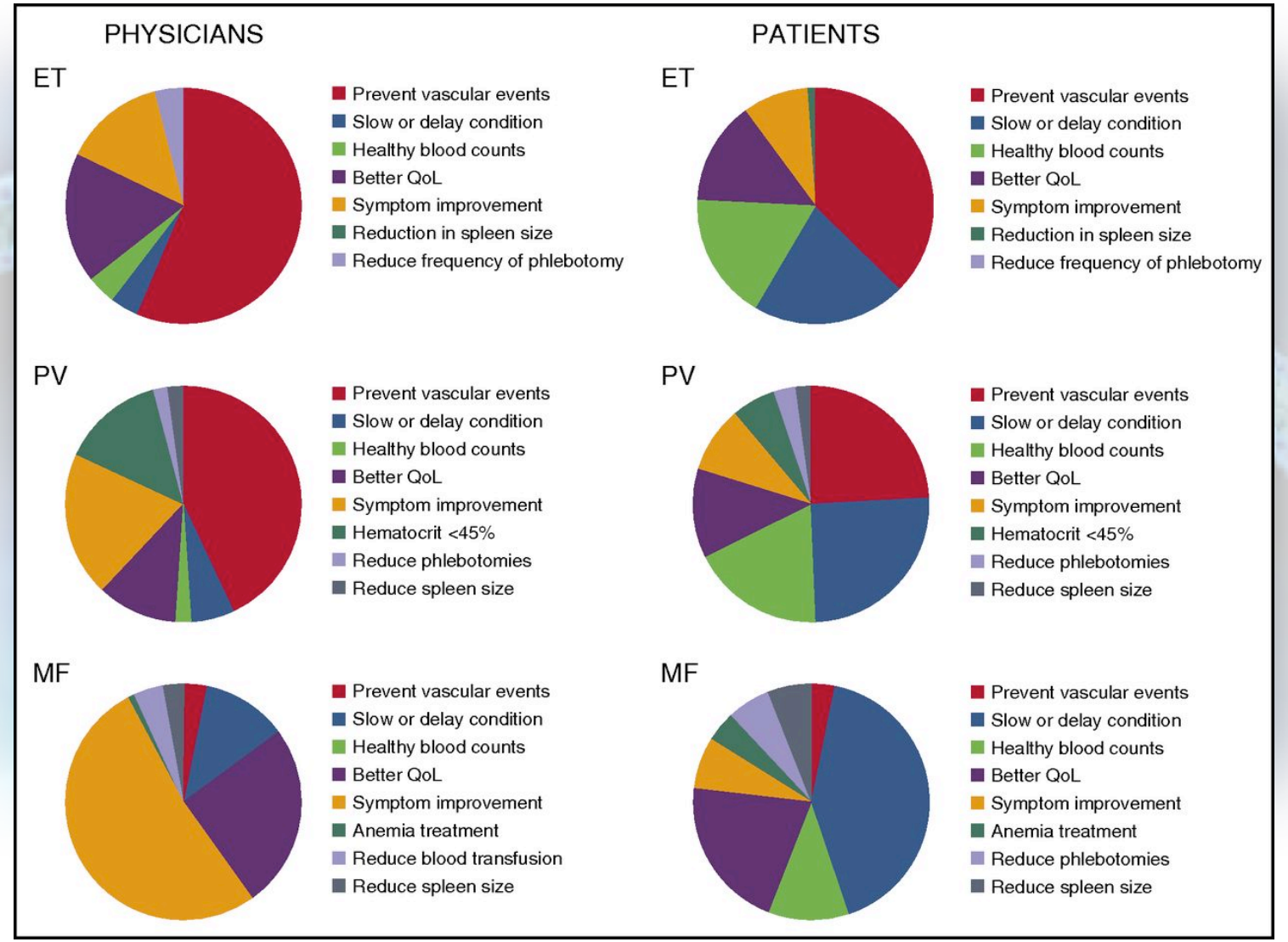
Francisco Cervantes Blood 2014;124:2635-2642

Conventional and molecular risk factors for patients with MPNs

Essential thrombocythemia	Polycythemia vera	Primary myelofibrosis
Thrombosis: • previous thrombosis • age ≥ 60 years • *JAK2* (V617F)	***Thrombosis:*** • previous thrombosis • age ≥ 60 years	***Survival & leukemic transformation:*** • age >65 years • presence of constitutional symptoms • anemia (Hb <10 g/dL) • leukocytosis (WBC count >25 x 10^9/L) • thrombocytopenia (<100 x 10^9/L) • circulating blasts (≥1%) • degree of bone marrow fibrosis • unfavorable karyotype • driver mutation (triple negative vs *JAK2/MPL* vs *CALR* mutation) • co-operating mutations in myeloid genes
Bleeding: • previous major bleeding • high PLT count (≥1500 x 10^9/L)	***Myelofibrotic transformation:*** • *JAK2* (V617F)-mutant allele burden>50% • co-operating mutations in myeloid genes	
Polycythemic transformation: • *JAK2* (V617F)	***Leukemic transformation:*** • co-operating mutations in myeloid genes	
Myelofibrotic transformation: • *CALR* mutation • co-operating mutations in myeloid genes	***Survival:*** • previous thrombosis • leukocytosis • co-operating mutations in myeloid genes	
Leukemic transformation: • co-operating mutations in myeloid genes		
Survival: • previous thrombosis • leukocytosis • co-operating mutations in myeloid genes		

Elisa Rumi, and Mario Cazzola Blood 2017;129:680-692

Priorities for MPN Treatment



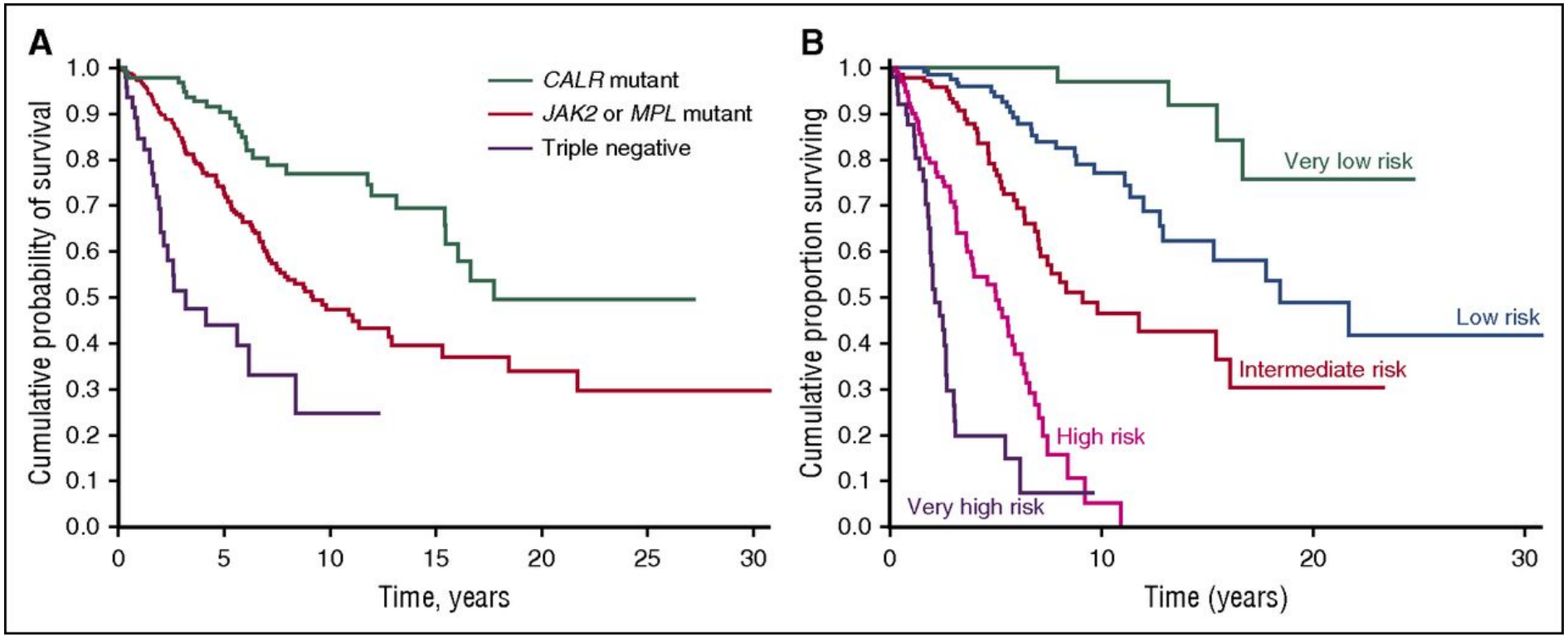
HSCT options for patients with MF

Table 1 Recommendations on selection and preparation measures for patients with MF referred to HSCT, as developed by the ELN/IWG-MRT panel.

Recommended	Indicated*	Not recommended
PATIENT SELECTION		
- Patients with intermediate-2 or high-risk disease according to IPSS, DIPSS or DIPSS-plus, and age <70 years.	- Patients with intermediate-1-risk disease and age <65 years if they present with either refractory, transfusion-dependent anemia, or >2% blasts in peripheral blood or adverse cytogenetics.	- Patients with low-risk disease.
	- Patients with intermediate 1-risk disease if they are triple negative or ASXL1 positive, or both.	- Patients in blast transformation.
	- Patients in blast transformation after achieving a partial or complete remission of leukemia with debulking therapy.	
PRETRANSPLANT MANAGEMENT		
-Iron chelation therapy in severely iron overloaded patients only.	- Ruxolitinib treatment for patients with a symptomatic spleen and/or constitutional symptoms.	- Splenic irradiation - Splenectomy (case-by-case decision)

*, means not a strong recommendation but case-by-case approach.

Kaplan-Meier analysis of survival of PMF patients stratified according to their driver mutation or a clinical-molecular prognostic model that includes IPSS variables and driver mutation



Elisa Rumi, and Mario Cazzola Blood 2017;129:680-692

References

- Rumi, E., & Cazzola, M. (2017). Diagnosis, risk stratification, and response evaluation in classical myeloproliferative neoplasms. Blood, 129(6), 680-692. Accessed March 15, 2017. https://doi.org/10.1182/blood-2016-10-695957.
- Vainchenker, W., & Kralovics, R. (2017). Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. Blood, 129(6), 667-679. Accessed March 15, 2017. https://doi.org/10.1182/blood-2016-10-695940.
- Cervantes, F. (2014). How I treat myelofibrosis. Blood, 124(17), 2635-2642. Accessed March 15, 2017. https://doi.org/10.1182/blood-2014-07-575373.
- Tefferi, A., & Pardanani, A. (2015). Myeloproliferative neoplasms: A contemporary review. *JAMA Oncology, 1*(1), 97-105. doi:10.1001/jamaoncol.2015.89
- Vannucchi, A. M., & Harrison, C. (2016). Emerging treatments for classical myeloproliferative neoplasms. Blood, (), blood-2016-10-695965. Accessed March 15, 2017. https://doi.org/10.1182/blood-2016-10-695965.
- https://en.wikipedia.org/wiki/Myeloproliferative_neoplasm
- https://en.wikipedia.org/wiki/Janus_kinase_2
- https://en.wikipedia.org/wiki/Myelofibrosis
- http://www.mpnconnect.com/myelofibrosis.aspx
- https://imagebank.hematology.org/