

Come nascono le sindromi mieloproliferative croniche

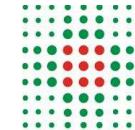
Elena Masselli

Ricercatore Universitario, Anatomia Umana – Università di Parma
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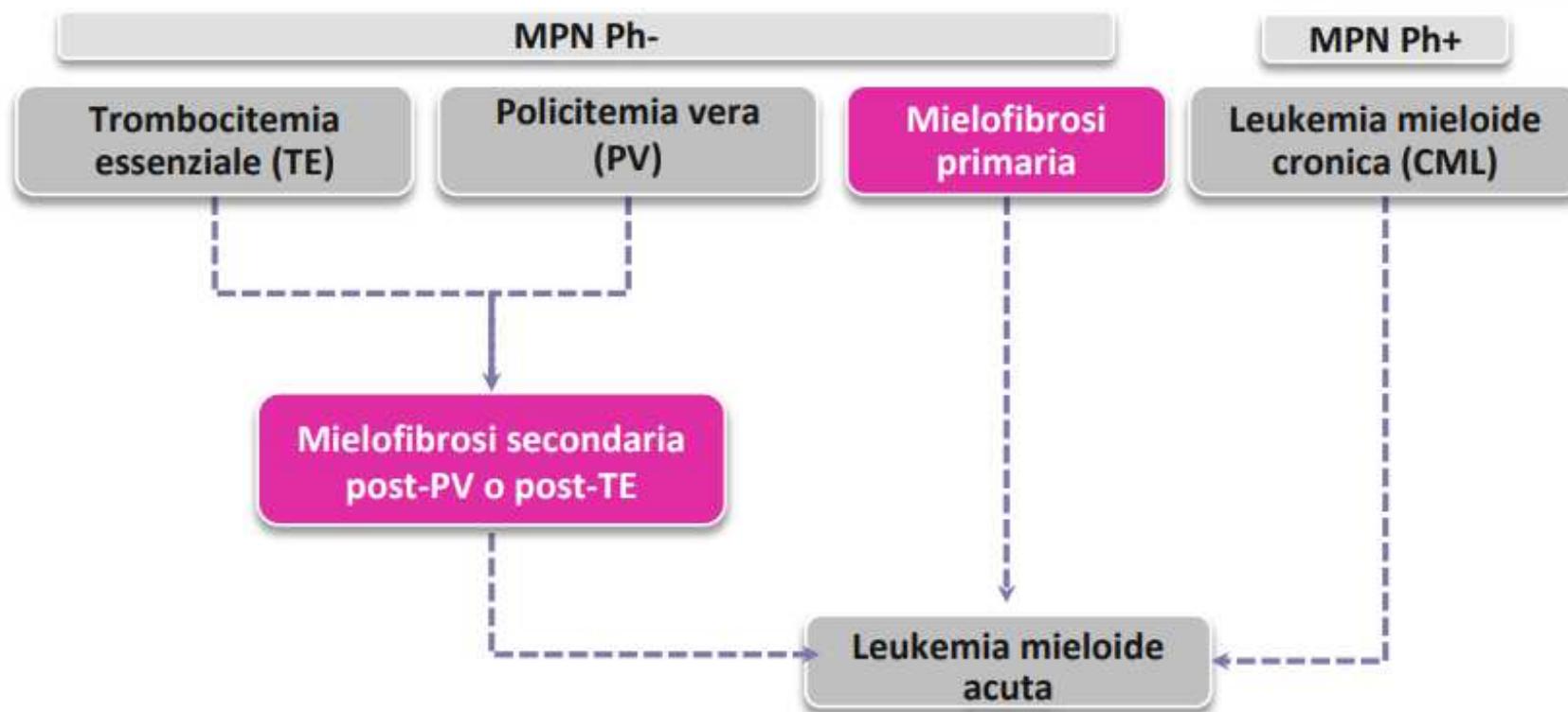
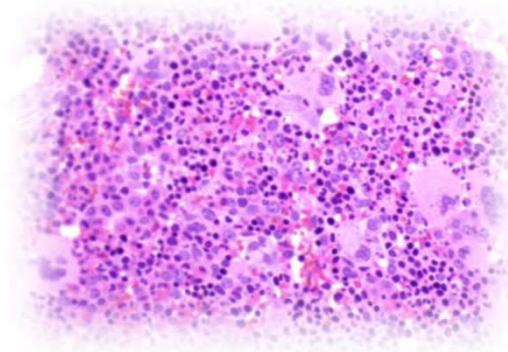
**UNIVERSITÀ
DI PARMA**

DIPARTIMENTO DI MEDICINA E CHIRURGIA

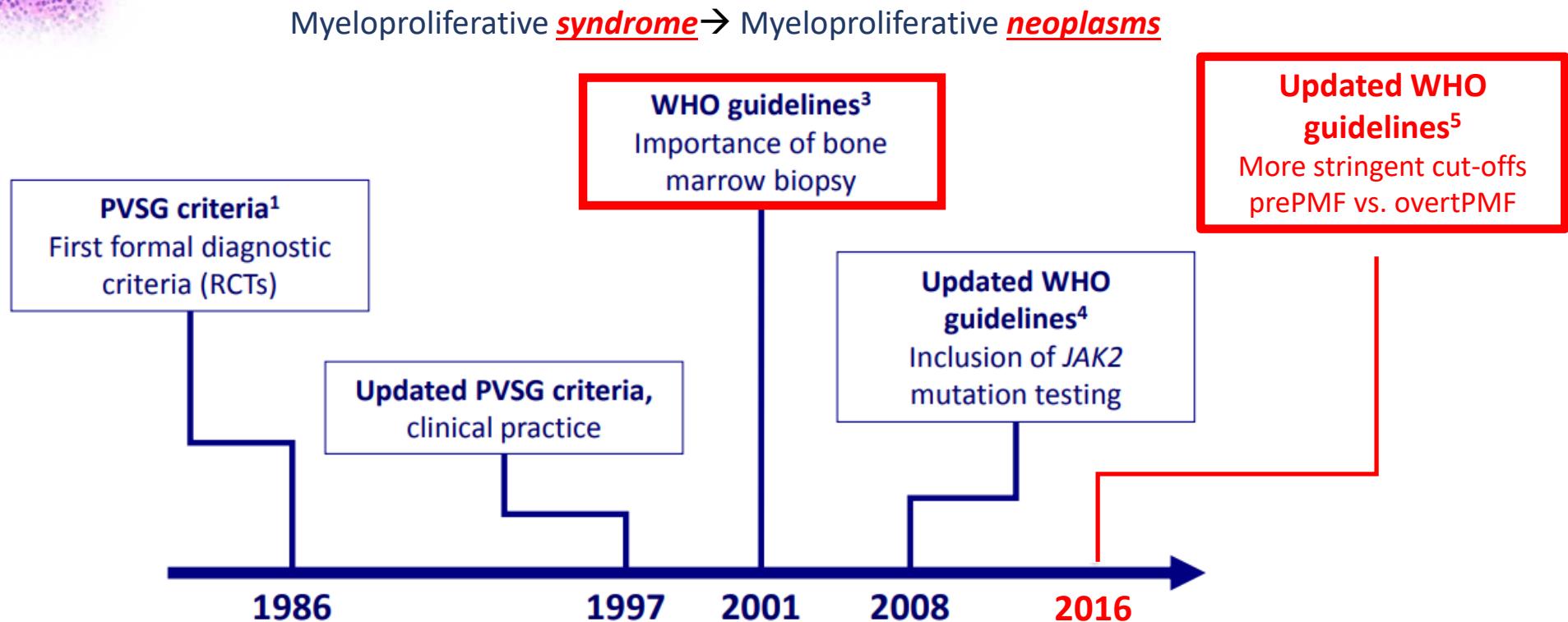


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Neoplasie Mieloproliferative croniche (MPN) Ph-negative



Evoluzione dei criteri diagnostici per le MPN



PVSG: Polycythaemia Vera Study Group
WHO: World Health Organization

1. Murphy et al. Semin Hematol 1986
2. Murphy et al. Semin Hematol 1997
3. Vardiman et al. Blood 2002
4. Tefferi et al. Blood 2007
5. Arber et al. Blood 2016

Le MPN sono malattie rare

Policitemia
Vera

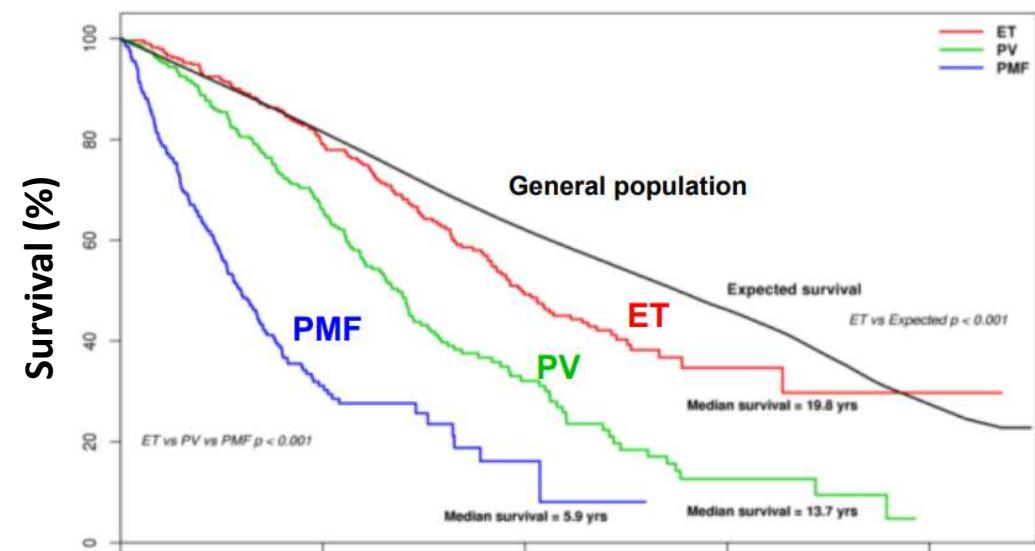
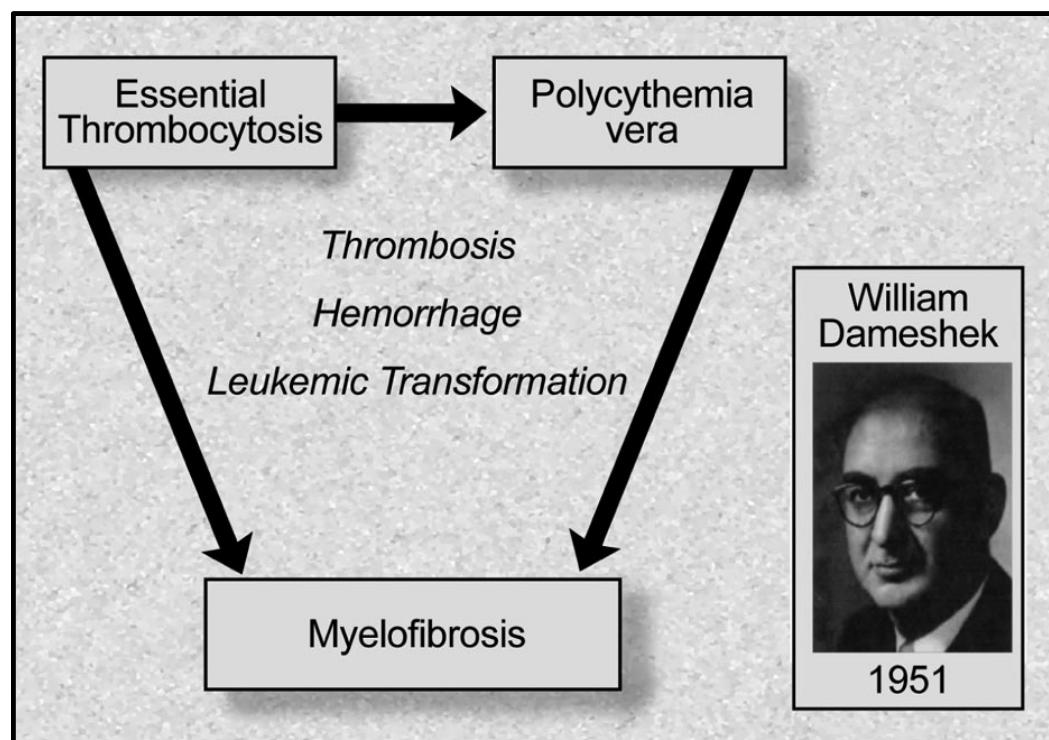
Trombocitemia
essenziale

Mielofibrosi

ORPHA:729	Vaquez disease	OMIM: 263300
<i>Classification level: Disorder</i>		
<i>Synonym(s):</i>		
Acquired primary erythrocytosis		
Osler-Vaquez disease		
PV		
Polycythemia rubra vera		
ORPHA:3318	Inheritance: Multigenic/multifactorial or Not applicable	UMLS: C0040028
<i>Classification level: Disorder</i>		
<i>Synonym(s):</i>		
ET		
Essential thrombocythosis		
<i>Prevalence: 1-5 /10 000</i>		
ORPHA:824	Age of onset: All ages	MeSH: D013920
<i>Classification level: Disorder</i>		
<i>Synonym(s):</i>		
Agnogenic myeloid metaplasia		
Idiopathic myelofibrosis		
Myelofibrosis with myeloid metaplasia		
Osteomyelofibrosis		
	<i>ICD-10: D47.3</i>	GARD: 6594
	OMIM: 187950 601977 614521	MedDRA: 10015493
	Prevalence: 1-9 /100 000	OMIM: 254450
	Inheritance: Not applicable	UMLS: C0001815 C0026987
	Age of onset: Adult	MeSH: -
	ICD-10: D47.4	GARD: 8618
		MedDRA: -

<https://www.orpha.net/>

MPN: spettro clinico e sopravvivenza



Storia naturale delle MPN

Cause di morte:

- Progressione a leucemia acuta
- Eventi cardiovascolari
- Conseguenze della/e citopenia/e (infezioni, sanguinamenti)

	Myelofibrosis	Leukaemia
ET	4%	2%*, 3.3%"
PV	6%	7%
PMF		~20%

Cumulative risk at 15 years; * ET diagnosed using PVSG criteria, " ET diagnosed by WHO criteria

*Cumulative risk @15y *ET diagnosed according PVSG criteria; " ET diagnosed according to WHO criteria*

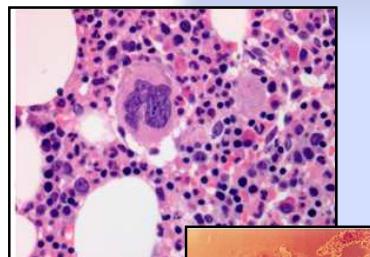
Policitemia Vera



Louis Henri Vaquez- 1892

«Erythremia» with hepatosplenomegaly

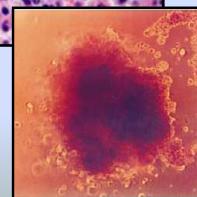
BM hypercellularity



Hepatosplenomegaly



CFU-E & BFU-E
autonomous cell growth

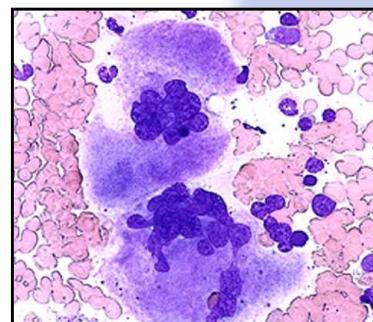


Pletoric face

Trombocitemia essenziale

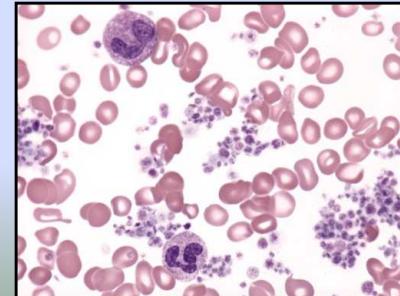
Epstein & Goedel - 1934

«hemorrhagic thrombocythemia»: permanent elevation of plt> 3-times normal,
hyperplasia of megakaryocytes, and the tendency for venous thrombosis and
spontaneous hemorrhage



MK hyperplasia

Thrombocythosis

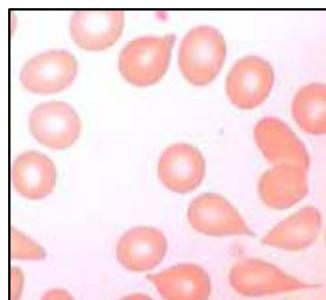


Thrombosis/bleeding

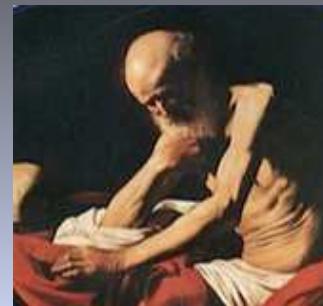
Mielofibrosi

Gustav Heuck- 1879

*two pts with massive splenomegaly, circulating RBCs,
increased WBCs and marrow fibrosis*



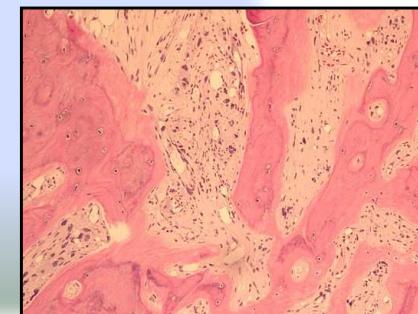
Anemia



Massive splenomegaly
cachexia



Leukoerythroblastic blood film



BM fibrosis

THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia

Daniel A. Arber,¹ Attilio Orazi,² Robert Hasserjian,³ Jürgen Thiele,⁴ Michael J. Borowitz,⁵ Michelle M. Le Beau,⁶ Clara D. Bloomfield,⁷ Mario Cazzola,⁸ and James W. Vardiman⁹

“The revision of the fourth edition follows the philosophy of the third and fourth editions to incorporate clinical features, morphology, immunophenotyping, cytogenetics, and molecular genetics to define disease entities of clinical significance”

- ✓ Inclusion of recently identified **molecular markers** with diagnostic, prognostic and physio-pathologic relevance
- ✓ Improvement of the **characterization and standardization of morphological features**, particularly of BCR-ABL1-neg MPNs
 - ✓ Integrated approach

Classificazione WHO

2008

vs

2016

Myeloproliferative neoplasms (MPN)

Chronic myelogenous leukemia, *BCR-ABL1*-positive

Chronic neutrophilic leukemia

Polycythemia vera

Primary myelofibrosis

Essential thrombocythemia

Chronic eosinophilic leukemia, not otherwise specified

Mastocytosis

Myeloproliferative neoplasms, unclassifiable

Myeloproliferative neoplasms, unclassifiable

Mastocytosis

Chronic eosinophilic leukemia, not otherwise specified

Myeloproliferative neoplasms (MPN)

Chronic myeloid leukemia (CML), *BCR-ABL1*⁺

Chronic neutrophilic leukemia (CNL)

Polycythemia vera (PV)

Primary myelofibrosis (PMF)

PMF, prefibrotic/early stage

PMF, overt fibrotic stage

Essential thrombocythemia (ET)

Chronic eosinophilic leukemia, not otherwise specified (NOS)

MPN, unclassifiable

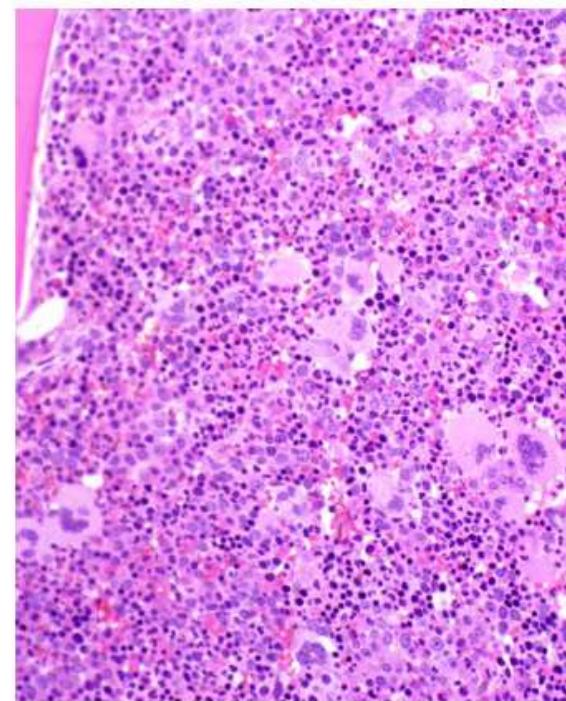
Criteri diagnostici Policitemia vera - WHO 2008

Major criteria

1. Hemoglobin **>18.5 g /dL** in men, **16.5 g/dL** in women or other evidence of increased red cell volume
2. Presence of JAK2 V617F or other functionally similar mutation such as JAK2 exon 12 mutation

Minor criteria

1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range from normal
3. Endogenous erythroid colony formation in vitro



- Diagnosis requires the presence **of both major criteria and one minor criterion**, or the presence of the first major criterion together with two minor criteria

Criteri diagnostici Policitemia vera - WHO 2016

Polycythemia Vera (PV)*		
Major criteria	1	Hemoglobin >16.5 g/dL (men) >16 g/dL (women) <i>or</i> Hematocrit >49% (men) >48% (women) Or increased red cell mass (>25% predicted value)
	2	Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
	3	Presence of JAK2 mutation (V617F or exon 12)
Minor criterion	1	Subnormal serum erythropoietin level

*PV diagnosis: all 3 major criteria or the first 2 major criteria and the minor criterion

Arber DA. et al. Blood. 2016

Policitemia vera - differenze WHO 2016 vs. 2008

1. Changes in the definition of increased red cell mass

- Hb >18.5 g/dL (men)
 >16.5 g/dL (women)
- RCM >25% normal value
- Hb or Hct >99th percentile of reference range
- Hb >17 g/dL (men), >15 g/dL (women) after increase ≥ 2 g/dL from baseline value without correction of iron deficiency

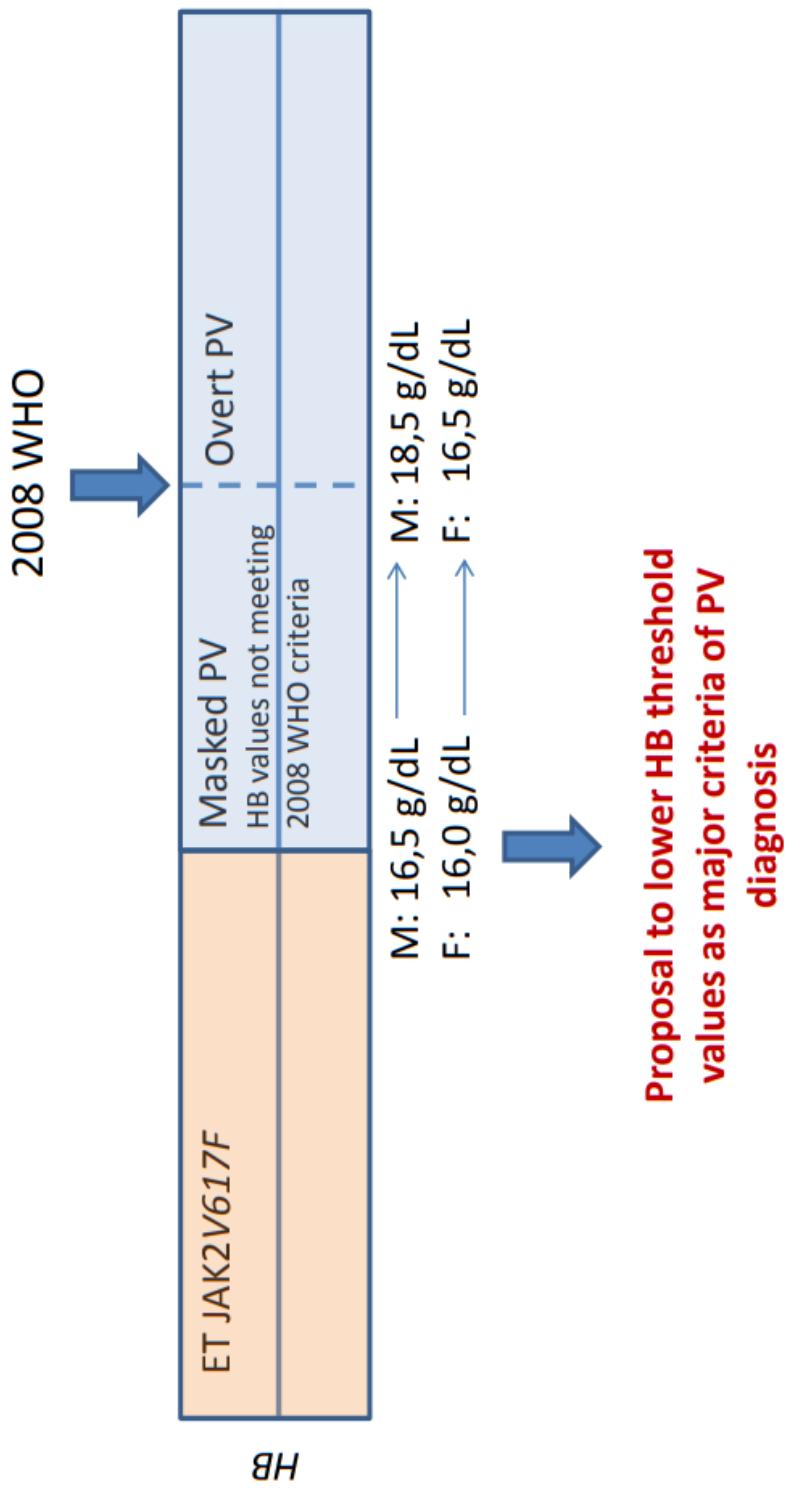


Hb >16.5 g/dL (men
 >16 g/dL (women)
or
Hct >49% (men)
 >48% (women)

2. Inclusion of bone marrow morphology as a major criterion

3. Removal of EEC growth as a minor criterion

Discrimination of ET and PV in JAK2V617F Patients by Hemoglobin Levels



Criteri diagnostici Mielofibrosi Primaria- WHO 2008

Major criteria

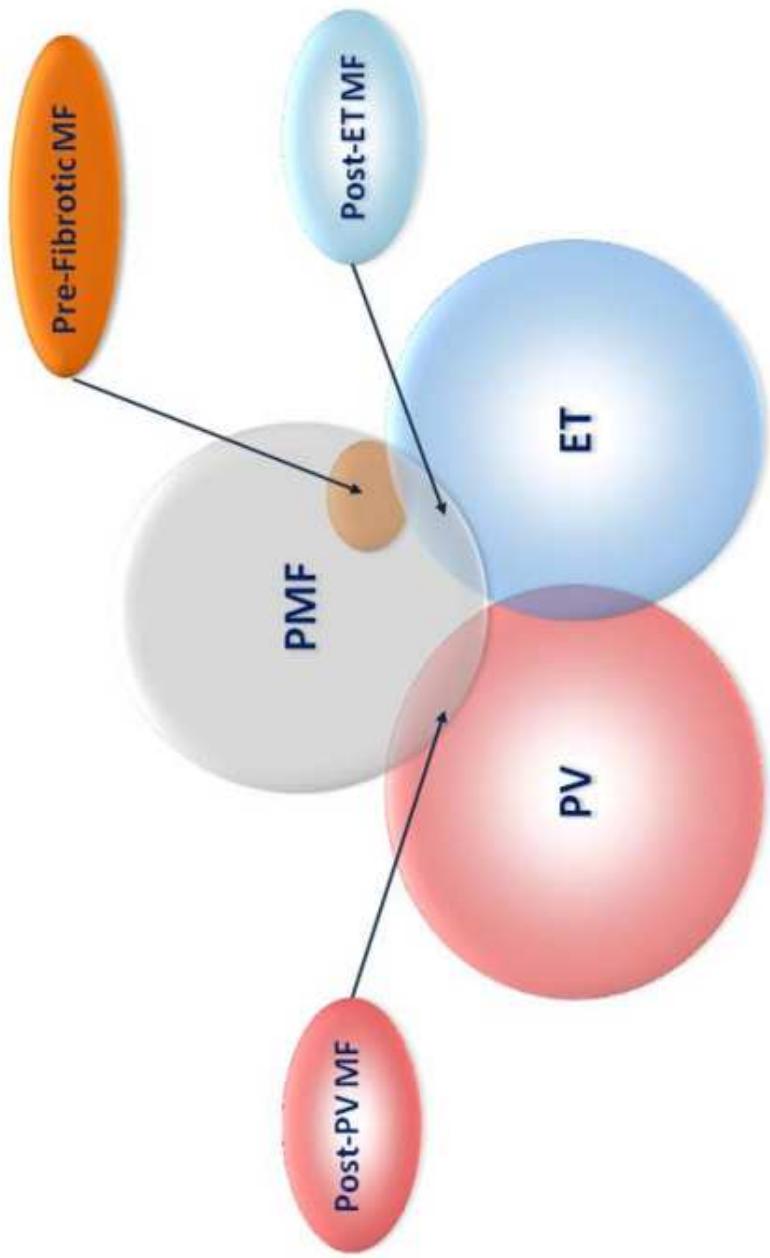
- **Megakaryocyte proliferation and atypia**, usually accompanied by either reticulin and/or collagen fibrosis,
or, in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (i.e. pre-fibrotic cellular-phase disease).
- Not meeting WHO criteria for PV, CML, MDS, or other myeloid neoplasm
- Demonstration of JAK2V617F or other clonal marker (e.g. MPLW515L/K),
or in the absence of a clonal marker, no evidence of bone marrow fibrosis due to underlying inflammatory or other neoplastic disease

Minor criteria

- Leukoerythroblastosis
- Increase in serum lactate dehydrogenase level
- Anemia
- Palpable splenomegaly

Diagnosis requires meeting all three major criteria and two minor criteria

Myelofibrosis: an Heterogeneous Disease



Criteri diagnostici prePMF - WHO 2016

Table 6. WHO criteria for prePMF

WHO prePMF criteria

Major criteria

1. Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1*, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis
2. Not meeting the WHO criteria for *BCR-ABL1*⁺ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,† or absence of minor reactive BM reticulin fibrosis‡

Minor criteria

Presence of at least 1 of the following, confirmed in 2 consecutive determinations:

- a. Anemia not attributed to a comorbid condition
- b. Leukocytosis $\geq 11 \times 10^9/L$
- c. Palpable splenomegaly
- d. LDH increased to above upper normal limit of institutional reference range

Diagnosis of prePMF requires meeting all 3 major criteria, and at least 1 minor criterion

*See Table 8.

†In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (eg, *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.

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

Criteri diagnostici overtPMF - WHO 2016

Table 7. WHO criteria for overt PMF

WHO overt PMF criteria

Major criteria

1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3*
2. Not meeting WHO criteria for ET, PV, *BCR-ABL1*⁺ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker,† or absence of reactive myelofibrosis‡

Minor criteria

Presence of at least 1 of the following, confirmed in 2 consecutive determinations:

- a. Anemia not attributed to a comorbid condition
- b. Leukocytosis $\geq 11 \times 10^9/L$
- c. Palpable splenomegaly
- d. LDH increased to above upper normal limit of institutional reference range
- e. Leukoerythroblastosis

Diagnosis of overt PMF requires meeting all 3 major criteria, and at least 1 minor criterion

*See Table 8.

†In the absence of any of the 3 major clonal mutations, the search for the most frequent accompanying mutations (eg, *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*) are of help in determining the clonal nature of the disease.

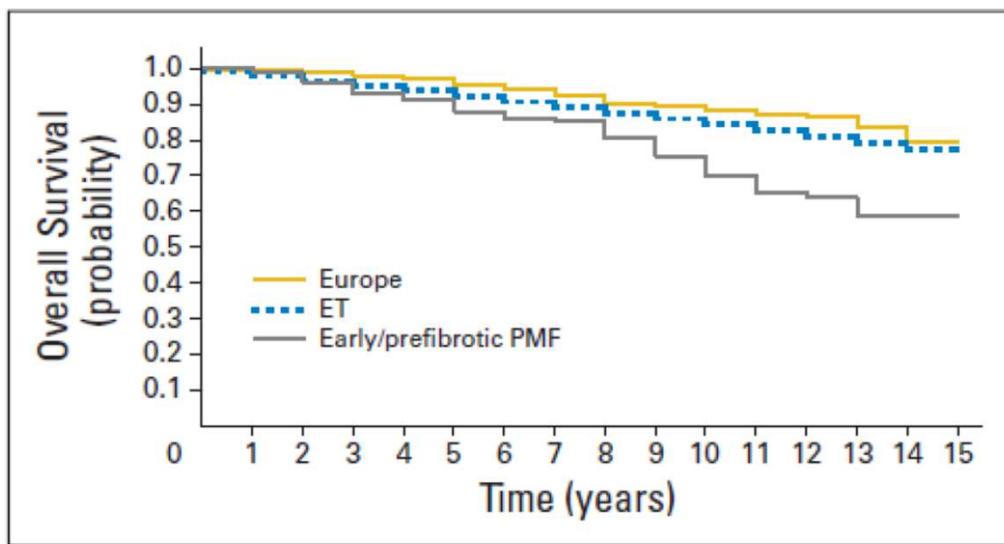
‡BM fibrosis secondary to infection, autoimmune disorder, or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

Diagnosi di prePMF: perché è rilevante

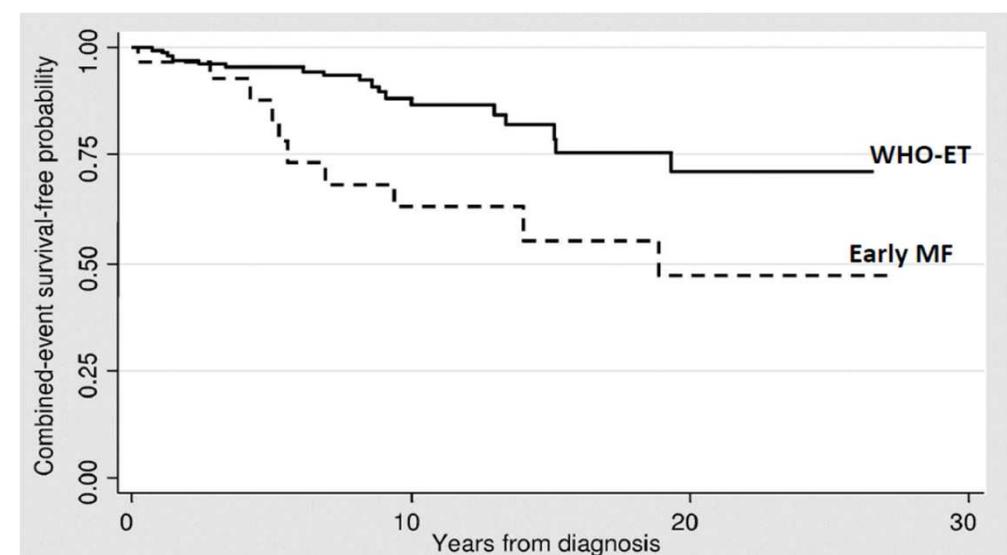
	ET (n=891)	PMF (n=180)	P value
Age, years, median (range)	56 (13-91)	57 (21-88)	0.66
Male/Female	370/521	74/106	0.92
Follow-up, years	6.2 (0-27)	7.0 (0-27.2)	0.30
WBC, x 10 ⁹ /L, median (range)	8.6 (2.5-53.4)	9.7 (4.8-24.2)	< 0.001
Hb, g/dL, median (range)	14.1 (6.9-18.0)	13.8 (6.9-16.7)	0.01
PLT, x 10 ⁹ /L, median (range)	774 (291-3920)	902 (462-3401)	0.002
LDH (n=519), mU/mL median (range)	298 (113-1070)	429 (70-1517)	< 0.001
CD34 ⁺ (N=246) /mcL, median (range)	2 (0-15.2)	4.7 (0-60)	0.03
JAK2 (V617F)-pos (n=805)	422 (61%)	67 (58%)	0.56
Fibrosis (n=968)	23 (3%)	38 (22%)	< 0.001
Splenomegaly	146 (16%)	41 (23%)	0.04

Diagnosi di prePMF: perché è rilevante

Overall survival

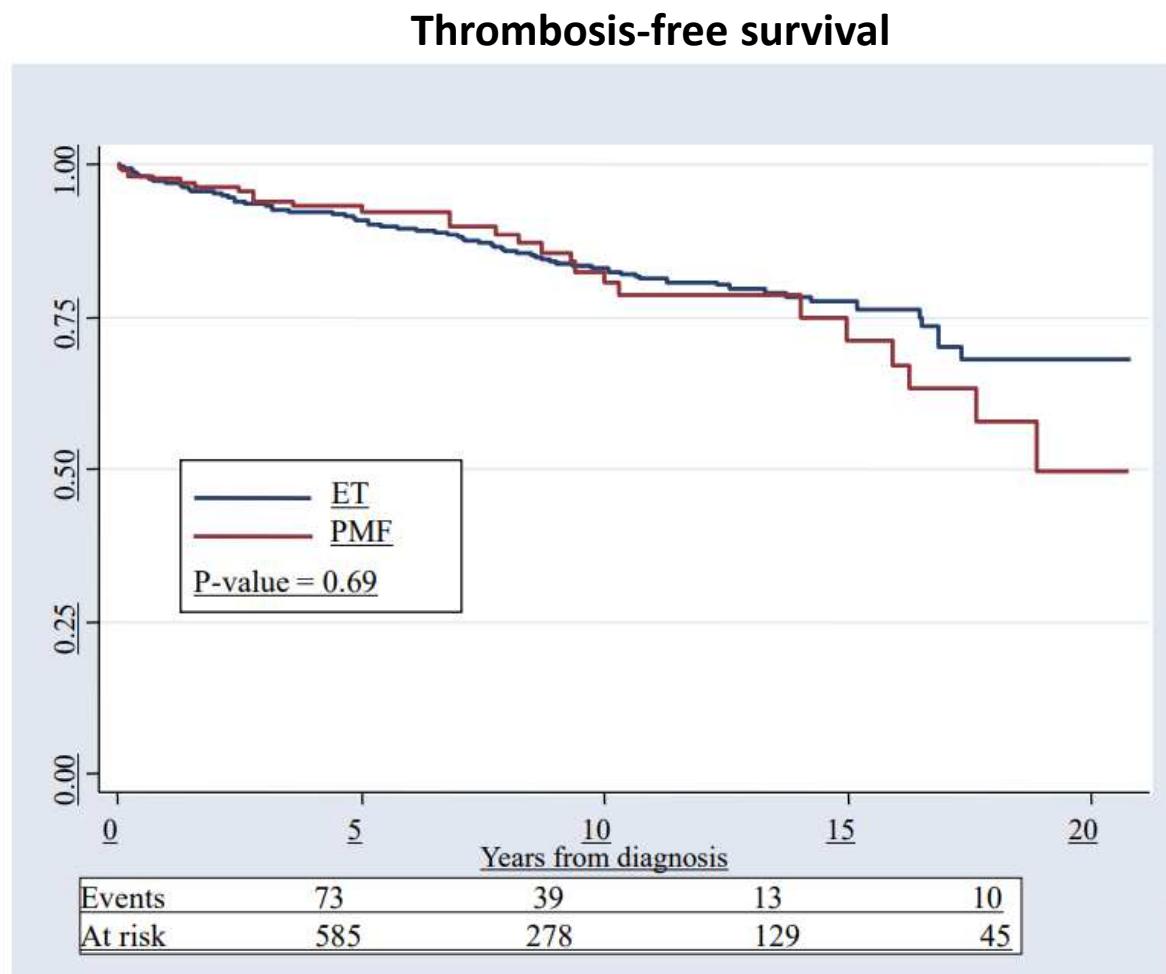


Composite outcome
thrombosis, bleeding, and evolution to overt MF



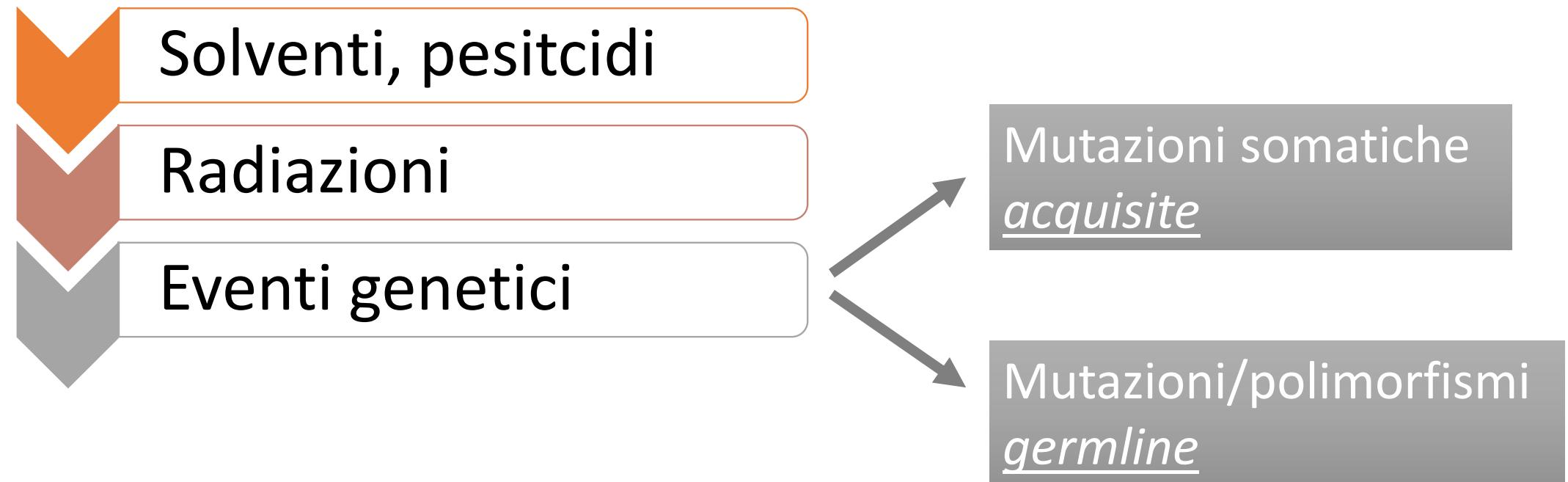
Barbui T et al, JCO 2011

Diagnosi di prePMF: perché è rilevante

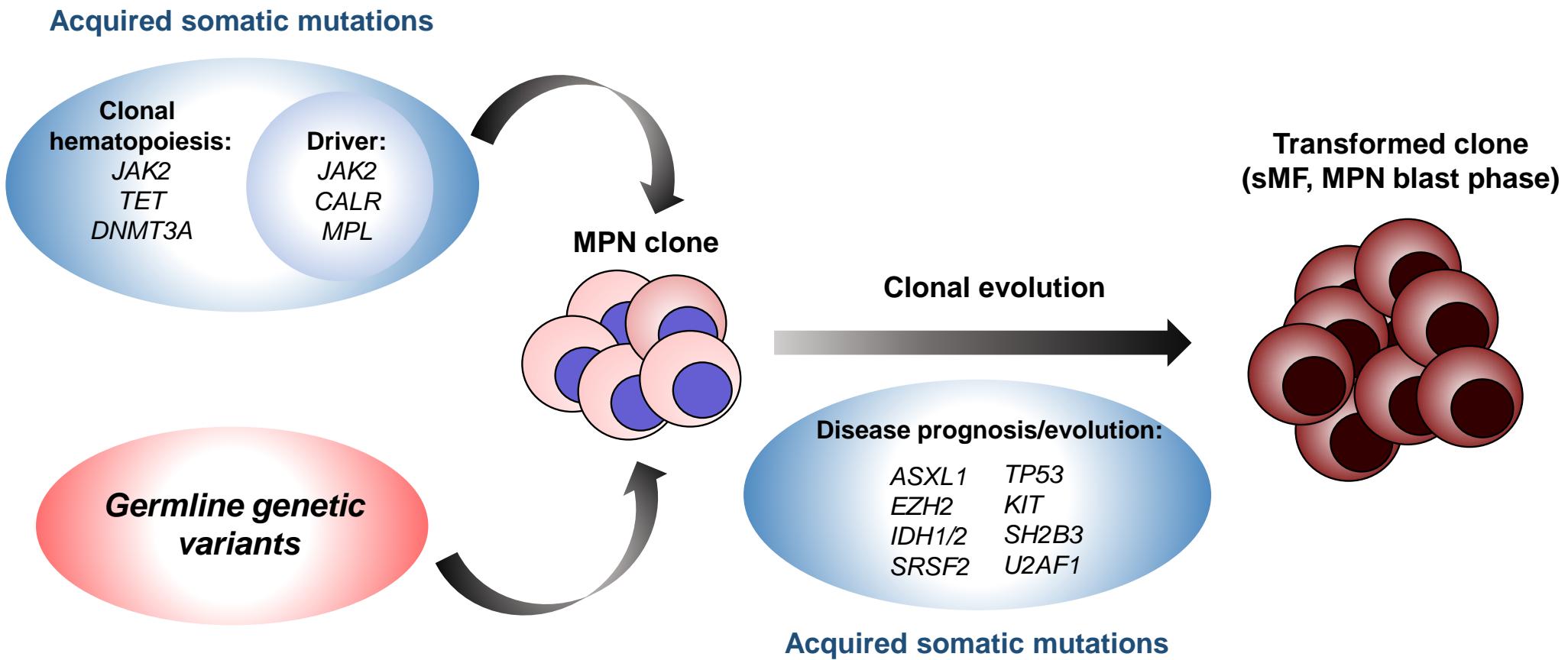


Barbui T et al, JCO 2011

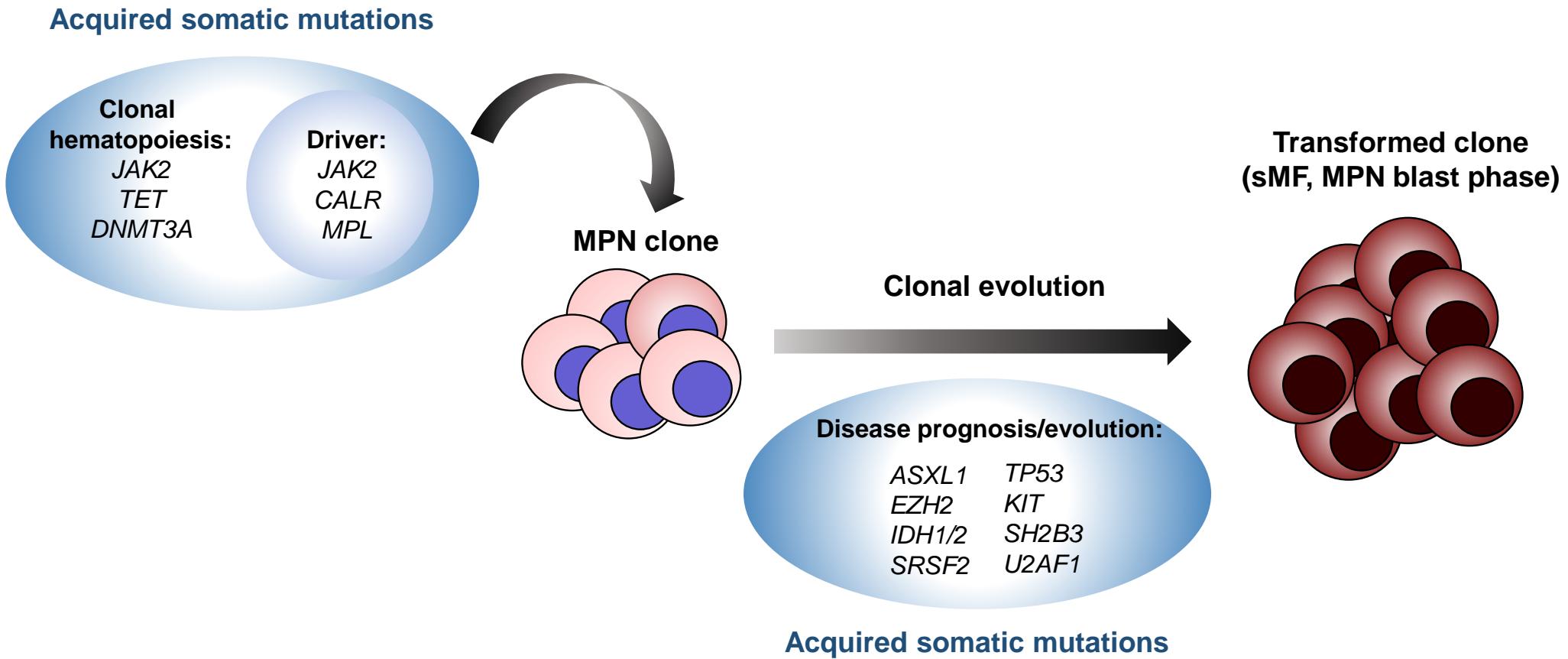
MPN - Eziologia



Basi genetiche delle MPN



Basi genetiche delle MPN: mutazioni somatiche



Mutazioni somatiche

Mutazioni «Driver»

Signaling
• JAK2V617F
• JAK2 exon 12
• MPLW515
• CALR type1 & type2

Mutazioni «Non-Driver»

Signaling	Epigenetics	mRNA splicing	Transcription
• CBL	• TET2	• SF3B1	• CUX1
• LNK	• ASXL1	• SRSF2	• RUNX1
• SOCS	• EZH2	• U2AF1	• P53
• CSF3R	• DNMT3A	• ZSRS2	• CEBPA
• SETBP1	• IDH1/2		
• KIT			
• NF1			

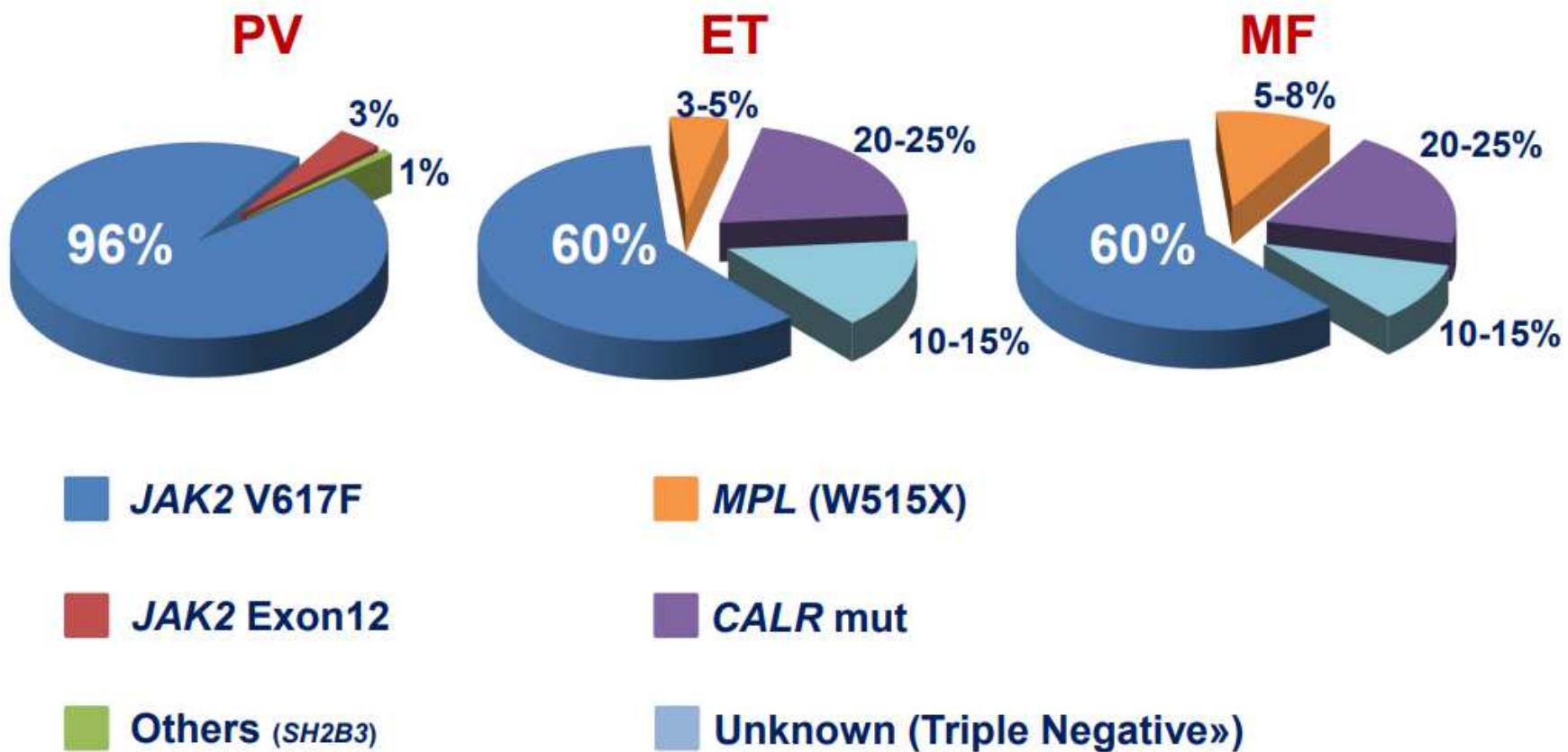
Mutazioni «DRIVER»

Signaling

- **JAK2V617F**
- **JAK2 exon 12**
- **MPLW515**
- **CALR type1 & type2**

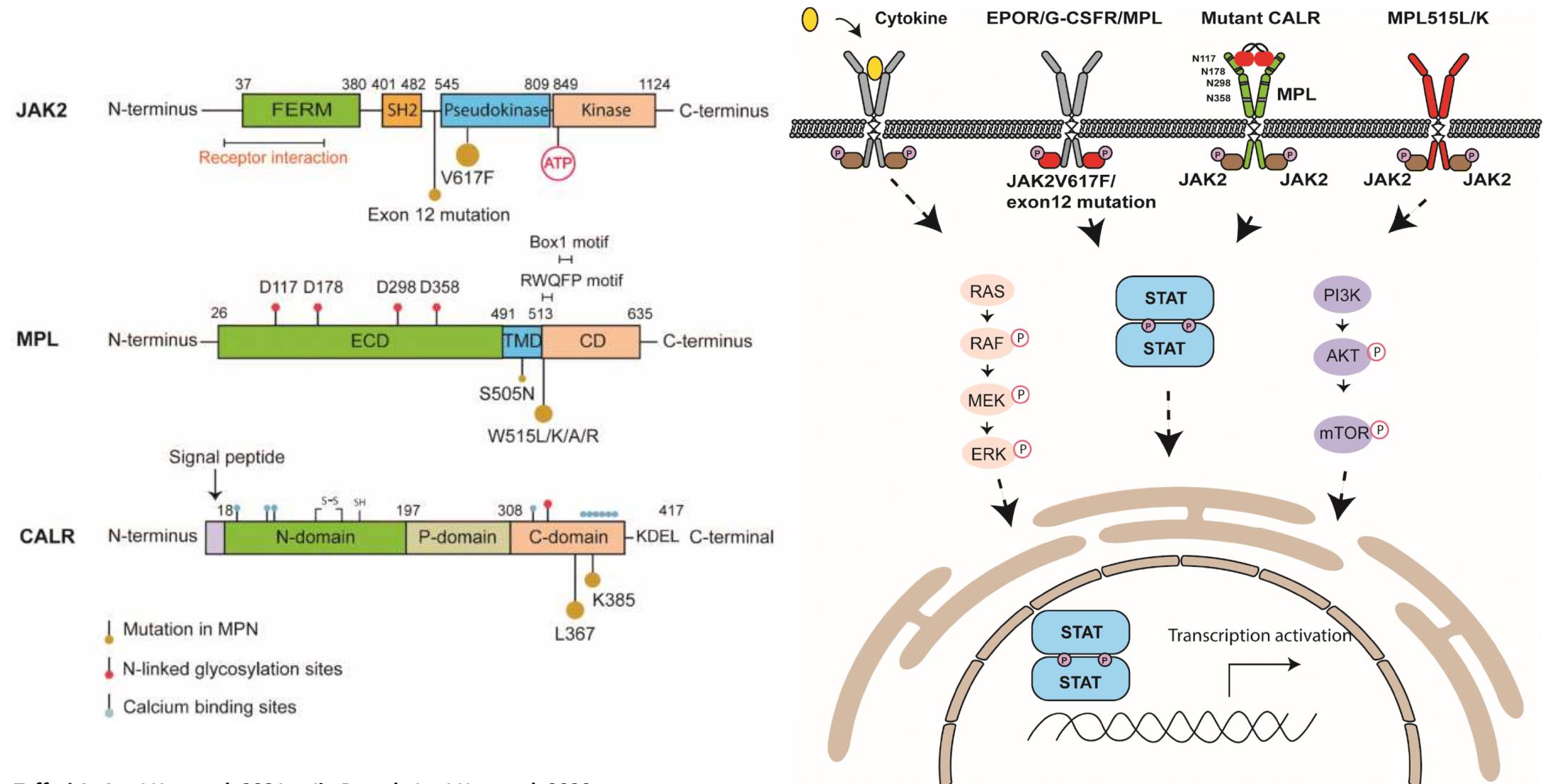
- Pannello mutazionale «base» in MPN sospetta/confermata
- Mutualmente esclusive
- JAK2V617F → CALR → MPL
- JAK2 ex12 solo PV
- Indagine molecolare tramite PCR

Mutazioni «DRIVER»: frequenza



Klampfl T, et al. *NEJM* 2013;369(25):2379-90; Nangalia J, et al. *NEJM* 2013;369(25):2391-405.

Mutazioni «DRIVER»: meccanismo d'azione



Mutazioni somatiche «NON-DRIVER»

Signaling	Epigeneti cs	mRNA splicing	Transcript
• CBL	• TET2	• SF3B1	• CUX1
• LNK	• ASXL1	• SRSF2	• RUNX1
• SOCS	• EZH2	• U2AF1	• P53
• CSF3R	• DNMT3A	• ZSRS2	• CEBPA
• SETBP1	• IDH1/2		
• KIT			
• NF1			

- Pannello mutazionale di II livello in MPN (confermata)
- Relativamente rare
- Indagine molecolare tramite Next Generation Sequencing
- Ruolo prognostico nella MF, no in PV ed ET

Mutazioni somatiche «NON-DRIVER»: frequenza

LNK (as in Links) a.k.a. SH2B3 (a membrane-bound adaptor protein) <i>MPN-associated mutations were monoallelic and involved exon 2</i>	12q24.12	PV ~ rare ET ~ rare PMF ~ rare BP-MPN ~ 10%	DNMT3A (DNA cytosine methyltransferase 3a) <i>Most frequent mutations affect amino acid R882</i>	2p23	PV ~7% PMF ~7% BP-MPN ~14%
TET2 (TET oncogene family member 2) <i>Mutations involve several exons</i>	4q24	PV ~16% ET ~ 5% PMF ~17% BP-MPN ~17%	CBL (Casitas B-lineage lymphoma proto-oncogene) <i>Exon 8/9 mutations</i>	11q23.3	PV ~ rare ET ~ rare MF ~6%
ASXL1 (Additional Sex Combs-Like 1) <i>Exon 12 mutations</i>	20q11.1	ET ~3% PMF ~13% BP-MPN ~18%	RAS (Rat sarcoma viral oncogene homolog) KRAS-Kirsten RAS; HRAS-Harvey RAS; <i>NRAS-Neuroblastoma RAS: Codons 12, 13 or 61</i>	12p12.1 11p15.5 1p13.2S	PV ~ rare ET ~ 1% MF ~5%
IDH1/IDH2 (Isocitrate dehydrogenase) <i>Exon 4 mutations</i>	2q33.3/ 15q26.1	PV ~2% ET ~ 1% PMF ~4% BP-MPN ~20%	IKZF1 (IKAROS family zinc finger 1) <i>Mostly deletions including intragenic</i>	7p12	CP-MPN ~ rare BP-MPN ~19%
EZH2 (enhancer of zeste homolog 2) <i>Mutations involve several exons</i>	7q36.1	PV ~3% PMF ~7% MDS ~6%	TP53 (tumor protein p53) <i>Exons 4 through 9</i>	17p13.1	PMF ~ 4% BP-MPN ~ 27%
			SF3B1 (splicing factor 3B subunit 1)	2q33.1	PMF ~ 7%
			SRSF2 (serine/arginine-rich splicing factor 2) <i>Exon 2</i>	17q25.1	PMF ~ 17%
			U2AF1(U2 Small Nuclear RNA Auxiliary Factor 1)	21q22.3	PMF ~ 16%

Mutations in MPN

What do they mean, how can we use them

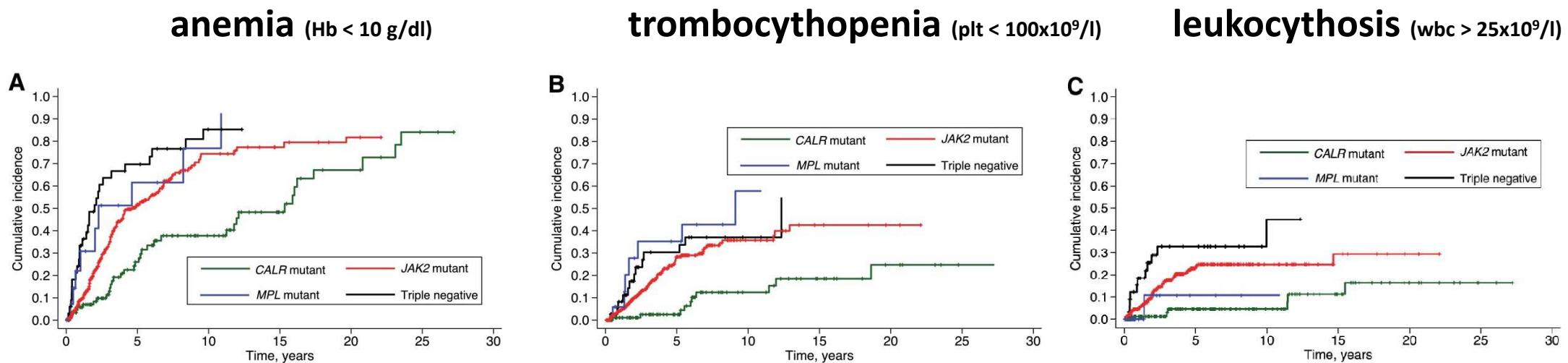


- Diagnosi → marker di CLONALITA'
- Valutazione RISCHIO TROMBOTICO
- PROGNOSI (mielofibrosi)

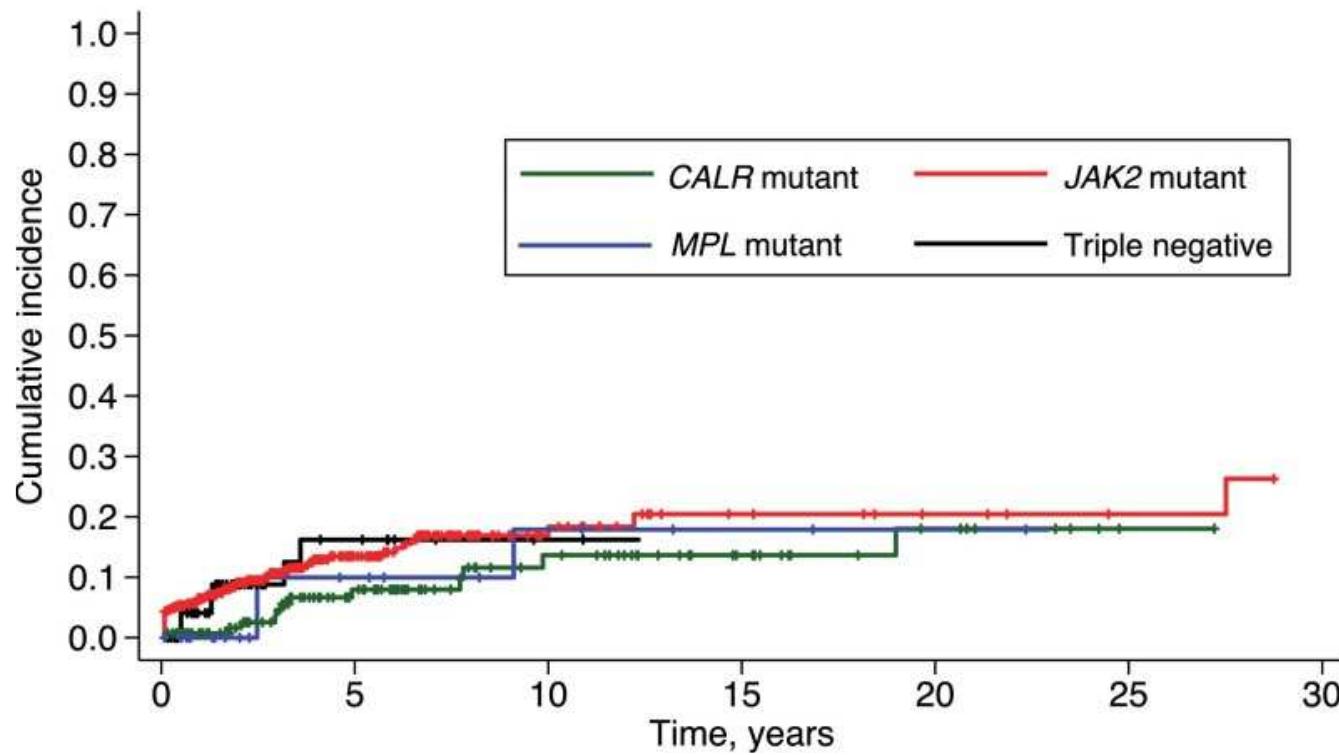


Prognosi: PMF CALR^{mut} ha un fenotipo meno severo

- n. 617 PMF

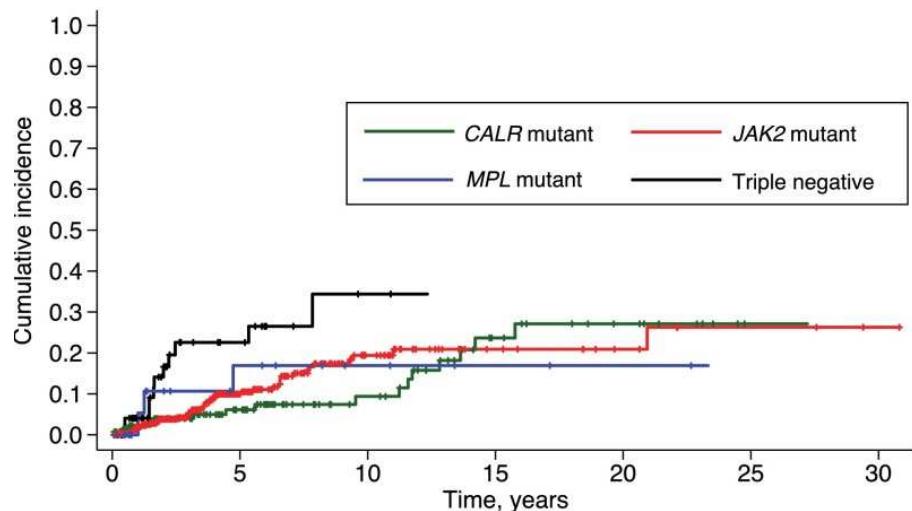


Trombosi: JAK2^{mut} PMF ha un rischio trombotico maggiore

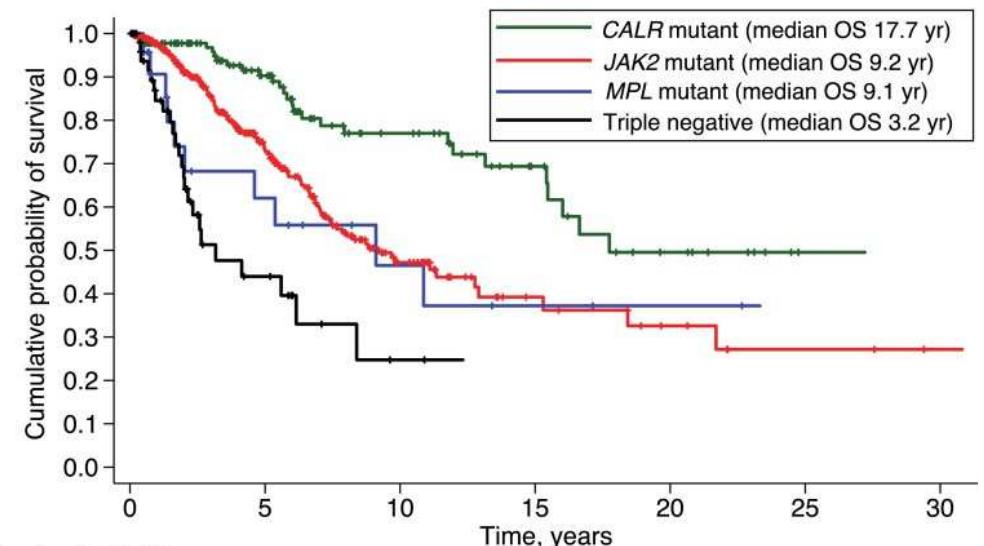


Prognosi: PMF triplo-negative ha la prognosi peggiore

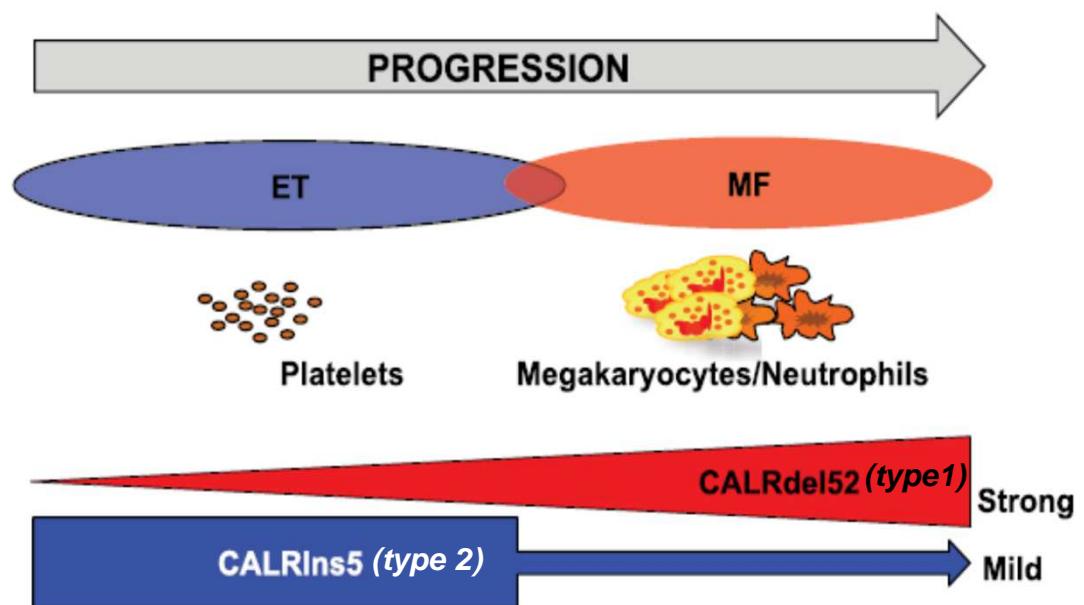
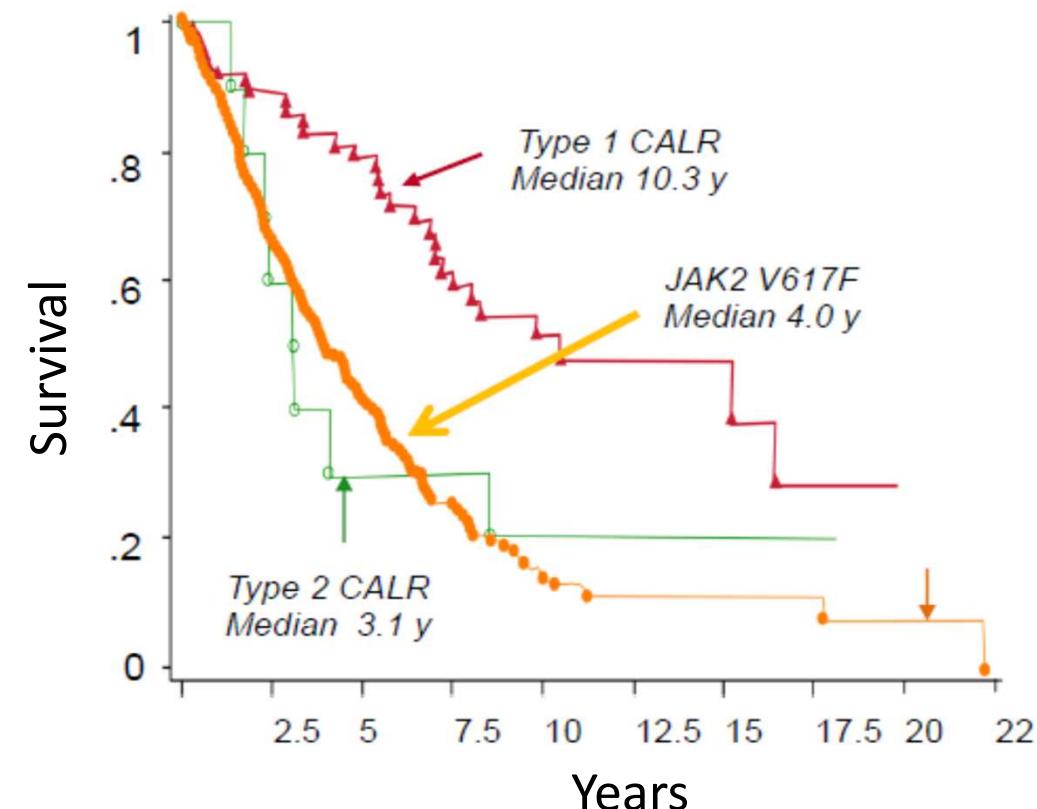
Leukemic transformation



Overall Survival



Prognosis: CALR type-1 vs. CALR type-2 PMF

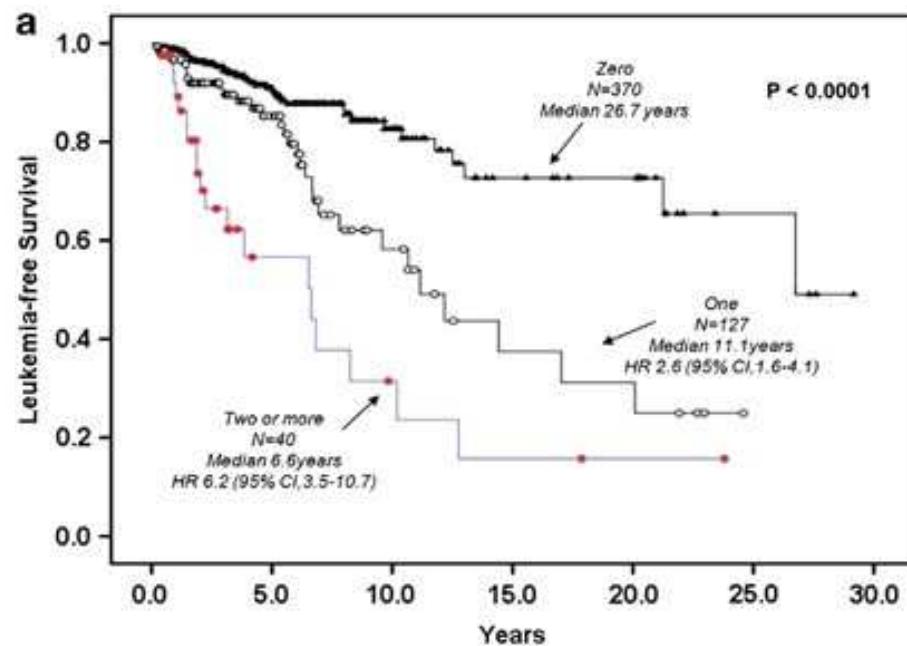
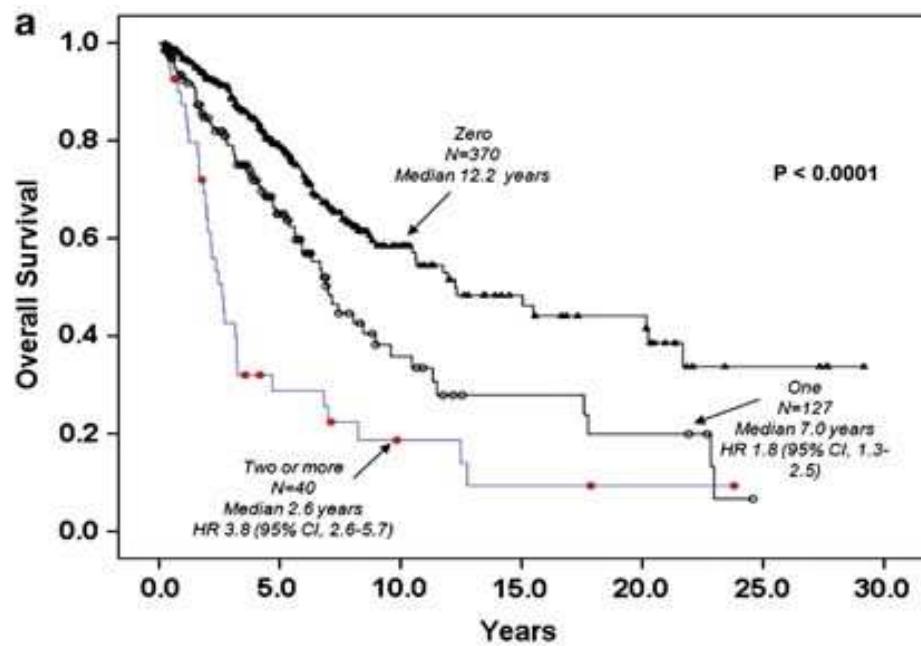


Impatto delle mutazioni ad alto rischio nella PMF

- n. 797 PMF

High molecular risk (HMR) mutations

- ASXL1
- EZH2
- IDH1/2
- SRSF2



Stratificazione del rischio: score prognostici «clinici»

Variable	IPSS	DIPSS	DIPSS-plus
Age >65 y	✓	✓	If DIPSS:
Constitutional symptoms	✓	✓	Low= 0
Hemoglobin <10 g/dL	✓	✓	Int-1= 1
Leukocyte count >25x10 ⁹ /L	✓	✓	Int-2=2
Circulating blasts <u>></u> 1%	✓	✓	High= 3
Platelet count <100x10 ⁹ /L			✓
RBC transfusion need			✓
Unfavorable karyotype +8,-7/7q-,i(17q),inv(3), -5/5q-,12p-, 11q23 rearr.			✓

Cervantes F et al, Blood.2009

Passamonti F, et al. Blood. 2010

Gangat N, et al. J Clin Oncol.201

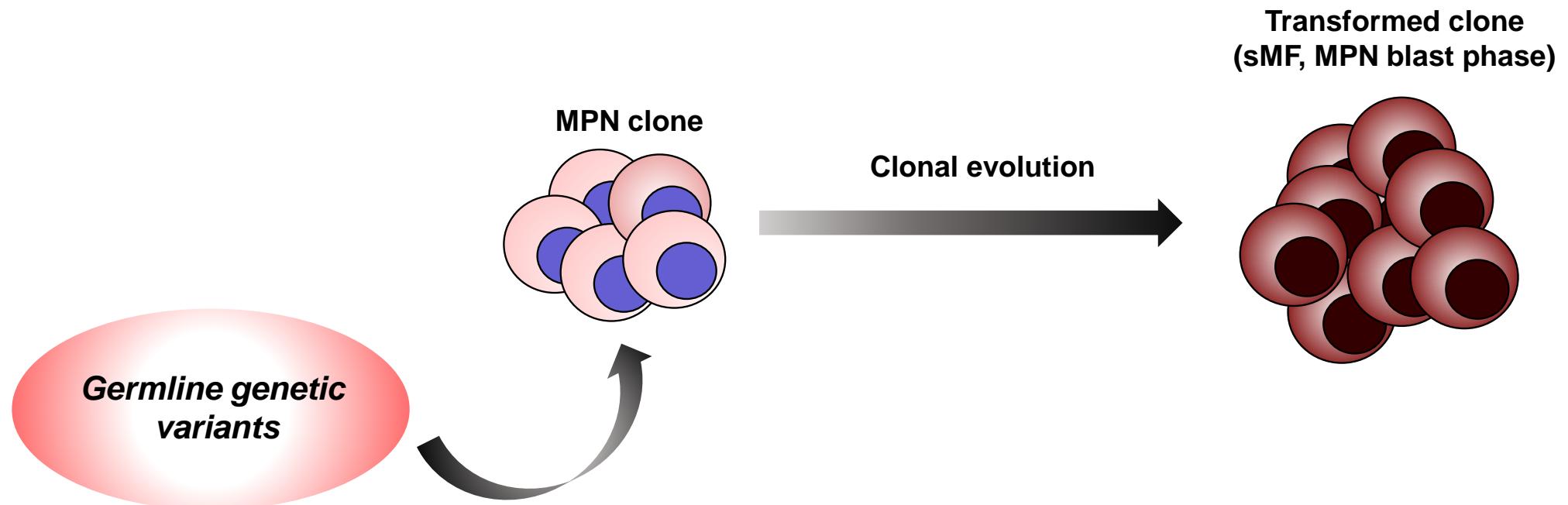
Stratificazione del rischio: score prognostici «molecolari»

MIPSS70 (3-tiered)		MIPSS70+ version 2.0 (5-tiered)		GIPSS (4-tiered)
Genetic variables	Clinical variables	Genetic variables	Clinical variables	Genetic variables
One HMR mutation (1 point)	Hemoglobin < 10 g/dL (1 point)	VHR karyotype (4 points)	Severe anemia (2 points)	VHR karyotype (2 points)
≥ 2 HMR mutations (2 points)	Leukocytes $> 25 \times 10^9/L$ (2 points)	Unfavorable karyotype (3 points)	Moderate anemia (1 point)	Unfavorable karyotype (1 point)
Type 1/like CALR absent (1 point)	Platelets $< 100 \times 10^9/L$ (2 points)	≥ 2 HMR mutations (3 points)	Circulating blasts $\geq 2\%$ (1 point)	Type 1/like CALR absent (1 point)
	Circulating blasts $\geq 2\%$ (1 point)	One HMR mutation (2 points)	Constitutional symptoms (2 points)	ASXL1mutation (1 point)
	Constitutional symptoms (1 point)	Type 1/like CALR absent (2 points)		SRSF2 mutation (1 point)
	Bone marrow fibrosis grade ≥ 2 (1 point)			U2AF1Q157 mutation (1 point)
Very low risk (median survival)		Zero points (not reached)		
Low risk (median survival)	0-1 points (not reached)	1-2 points (16.4 y)		Zero points (26.4 y)
Intermediate-1 risk (median survival)				One point (8 y)
Intermediate risk (median survival)	2-4 points (6.3 y)	3-4 points (7.7 y)		
Intermediate-2 risk (median survival)				Two points (4.2 y)
High risk (median survival)	≥ 5 points (3.1 y)	5-8 points (4.1 y)		≥ 3 points (2 y)
Very high risk (median survival)		≥ 9 points (1.8 y)		

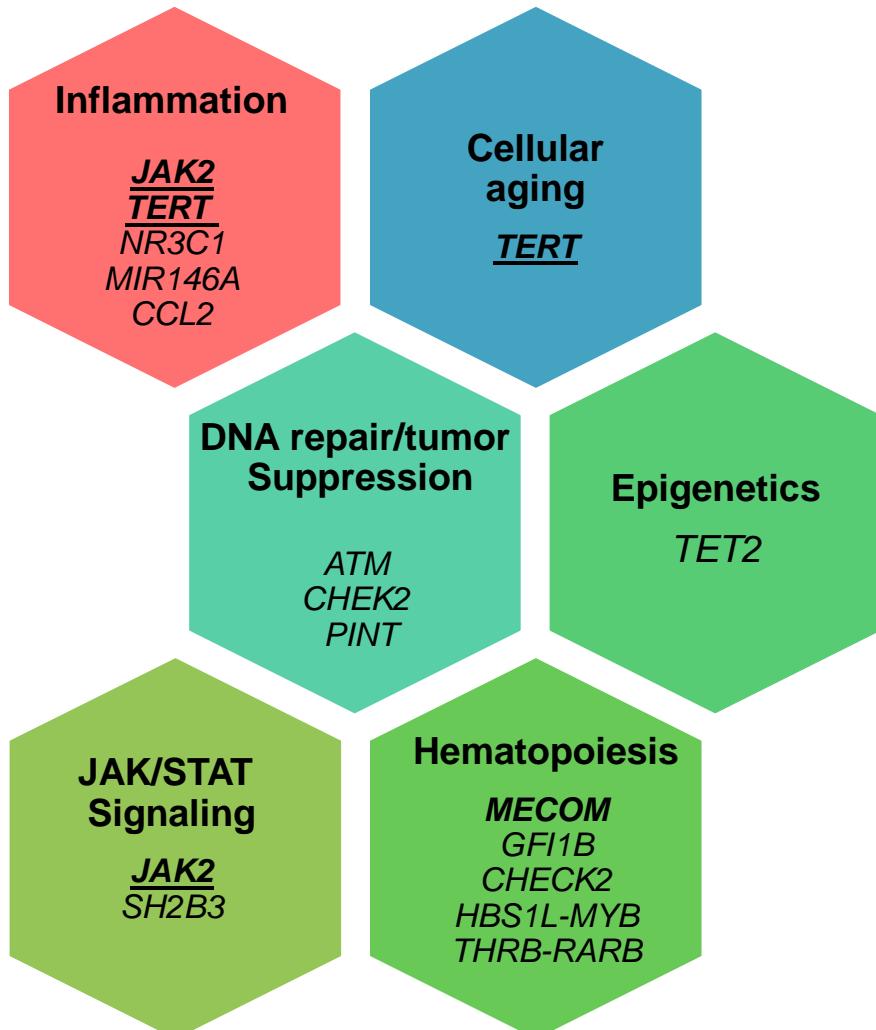
Note: Severe anemia, Hemoglobin <8 g/dL in women and < 9 g/dL in men; Moderate anemia, Hemoglobin 8-9.9 in women and 9-10.9 in men.

Abbreviations: GIPSS, genetically-inspired prognostic scoring system. Survival quotes are for all age groups; HMR, high molecular risk mutations include ASXL1, SRSF2, EZH2, IDH1, IDH2 and, in addition, for GIPSS and MIPSS70+ version 2.0, U2AF1Q157; MIPSS70, mutation-enhanced international prognostic system for transplant-age patients (age ≤ 70 years); MIPSS70+ version 2.0, mutation and karyotype enhanced international prognostic system. Survival quotes are for age ≤ 70 years; VHR, very high risk karyotype.

Basi genetiche delle MPN: varianti «germline»

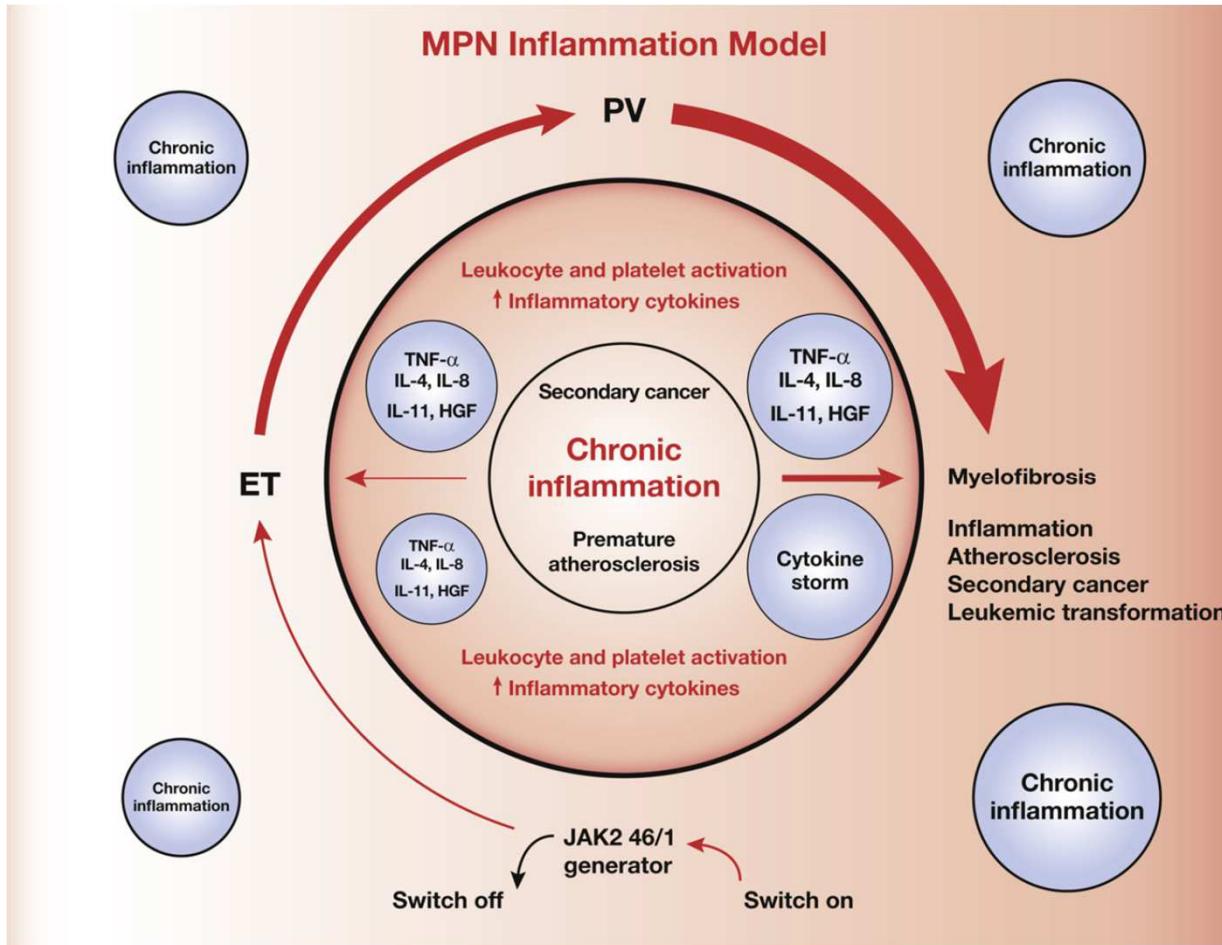


Impatto biologico delle varianti *germline* associate ad un aumentata suscettibilità alle MPN



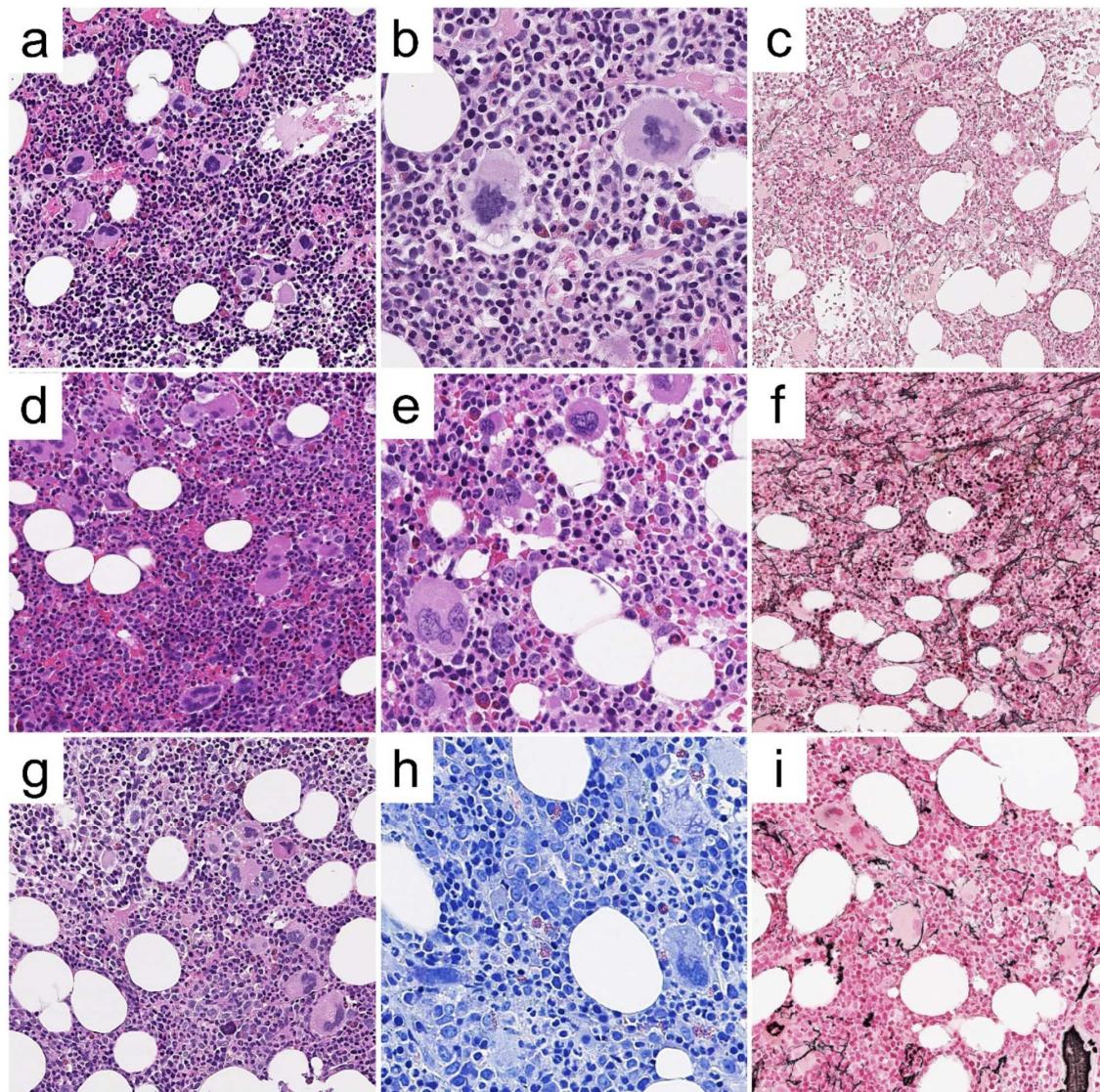
- Aumentato rischio di sviluppare una MPN (incluse forme familiari)
- Modulano il fenotipo
- Influenzano la risposta alla terapia

Ruolo dell'infiammazione: MPN come disordini «oncoinfiammatori»

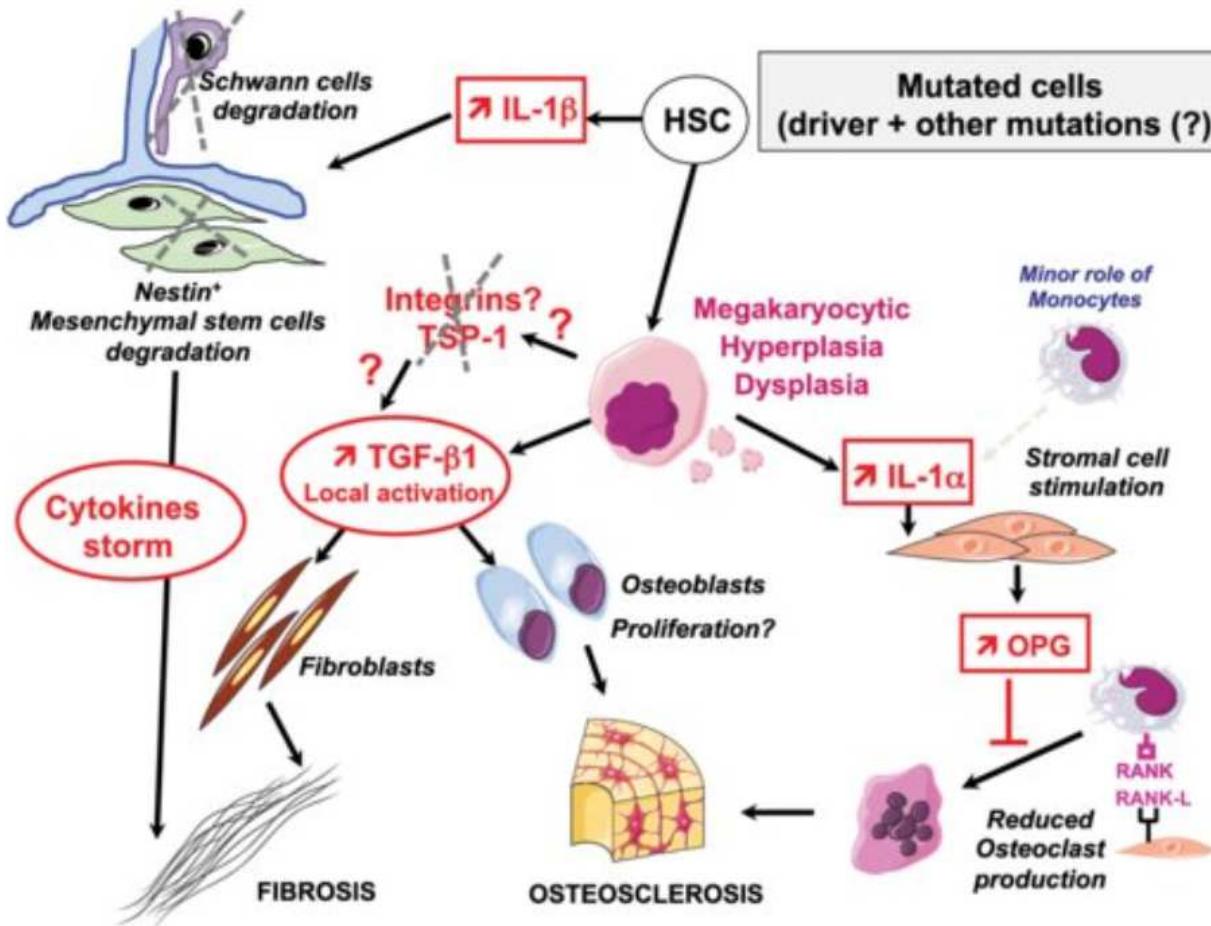


Ruolo del clone megacariocitario nelle MPN

MPNs are characterized by **MK hyperplasia**, which is associated with an **aberrant inflammatory and metabolic signature**

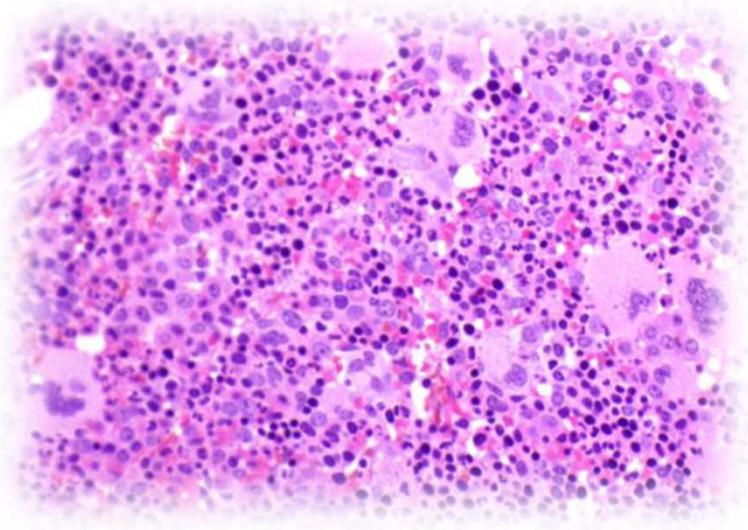


Ruolo del clone megacariocitario nelle MPN



Vancheinker W. et al. F1000, 2016

MPN



- ✓ Rare neoplasie del sangue
- ✓ Mutazioni geniche somatiche e varianti germline
- ✓ Ruolo dell'infiammazione cronica e del clone megacarocitario
- ✓ Mutazioni geniche somatiche usate per la diagnosi, la definizione del rischio trombotico e la prognosi