"Diagnostiquer les néoplasies myéloprolifératives: quels tests de laboratoire ?"

Dr Frédéric Lambert
Laboratoire de biologie moléculaire hémato-oncologiques (BMH)
Service de Génétique Humaine / UniLab Lg
CHU de LIEGE

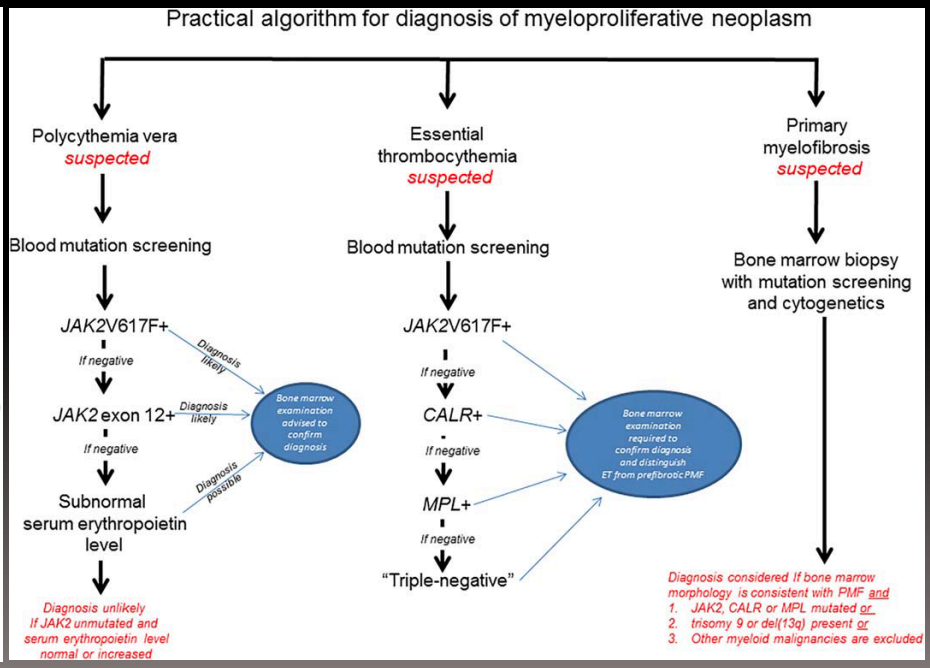
Mots d'ordre….

Be clear, clear, clear !
Keep it simple, simple, simple (with all respect for our patients) !

Pr Timothy Devos, KUL

Le diagnostic des néoplasies myéloprolifératives (NMP) ?

Une démarche logique, rationnelle, basée sur la collecte séquentielle d'éléments objectifs (preuves)…



Tefferi, Barbui, Am J Hematol 2017; 92: 95–108

Programme

I. Les “néoplasies myéloprolifératives“, qu’est-ce que c’est ?
II. La démarche diagnostique (OMS, PVSG,…);
III. Les outils du diagnostic;
IV. Quels buts pour ces examens (diagnostic; choix du traitement, suivi de la maladie…)
V. Conclusions.

Les néoplasies myéloprolifératives (NMP)

Pour comprendre la démarche diagnostique, il faut connaître:

- *les mécanismes normaux de la formation des cellules sanguines et*
- *les anomalies qui conduisent au développement des maladies…*

Plan

- Les "néoplasies myéloprolifératives (NMP)", qu'est-ce que c'est ?
- La démarche diagnostique (OMS, PVSG,…)
- Les outils du diagnostic:
  - La « prise de sang »
  - La ponction de moelle osseuse hématopoïétique
  - La biopsie de moelle osseuse hématopoïétique
  - Les examens des chromosomes
  - Les examens de gènes
  - Les tests biochimiques (dosage d'érythropoïétine)
- Quels buts pour ces examens (diagnostic; choix du traitement, suivi de la maladie…)
- Conclusions

NMP: un groupe hétérogène de maladies

WHO myeloid neoplasm and acute leukemia classification 2016

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)

NMP classiques, « Phi-négatives »

- Chronic eosinophilic leukemia, not otherwise specified (NOS)
- MPN, unclassifiable

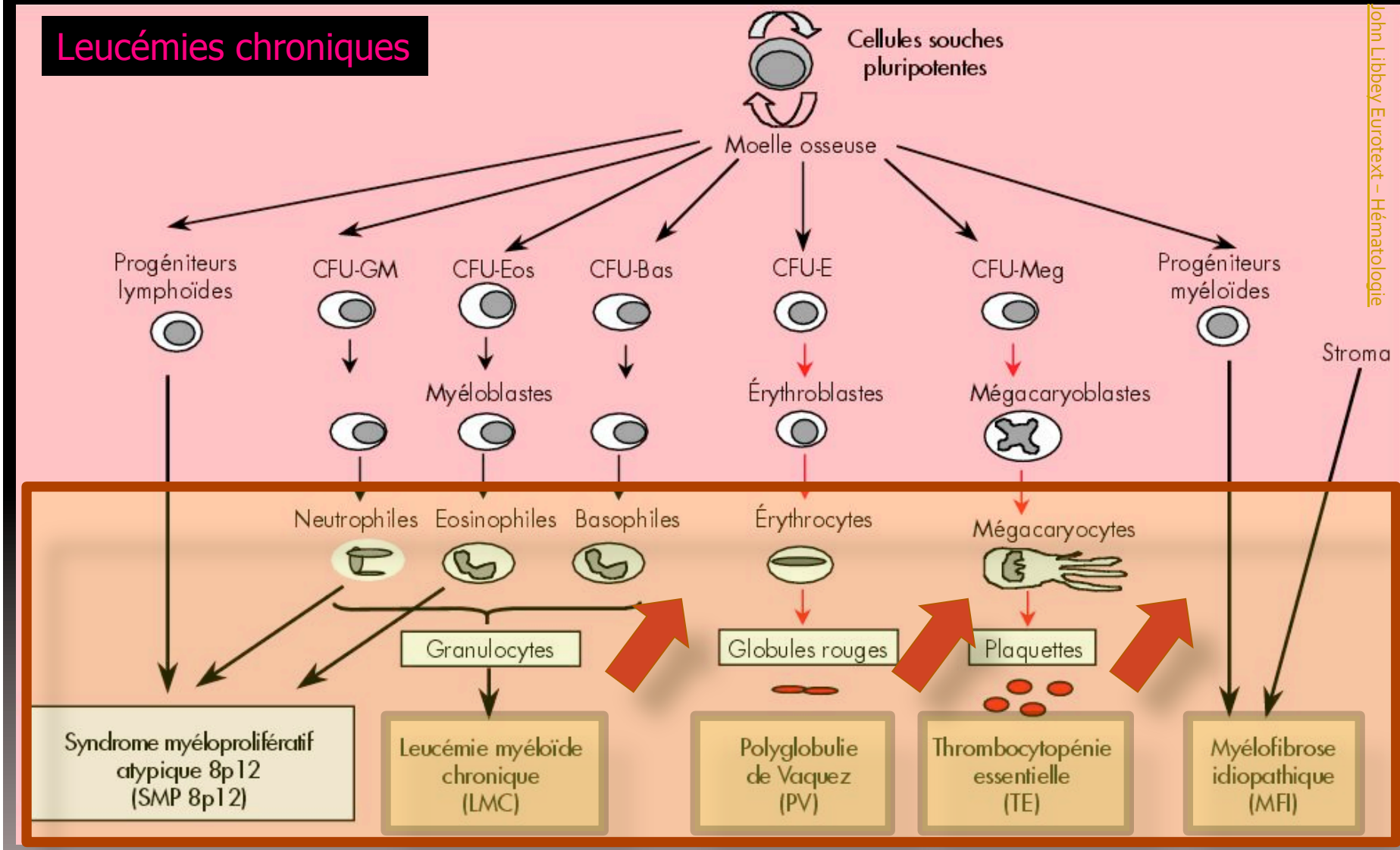
Mastocytosis

Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*, or with *PCM1-JAK2*

- Myeloid/lymphoid neoplasms with *PDGFRA* rearrangement
- Myeloid/lymphoid neoplasms with *PDGFRB* rearrangement
- Myeloid/lymphoid neoplasms with *FGFR1* rearrangement
- *Provisional entity: Myeloid/lymphoid neoplasms with PCM1-JAK2*

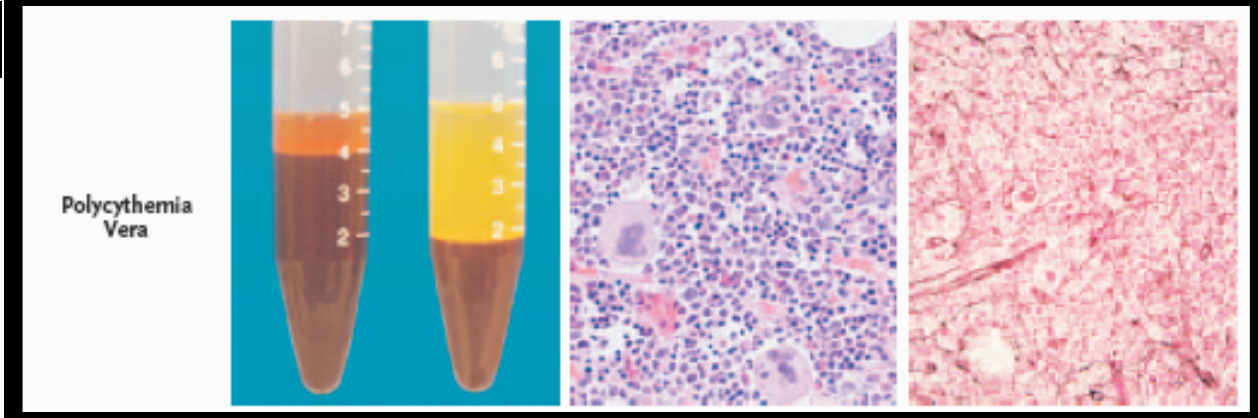
Arber et al, BLOOD, 19 MAY 2016

Partageant des caractéristiques communes: accumulation de cellules sanguines « matures »

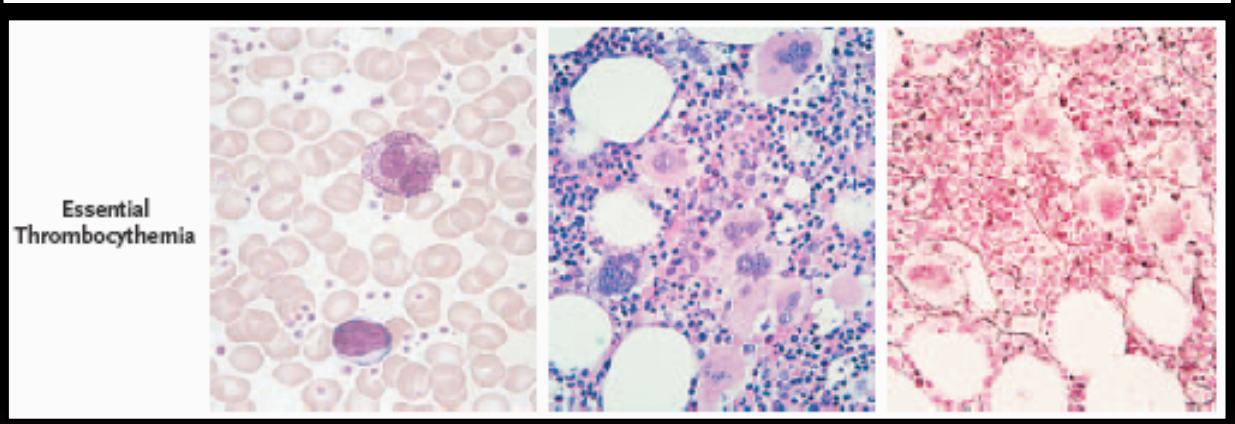


Adapted from John Libbey Eurotext – Hématologie

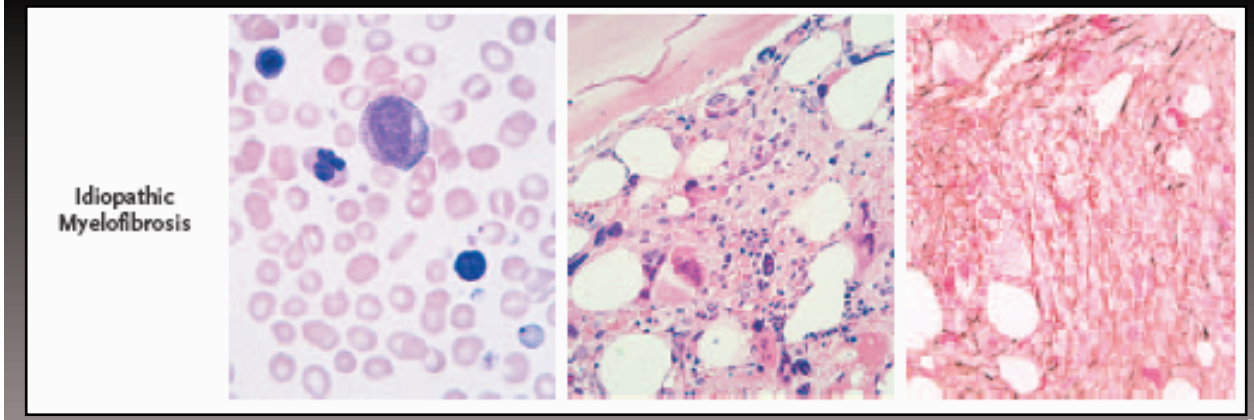
NMP classiques, « Philadelphia-négatives »:



Maladie de Vaquez



Thrombocythémie essentielle

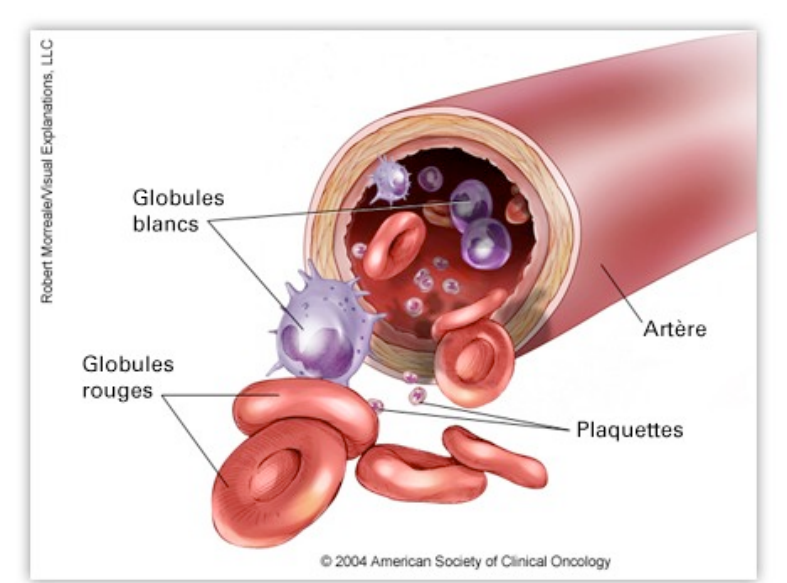


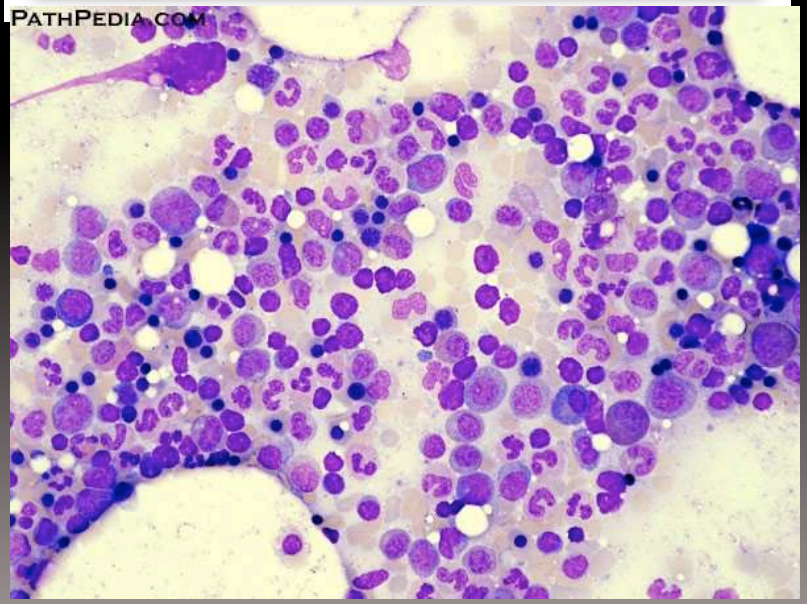
Myélofibrose primitive

Campbell, N Engl J Med 355;23 december 7, 2006

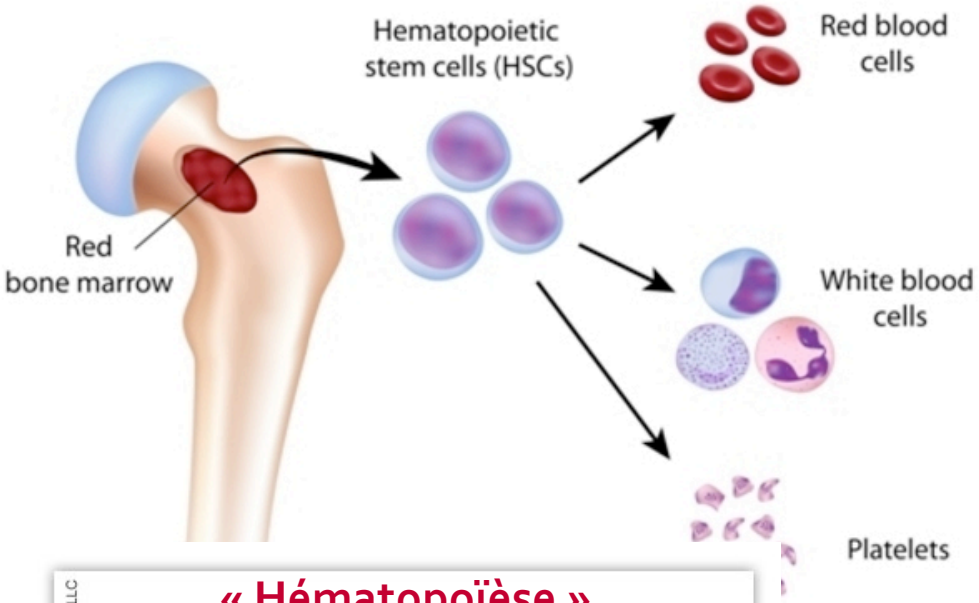
Formation des cellules sanguines:

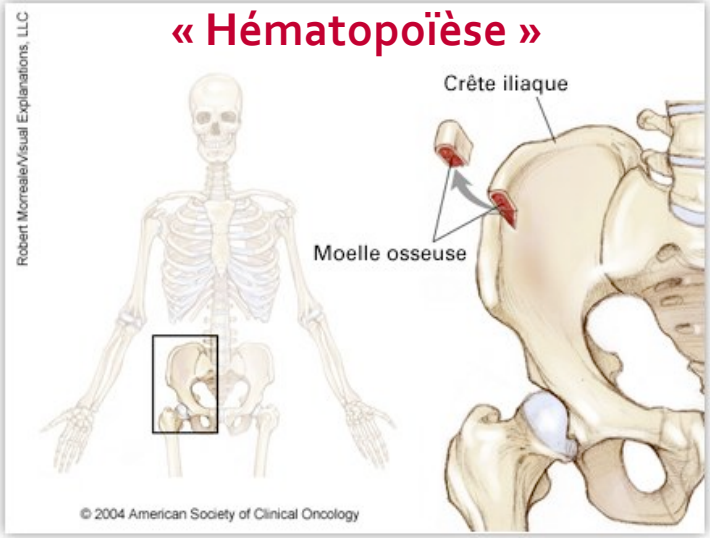
La moelle osseuse hématopoïétique





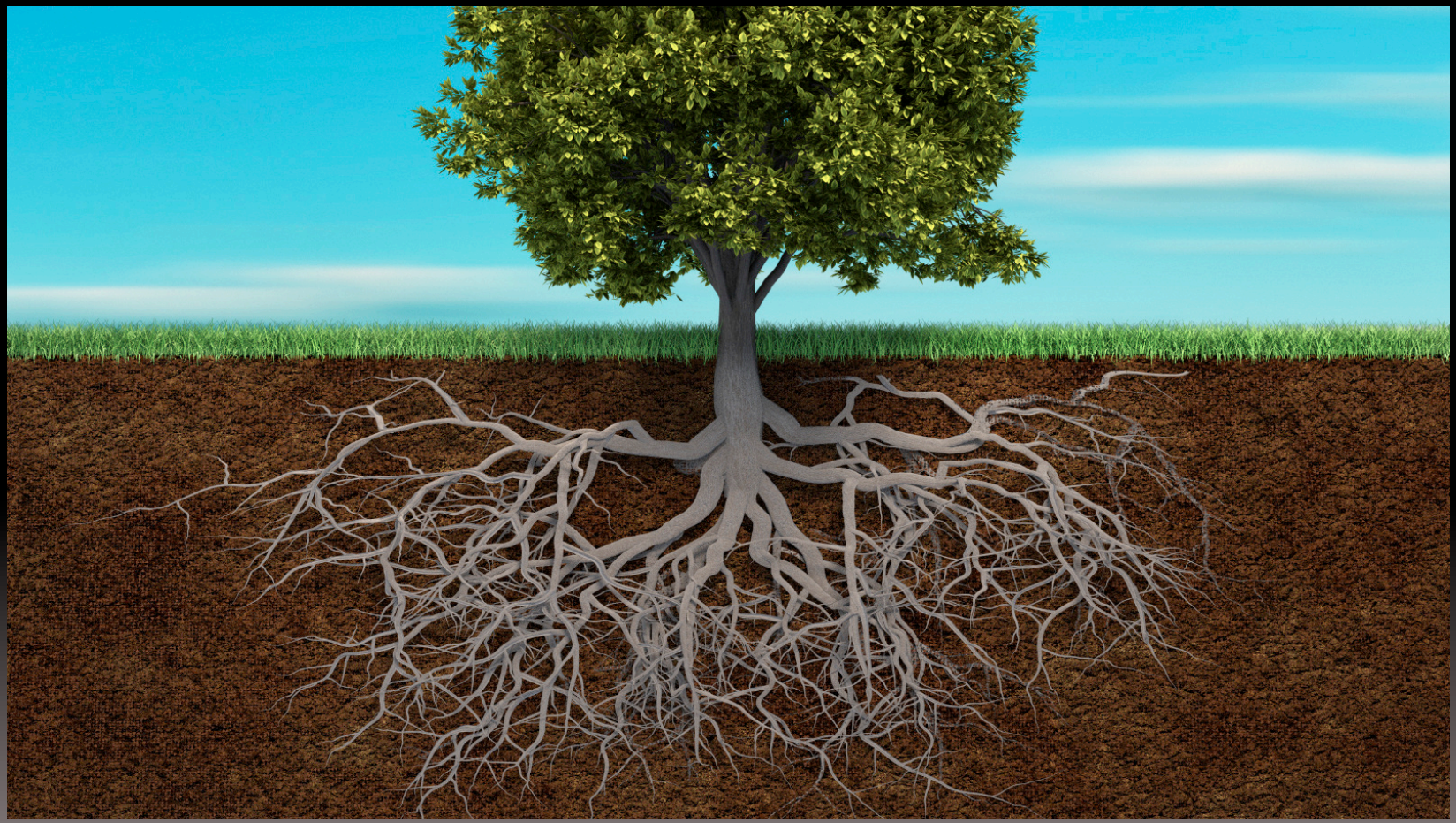
conversantbio.com





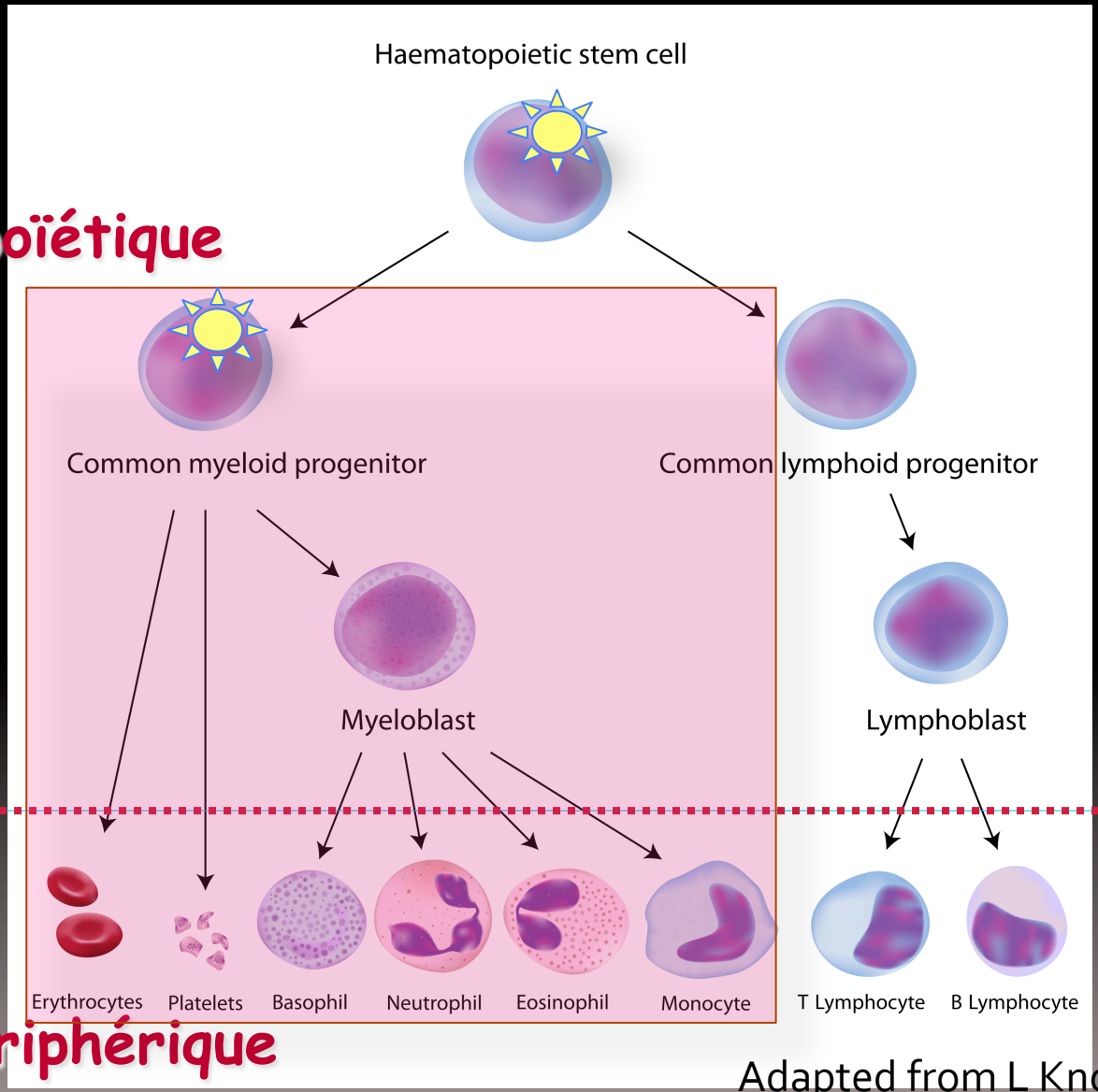
Formation des cellules sanguines:

L'hématopoïèse et la moelle osseuse hématopoïétique



Formation des cellules sanguines: L'hématopoïèse

Moëlle hématopoïétique



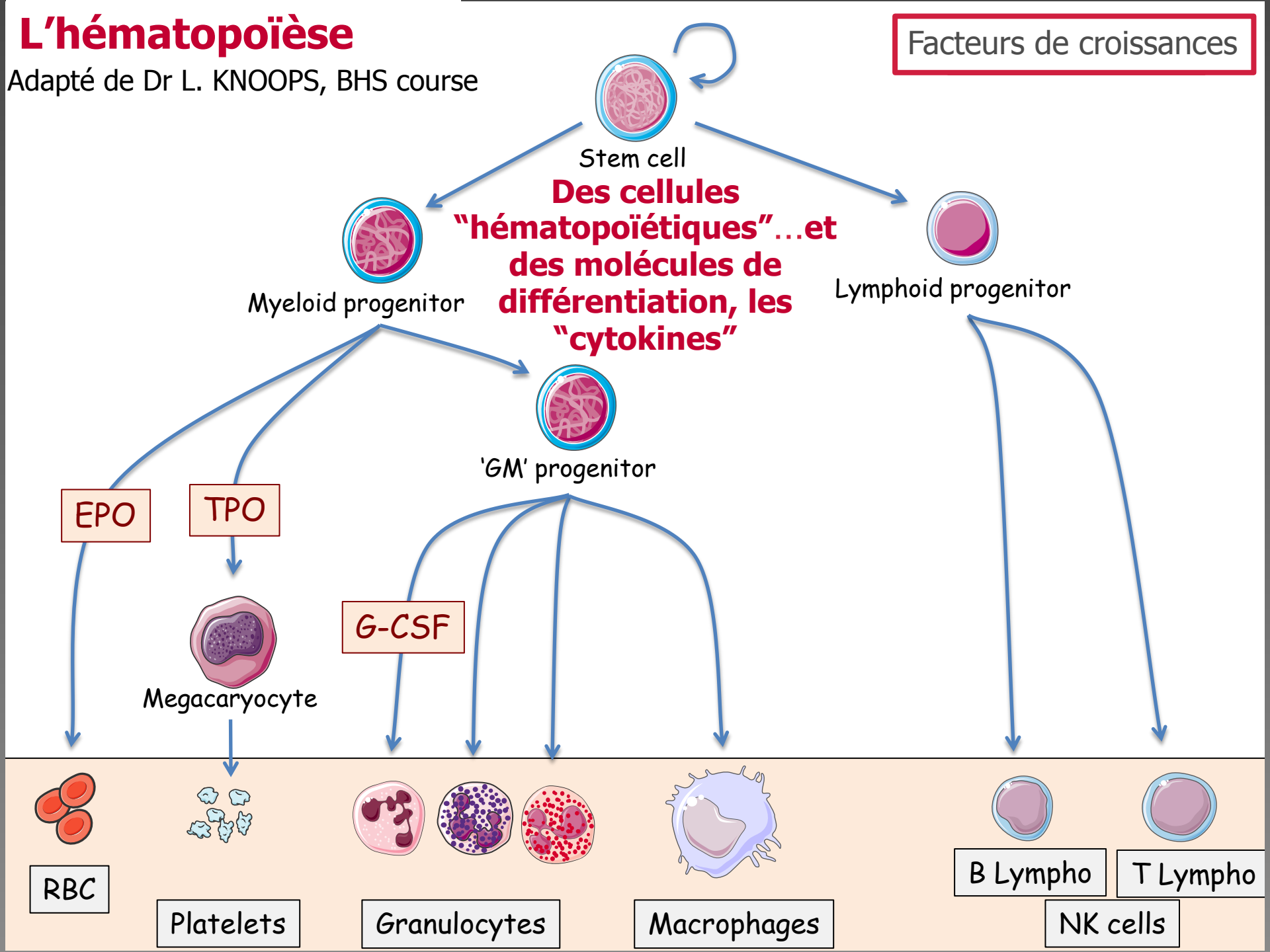
Sang périphérique

Adapted from L Knoops, BHS course

L'hématopoïèse

Adapté de Dr L. KNOOPS, BHS course

Facteurs de croissances



L'hématopoïèse: des cellules "hématopoïétiques"…et des molécules de différentiation, les "cytokines"

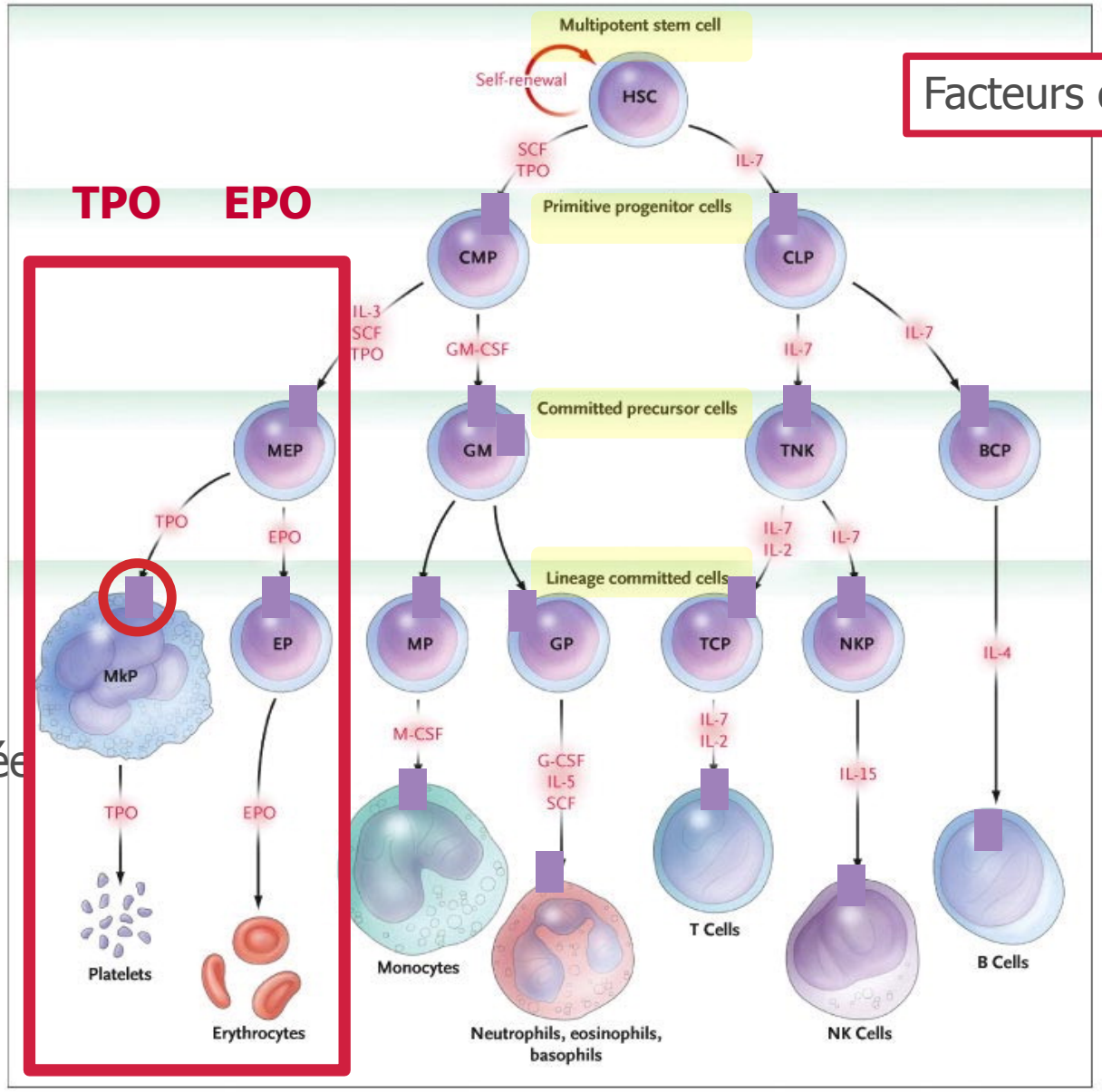
Cell. souches. Multipotentes

Cell. progénitrices primitives

Précurseurs engagés

Cellules engagées dans une lignée

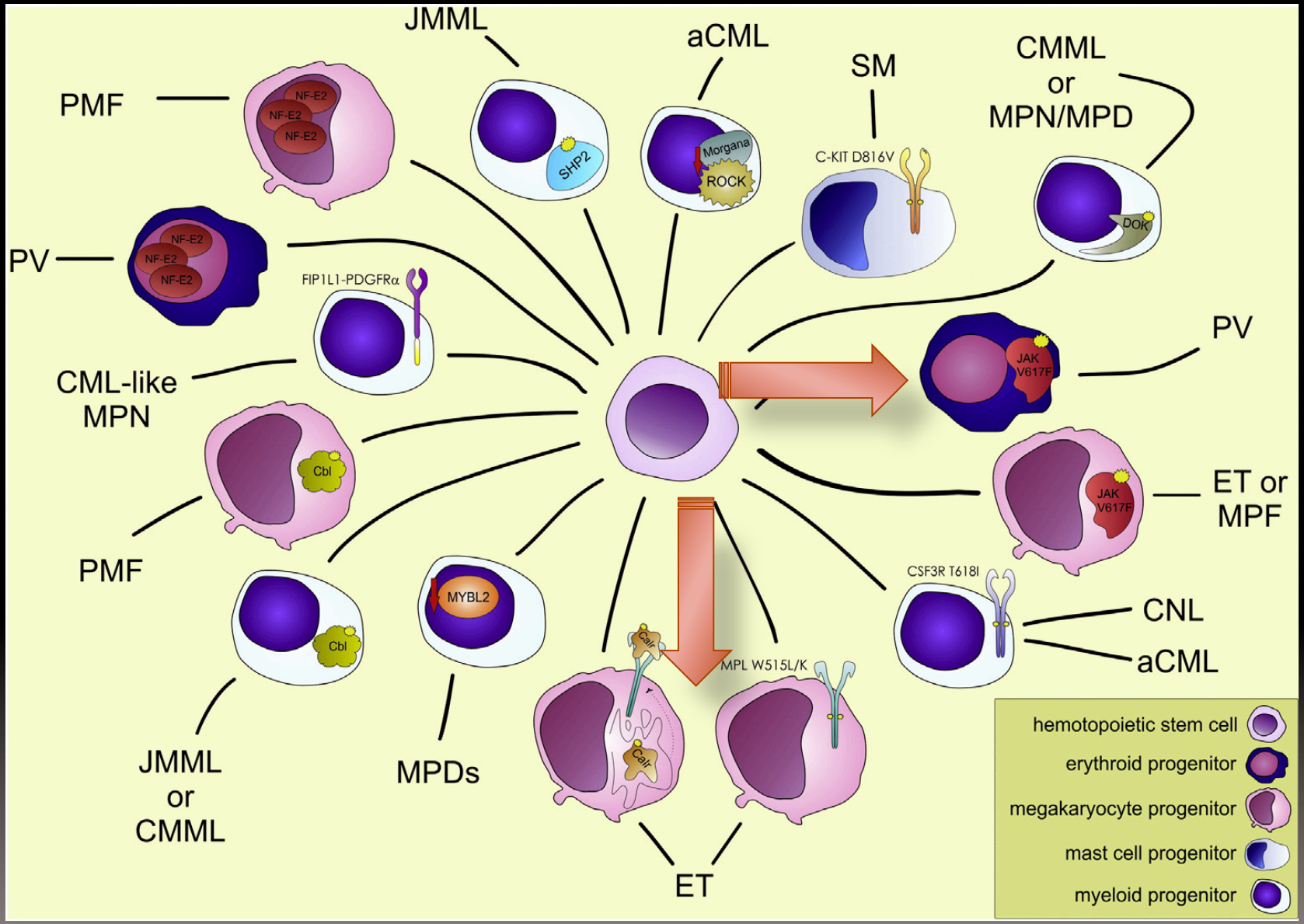
Cellules sanguines matures



Facteurs de croissances

Kaushansky K. N Engl J Med 2006;354:2034-2045.

Origines de la production « ANORMALE » des cellules sanguines:



Adapted from Morotti A, et al., Blood Rev (2016), http://dx.doi.org/10.1016/j.blre.2016.11.004

Anomalies de la production des cellules sanguines: Les néoplasies myéloprolifératives

Plan

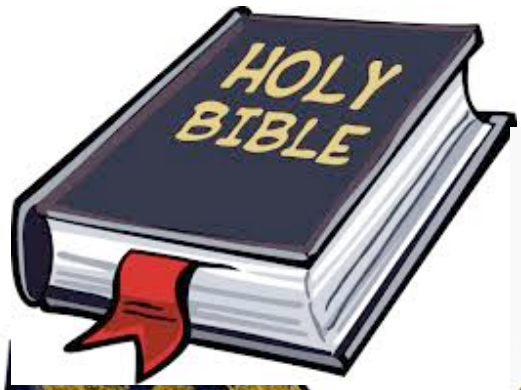
- Les "syndromes myéloprolifératifs", qu'est-ce que c'est ?
- La démarche diagnostique (OMS, PVSG,…)
- Les outils du diagnostic:
  - L'examen clinique et l' "interrogatoire " (anamnèse)
  - La « prise de sang »
  - La ponction de moelle osseuse hématopoïétique
  - La biopsie de moelle osseuse hématopoïétique
  - Les tests biochimiques (dosage d'érythropoïétine)
  - La cytométrie en flux
  - Les examens des chromosomes
  - Les examens de gènes
- Quels buts pour ces examens (diagnostic; choix du traitement; suivi de la maladie…)
- Conclusions

2016

The ~~2008~~ revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes

James W. Vardiman,[1] Jüergen Thiele,[2] Daniel A. Arber,[3] Richard D. Brunning,[4] Michael J. Borowitz,[5] Anna Porwit,[6] Nancy Lee Harris,[7] Michelle M. Le Beau,[8] Eva Hellström-Lindberg,[9] Ayalew Tefferi,[10] and Clara D. Bloomfield[11]

2009 114: 937-951
Prepublished online Apr 8, 2009;
doi:10.1182/blood-2009-03-209262



Review Series

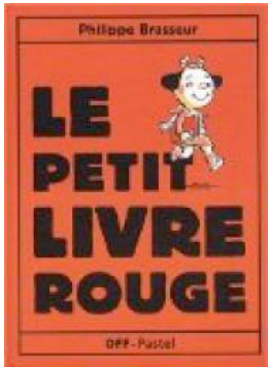
THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

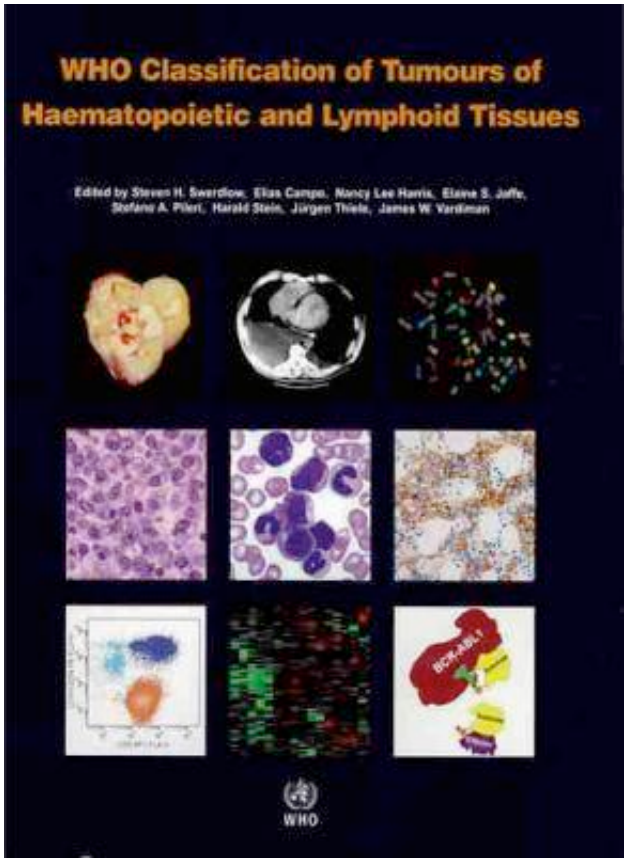
The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia

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. Vardiman[9]

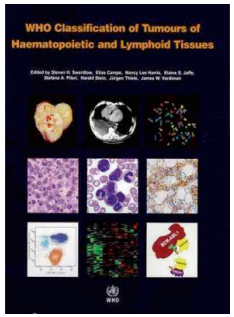
[1]Department of Pathology, Stanford University, Stanford, CA; [2]Department of Pathology, Weill Cornell Medical College, New York, NY; [3]Department of Pathology, Massachusetts General Hospital, Boston, MA; [4]Institute of Pathology, University of Cologne, Cologne, Germany; [5]Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD; [6]Section of Hematology/Oncology, University of Chicago, Chicago, IL; [7]Comprehensive Cancer Center, James Cancer Hospital and Solove Research Institute, The Ohio State University, Columbus, OH; [8]Department of Molecular Medicine, University of Pavia, and Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; and [9]Department of Pathology, University of Chicago, Chicago, IL

The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues was last updated in 2008. Since then, there have been numerous advances in the identification of unique biomarkers associated with some myeloid neoplasms and acute leukemias, largely derived from gene expression analysis and next-generation sequencing that can significantly improve the diagnostic criteria as well as the prognostic relevance of entities currently included in the WHO classification and that also suggest new entities that should be added. Therefore, there is a clear need for a revision to the current classification. The revisions to the categories of myeloid neoplasms and acute leukemia will be published in a monograph in 2016 and reflect a consensus of opinion of hematopathologists, hematologists, oncologists, and geneticists. The 2016 edition represents a revision of the prior classification rather than an entirely new classification and attempts to incorporate new clinical, prognostic, morphologic, immunophenotypic, and genetic data that have emerged since the last edition. The major changes in the classification and their rationale are presented here. (*Blood*. 2016; 127(20):2391-2405)





Practical algorithm for diagnosis of myeloproliferative neoplasm



Polycythemia vera *suspected*

Blood mutation screening

*JAK2*V617F+

If negative

JAK2 exon 12+

If negative

Subnormal serum erythropoietin level

Diagnosis unlikely
If JAK2 unmutated and serum erythropoietin level normal or increased

Diagnosis likely (*JAK2*V617F+)

Diagnosis likely (*JAK2* exon 12+)

Diagnosis possible (Subnormal serum erythropoietin level)

Bone marrow examination advised to confirm diagnosis

Essential thrombocythemia *suspected*

Blood mutation screening

*JAK2*V617F+

If negative

CALR+

If negative

MPL+

If negative

"Triple-negative"

Bone marrow examination required to confirm diagnosis and distinguish ET from prefibrotic PMF

Primary myelofibrosis *suspected*

Bone marrow biopsy with mutation screening and cytogenetics

Diagnosis considered If bone marrow morphology is consistent with PMF and
1. *JAK2, CALR or MPL mutated or*
2. *trisomy 9 or del(13q) present or*
3. *Other myeloid malignancies are excluded*

Tefferi, Barbui, Am J Hematol 2017; 92: 95–108

La démarche diagnostique des NMP:
La collecte minutieuse d'éléments objectifs (preuves)

La démarche diagnostique des NMP:

Un travail collaboratif

University of Michigan Comprehensive Cancer Center

Le recours à de nouvelles technologies

2016 Revised WHO Diagnostic Criteria for Myeloproliferative Neoplasms

Arber et al. Blood 2016;127:2391

Critères majeurs/mineurs

		Polycythemia Vera (PV)	Essential Thrombocythemia (ET)	Primary Myelofibrosis (PMF) (overt)	Primary Myelofibrosis (prefibrotic) (prePMF)
Major criteria	1	Hemoglobin (Hgb) >16.5 g/dL (men) >16 g/dL (women) or Hematocrit >49% (men) >48% (women) or ↑ red cell mass >25% above mean	1 Platelet count ≥450 x 10^9/L	1 Megakaryocyte proliferation and atypia*** and ≥ grade 2 reticulin/collagen fibrosis ***megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering	Megakaryocyte proliferation and atypia*** and ≤ grade 1 reticulin/collagen fibrosis, increased cellularity, granulocytic proliferation and decreased erythropoiesis
	2	Bone marrow (BM) tri-lineage myeloproliferation with pleomorphic mature megakaryocytes* Dispensable si Hb >18,5/16,5 ou Ht>55,(/49,5	2 BM megakaryocyte proliferation with large and mature morphology and hyper-lobulated nuclei. Reticulin fibrosis grade should be ≤1	2 Not meeting WHO criteria for other myeloid neoplasm	Not meeting WHO criteria for other myeloid neoplasm
	3	Presence of *JAK2* mutation	3 Not meeting WHO criteria for other myeloid neoplasms	3 Presence of *JAK2*, *CALR* or *MPL* mutation or presence of another clonal marker or absence of evidence for reactive bone marrow fibrosis	Presence of *JAK2*, *CALR* or *MPL* mutation or presence of another clonal marker or absence of evidence for reactive bone marrow fibrosis
	4		Presence of *JAK2*, *CALR* or *MPL* mutation		
Minor criteria	1.	Subnormal serum erythropoietin level	1. Presence of a clonal marker or absence of evidence for reactive thrombocytosis	1 1. Anemia not otherwise attributed 2. Leukocytosis ≥11 x 10^9/L 3. Palpable splenomegaly 4. Increased lactate dehydrogenase (LDH), above upper normal limit 5. Leukoerythroblastosis	1. Anemia not otherwise attributed 2. Leukocytosis ≥11 x 10^9/L 3. Palpable splenomegaly 4. Increased lactate dehydrogenase (LDH), above upper normal limit

Points

3M ou 2M+1m	4M ou 3M + 1m	3M+ min 1m	3M+ min 1m

Tefferi A et al., American Journal of Hematology, Vol. 92, No. 1, January 2017

Plan

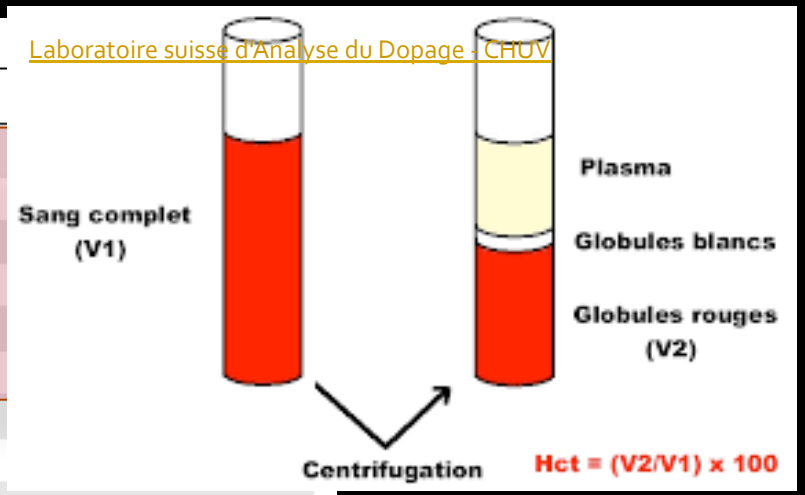
- Les "syndromes myéloprolifératifs", qu'est-ce que c'est ?
- La démarche diagnostique (OMS, PVSG,…)
- Les outils du diagnostic:
  - L'examen clinique et l' "interrogatoire " (anamnèse)
  - La « prise de sang »
  - La ponction de moelle osseuse hématopoïétique
  - La biopsie de moelle osseuse hématopoïétique
  - Les tests biochimiques (dosage d'érythropoïétine)
  - La cytométrie en flux
  - Les examens des chromosomes
  - Les examens de gènes
- Quels buts pour ces examens (diagnostic: choix du traitement, suivi de la maladie…)
- Conclusions

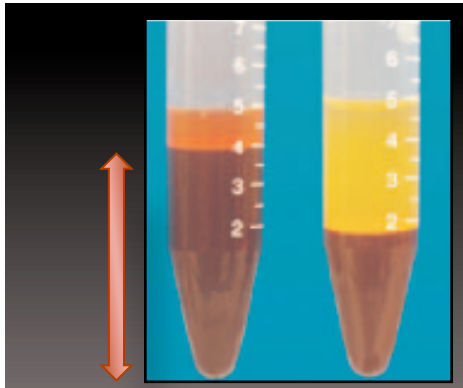
Démarche diagnostique :
l'exemple de la « maladie de Vaquez » ou Polycythemia Vera »

Hématocrite

Table 4. WHO criteria for PV

WHO PV criteria
Major criteria
1. Hemoglobin >16.5 g/dL in men
Hemoglobin >16.0 g/dL in women
or,
Hematocrit >49% in men
Hematocrit >48% in women
or,
increased red cell mass (RCM)*
2. BM 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*V617F or *JAK2* exon 12 mutation
Minor criterion
Subnormal serum erythropoietin level
Diagnosis of PV requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion†





Arber et al, BLOOD, 19 MAY 2016 x VOLUME 127, NUMBER 20

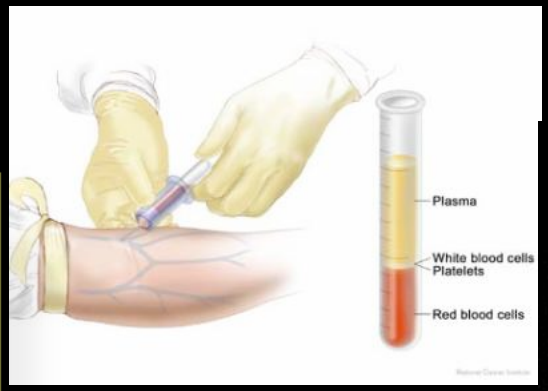
La numération sanguine automatisée

Polycythemia Vera (PV)

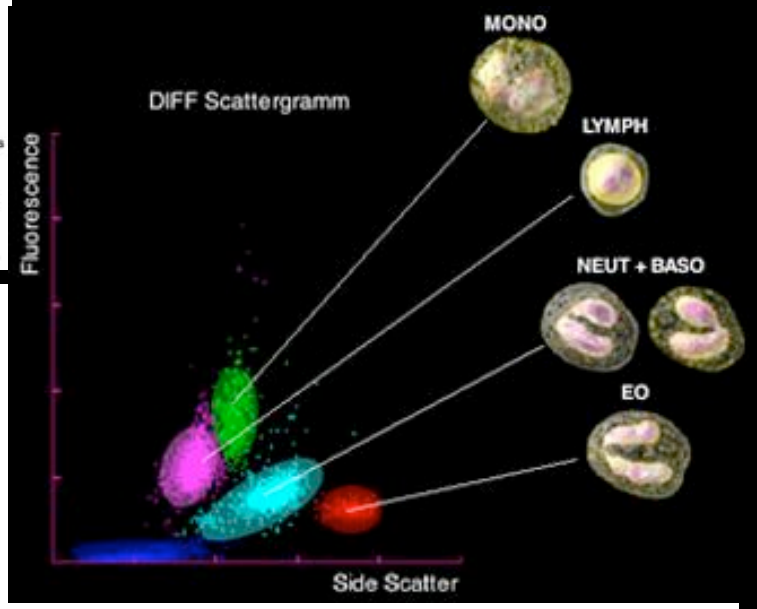
Major criteria	1	Hemoglobin (Hgb) >16.5 g/dL (men) >16 g/dL (women) or Hematocrit >49% (men) >48% (women) or ↑ red cell mass >25% above mean
	2	Bone marrow (BM) tri-lineage myeloproliferation with pleomorphic mature megakaryocytes*
	3	Presence of *JAK2* mutation
Minor criteria	1.	Subnormal serum erythropoietin level

Tefferi, AJH, Vol. 92, January 2017

PV diagnosis requires meeting all three major criteria or the first two major criteria and one minor criterion.
*BM biopsy may not be not required if Hb >18.5 g/dL in men or 16.5 in women (Hct >55.5 in men and 49.5 in women)



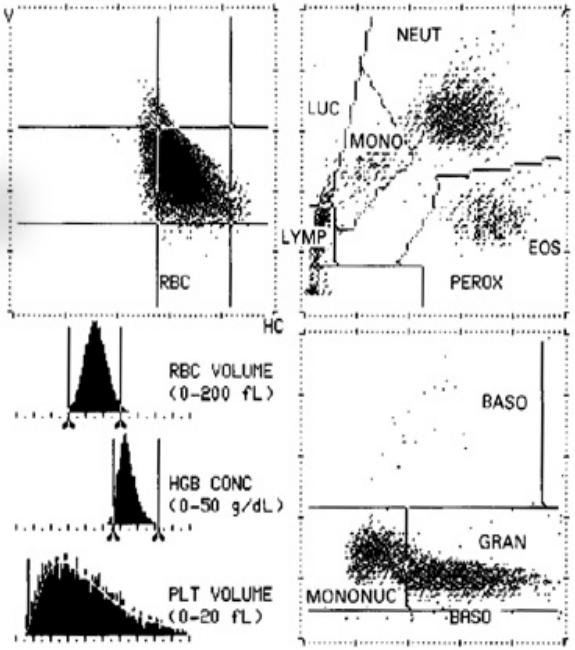
« Prise de sang »



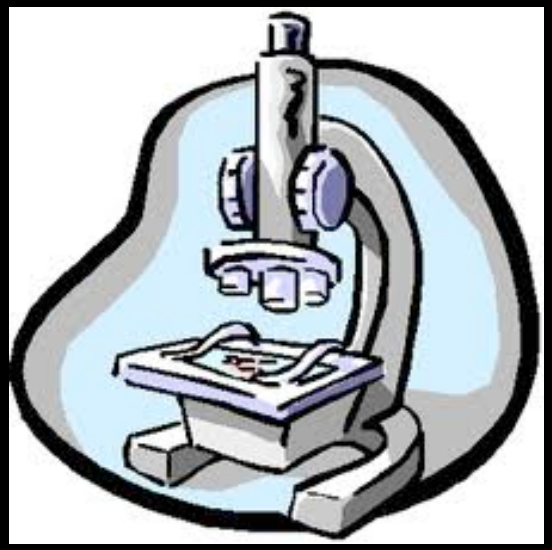
Comptage

Formule

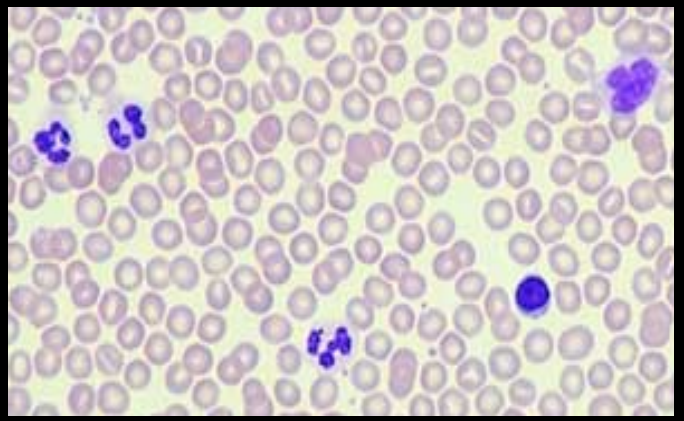
SEQ#	0001037 (0012)		
TIME	10:34 22/08/94		
SYS#	001		
ID	000000141982		
	CBC		
	6.42	x10³/L	WBC
	4.73	x10¹²/L	RBC
	13.9	g/dL	HGB
	.437		HCT
	92.4	fL	MCV
	29.5	pg	MCH
	31.9	g/dL	MCHC
H	14.2	%	RDW
H	2.58	g/dL	HDW
	227	x10³/L	PLT
	7.8	fL	MPV
	53.8	%	PDW
	.18	%	PCT
RBC FLAGS			0000
	%	DIFF	x10³/L
	48.8	NEUT	3.13
	30.7	LYMP	1.97
	5.9	MONO	.38
H	11.8	EOS H	.75
	.9	BASO	.06
	2.0	LUC	.13
	LI		2.00
	MPXI		-3.0
WBC FLAGS			0000



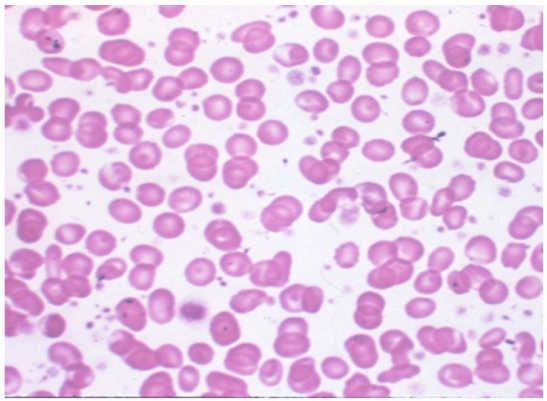
L'examen au microscope du frottis sanguin



Frottis sanguin normal

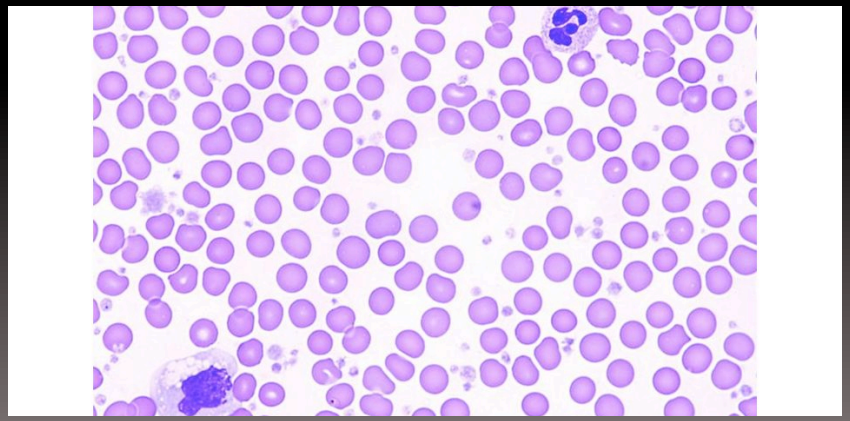


Frottis sanguins pathologiques



This peripheral blood smear from a patient with essential thrombocythemia shows increased numbers of platelets, including some large forms. (H and E, 400x)

Paquettes ↗



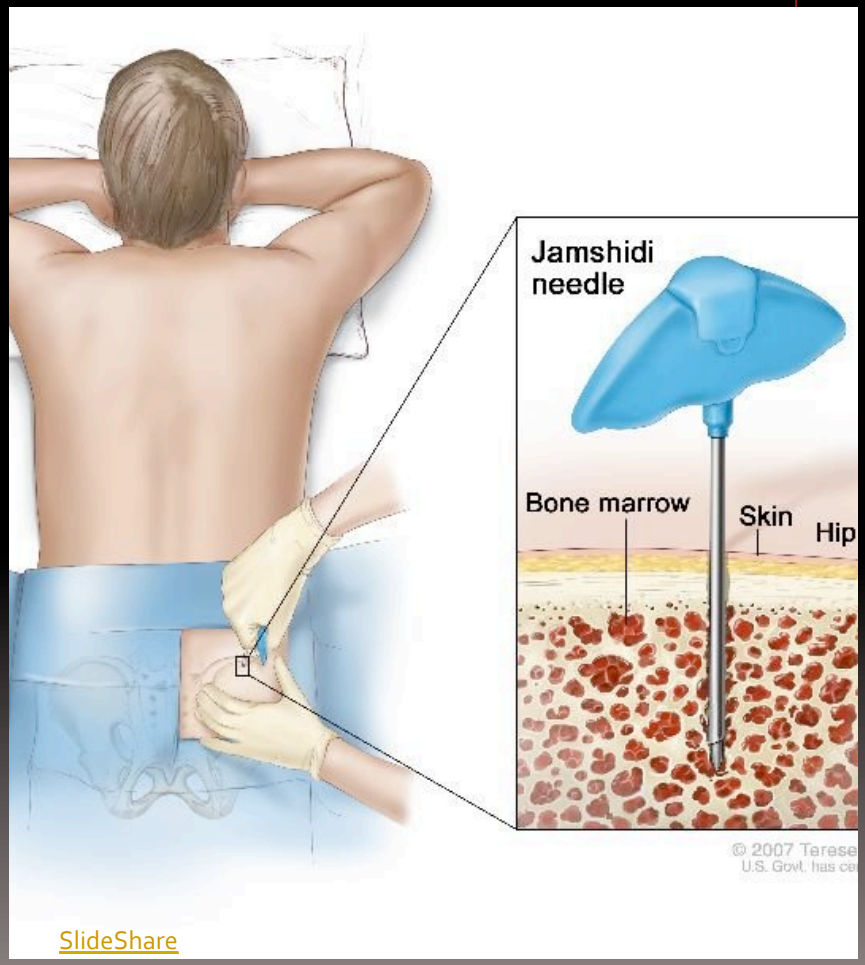
formes anormales gb

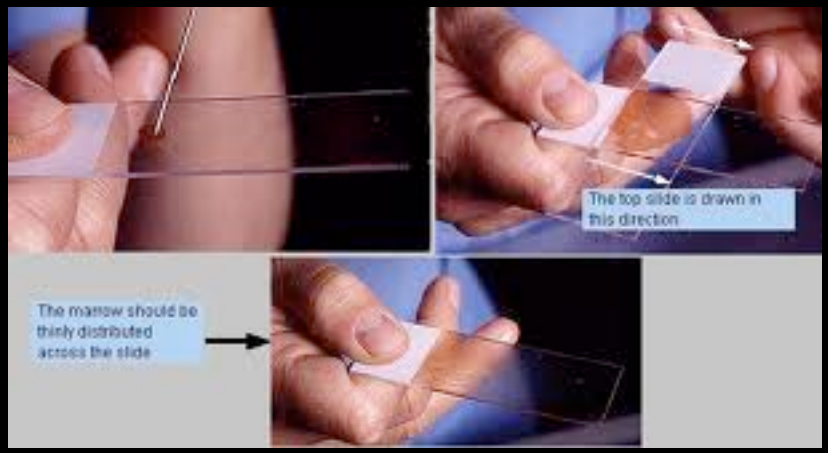
from the web

Plan

- Les "syndromes myéloprolifératifs", qu'est-ce que c'est ?
- La démarche diagnostique (OMS, PVSG,…)
- Les outils du diagnostic:
  - L'examen clinique et l' "interrogatoire " (anamnèse)
  - La « prise de sang »
  - La ponction de moelle osseuse hématopoïétique
  - La biopsie de moelle osseuse hématopoïétique
  - Les tests biochimiques (dosage d'érythropoïétine)
  - La cytométrie en flux
  - Les examens des chromosomes
  - Les examens de gènes
- Quels buts pour ces examens (diagnostic; choix du traitement; suivi de la maladie…)
- Conclusions

La ponction médullaire et l'examen du médullogramme

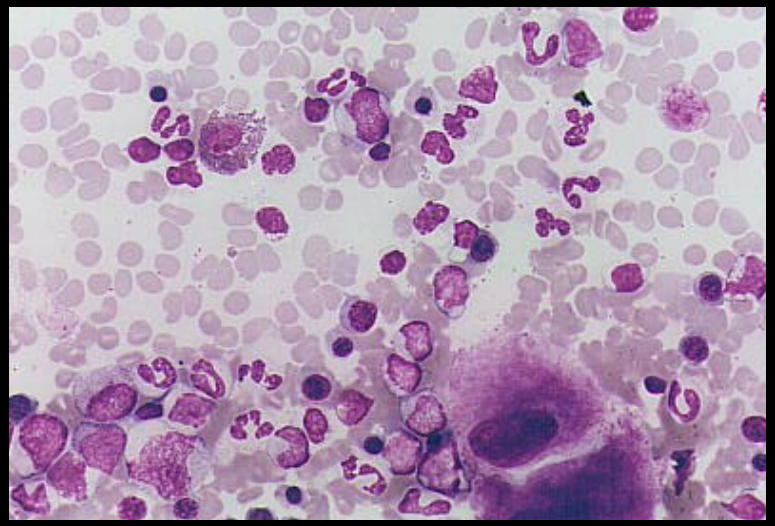




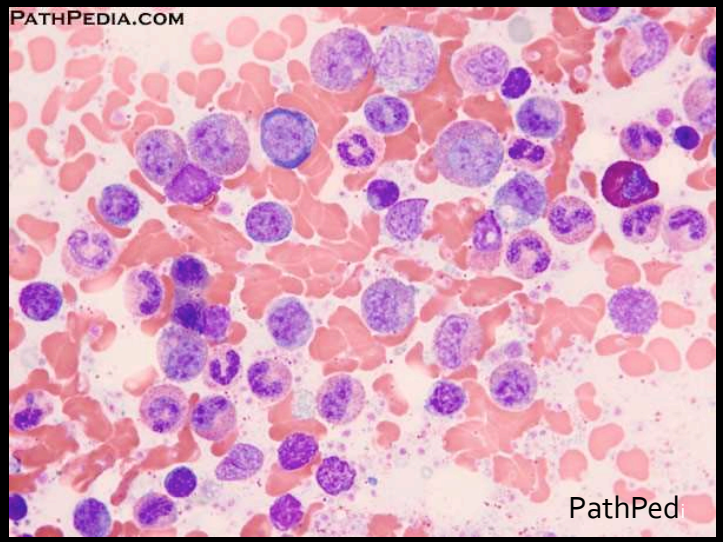
coloration H&E

Microscopie: l'examen du frottis de moelle

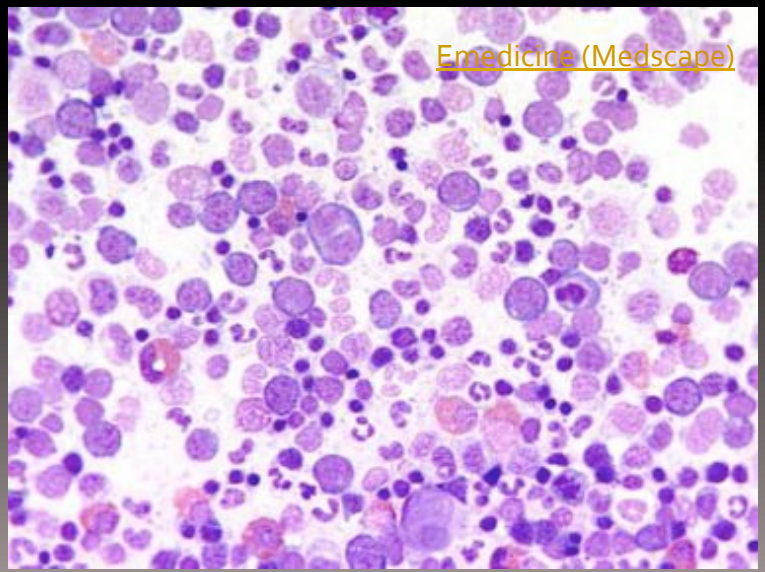
Frottis moelle osseuse normale



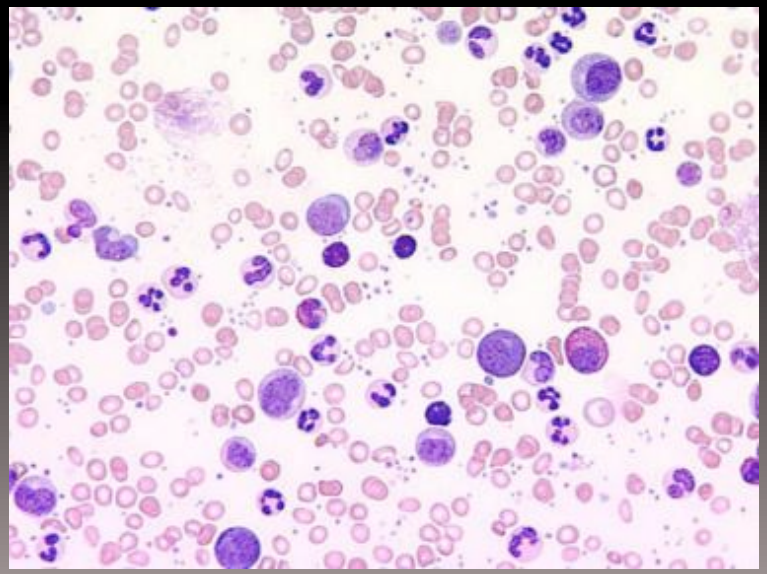
Thrombocythémie essentielle



Polycythémie vraie



Leucémie myéloïde chronique



Essential thrombocythemia

Major criteria

1. Platelet count ≥ 450 x 10^9/L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left-shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for *BCR-ABL1*-positive chronic myeloid leukemia, polycythemia vera, primary myelofibrosis, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2*, *CALR* or *MPL* mutation

Minor criterion

- Presence of a clonal marker or absence of evidence for reactive thrombocytosis

Diagnosis of ET requires meeting all four major criteria or the first 3 major criteria and the minor criterion

Elisa Rumi, Mario Cazzola, Blood, 2016, DOI 10.1182/blood-2016-10-695957

Ponction de moelle hématopoïétique, buts:

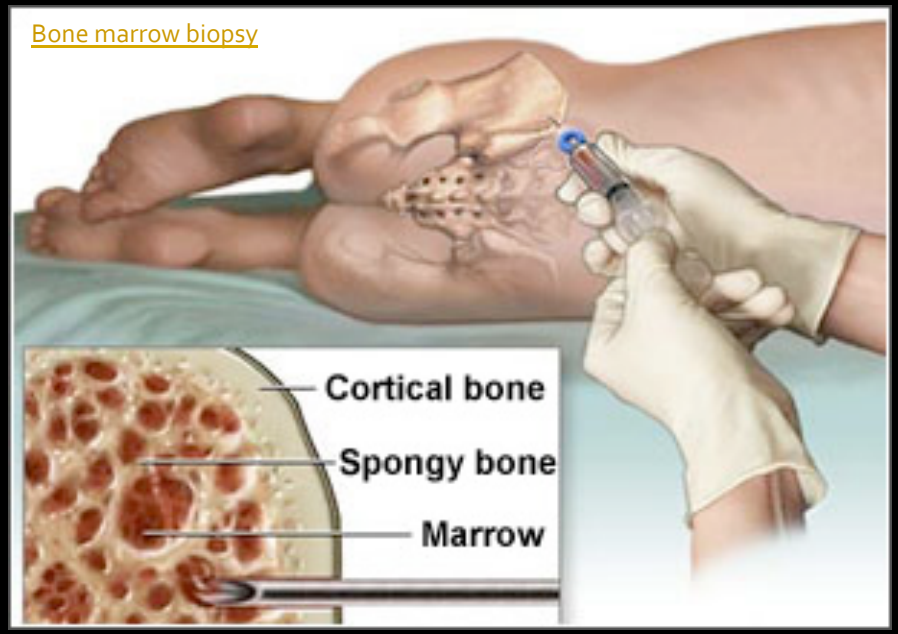
- Rechercher une maladie hématologique autre qu'une NMP (SMP); i.e syndrome myélodysplasique (SMD), LMC,…

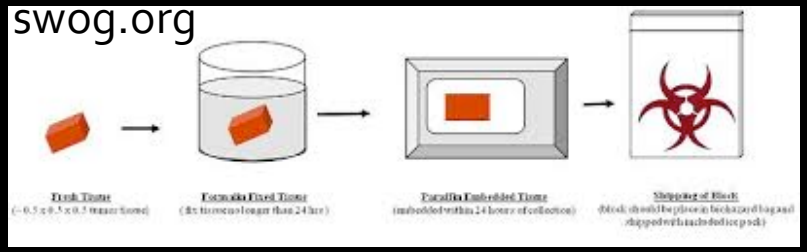
- Prélever de la moelle hématopoïétique pour réaliser une analyse des chromosomes (caryotype) et rechercher des anomalies associée à des diagnostics particuliers (i.e LMC)

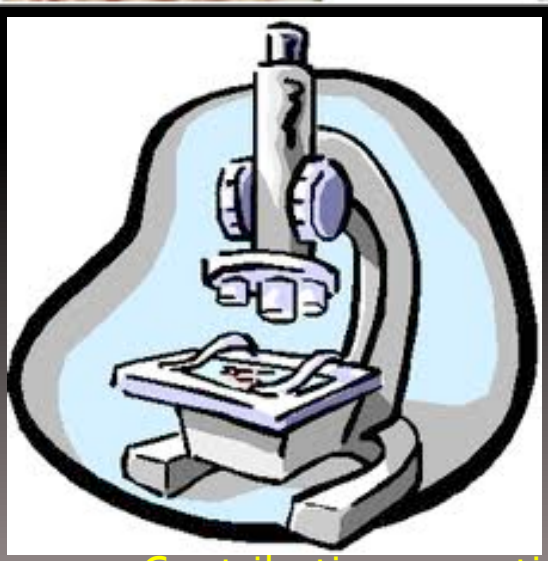
Plan

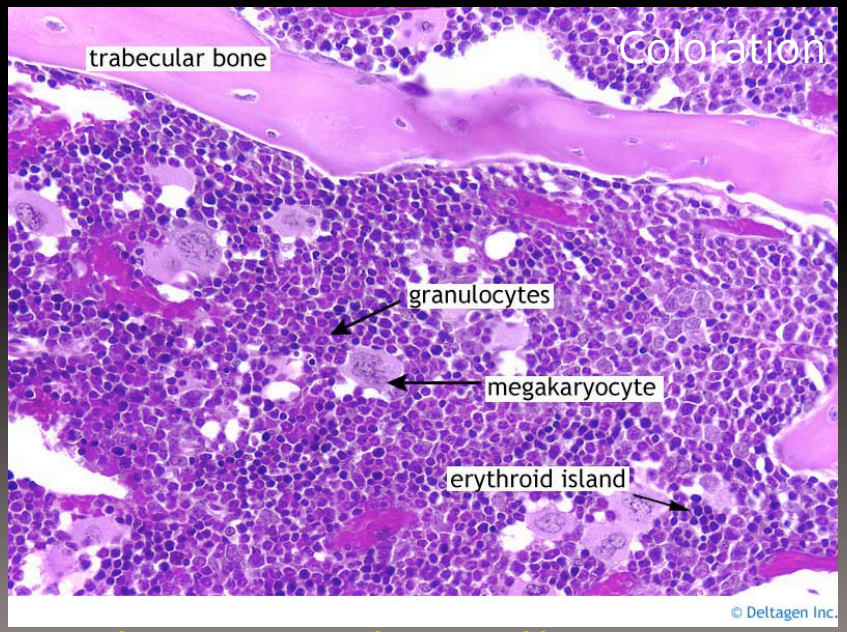
- Les "syndromes myéloprolifératifs", qu'est-ce que c'est ?
- La démarche diagnostique (OMS, PVSG,…)
- Les outils du diagnostic:
  - L'examen clinique et l' "interrogatoire " (anamnèse)
  - La « prise de sang »
  - La ponction de moelle osseuse hématopoïétique
  - La biopsie de moelle osseuse hématopoïétique
  - Les tests biochimiques (dosage d'érythropoïétine)
  - La cytométrie en flux
  - Les examens des chromosomes
  - Les examens de gènes
- Quels buts pour ces examens (diagnostic; choix du traitement; suivi de la maladie…)
- Conclusions

Microscopie: l'examen de la biospie ostéo-médullaire médullaire









Contribution essentielle au diagnostic des néoplasies myéloprolifératives (NMP)

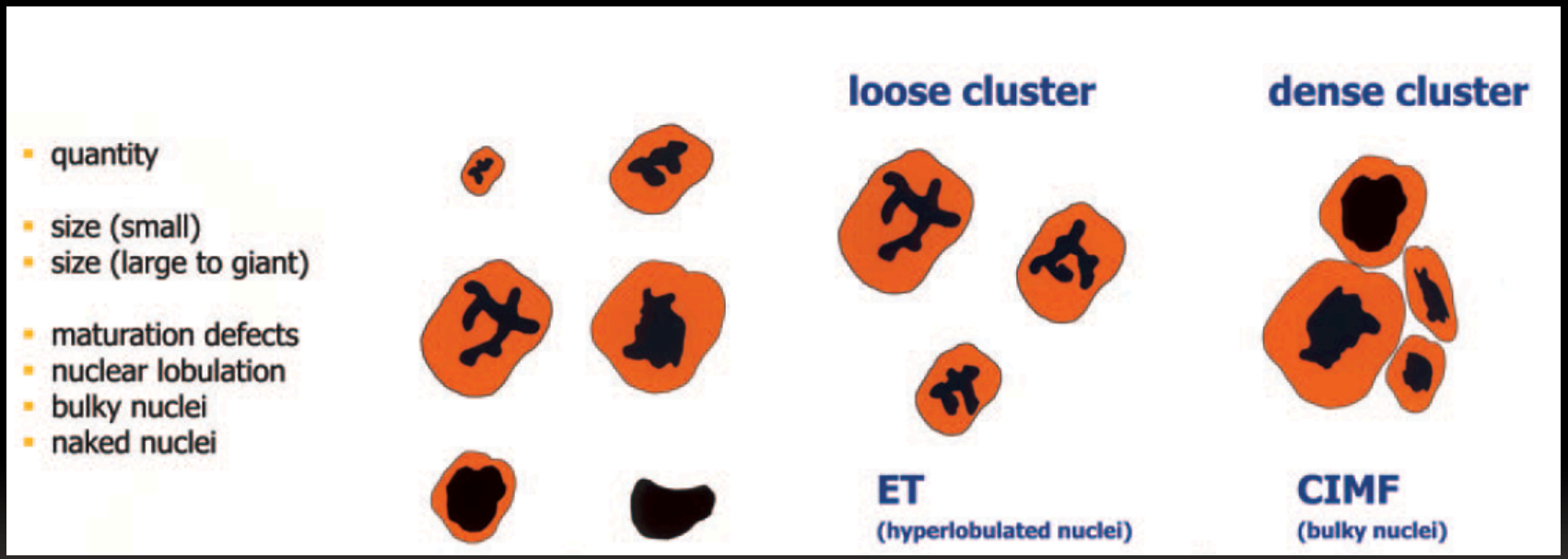
Table 4. WHO criteria for PV

WHO PV criteria
Major criteria
1. Hemoglobin >16.5 g/dL in men
Hemoglobin >16.0 g/dL in women
or,
Hematocrit >49% in men
Hematocrit >48% in women
or,
increased red cell mass (RCM)*
2. BM 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*V617F or *JAK2* exon 12 mutation
Minor criterion
Subnormal serum erythropoietin level
Diagnosis of PV requires meeting either all 3 major criteria, or the first 2 major criteria and the minor criterion†

Arber et al, BLOOD, 19 MAY 2016 x VOLUME 127, NUMBER 20

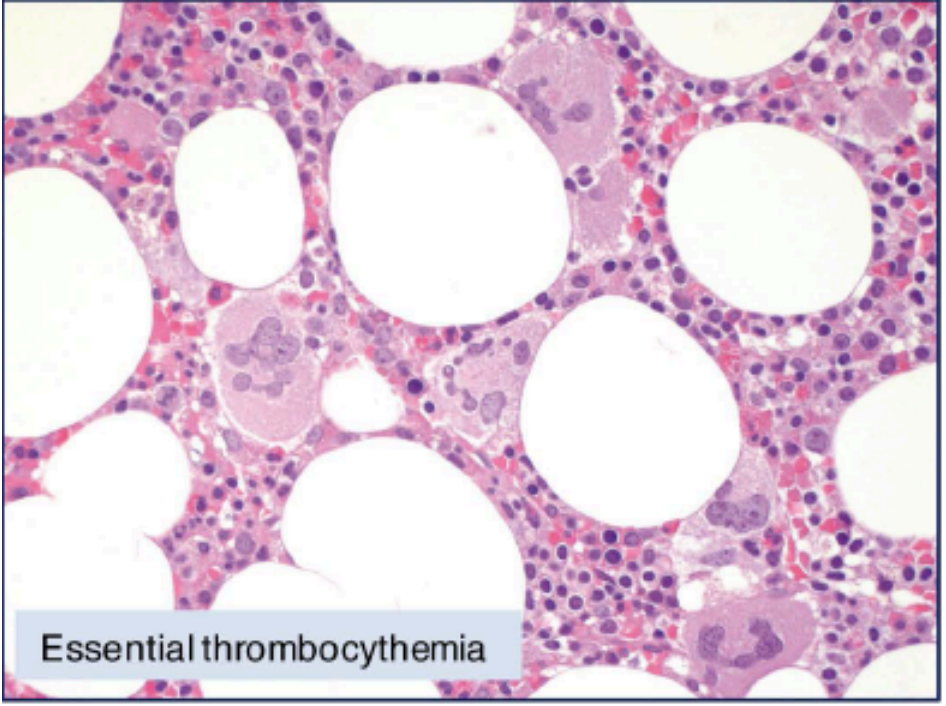
Microscopie: l'examen de la biospie ostéo-médullaire médullaire

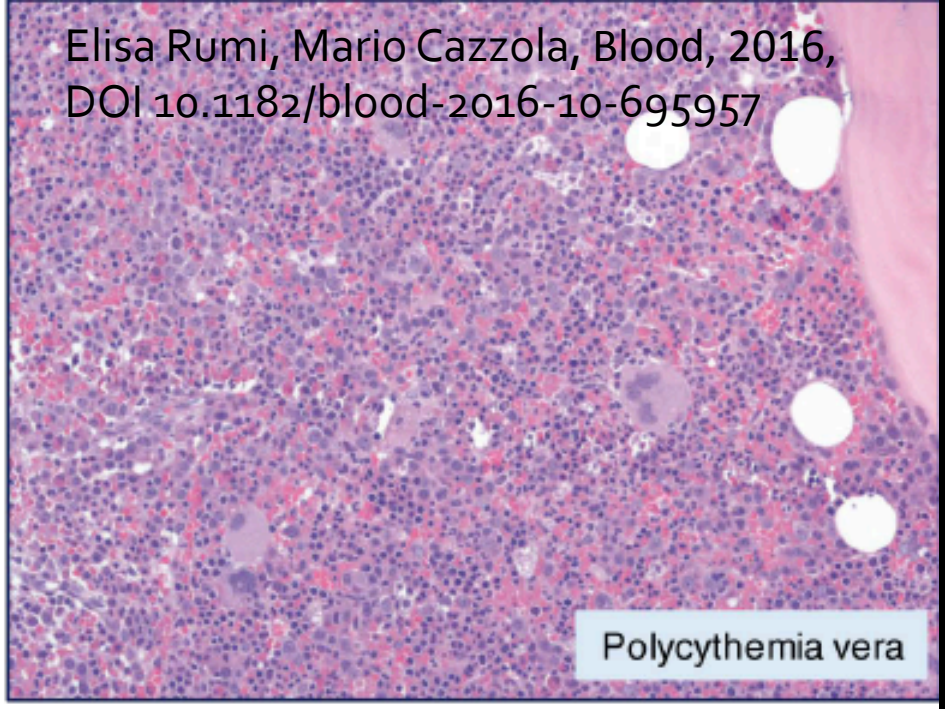
Morphologie des mégakaryocytes (plaquettes)

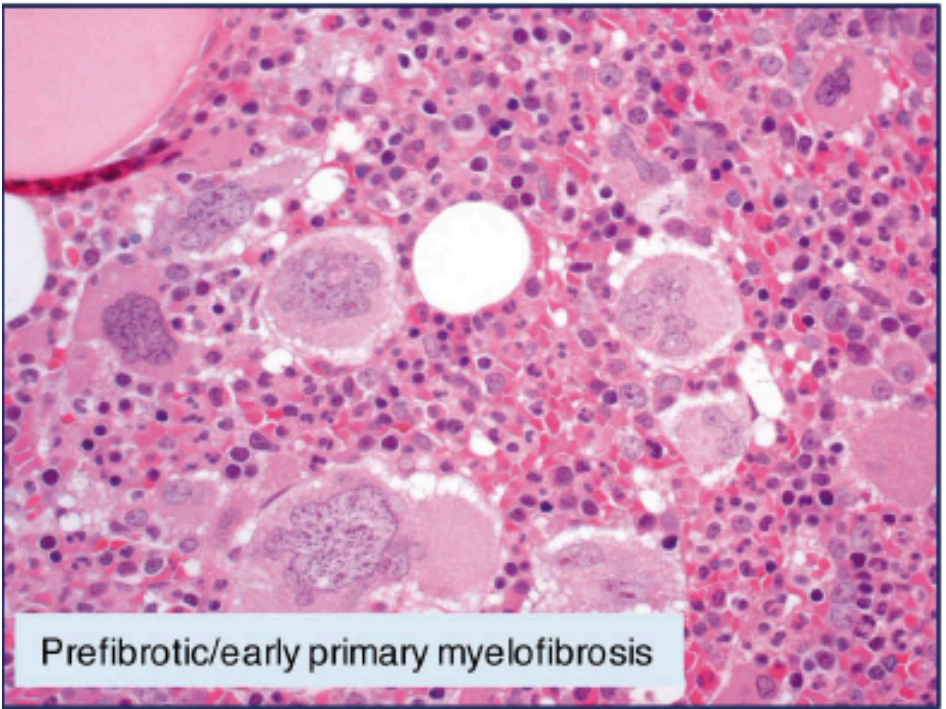


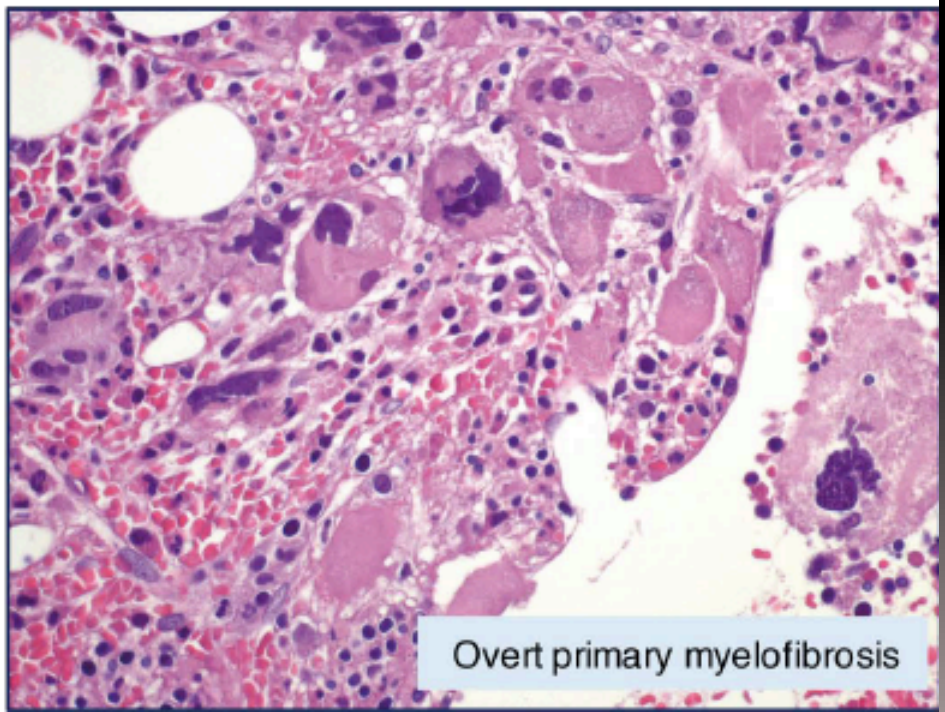
Contribution *essentielle* au diagnostic différentiel des NMP (TE, PMF, préfibrotic PMF)

J. Thiele, H.M. Kvasnicka1 and V. Diehl, Histol Histopathol (2005) 20: 633-644

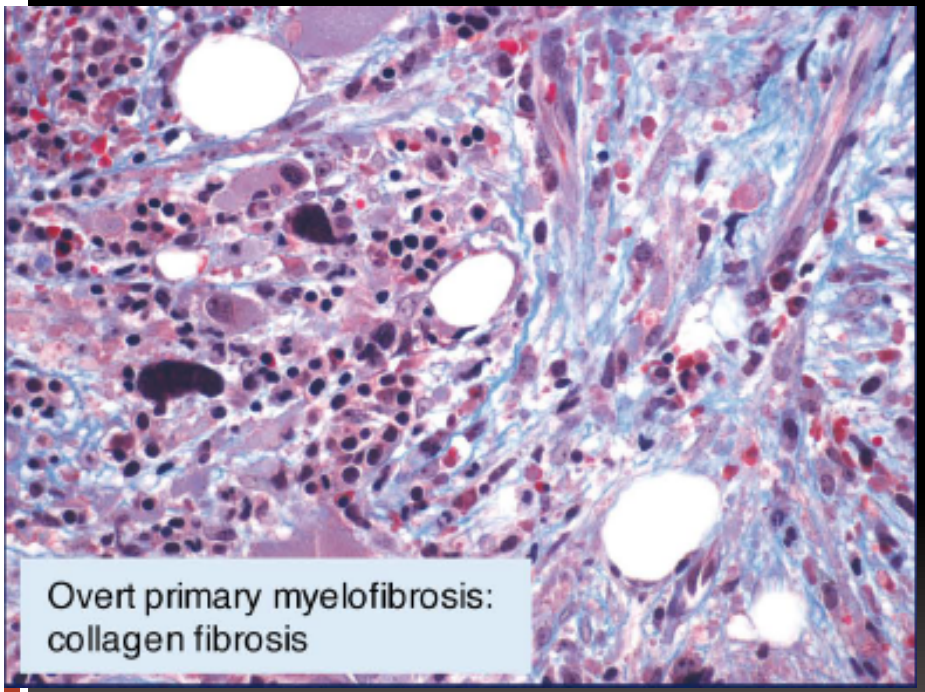

Essential thrombocythemia


Elisa Rumi, Mario Cazzola, Blood, 2016,
DOI 10.1182/blood-2016-10-695957
Polycythemia vera


Prefibrotic/early primary myelofibrosis


Overt primary myelofibrosis

La biopsie ostéo-médullaire: **Evaluation du degrés de fibrose médullaire**



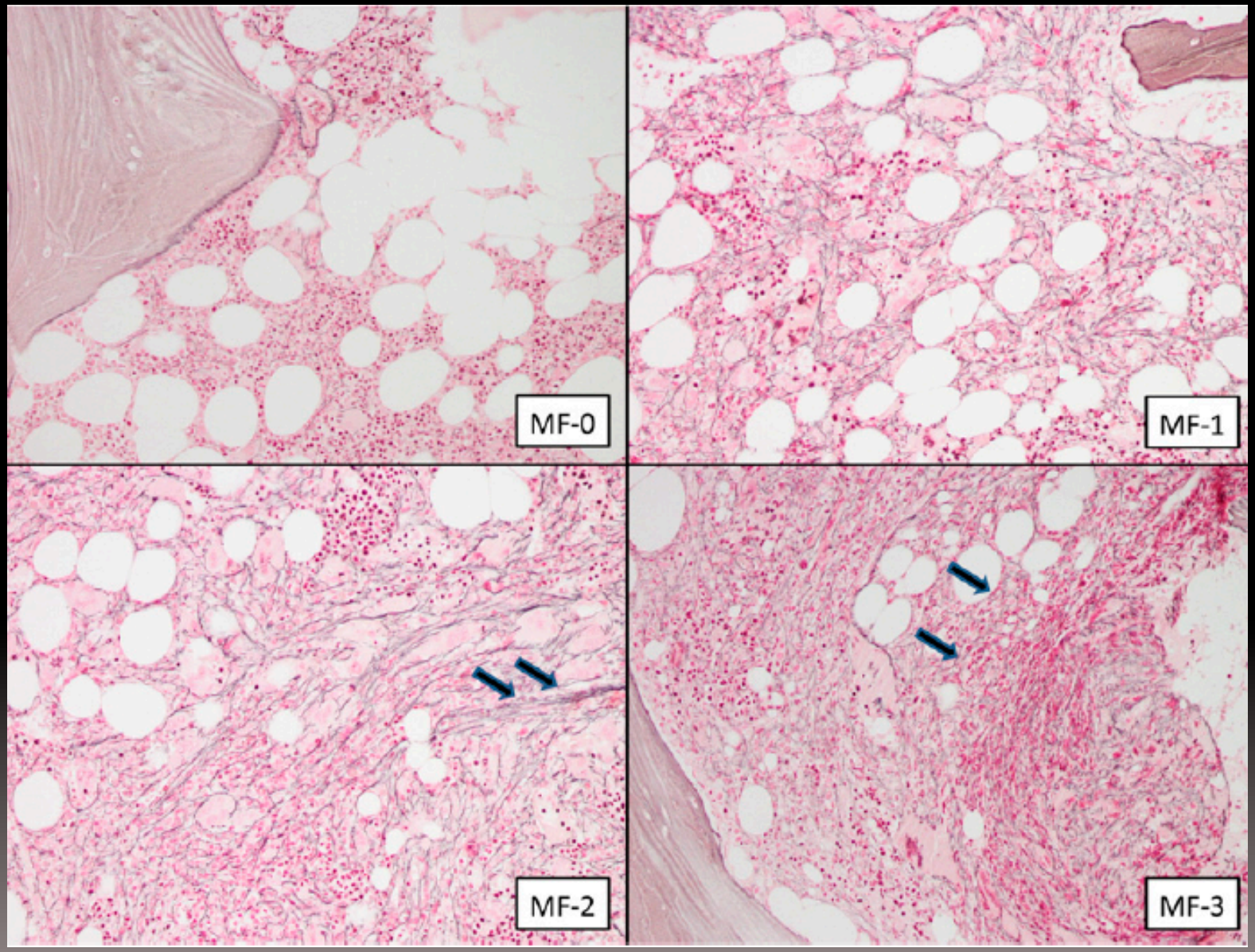
Overt primary myelofibrosis: collagen fibrosis

Table 3. Semiquantitative grading of BM-F

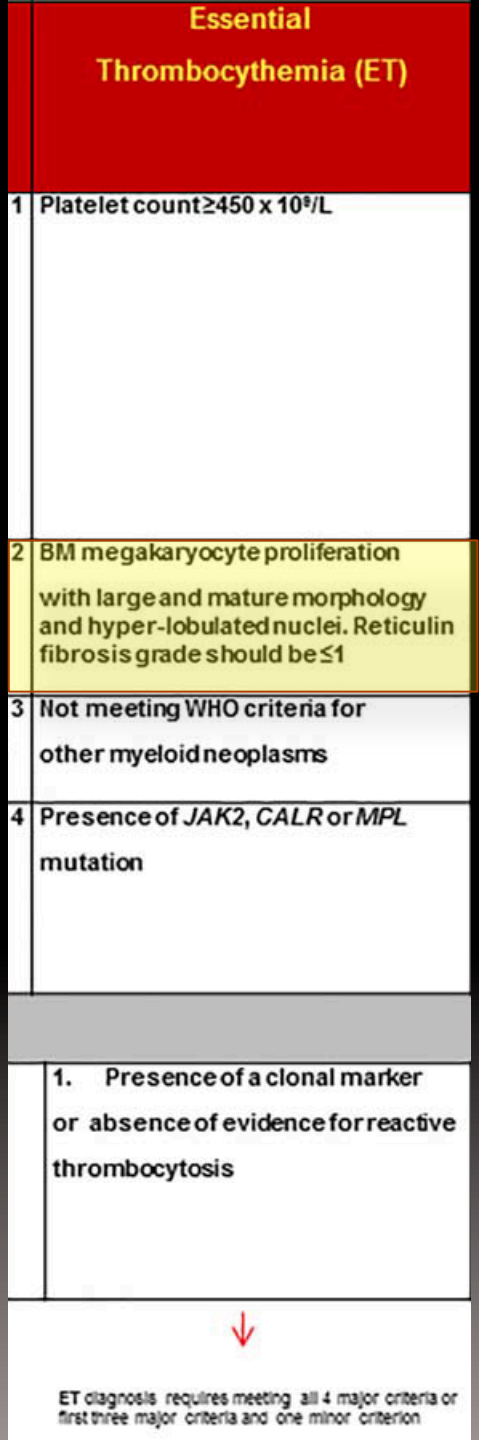
Grading	
MF-0	Scattered linear reticulin with no intersections (crossovers) corresponding to normal BM
MF-1	Loose network of reticulin with many intersections, especially in perivascular areas
MF-2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of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

Fiber density should be assessed only in hematopoietic areas.
*In grades MF-2 or MF-3, an additional trichrome stain is recommended.

L'évaluation du degrés de fibrose



Francesco Passamonti, Hematology, 2016



La biopsie médullaire permet de caractériser la morphologie des mégakaryocytes et de quantifier la fibrose médullaire

Tefferi, AJH, Vol. 92, January 2017

La biopsie médullaire permet de caractériser la morphologie des mégakaryocytes et de quantifier *la fibrose médullaire*

	Primary Myelofibrosis (PMF) (overt)	Primary Myelofibrosis (prefibrotic) (prePMF)
1	Megakaryocyte proliferation and atypia*** and ≥ grade 2 reticulin/collagen fibrosis ***megakaryocytes with aberrant nuclear/cytoplasmic ratio and hyperchromatic and irregularly folded nuclei and dense clustering	Megakaryocyte proliferation and atypia*** and ≤ grade 1 reticulin/collagen fibrosis, Increased cellularity, granulocytic proliferation and decreased erythropoiesis
2	Not meeting WHO criteria for other myeloid neoplasm	Not meeting WHO criteria for other myeloid neoplasm
3	Presence of *JAK2*, *CALR* or *MPL* mutation or presence of another clonal marker or absence of evidence for reactive bone marrow fibrosis	Presence of *JAK2*, *CALR* or *MPL* mutation or presence of another clonal marker or absence of evidence for reactive bone marrow fibrosis
1	1. Anemia not otherwise attributed 2. Leukocytosis ≥11 x 10^9/L 3. Palpable splenomegaly 4. Increased lactate dehydrogenase (LDH), above upper normal limit 5. Leukoerythroblastosis	1. Anemia not otherwise attributed 2. Leukocytosis ≥11 x 10^9/L 3. Palpable splenomegaly 4. Increased lactate dehydrogenase (LDH), above upper normal limit
	↓	↓
	PMF diagnosis requires meeting all 3 major criteria and at least one minor criterion	prePMF diagnosis requires meeting all 3 major criteria and at least one minor criterion

Tefferi, AJH, Vol. 92, January 2017

Le difficile diagnostic différentiel entre TE et PMF pré-fibrotique

WHO-Classification

hypercellular

clusters of hypolobulated megakaryocytes

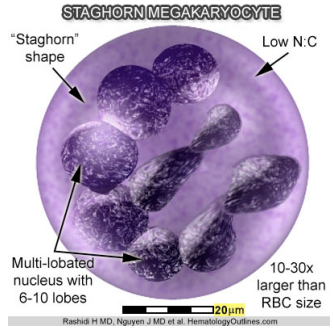
increased granulopoiesis

decreased erythropoiesis

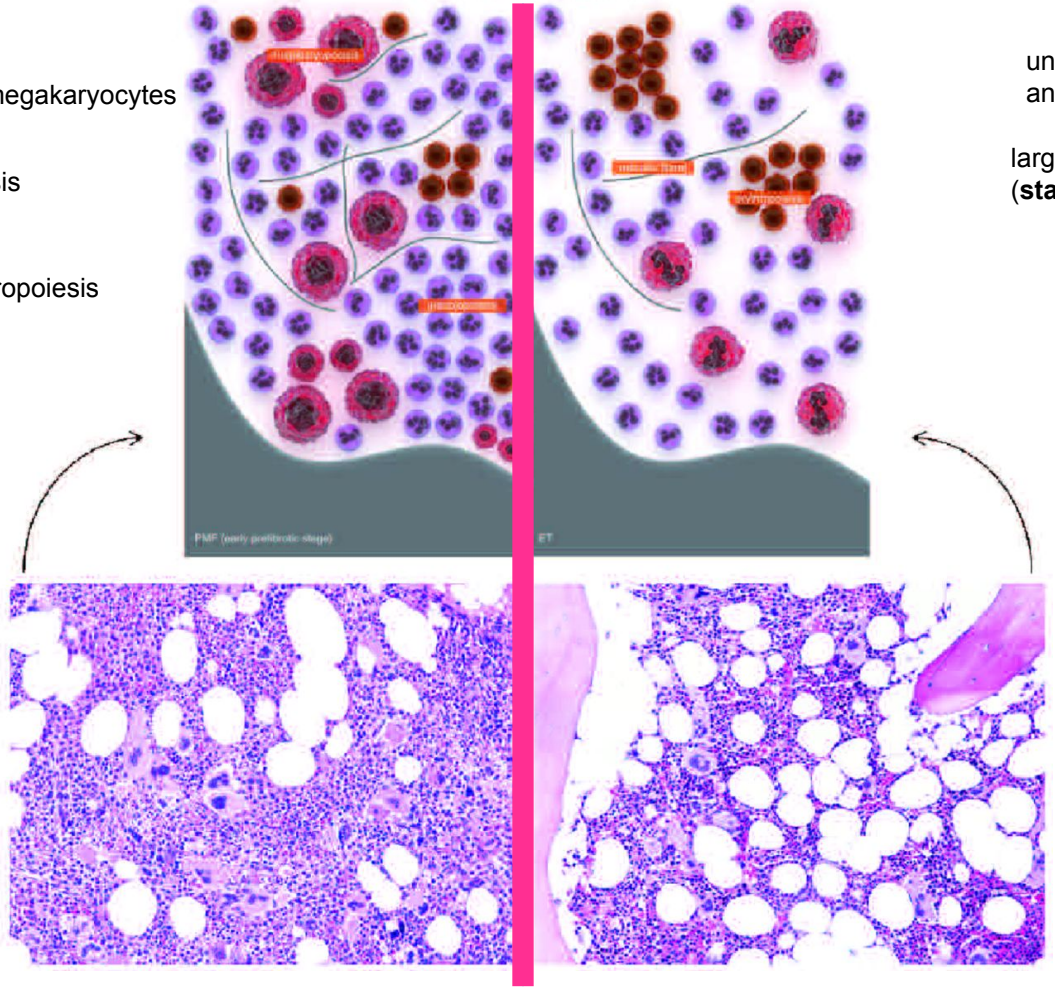
less cellular

unremarkable erythropoiesis and granulopoiesis

large hyperlobulated (**staghorn-like)** megakaryocytes



HematologyOutlines - Atlas



pré-PMF

ET

http://hematologyoutlines.com /atlas_topics/61.html

John O. Mascarenhas et al. Haematologica 2013;98:1499-1509

haematologica
Journal of the European Hematology Association
Published by the Ferrata Storti Foundation

Thiele J et al., *Blood. 2011;117(21):5710–8.*

Myélofibrose primitive *versus* secondaire
post-polycythemia/thrombocythemia

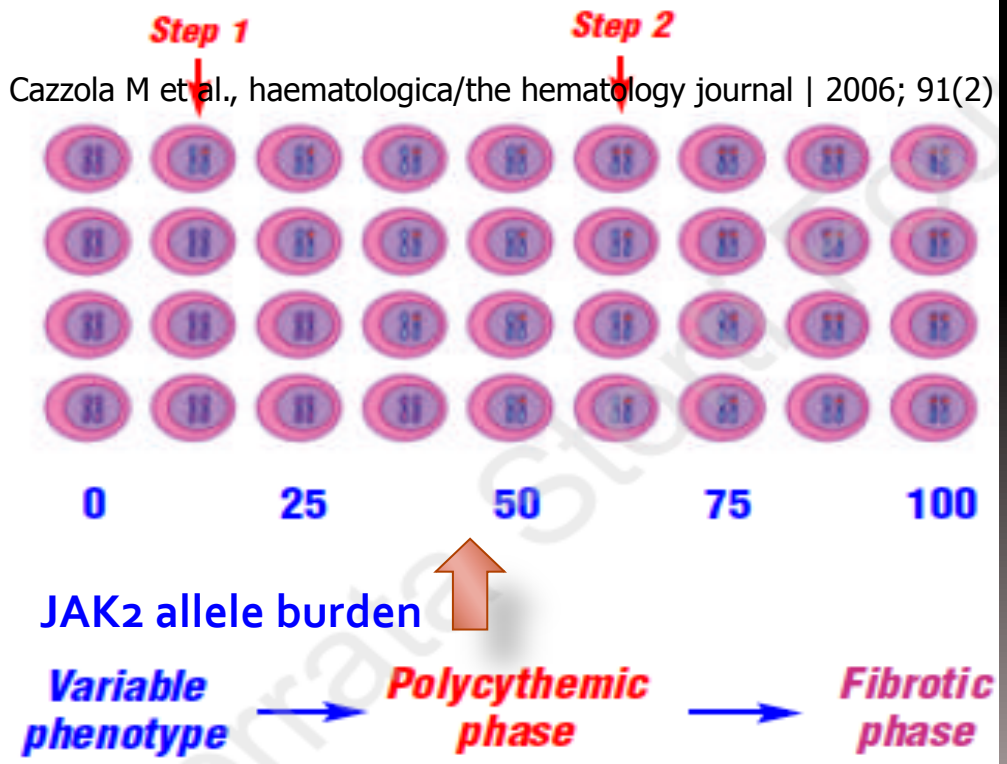
Table II. Diagnostic criteria for primary myelofibrosis: diagnosis requires A1 + A2 and any two B criteria.

A1	Bone marrow fibrosis ≥3 (on 0–4 scale).
A2	Pathogenetic mutation (e.g. in *JAK2* or *MPL*), or absence of both *BCR-ABL1* and reactive causes of bone marrow fibrosis
B1	Palpable splenomegaly
B2	Unexplained anaemia
B3	Leuco-erthroblastosis
B4	Tear-drop red cells
B5	Constitutional symptoms*
B6	Histological evidence of extramedullary haematopoiesis

Table III. Diagnostic criteria for post-PV and post-ET myelofibrosis: diagnosis requires A1 + A2 and any two B criteria.

A1	Bone marrow fibrosis ≥3 (on 0–4 scale)
A2	Previous diagnosis of ET or PV
B1	New palpable splenomegaly or increase in spleen size of ≥5 cm
B2	Unexplained anaemia with 20 g/l decrease from baseline haemoglobin
B3	Leuco-erythroblastic blood film.
B4	Tear-drop red cells
B5	Constitutional symptoms*
B6	Histological evidence of extramedullary haematopoiesis.

Cazzola M et al., haematologica/the hematology journal | 2006; 91(2)



Reilly J et al., British Journal of Haematology, 2012

Plan

- Les "Néoplasies myéloprolifératives", qu'est-ce que c'est ?
- La démarche diagnostique (OMS, PVSG,…)
- Les outils du diagnostic:
  - L'examen clinique et l' "interrogatoire " (anamnèse)
  - La ponction de moelle osseuse hématopoïétique
  - La biopsie de moelle osseuse hématopoïétique
  - Les tests biochimiques (dosage d'érythropoïétine)
  - La cytométrie en flux
  - Les examens des chromosomes
  - Les examens de gènes
- Quels buts pour ces examens (diagnostic; choix du traitement, suivi de la maladie…)
- Conclusions

Pourquoi examiner les chromosomes (caryotypage)?

Essential thrombocythemia

Major criteria

1. Platelet count ≥ 450 x 10^9/L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left-shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for *BCR-ABL1*-positive chronic myeloid leukemia, polycythemia vera, primary myelofibrosis, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2*, *CALR* or *MPL* mutation

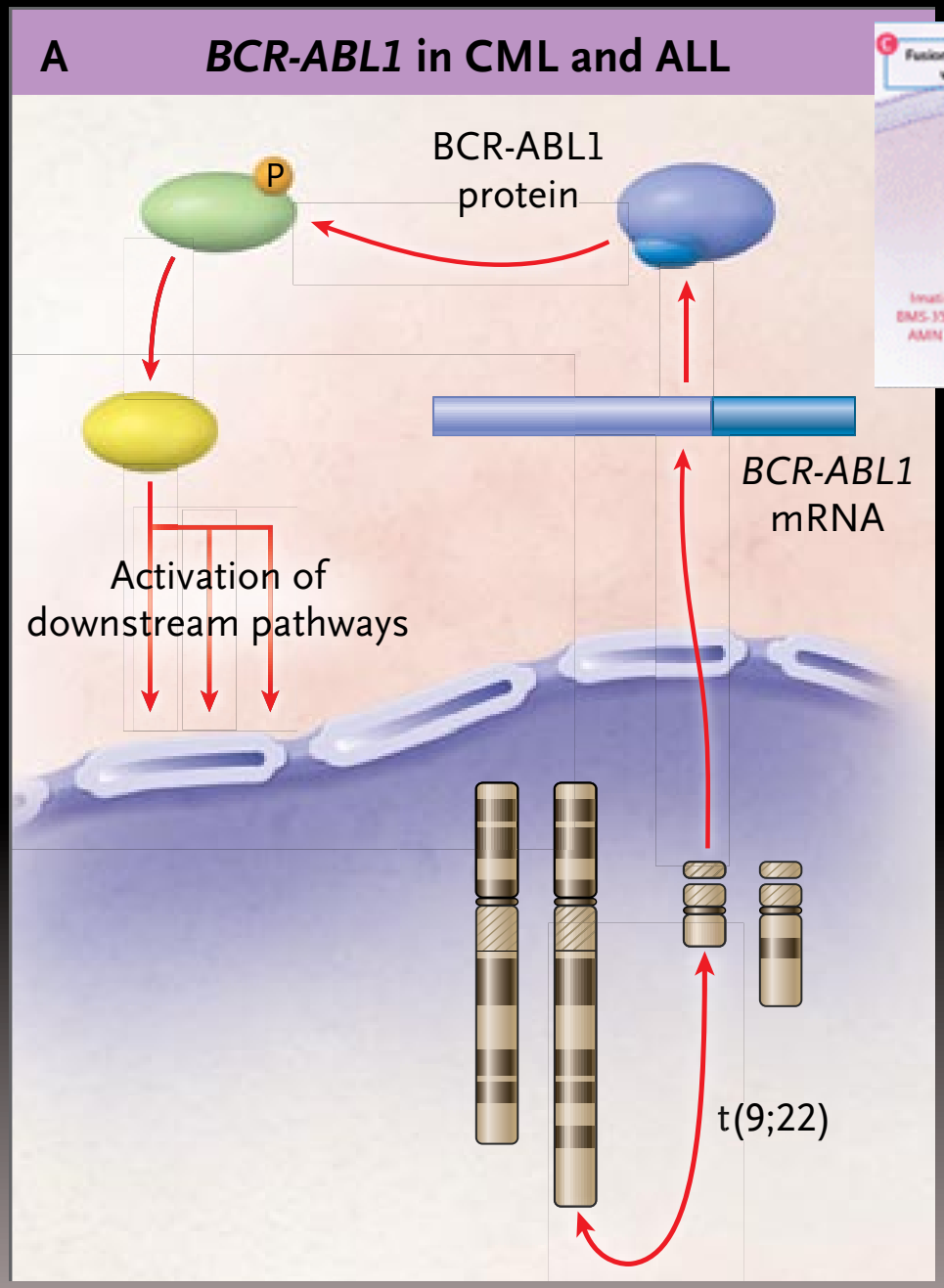
Minor criterion

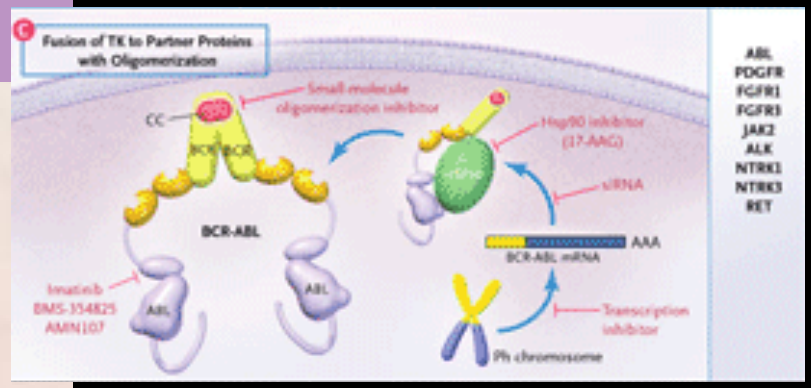
- Presence of a clonal marker or absence of evidence for reactive thrombocytosis

Diagnosis of ET requires meeting all four major criteria or the first 3 major criteria and the minor criterion

Elisa Rumi & Mario Cazzola, Blood, 2016, DOI 10.1182/blood-2016-10-695957

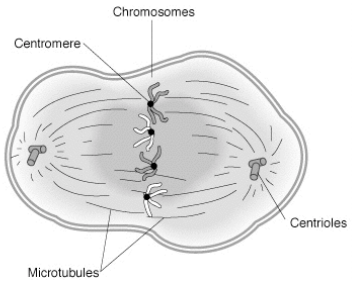
La translocation (9;22) caractéristique des LMC





La cytogénétique: le caryotypage

moelle hématopoïétique



add tissue sample

add chemical to stimulate mitosis

Culture in a growth medium

incubate for 2-3 days

add chemical to stop mitosis in metaphase

transfer cells to tube and centrifuge to concentrate in layers

transfer to tube containing fixative

put cells onto microscope slide

add stain to enhance chromosomes

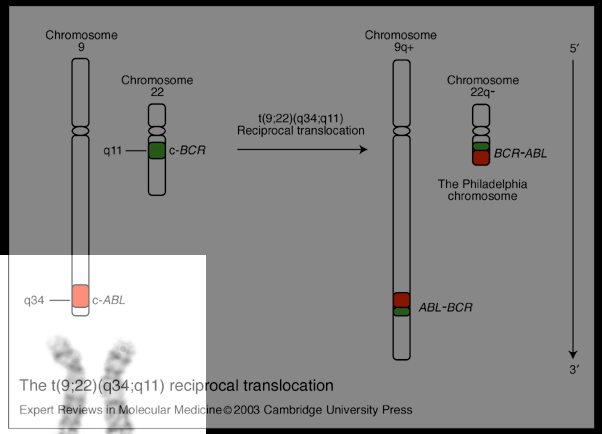
identify and photograph chromosomes

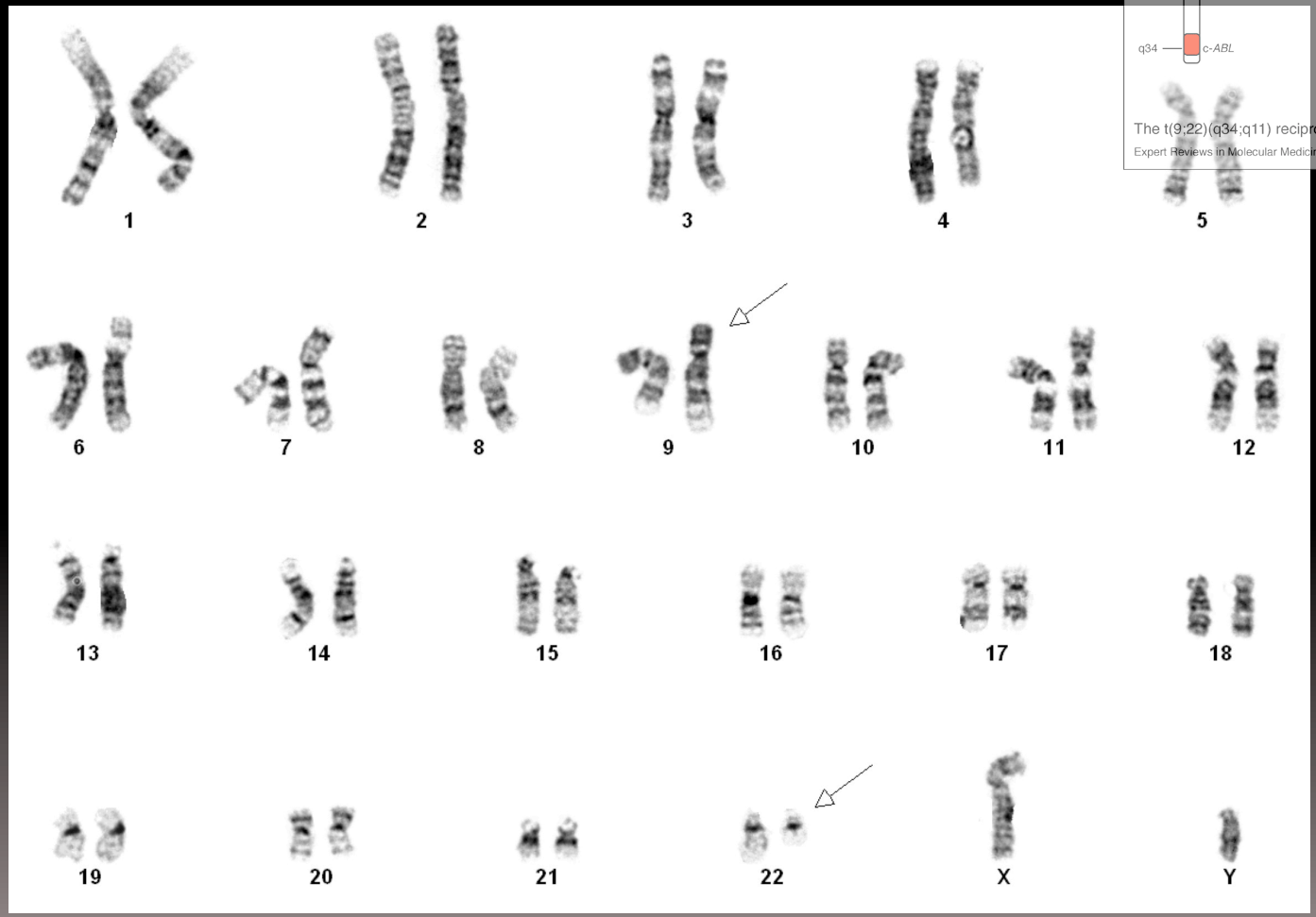
cut out chromosome pictures and arrange into karyotype

Adapted from N. Van Roy, BHS course

Anomalies du caryotype associées aux NMP: translocation balancée (9;22)

Exemple de la t(9;22)(q34;q11)

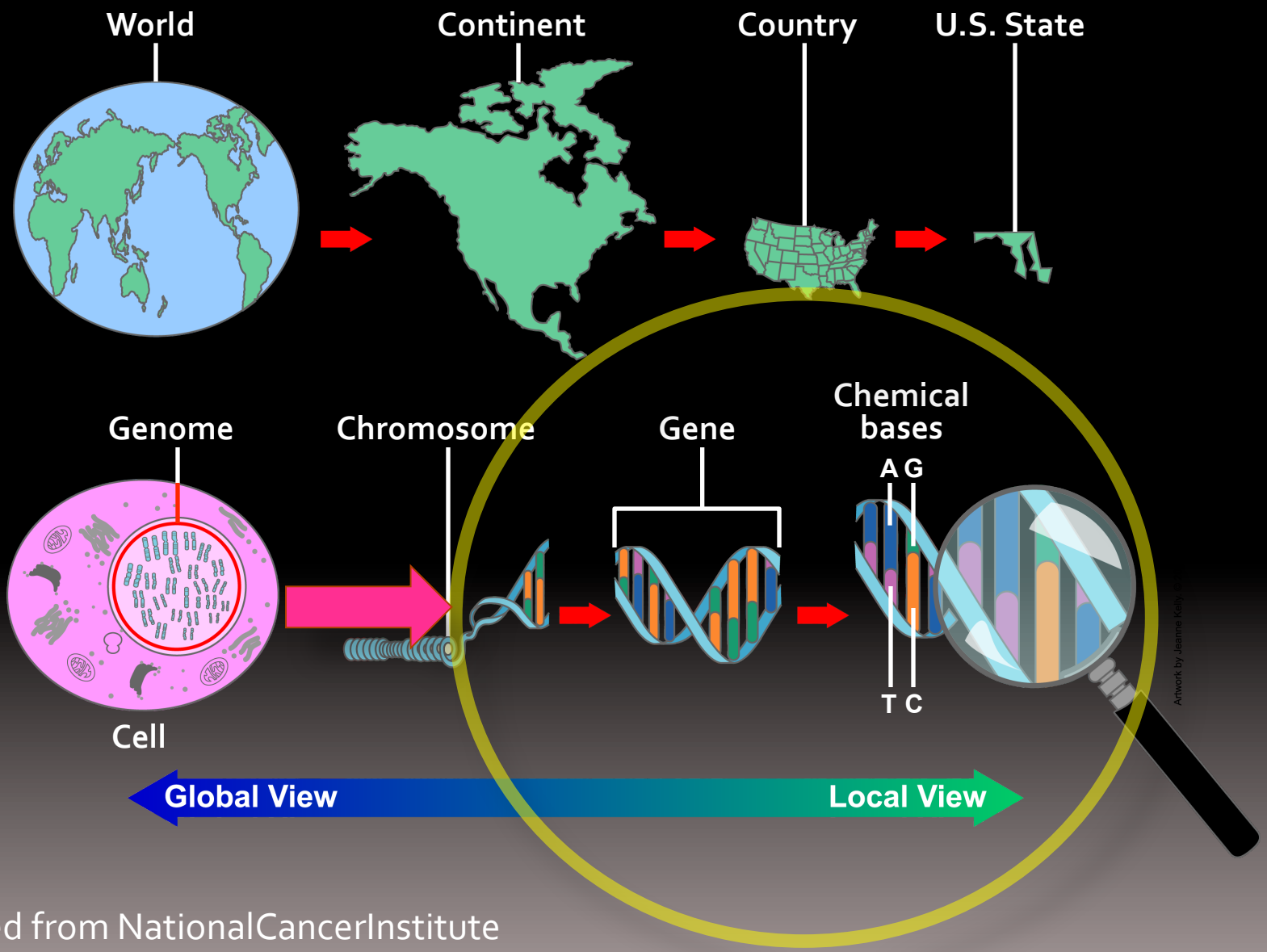




Plan

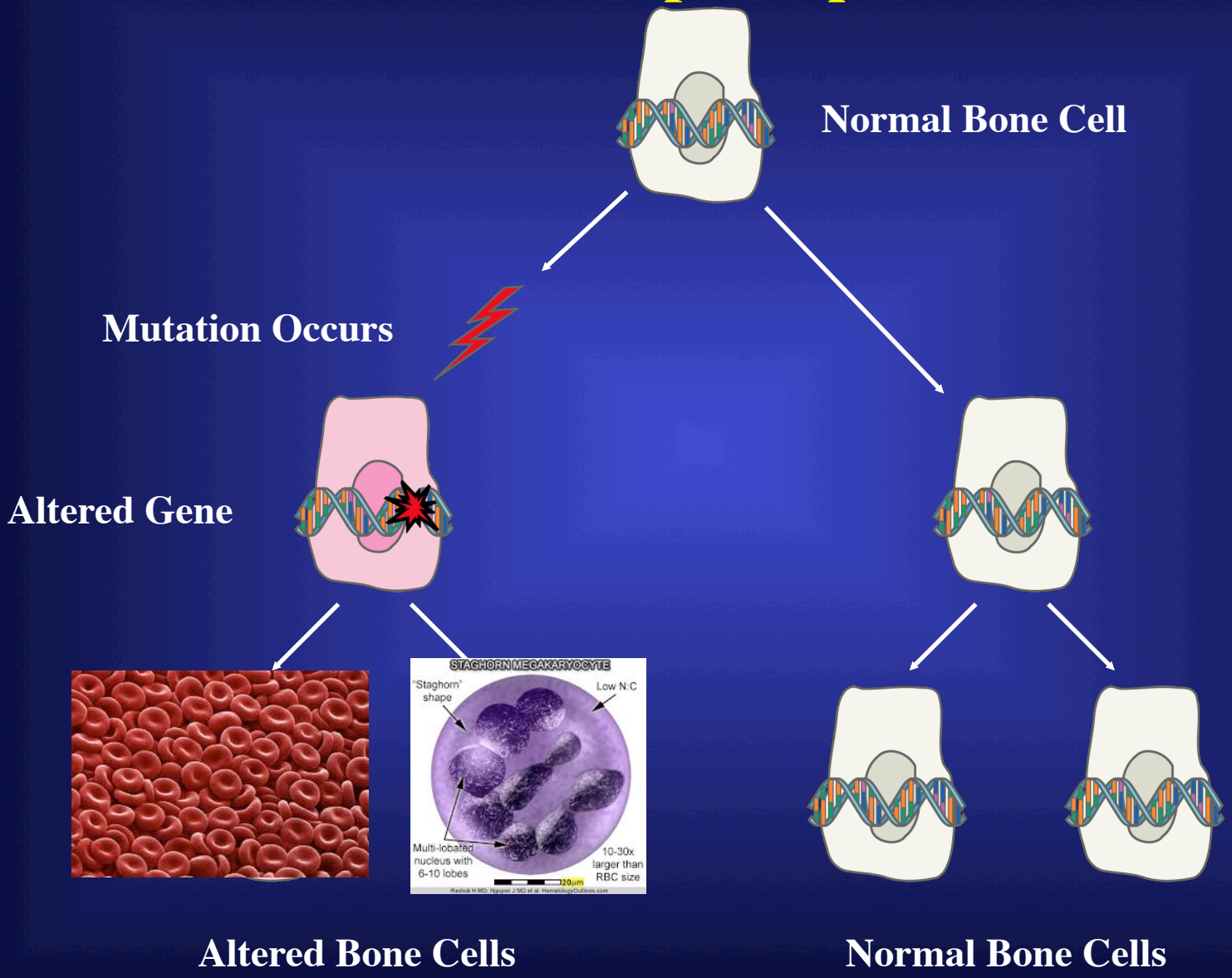
- Les "Néoplasies myéloprolifératives", qu'est-ce que c'est ?
- La démarche diagnostique (OMS, PVSG,…)
- Les outils du diagnostic:
  - L'examen clinique et l' "interrogatoire " (anamnèse)
  - La ponction de moelle osseuse hématopoïétique
  - La biopsie de moelle osseuse hématopoïétique
  - Les tests biochimiques (dosage d'érythropoïétine)
  - La cytométrie en flux
  - Les examens des chromosomes
  - Les examens de gènes
- Quels buts pour ces examens (diagnostic; choix du traitement; suivi de la maladie…)
- Conclusions

Les techniques de biologie moléculaire (gènes)

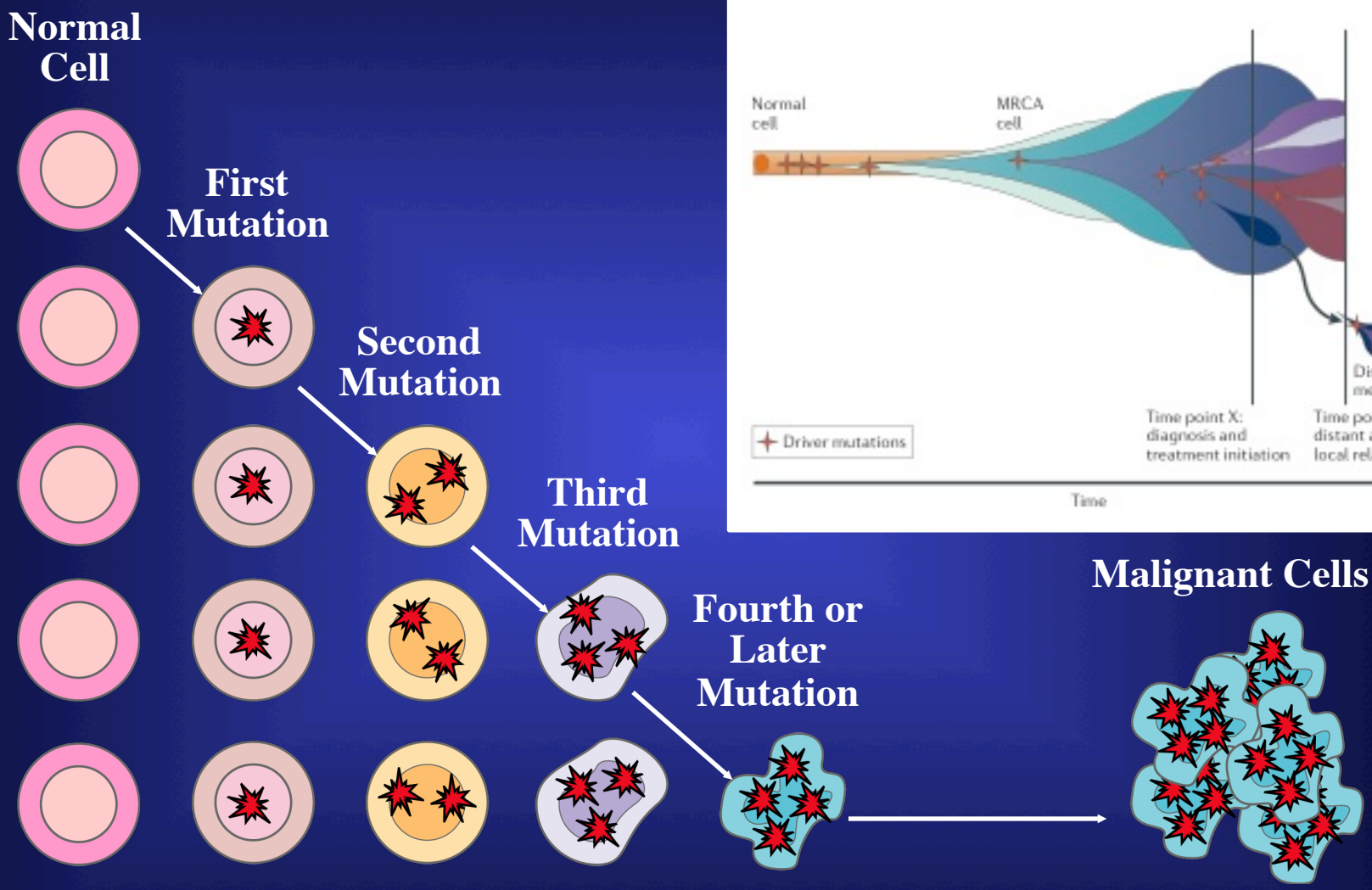


Adpated from NationalCancerInstitute

Mutations acquises par des cellules souches hématopoïétiques



Plusieures mutations coopèrent



Polycythemia vera

Major criteria

1. Hemoglobin >16.5 g/dL in men or >16.0 g/dL in women, OR hematocrit >49% in men or >48% in women, OR increased red cell mass (more than 25% above mean normal predicted value)
2. BM 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* (V617F) or *JAK2* exon 12 mutation

Minor criterion

- Subnormal serum erythropoietin level

Diagnosis of PV requires meeting all 3 major criteria or the first 2 major criteria and the minor criterion

Note. Criterion number 2 (BM biopsy) may not be required in cases with sustained absolute erythrocytosis: hemoglobin levels >18.5 g/dL in men (hematocrit, 55.5%) or >16.5 g/dL in women (hematocrit, 49.5%) if major criterion 3 and the minor criterion are present. However, initial myelofibrosis (present in up to 20% of patients) can only be detected by performing a BM biopsy; this finding may predict a more rapid progression to overt myelofibrosis (post-PV myelofibrosis)

Elisa Rumi, Mario Cazzola, Blood, 2016, DOI 10.1182/blood-2016-10-695957

Essential thrombocythemia

Major criteria

1. Platelet count ≥ 450 x 10^9/L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left-shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for *BCR-ABL1*-positive chronic myeloid leukemia, polycythemia vera, primary myelofibrosis, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2*, *CALR* or *MPL* mutation

Minor criterion

- Presence of a clonal marker or absence of evidence for reactive thrombocytosis

Diagnosis of ET requires meeting all four major criteria or the first 3 major criteria and the minor criterion

Elisa Rumi, Mario Cazzola, Blood, 2016, DOI 10.1182/blood-2016-10-695957

Overt primary myelofibrosis

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 BCR-ABL1-positive chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, 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 reticulin fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic myelopathies)

Minor criteria

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

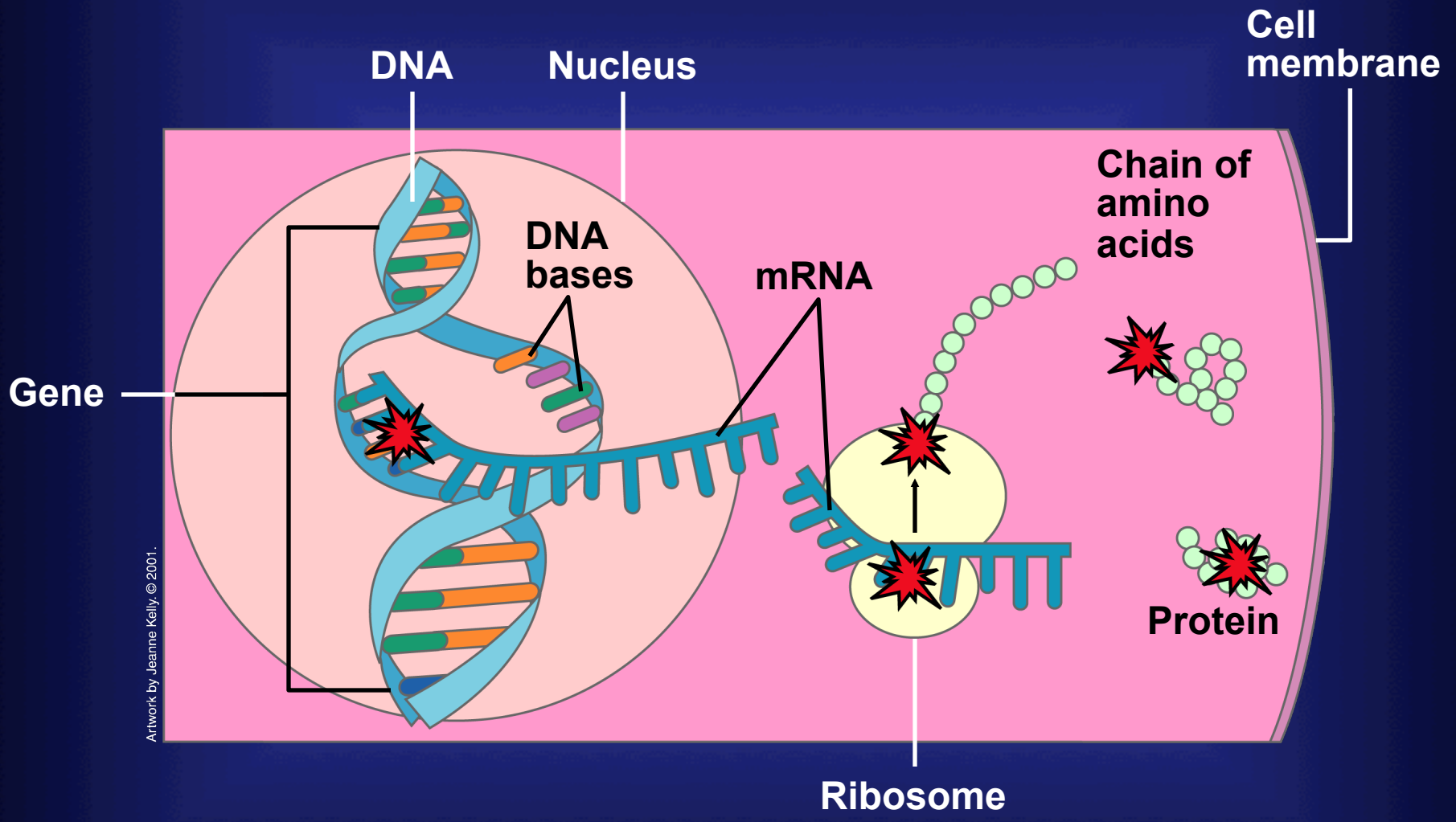
- Anemia not attributed to a comorbid condition
- Leukocytosis (WBC count ≥11 × 10^9/L)
- Palpable splenomegaly
- Lactate dehydrogenase (LDH) level increased to above upper normal limit of institutional reference range

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

Note. 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.

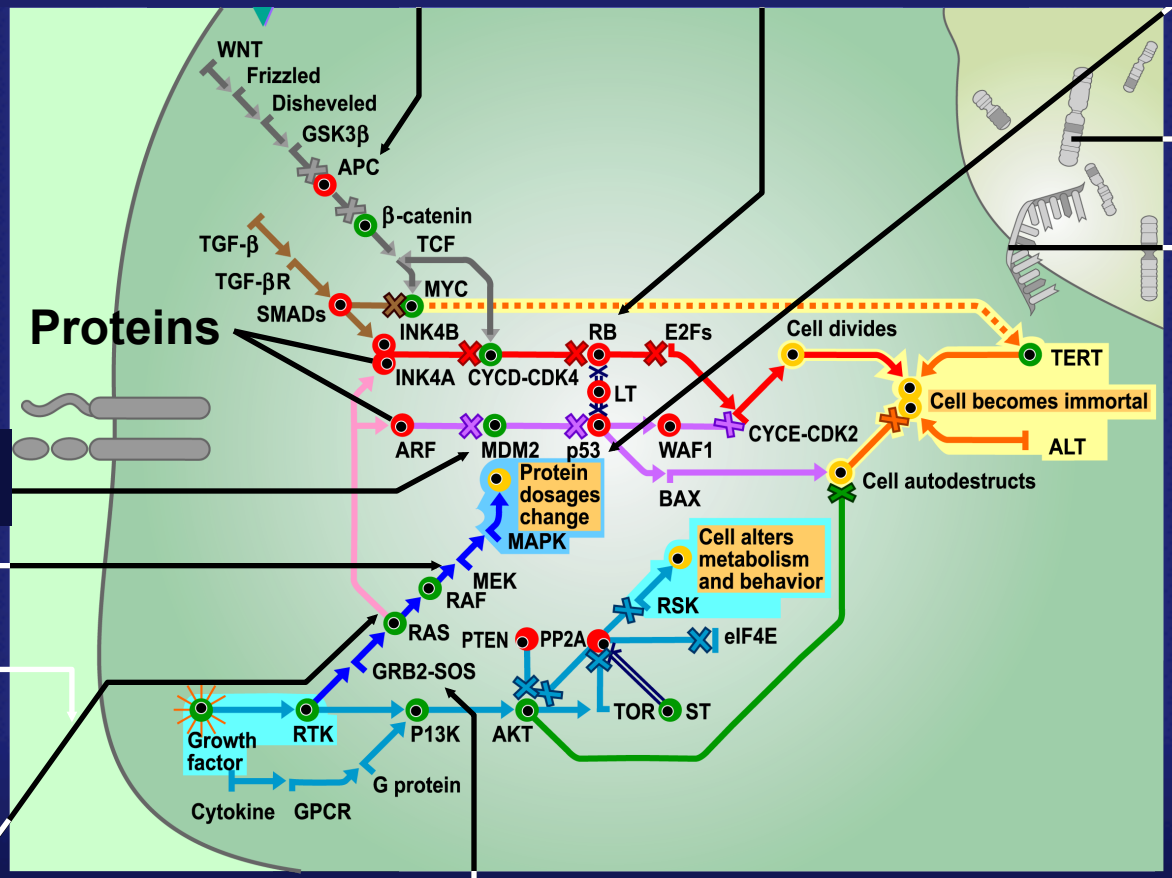
Elisa Rumi, Mario Cazzola, Blood, 2016, DOI 10.1182/blood-2016-10-695957

Gènes mutés -> Protéines modifiées

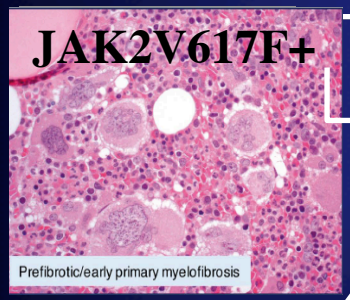


NMP: A Communication Failure

Les cytokines transmettent leurs signaux via une « voie de signalisation, la voie « JAK/STAT »

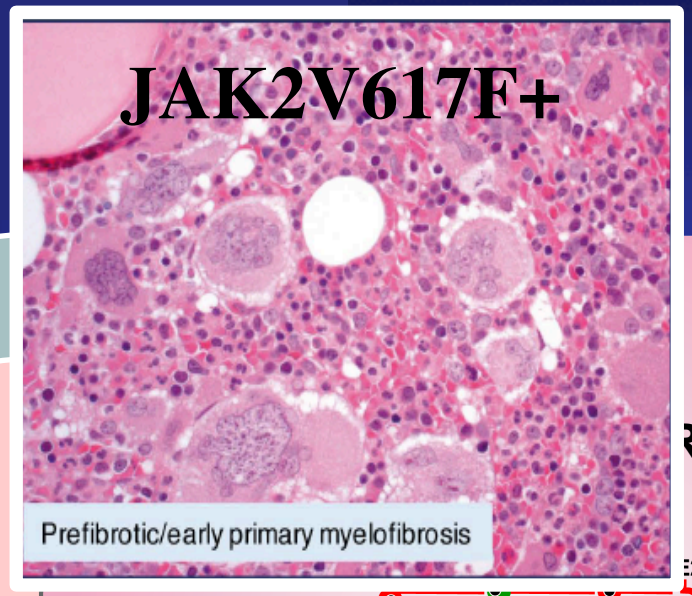


EPO/TPO



NMP

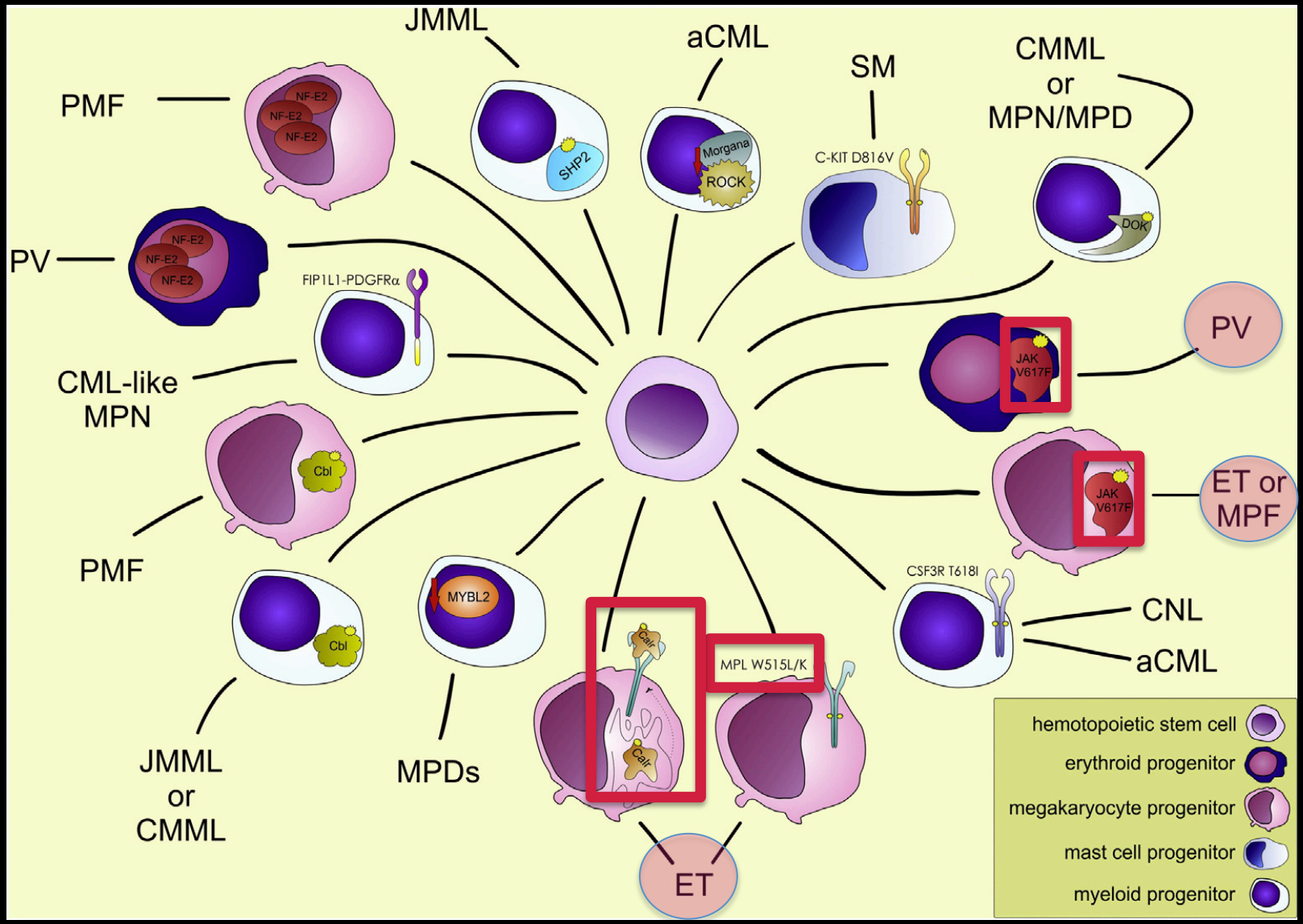
NMP: A Communication Failure



Some communications pathways

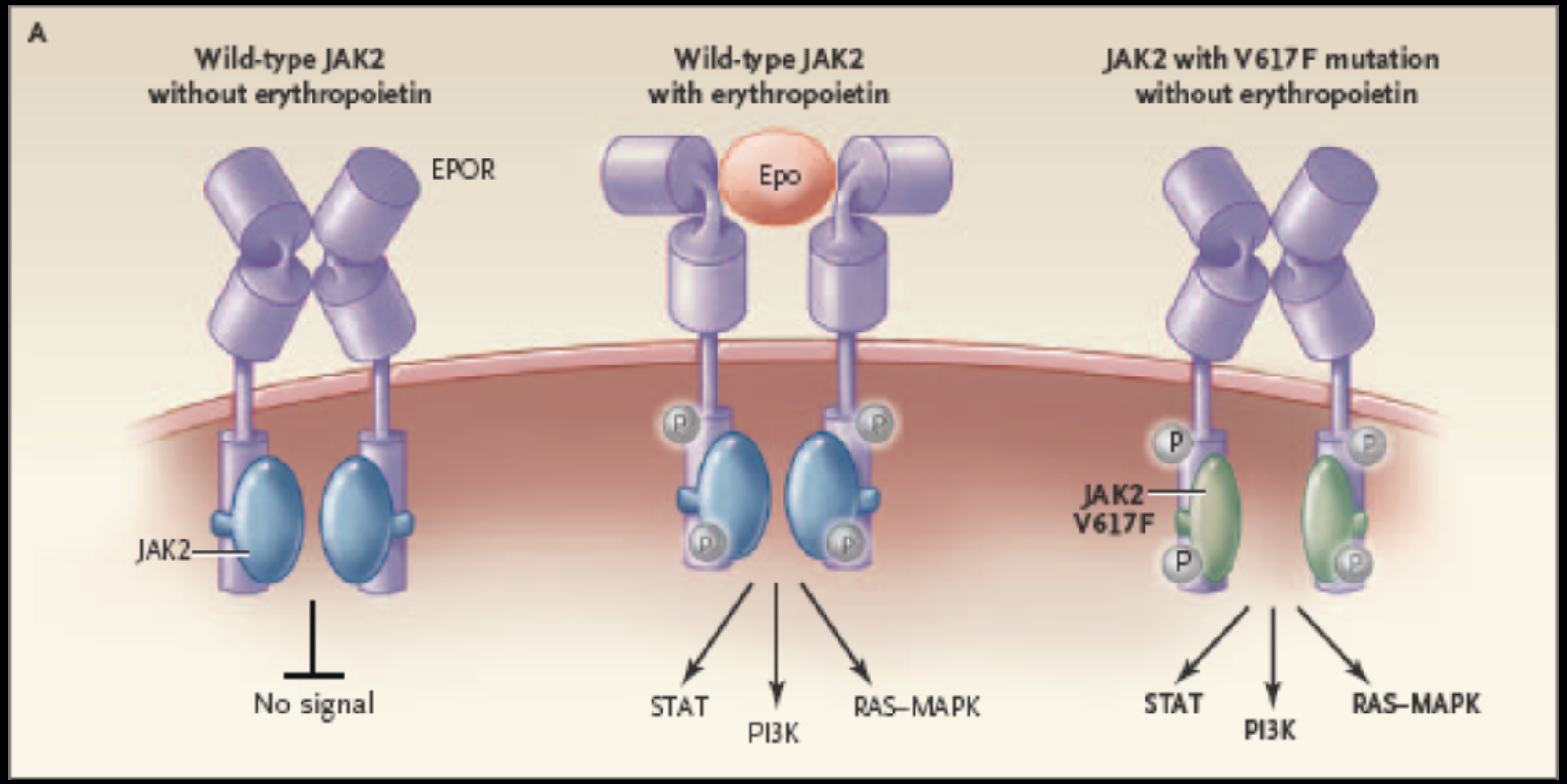
NATIONAL CANCER INSTITUTE

Origines de la production ANORMALES des cellules sanguines:



Morotti A, et al., Blood Rev (2016), http://dx.doi.org/10.1016/j.blre.2016.11.004

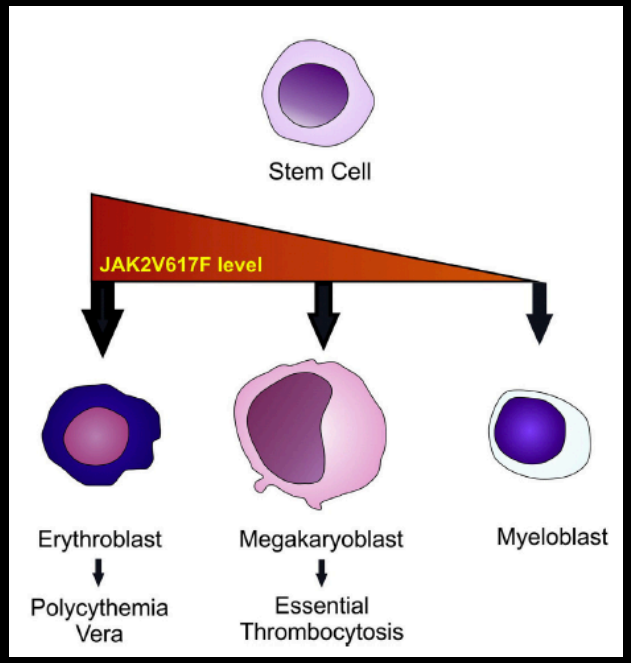
Physiopathologie moléculaire des NMP, l'impact de la mutation V617F *JAK2*

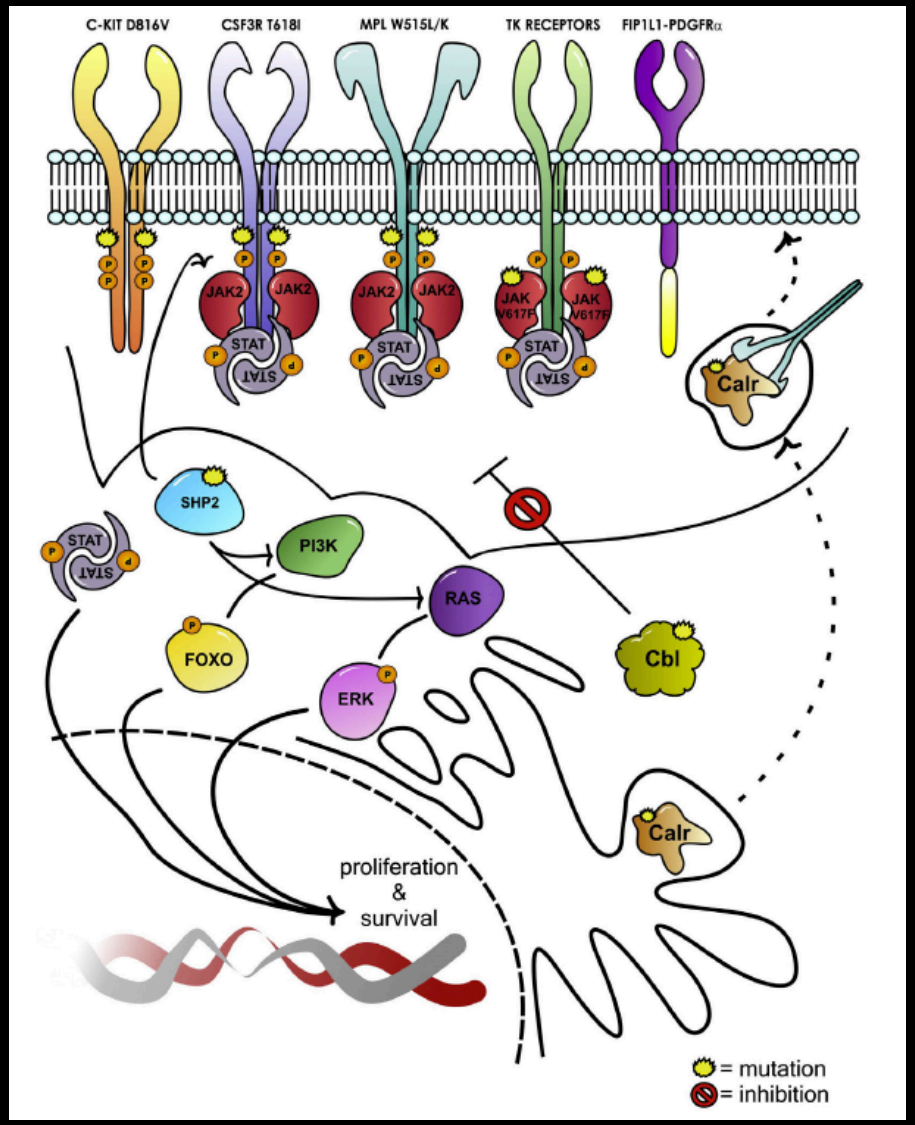


Activation de la voie de signalisation cellulaire JAK/STAT

Campbell, N Engl J Med 355;23 december 7, 2006

Physiopathologie moléculaire des NMP





Morotti A, et al, Blood Rev (2016), http://dx.doi.org/10.1016/j.blre.2016.11.004

Prefibrotic myelofibrosis

Major criteria

1. Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted bone marrow cellularity, granulocytic proliferation, and often decreased erythropoiesis
2. Not meeting WHO criteria for BCR-ABL1-positive chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, 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 reticulin fibrosis secondary to infection, autoimmune disorder or other chronic inflammatory conditions, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic myelopathies)

Minor criteria

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

- Anemia not attributed to a comorbid condition
- Leukocytosis (WBC count ≥11 × 10^9/L)
- Palpable splenomegaly
- Lactate dehydrogenase (LDH) level increased to above upper normal limit of institutional reference range

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

Note. 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.

Elisa Rumi, Mario Cazzola, Blood, 2016, DOI 10.1182/blood-2016-10-695957

Plan

- Les "syndromes myéloprolifératifs", qu'est-ce que c'est ?
- La démarche diagnostique (OMS, PVSG,…)
- Les outils du diagnostic:
  - L'examen clinique et l' "interrogatoire " (anamnèse)
  - la « prise de sang »
  - La ponction de moelle osseuse hématopoïétique
  - La biopsie de moelle osseuse hématopoïétique
  - La cytométrie en flux
  - Les examens des chromosomes
  - Les examens de gènes
  - Les tests biochimiques (dosage d'érythropoïétine)
- Quels buts pour ces examens (diagnostic; choix du traitement; suivi de la maladie…)
- Conclusions

Les tests biochimiques:

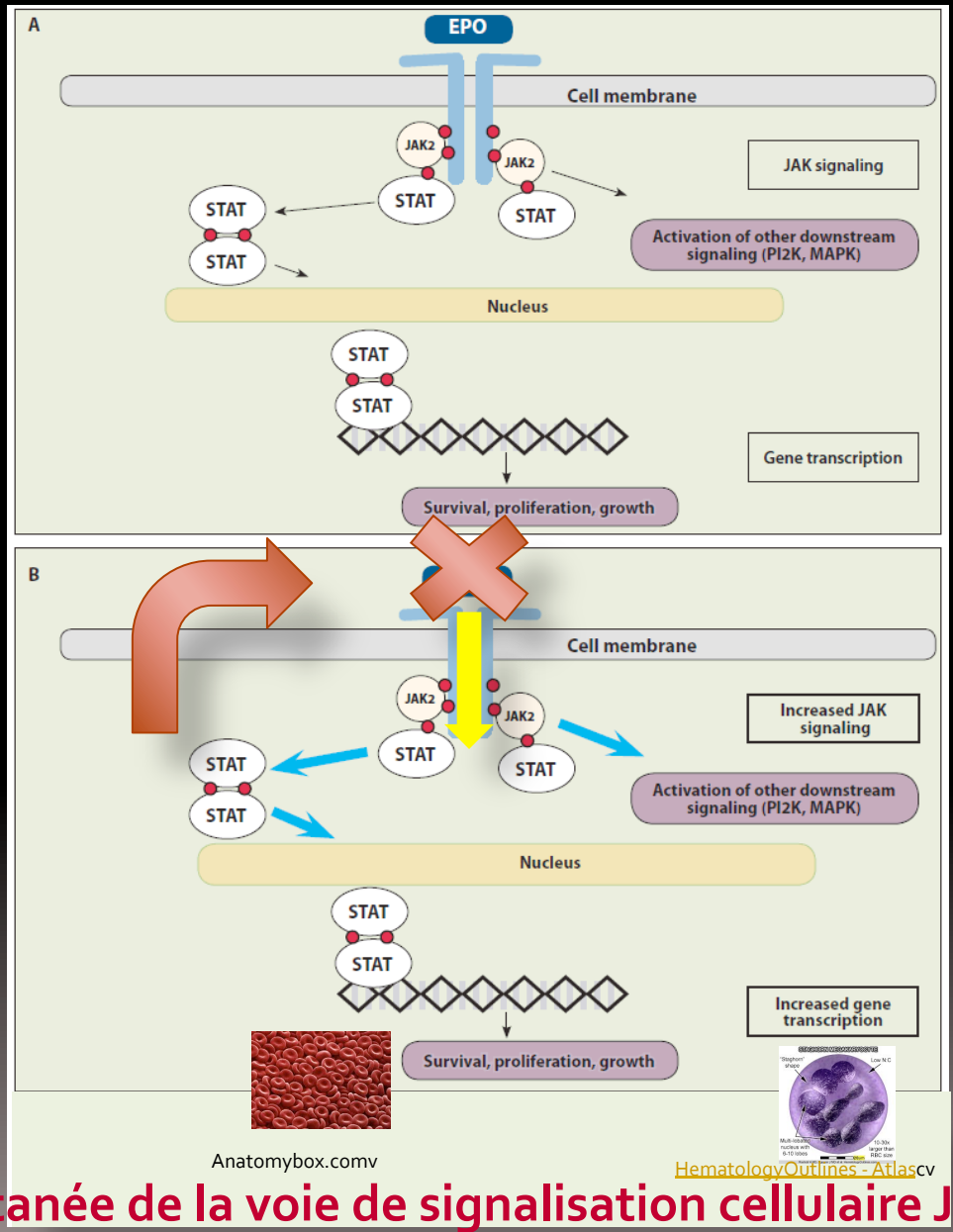
dosage d'érythropoïétine, lactate déshydrogènase (LDH)

	Polycythemia Vera (PV)	Essential Thrombocythemia (ET)		Primary Myelofibrosis (PMF) (overt)	Primary Myelofibrosis (prefibrotic) (prePMF)
Minor criteria	1. Subnormal serum erythropoietin level	1. Presence of a clonal marker or absence of evidence for reactive thrombocytosis	1	1. Anemia not otherwise attributed 2. Leukocytosis ≥11 x 10^9/L 3. Palpable splenomegaly 4. Increased lactate dehydrogenase (LDH), above upper normal limit 5. Leukoerythroblastosis	1. Anemia not otherwise attributed 2. Leukocytosis ≥11 x 10^9/L 3. Palpable splenomegaly 4. Increased lactate dehydrogenase (LDH), above upper normal limit

Tefferi A et al., American Journal of Hematology, Vol. 92, No. 1, January 2017

Physiopathologie moléculaire

Taux EPO sérique ↓



Anatomybox.comv

HematologyOutlines - Atlascv

Activation spontanée de la voie de signalisation cellulaire JAK/STAT

http://www.cancernetwork.com/oncology-journal/clarifying-use-ruxolitinib-patients-myelofibrosis

Plan

- Les "syndromes myéloprolifératifs", qu'est-ce que c'est ?
- La démarche diagnostique (OMS, PVSG,…)
- Les outils du diagnostic:
  - L'examen clinique et l' "interrogatoire " (anamnèse)
  - La « prise de sang »
  - La ponction de moelle osseuse hématopoïétique
  - La biopsie de moelle osseuse hématopoïétique
  - Les tests biochimiques (dosage d'érythropoïétine)
  - La cytométrie en flux
  - Les examens des chromosomes
  - Les examens de gènes
- Quels buts pour ces examens (diagnostic; choix du traitement, suivi de la maladie…)
- Conclusions

De l'empirisme (diagnostic)

à la médecine basée sur les évidences (« evidence based »)

Erythrocytose

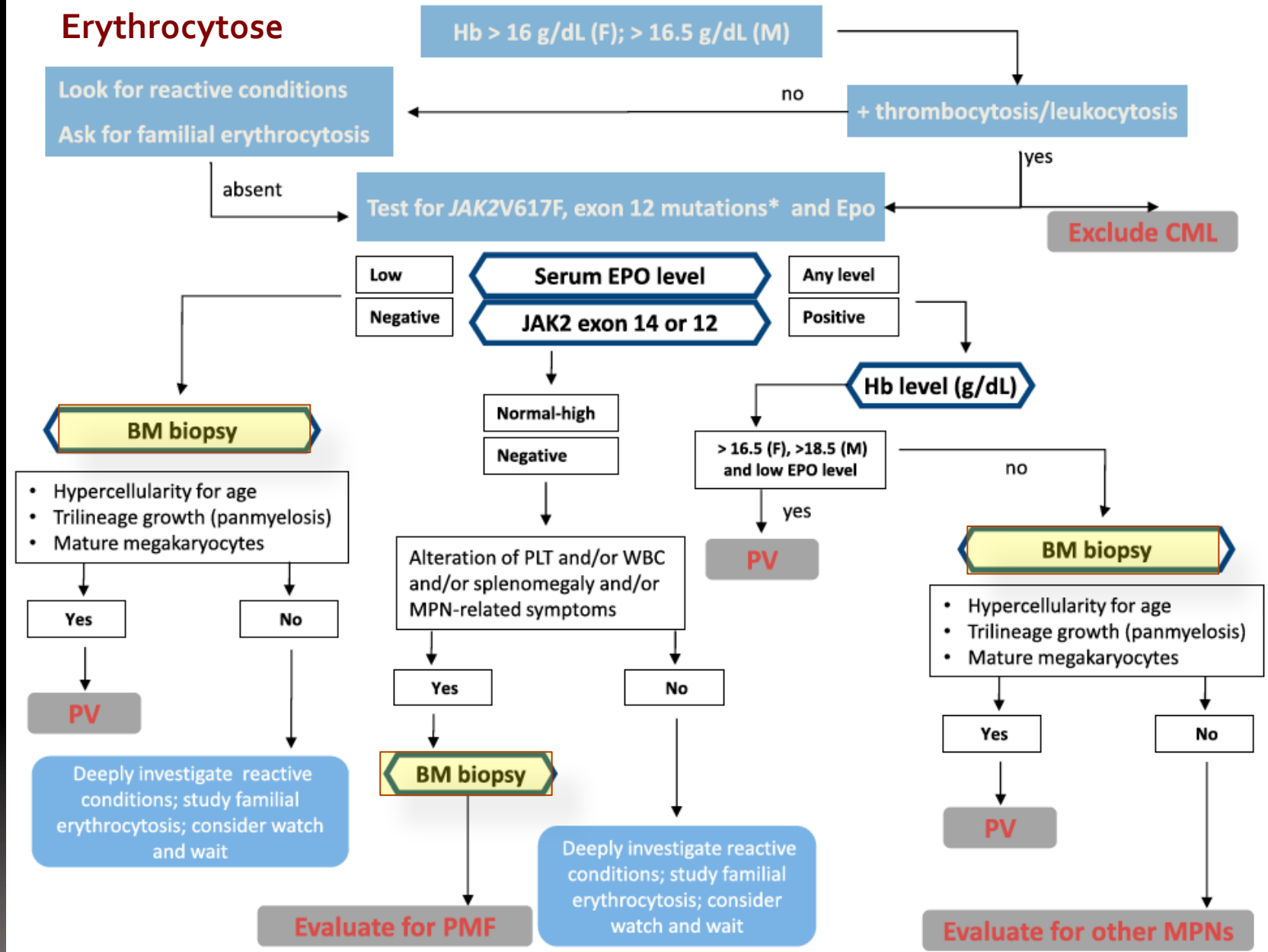


Figure 1. Algorithm for evaluation of MPN phenotype with erythrocytosis. *Test *JAK2* V617F first and exon 12 *JAK2* mutations if V617F is negative. CML, chronic myeloid leukemia; Epo, erythropoietin; F, female; M, male; MK, megakaryocytes; PLT, platelet; WBC, white blood cell.

Francesco Passamonti and Margherita Maffioli, Hematology 2016

Thrombocytose

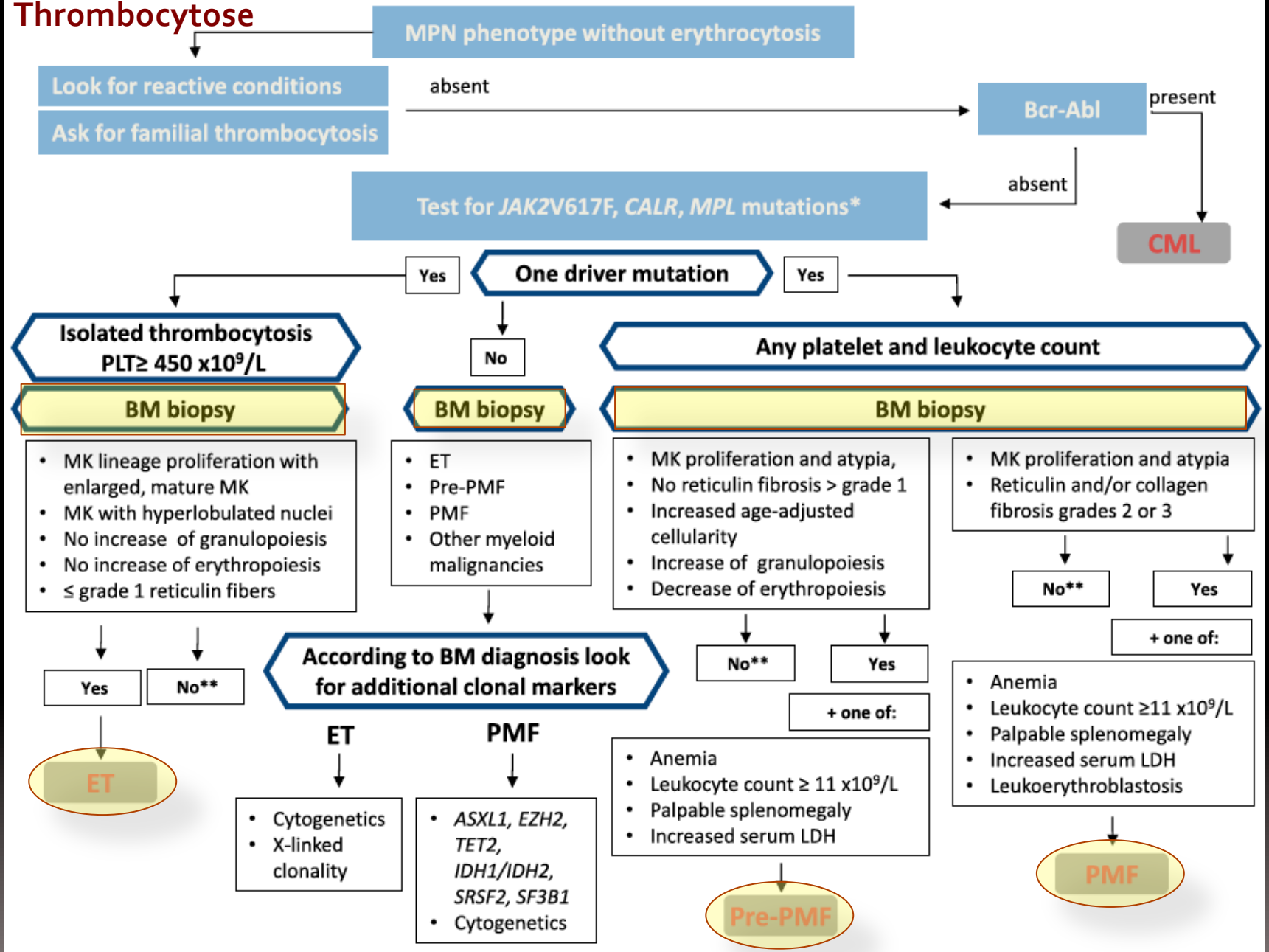


Figure 2. Algorithm for evaluation of MPN phenotype without erythrocytosis. *Test *JAK2* V617F first, *CALR* mutations if V617F is negative, and *MPL* mutations if *JAK2* and *CALR* are negative. **Evaluate for MPN, MDS, MDS/MPN, or other myeloid malignancies.

Francesco Passamonti and Margherita Maffioli, Hematology 2016

Un diagnostic précis: un préalable à une prise en charge personnalisée

Essential thrombocythemia

Thrombosis:
- previous thrombosis
- age ≥ 60 years
- *JAK2* (V617F)

Bleeding:
- previous major bleeding
- high PLT count (≥1500 x 10^9/L)

Polycythemic transformation:
- *JAK2* (V617F)

Myelofibrotic transformation:
- *CALR* mutation
- co-operating mutations in myeloid genes

Leukemic transformation:
- co-operating mutations in myeloid genes

Survival:
- previous thrombosis
- leukocytosis
- co-operating mutations in myeloid genes

Polycythemia vera

Thrombosis:
- previous thrombosis
- age ≥ 60 years

Myelofibrotic transformation:
- *JAK2* (V617F)-mutant allele burden >50%
- co-operating mutations in myeloid genes

Leukemic transformation:
- co-operating mutations in myeloid genes

Survival:
- previous thrombosis
- leukocytosis
- co-operating mutations in myeloid genes

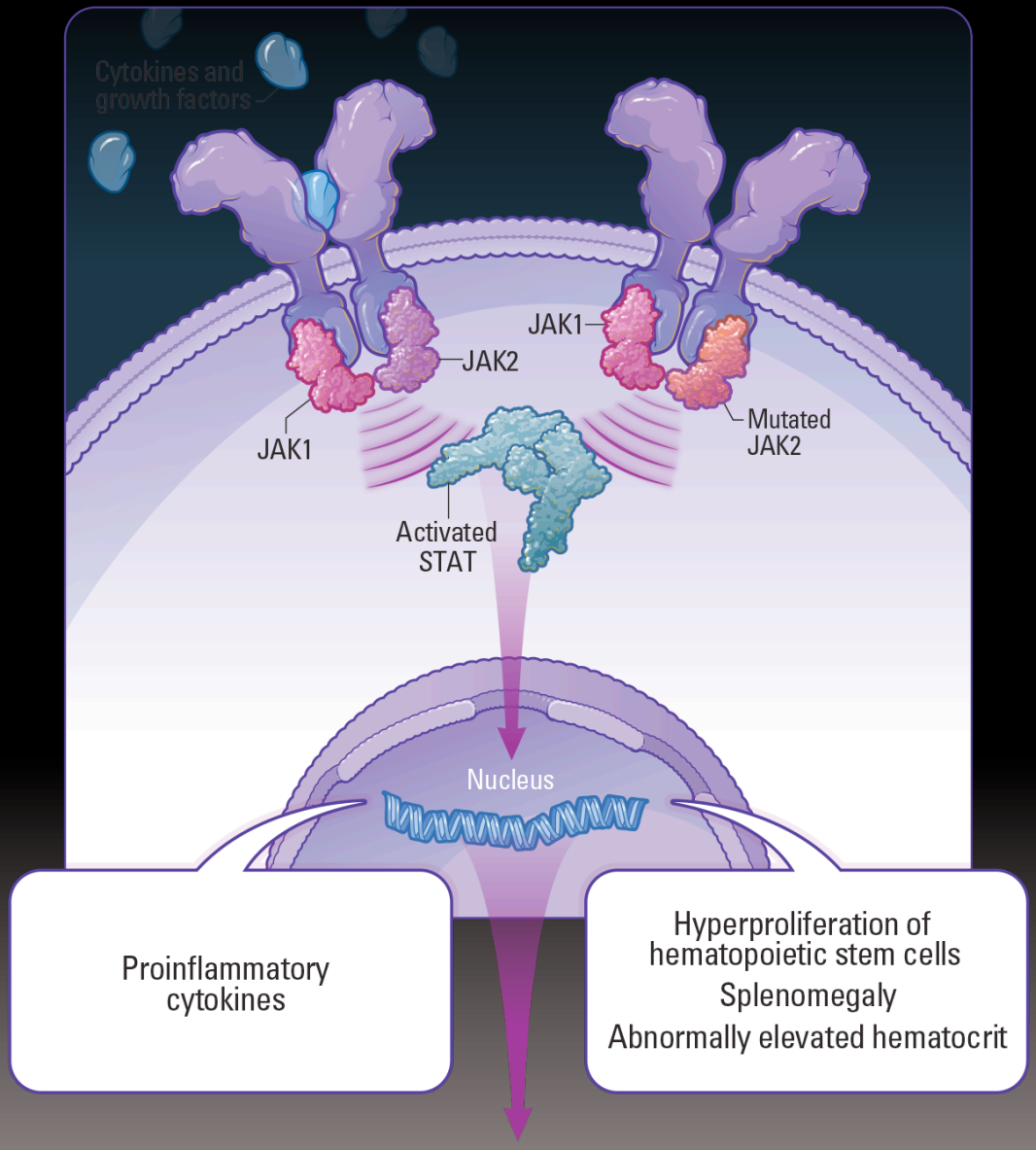
Primary myelofibrosis

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

Figure 5

E. Rumi and M. Cazzola, Blood, Prepublished online December 27, 2016

Les médicaments des thérapies « ciblées »



Jakafi.com

Plan

- Les "syndromes myéloprolifératifs", qu'est-ce que c'est ?
- La démarche diagnostique (OMS, PVSG,…)
- Les outils du diagnostic:
  - L'examen clinique et l' "interrogatoire " (anamnèse)
  - La « prise de sang »
  - La ponction de moelle osseuse hématopoïétique
  - La biopsie de moelle osseuse hématopoïétique
  - La cytométrie en flux
  - Les examens des chromosomes
  - Les examens de gènes
  - Les tests biochimiques (dosage d'érythropoïétine)
- Quels buts pour ces examens (diagnostic: choix du traitement, suivi de la maladie…)
- Conclusions

Conclusions

- Néoplasies myéloprolifératives = groupe hétérogène de maladies;
- Démarche diagnostique définie par OMS/PVSC/ BCSH;
- Un diagnostic précis permet de proposer un traitement adapté – personnalisé;
- Des facteurs pronostics et prédictifs cytogénétiques et moléculaires sont dorénavant intégrés aux décisions thérapeutiques aux cotés de l'histologie;
- Les perspectives sont d'adapter les traitements à chaque patient, selon son diagnostic précis et les anomalies spécifiques identifiées chez lui = « médecine personnalisée ».

MERCI POUR VOTRE ATTENTION
DANK U VOOR UW AANDACHT

QUESTIONS / VRAGEN ?