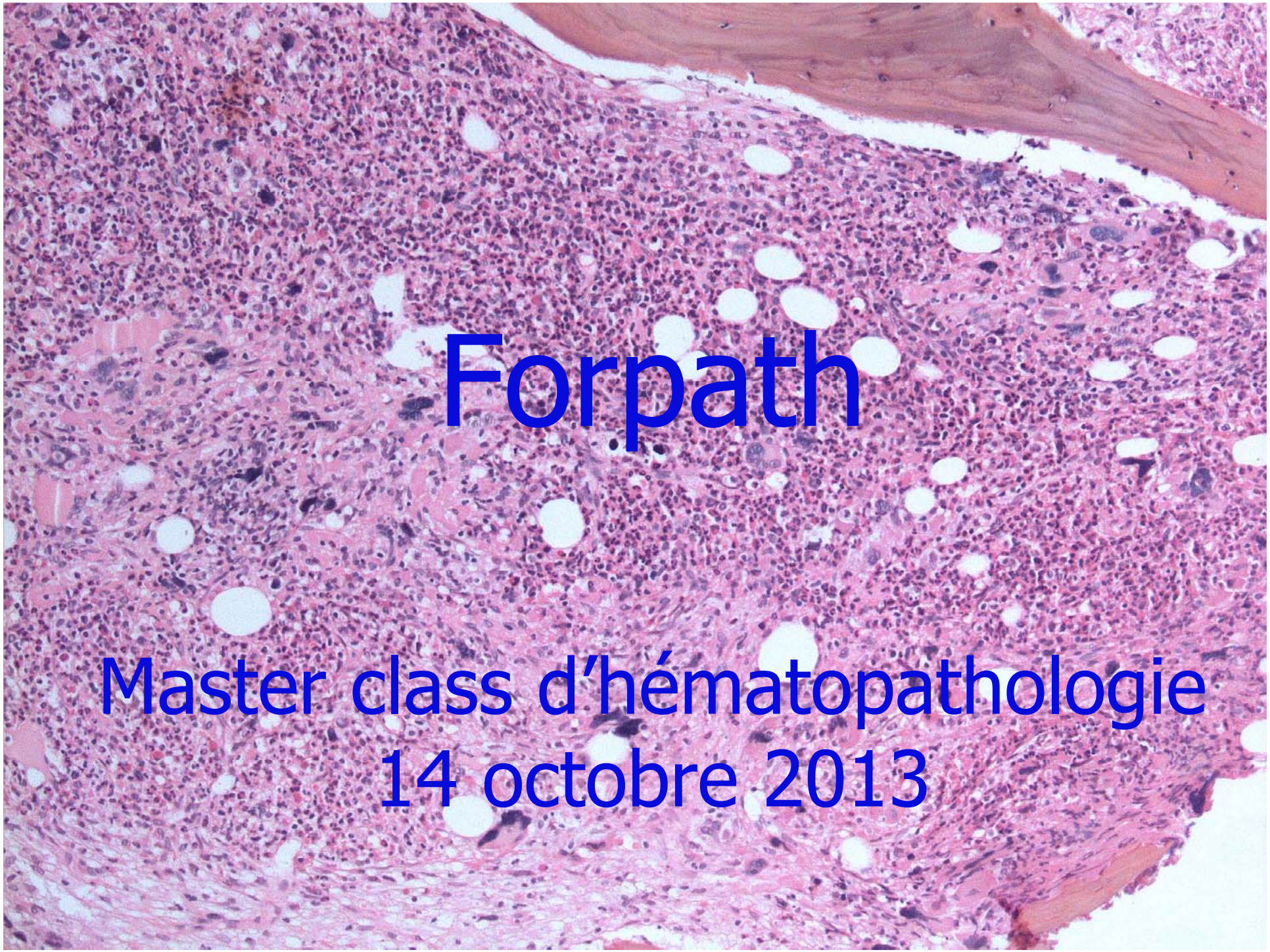


Forpath

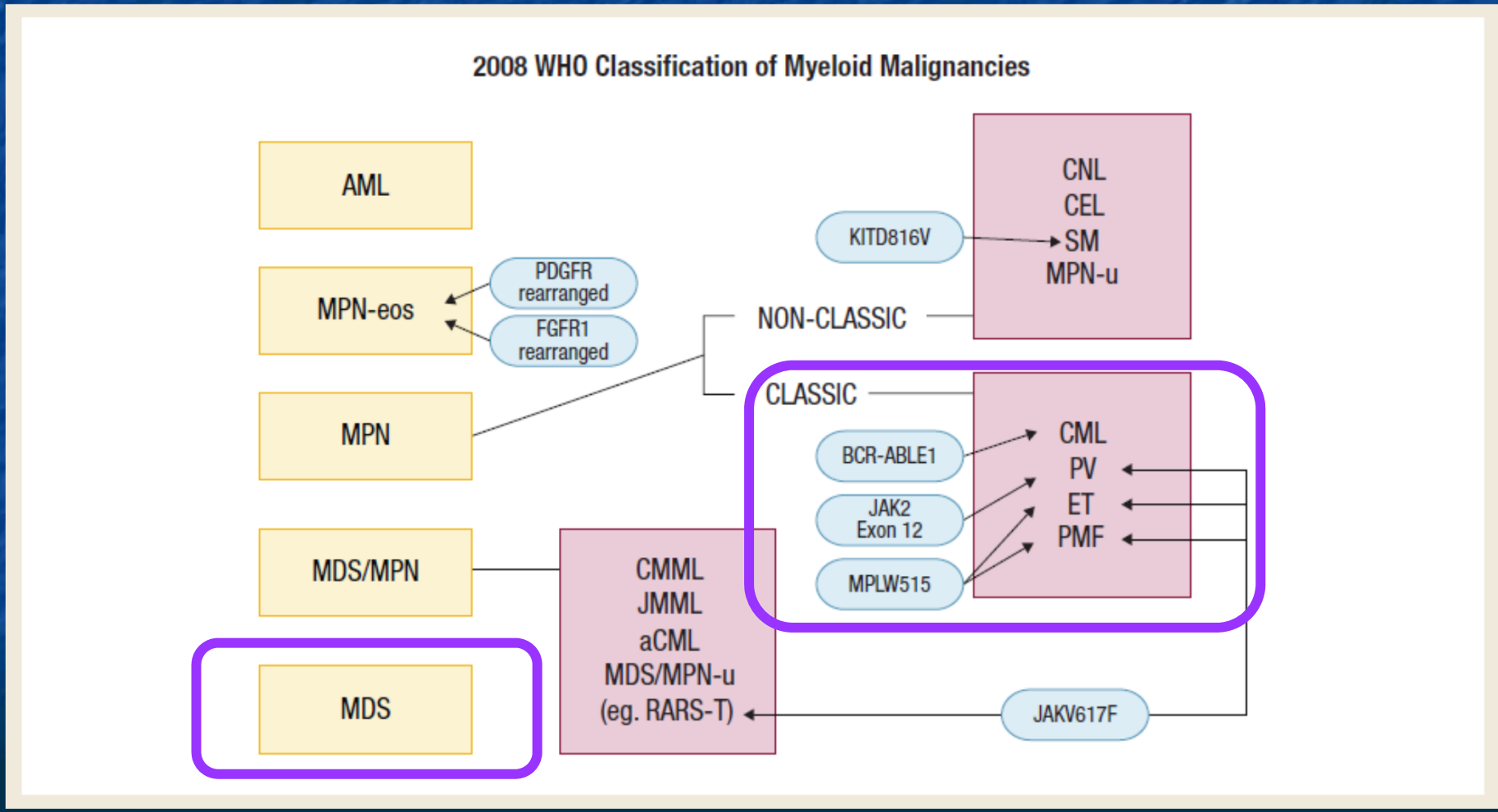
Master class d'hématopathologie
14 octobre 2013

Néoplasies myéloprolifératives Syndromes myélodysplasiques

Aspects morphologiques, moléculaires et génétiques

M.F. Dehou, B.Dessars

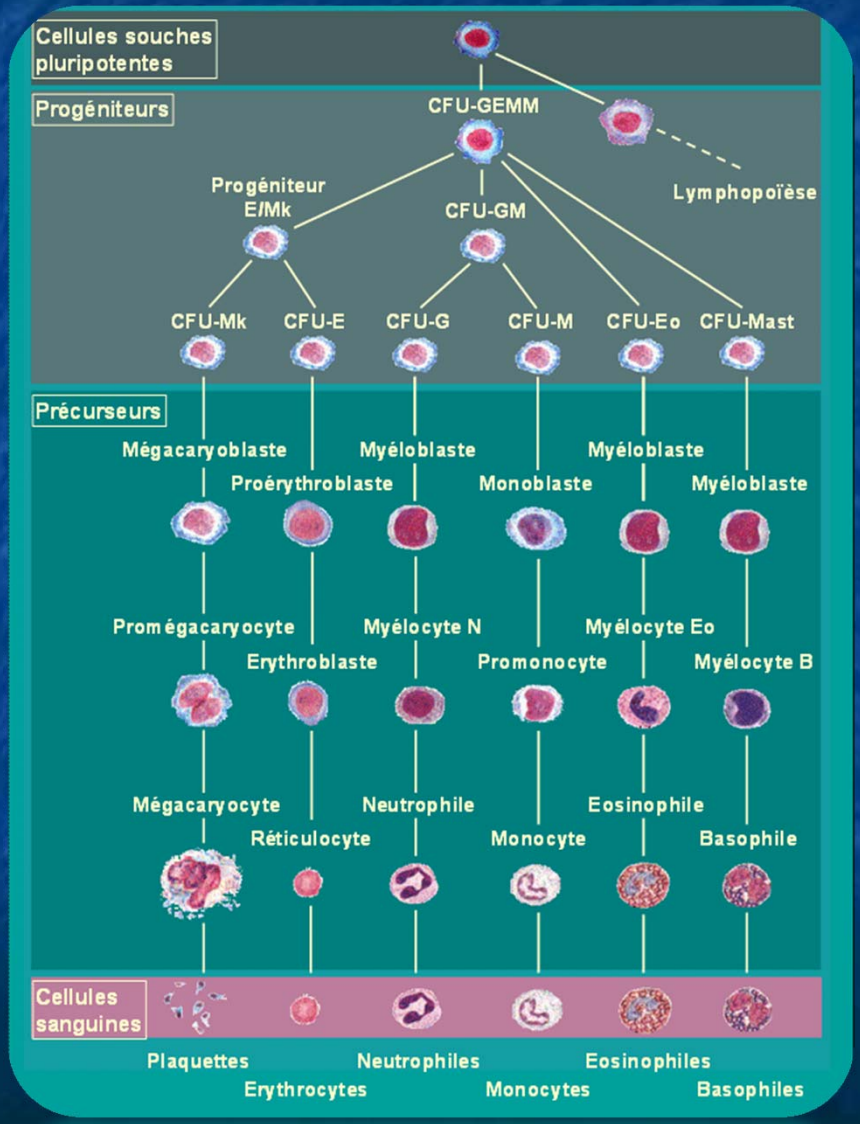
Hémopathies myéloïdes classification OMS 2008



Vakil E and Tefferi A, *Clinical Lymphoma, Myeloma & Leukemia* 2011, Vol.11, N° S1, S37-45

MPN

- LMC
  - Cellule souche pluripotente médullaire



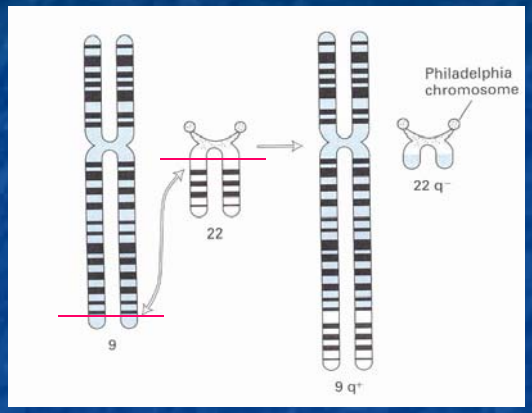
- Clinique :
  - 20-40% = asymptomatiques
  - Fatigue, perte de poids, sudations nocturnes, splénomégalie

  - Phase chronique :
    - leucocytose (>12x10E09/L) (neutrophiles)
    - neutrophiles à différents stades de maturation (pic myélo et PN segmentés)
    - Pas de dysplasie significative
    - En général <2% blastes PB
    - Monocytose, en général <3% (sauf p190)
    - PLT : N, augmentées, rarement diminuées

    → Phase accélérée

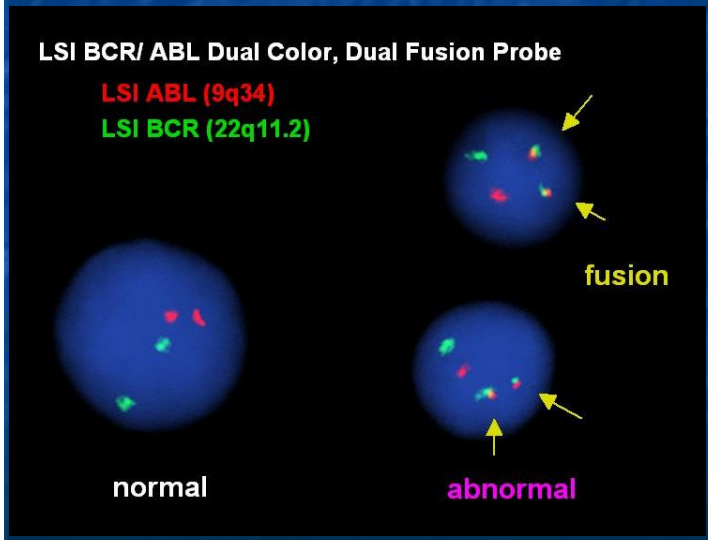
    → Phase blastique

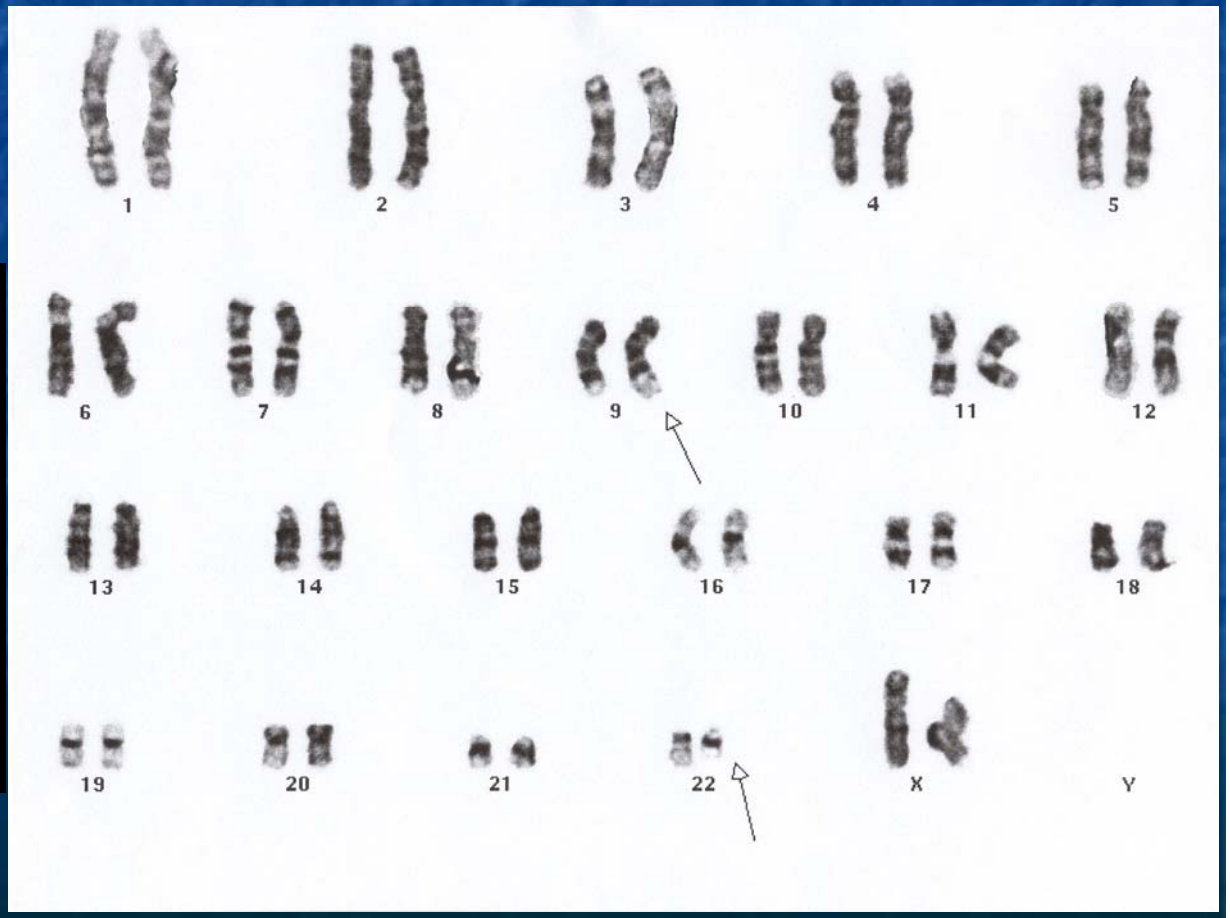
- Gène de fusion BCR-ABL1 sur le chr Ph :
  dans TOUS les cas par FISH (sang / moelle)



90-95% : t(9;22)(q34;q11)

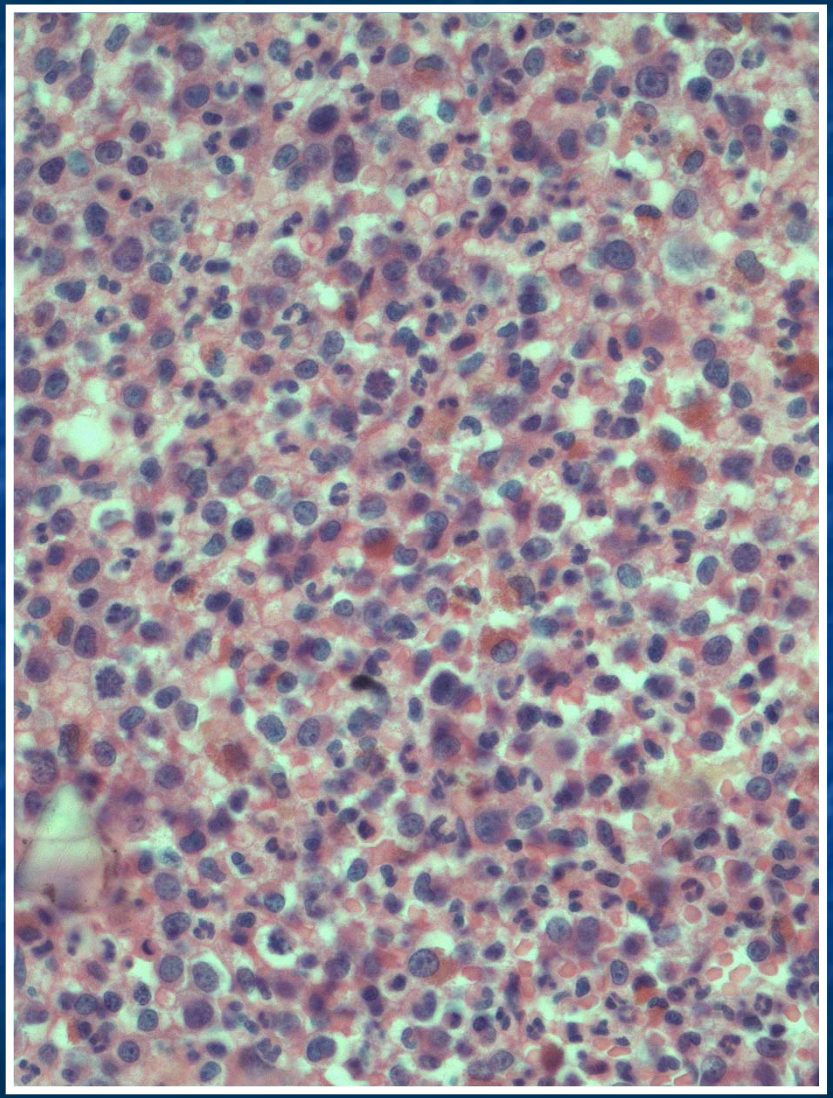
5-10%: transloc cryptique
variant (3è ou 4è chr)

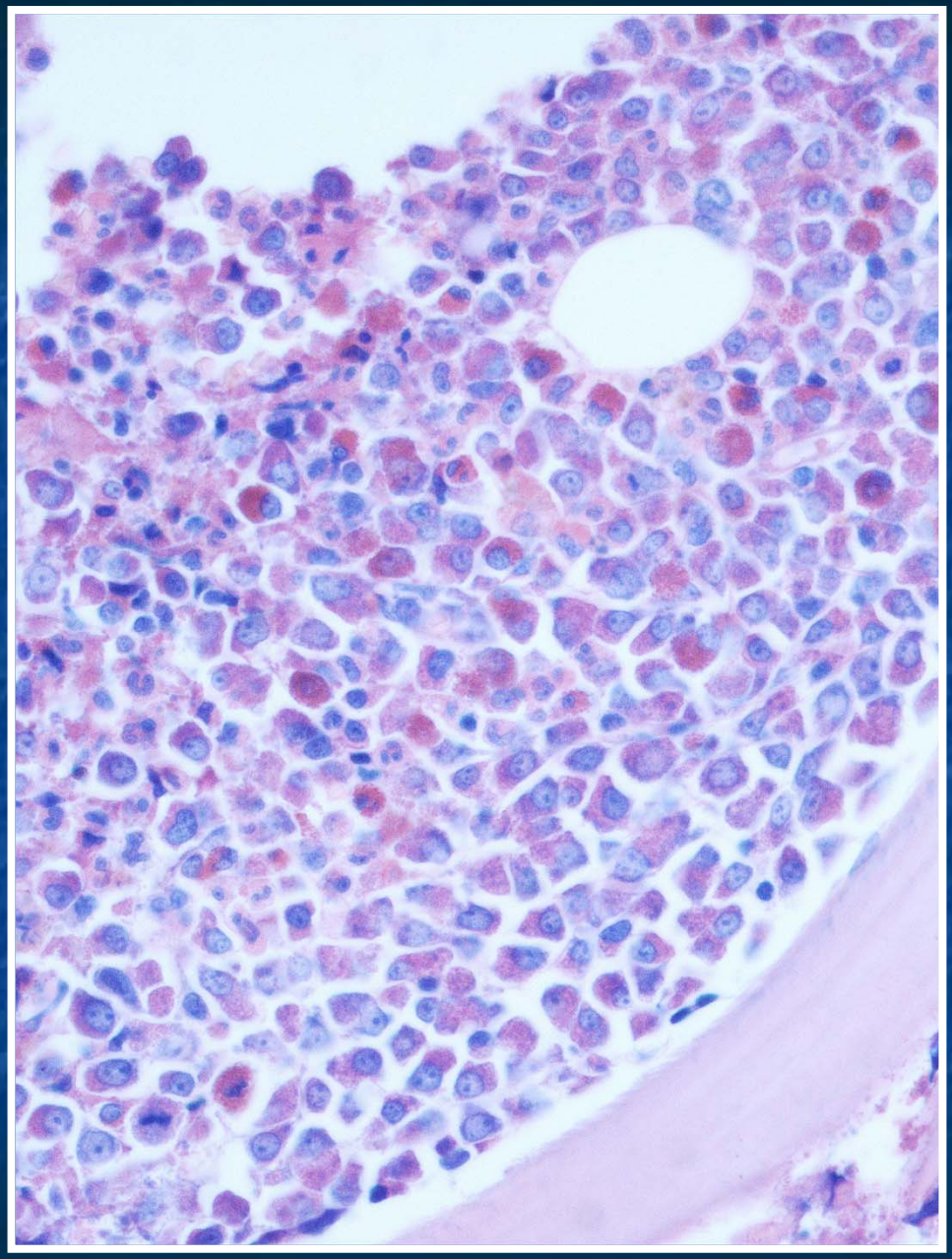


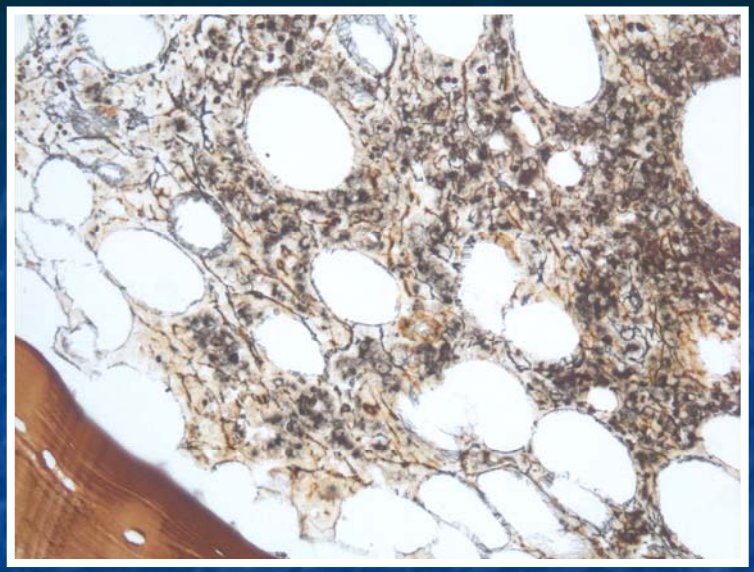


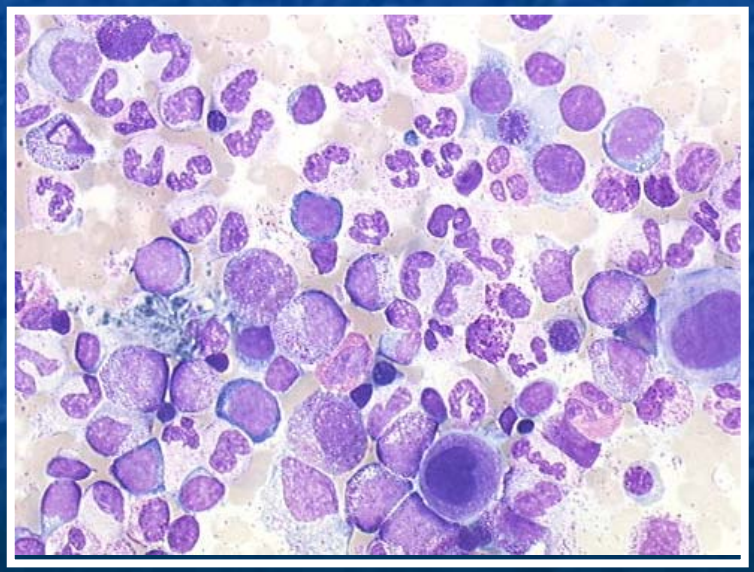
Morphologie

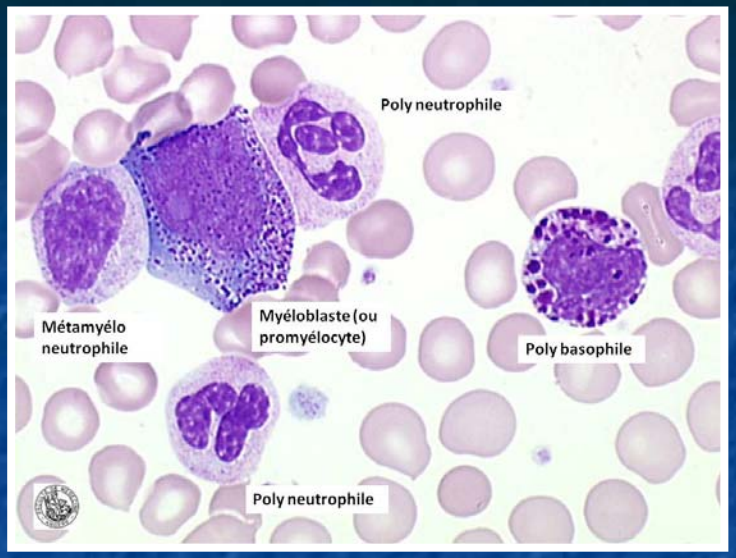
- Moelle hypercellulaire
- Prédominance de myélocytes et de neutrophiles
- Souvent élargissement de la zone paratrabéculaire de précurseurs myéloïdes
- Pas de signes évidents de dysplasie
- Erythropoièse svt un peu diminuée
- Mégacaryocytes : svt plus petits, en nb N ou lgt ↑



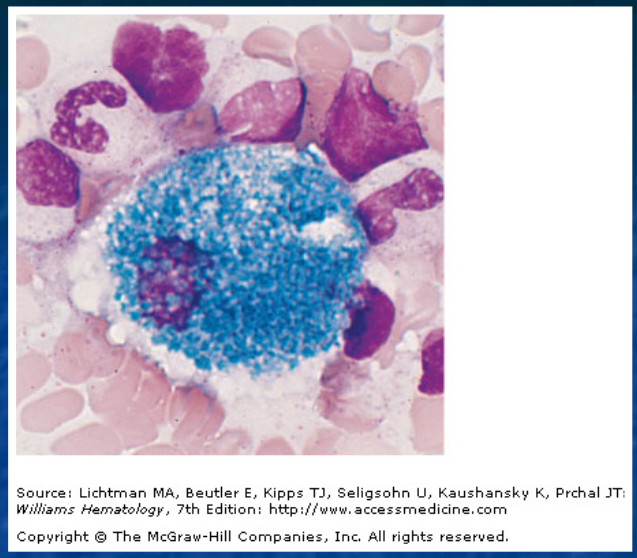






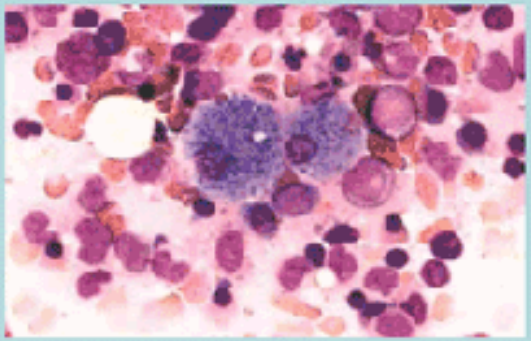


Excès de basophiles : sang périphérique, frottis médullaire, rarement vus dans la bom



Présence de macrophages en nb augmenté, dont des sea blue histiocytes

A pair of pseudo-Gaucher cells are seen in a bone marrow aspirate from a patient with CML



Maslak, P. ASH Image Bank 2002;2002:100469.
Copyright© 2002 American Society of Hematology.

BOM et LMC : intérêt

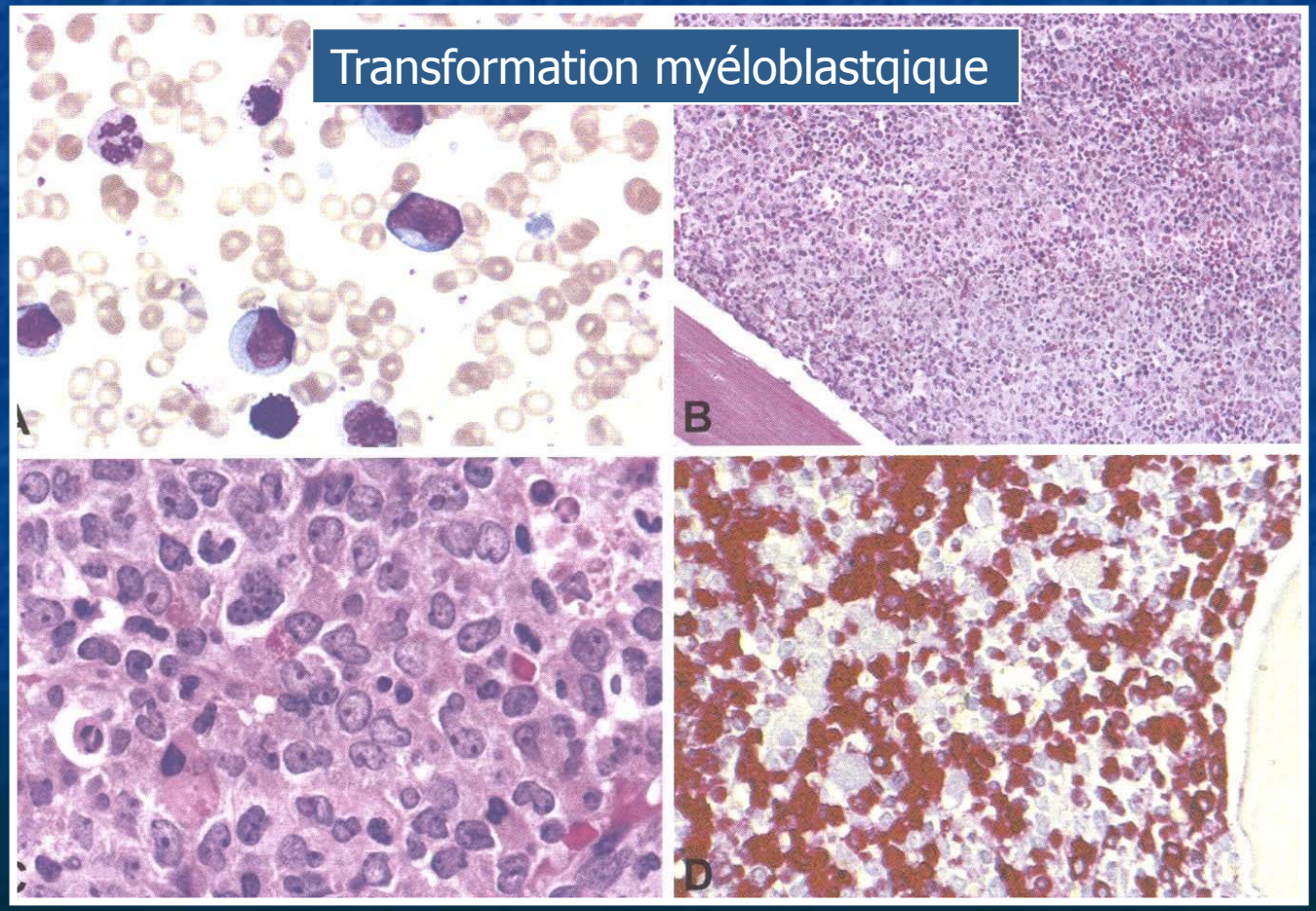
- Confirmation de l'hypercellularité médullaire
- Evaluation de la fibrose
- Evaluation du nb de blastes
- IHC : MPO, CD34, CD117
- Evolution

Evolution /progression

- Modifications liées au traitement par inhibiteurs de la tyrosine kinase
  - Réduction de la cellularité et en particulier de la granulopoièse
  - Normalisation de la mégacaryopoièse
  - Régression de la fibrose
  - Augmentation de l'apoptose avec diminution de l'activité proliférative
- Phase accélérée
- Phase blastique

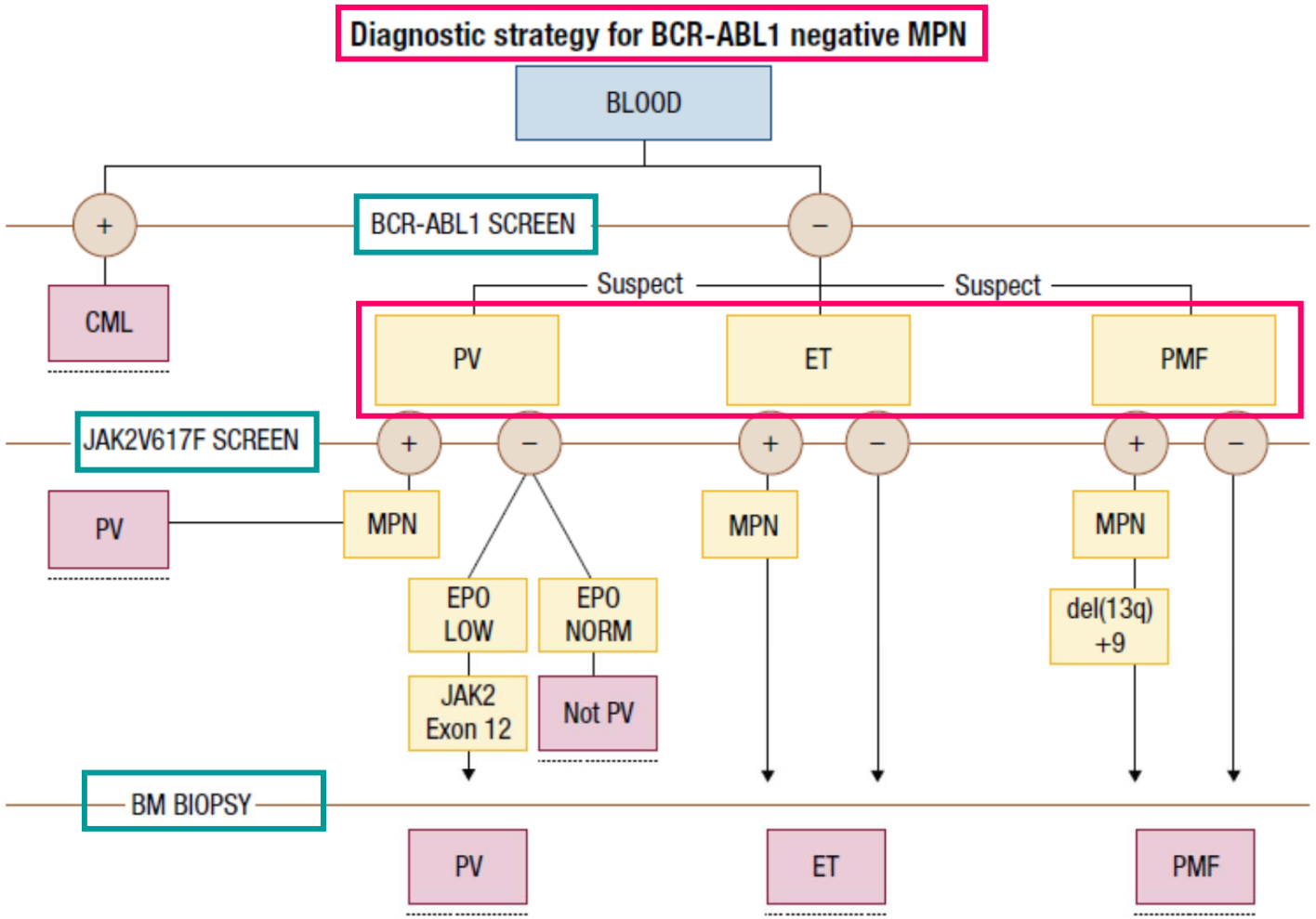
- Phase accélérée
  - Persistance ou augmentation des GB
  - Persistance ou augmentation de la splénomégalie
  - Persistance d'une thrombocytose
  - Persistance d'une thrombopénie
  - Modification moléculaire : apparition d'autres clones
  - Basophilie >20 %
  - 10%<Blastes>19% (CD34 !!)
  - Apparition de mégacaryocytes anormaux en groupe avec augmentation de la fibrose

- Phase blastique
  - 70 % : Lignée myéloïde (y compris Ebl, Mégaryobl)
  - 30% : lymphoblastique



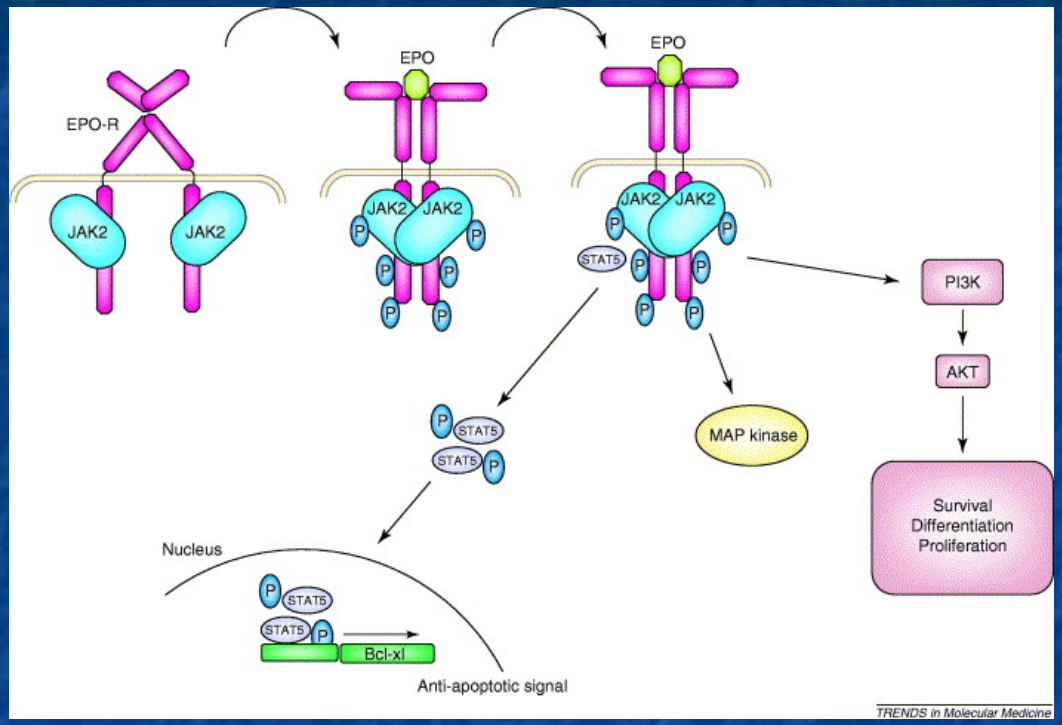
WHO 2008

PV, TE, PMF : JAK2^{V617F} et Biopsie

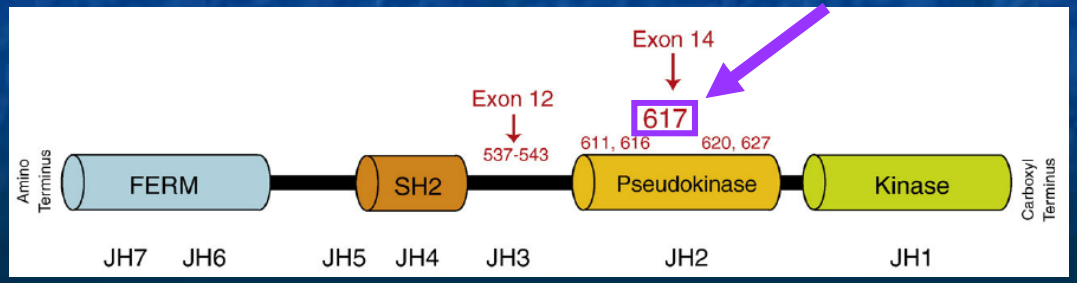


Vakil E and Tefferi A, *Clinical Lymphoma, Myeloma & Leukemia* 2011

JAK2



James C et al. *Trends Mol Med* 2005



Smith C.A. et al. *Hum Pathol* 2008

Expression JAK2^{V617F} :
- croissance cytokines-indépendante
- hypersensibilité aux cytokines

JAK2^{V617F} :
- >95% PV (cas restants = mut exon12)
- ~50% TE et PMF
- <5% HES, mastocytoses, LAM et RARS (mais ~50% RARS-T!)

- PV

Table 1 2008 World Health Organization Diagnostic Criteria for Polycythemia Vera

Diagnosis Requires Meeting Both Major Criteria OR the First Major Criteria and 2 Minor Criteria	
Major Criteria	1. Hemoglobin >18.5 g/dL (men), >16.5 g/dL (women) **OR** hemoglobin or hematocrit >99th percentile of reference range for age, sex, or altitude of residence **OR** red cell mass >25% above mean normal predicted **OR** hemoglobin >17 g/dL (men), >15 g/dL if representing a sustained increase of ≥2 g/dL from baseline that is not attributed to correction of iron deficiency 2. Presence of *JAK2V617F* or *JAK2* exon 12 mutation
Minor Criteria	1. Bone marrow trilineage myeloproliferation 2. Sub-normal serum erythropoietin levels 3. Endogenous erythroid colony growth

Vakil E and Tefferi A, *Clinical Lymphoma, Myeloma & Leukemia* 2011

PV

Anomalies cytogénétiques :

- 10-30%
- Peu/pas spécif :
  -Y, +8, +9 (la « + spécif »), gain ou dup(1q), 20q-, 13q-, 11q-, 3p-, 5q-, …
- Peu/pas pronostique (?5q- // transfo)

→ caryotype pas/peu utile si mutation JAK2

+9

20q-

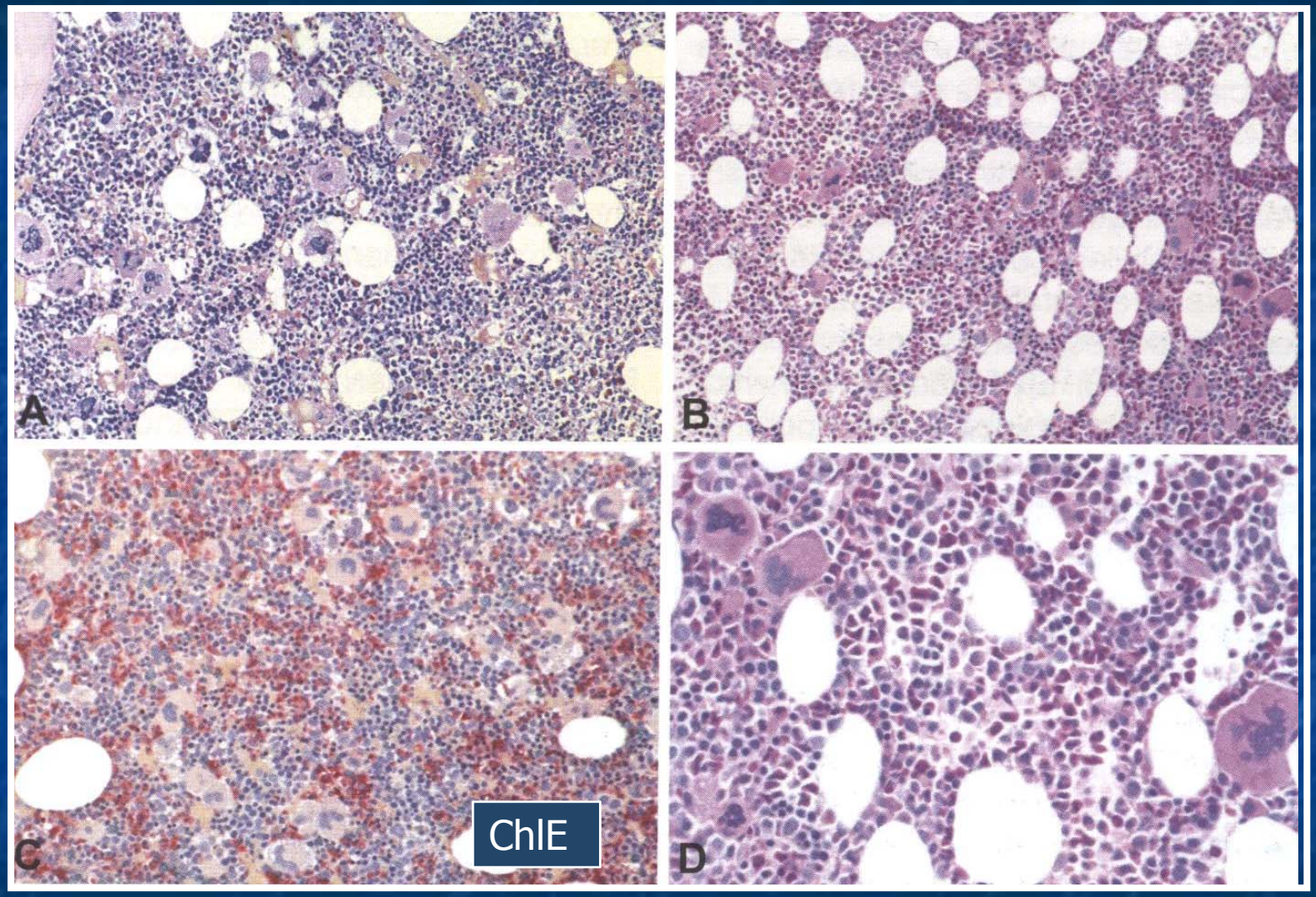
13q-

5q-

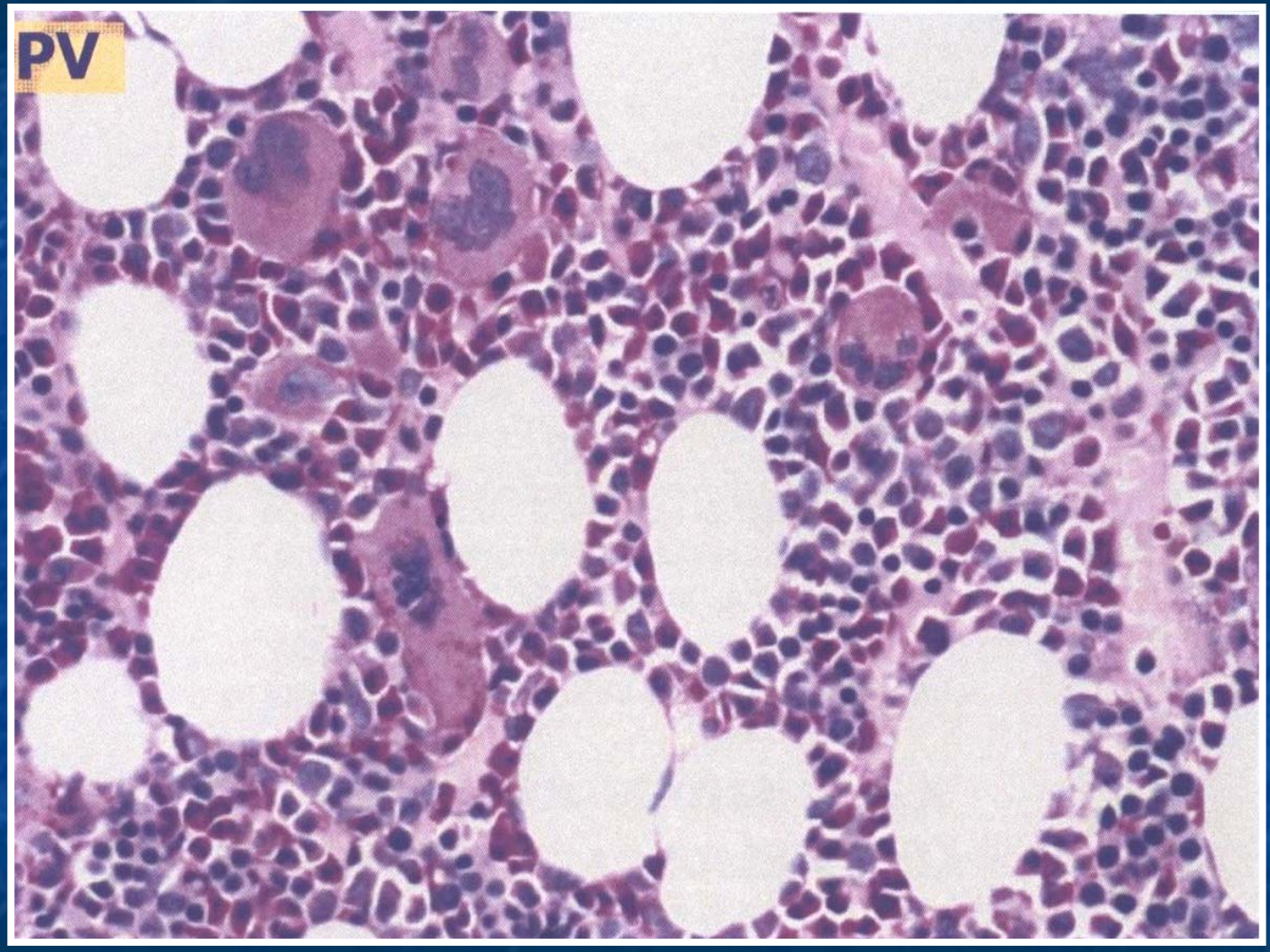
http://atlasgeneticsoncology.org/

Maladie de Vaquez

- Panmyélose : atteinte des lignées granulocytaire, erythroblastique et mégacaryocytaire
- Hypercellularité médullaire à la biopsie, en particulier sous corticale surtout de la lignée rouge et mégacaryocytaire
- Mégacaryocytes hyperlobés, svt en petits clusters péritrabeculaires
- Absence de Fer médullaire



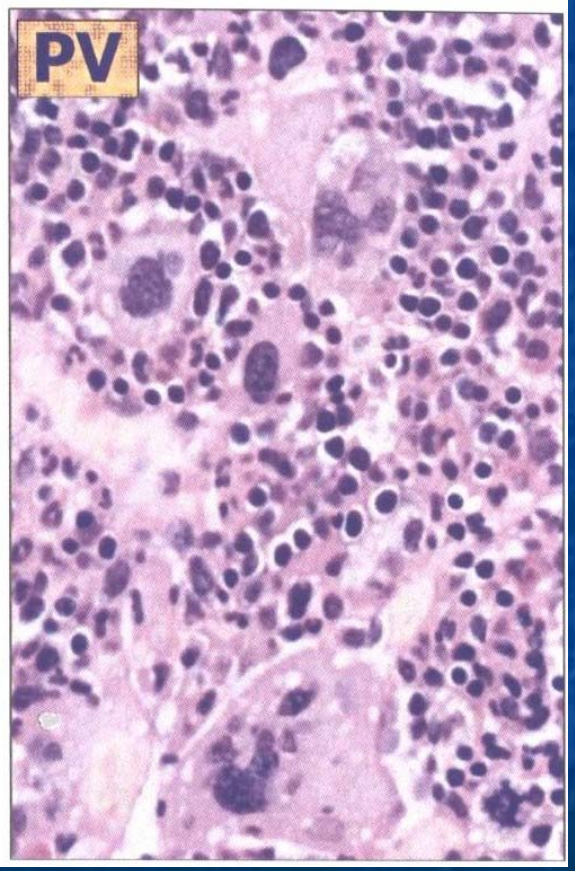
WHO 2008



9th Intern course of BM pathology Geneva 2009,
H.M. Kvasnicka Köln

Discrimination between initial PV and ET

Incidence of BM features (%)	PV	ET
Increase in		
▪ cellularity	**100**	0
▪ erythropoiesis	**89**	9
▪ granulopoiesis	**78**	21
Megakaryocytes		
▪ giant forms	25	**98**
▪ pleomorphous aspect (differences in size)	**97**	5



9th Intern course of BM pathology Geneva 2009,
H.M. Kvasnicka Köln

- PMF

Table 3 2008 World Health Organization Diagnostic Criteria for Primary Myelofibrosis

Diagnosis Requires Meeting All 3 Major Criteria AND 2 Minor Criteria	
Major Criteria	1. Megakaryocyte proliferation with atypia* in association with reticulin and/or collagen fibrosis **OR** in the absence of reticulin fibrosis, an increase in bone marrow cellularity associated with granulocyte proliferation and often decreased erythrocyte proliferation 2. Not meeting the World Health Organization criteria for chronic myelogenous leukemia, polycythemia vera, primary myelofibrosis, myelodysplatic syndrome, or other myeloid malignancies 3. Presence of *JAK2V617F* or other clonal makers **OR** no evidence of reactive marrow fibrosis*
Minor Criteria	1. Leukoerythroblastosis 2. Increased serum lactate dehydrogenase level 3. Anemia 4. Palpable splenomegaly

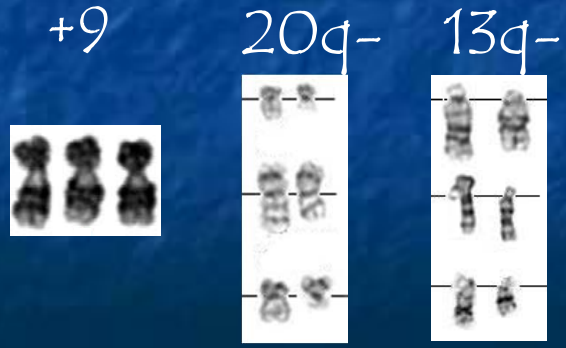
* The presence of reactive marrow fibrosis does not exclude a diagnosis of primary myelofribrosis in the presence of numerous other criteria.

Vakil E and Tefferi A, *Clinical Lymphoma, Myeloma & Leukemia* 2011

PMF

Anomalies cytogénétiques :

- ! Exclure Ph
- ≈ 30-50%
- Caryotype svt complexe :
  13q-, 20q-, dup(1q), +8, +9, 7q-, 5q-, 12p-, …
- Rôle pronostique :
  - ➢Caryotype défavorable = toute anomalie SAUF 13q- ou 20q- ou +9 isolée
  - ➢Si caryo défavorable : à considérer à « haut risque »
  - ➢Mais informatif que dans 30-50% …
  - ➢Evolution clonale // progression clinique : 90% d'anomalies si transformation



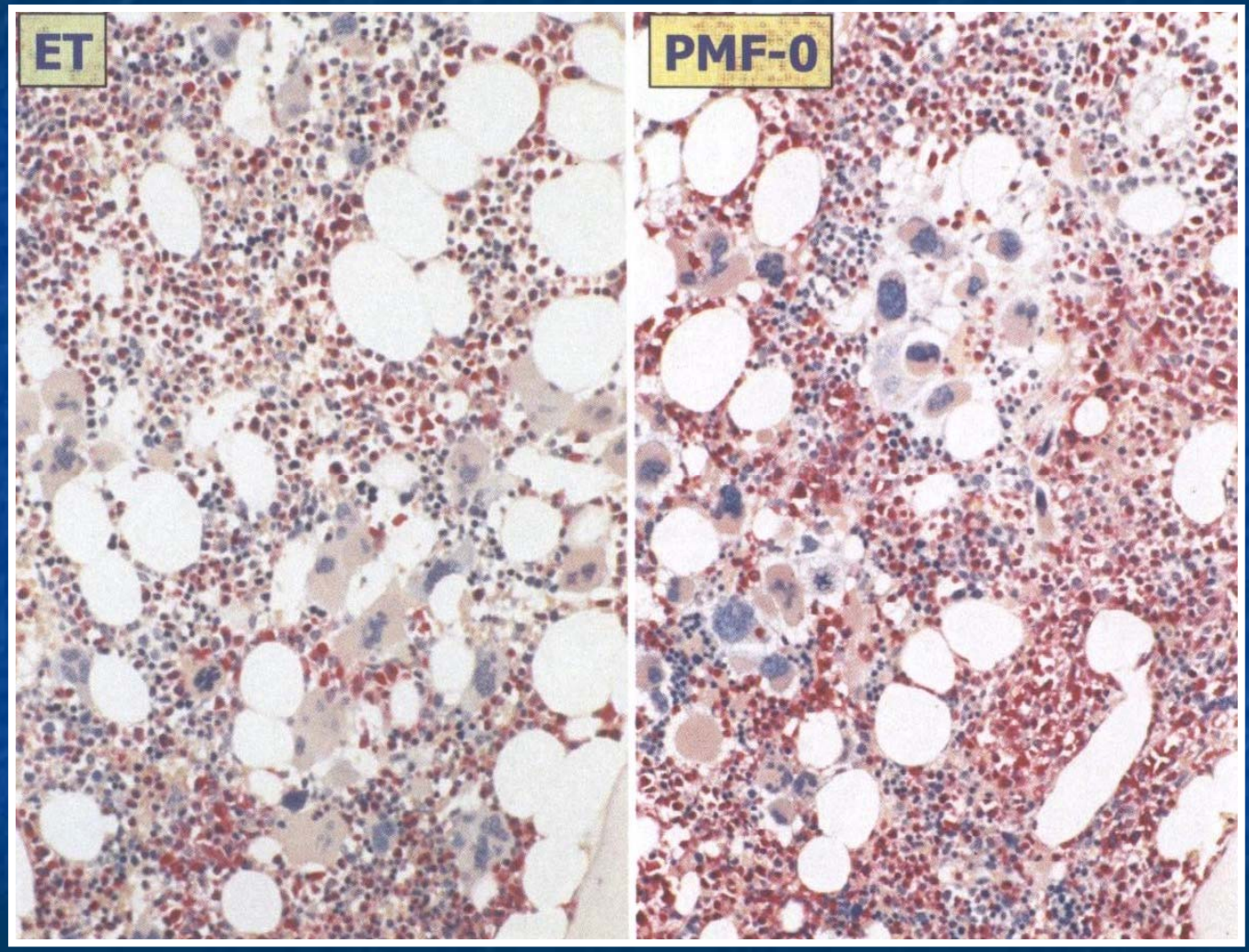
http://atlasgeneticsoncology.org/

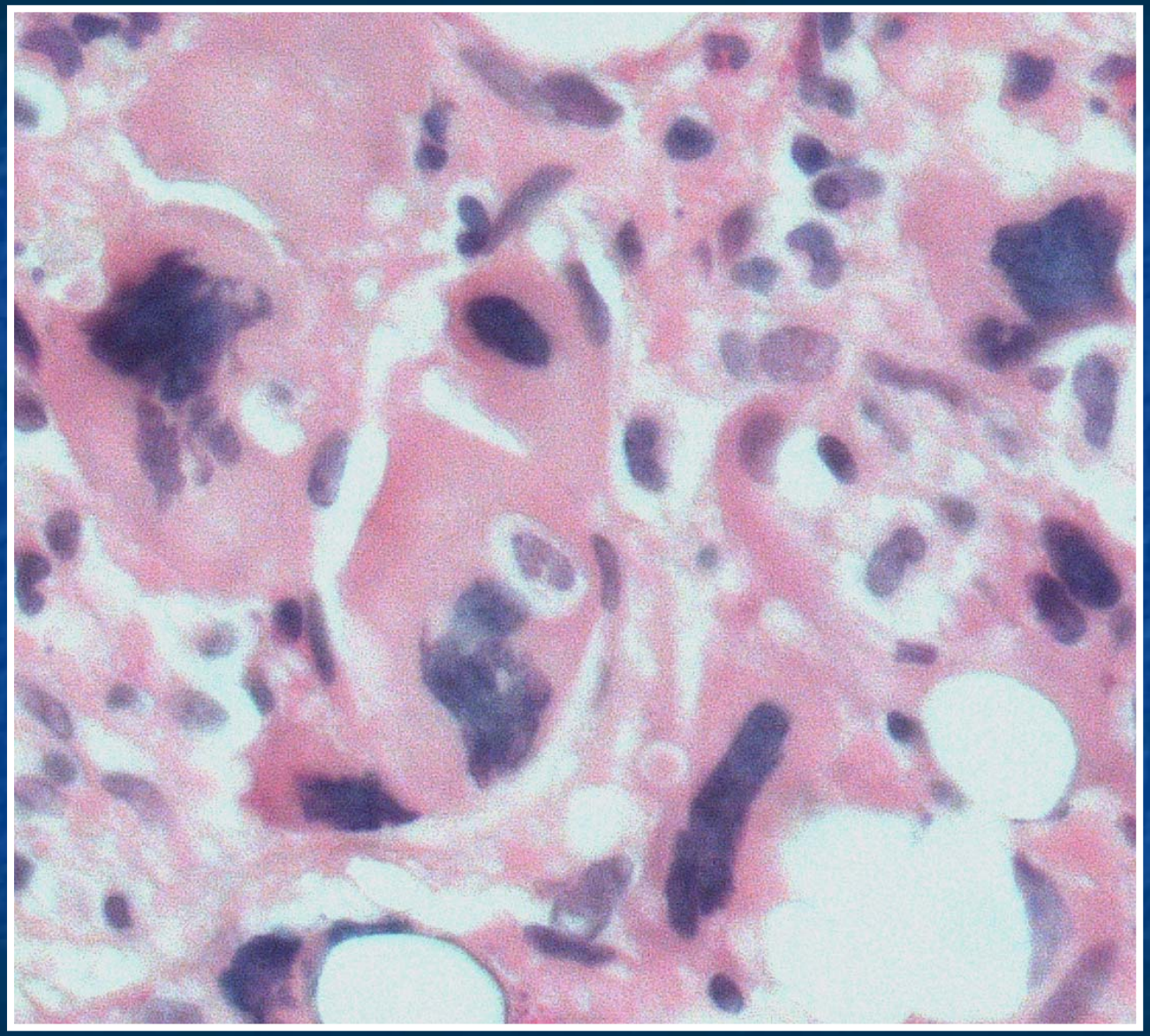
Myélofibrose idiopathique primitive

- Néoplasie myéloprolifératives induisant une fibrose massive associée à une hématopoièse extramédullaire
- Diagnostic d'exclusion des MF 2aires (autres MPD, Néoplasies solides, lésions inflammatoires ou toxiques
- Stade préfibrotique et fibrotique

Myelofibrose idiopathique primitive

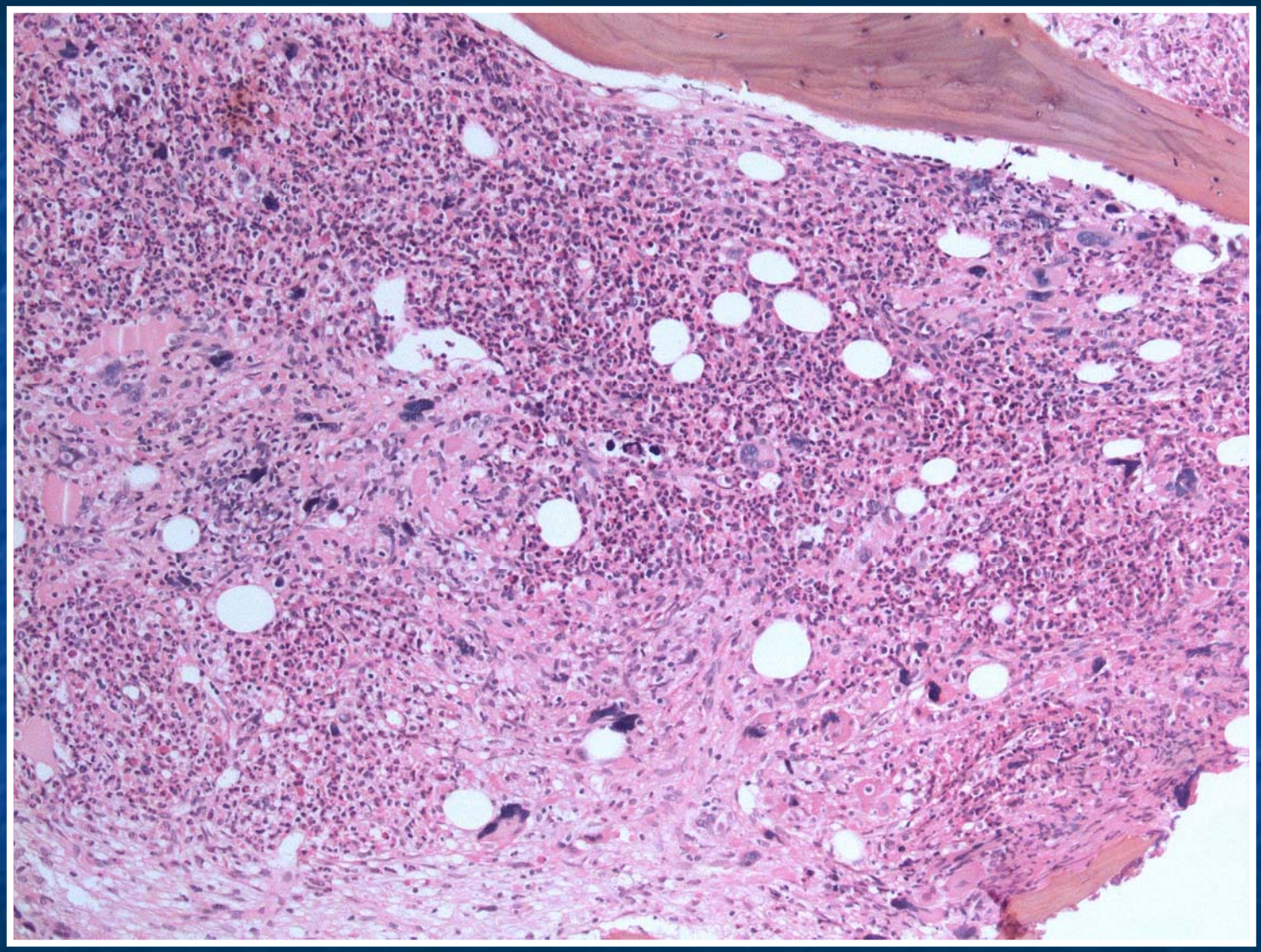
- Stade précoce(préfibrotique)
  - Hyperplasie granuleuse (shift à G, svt métamyélo)
  - Hyperplasie mégacaryocytaire:
    - Mégaryocytes atypiques
    - Clusters importants, svt ajacents aux sinus vasculaires et aux trabécules osseux
  - Fibrose réticulinique(importance de la coloration spécifique)
  - Erytropoièse svt réduite mais pfs formes jeunes prédominantes

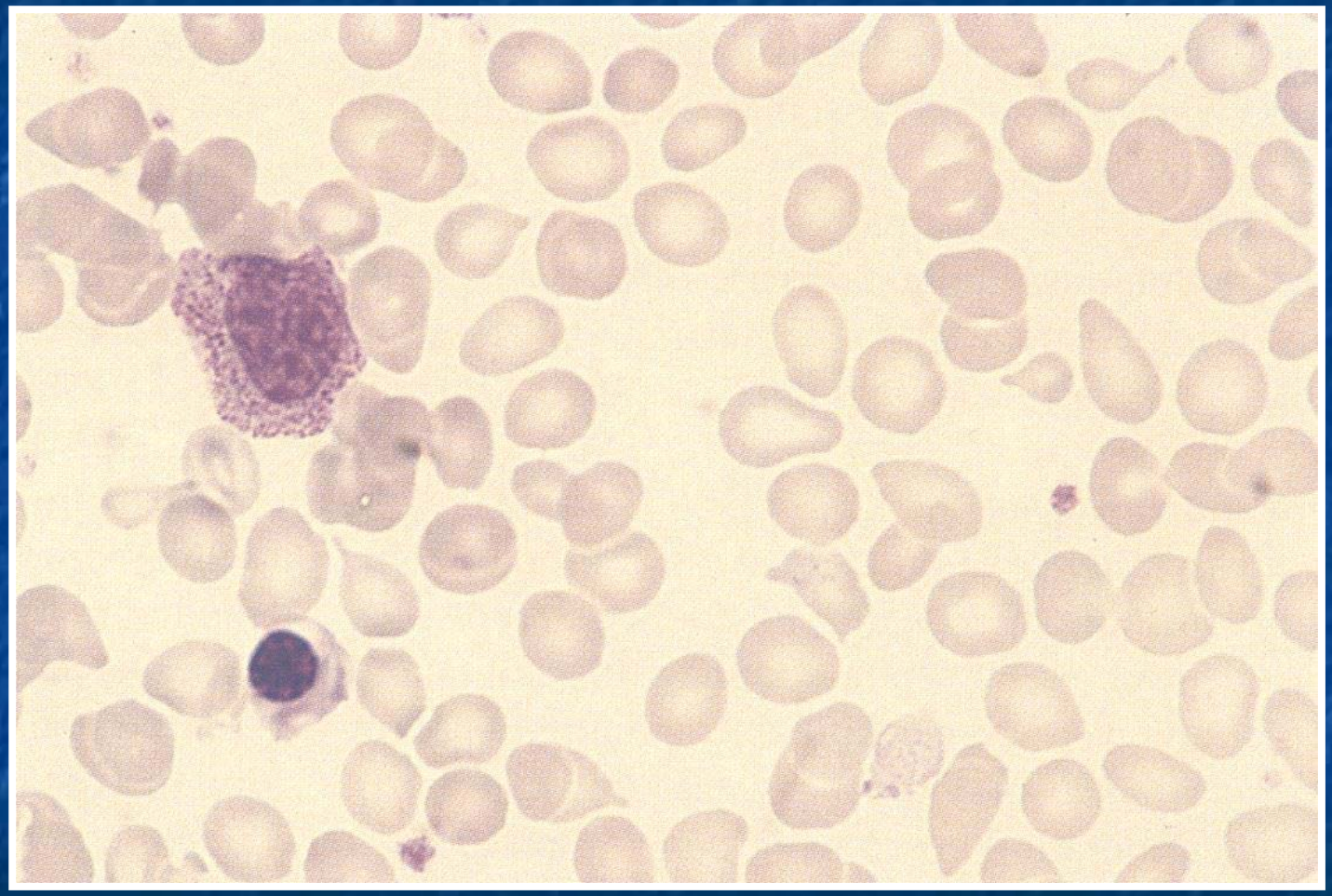

ET
PMF-0

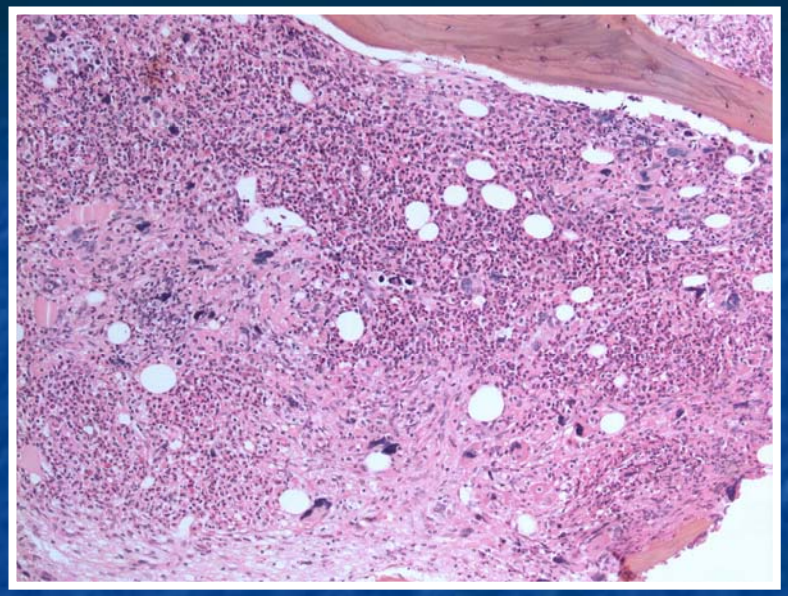


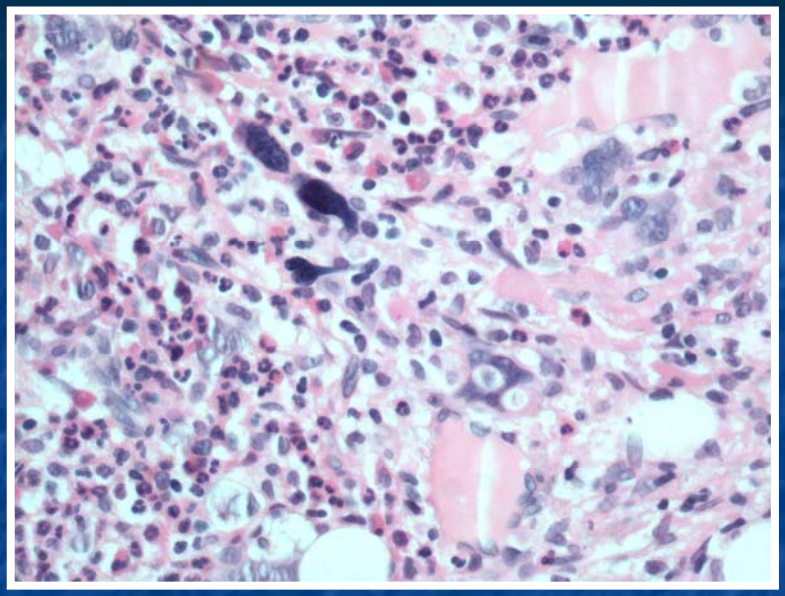
Myelofibrose idiopathique primitive

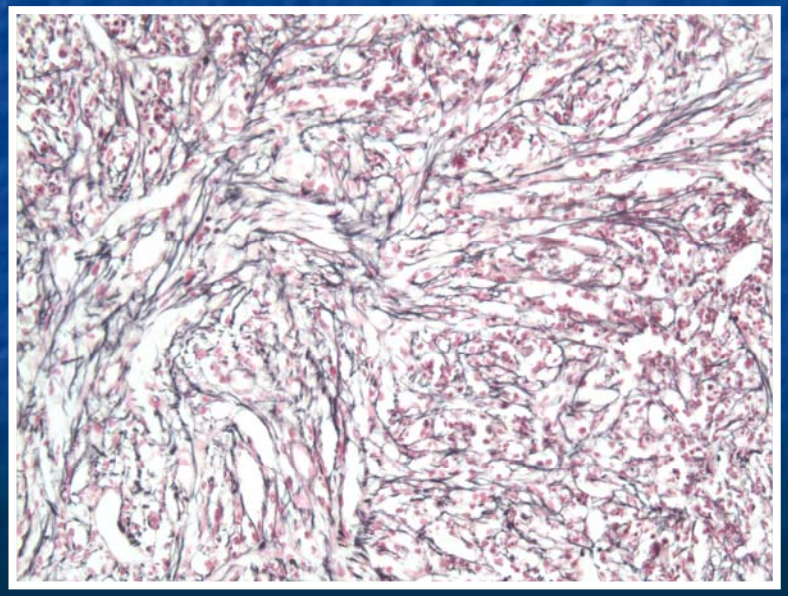
- Stade fibrotique
  - Myélofibrose mutilante évoluant vers l'ostéomyélosclérose
  - MO hypercellulaire évoluant vers MO Pauvre
  - Atypies mégacaryocytaires
  - Dilatation des sinus médullaires avec hématopoièse intraluminale
  - Hématopoièse extramédullaire

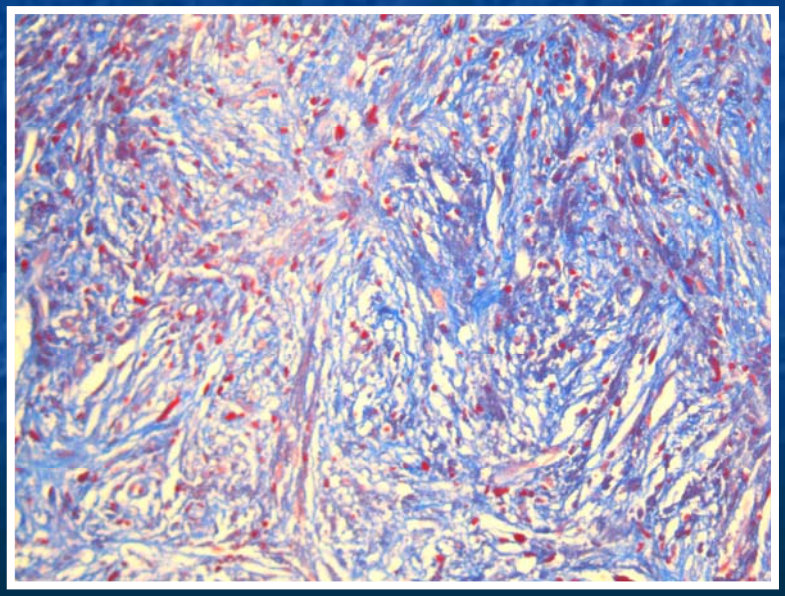












- TE

Table 2 2008 World Health Organization Diagnostic Criteria for Essential Thrombocythemia

Diagnosis Requires Meeting all 4 Major Criteria	
Major Criteria	1. Sustained platelet count $\geq 450 \times 10^9$/L before treatment 2. Bone marrow proliferation of enlarged, mature megakaryocytes 3. Not meeting the World Health Organization criteria for chronic myelogenous leukemia, polycythemia vera, primary myelofibrosis, myelodysplatic syndrome, or other myeloid malignancies 4. Presence of *JAK2V617F* or other clonal markers **OR** no evidence of reactive thrombocytosis*

* The presence of reactive thrombosis does not exclude a diagnosis of essential thrombocytosis in the presence of 3 other major criteria.

Vakil E and Tefferi A, *Clinical Lymphoma, Myeloma & Leukemia* 2011

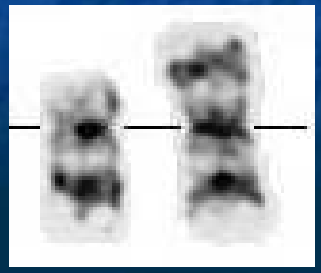
TE

Anomalies cytogénétiques :

- ! Exclure Ph
- ≈ 5-10%
- Peu/pas spécif :
  - -Y, +8, 20q-, +9, gain ou dup(1q), 13q-, -6, anomalie 9q, …
  - ! 17p- : svt // transformation aigue
  - ! 5q- : vérifier que pas « syndrome 5q- »
- Peu/pas pronostique (?5q- // transfo)

→ ? Caryotype : peu utile si mutation JAK2 et si vérification FISH que pas Ph

i(17q)

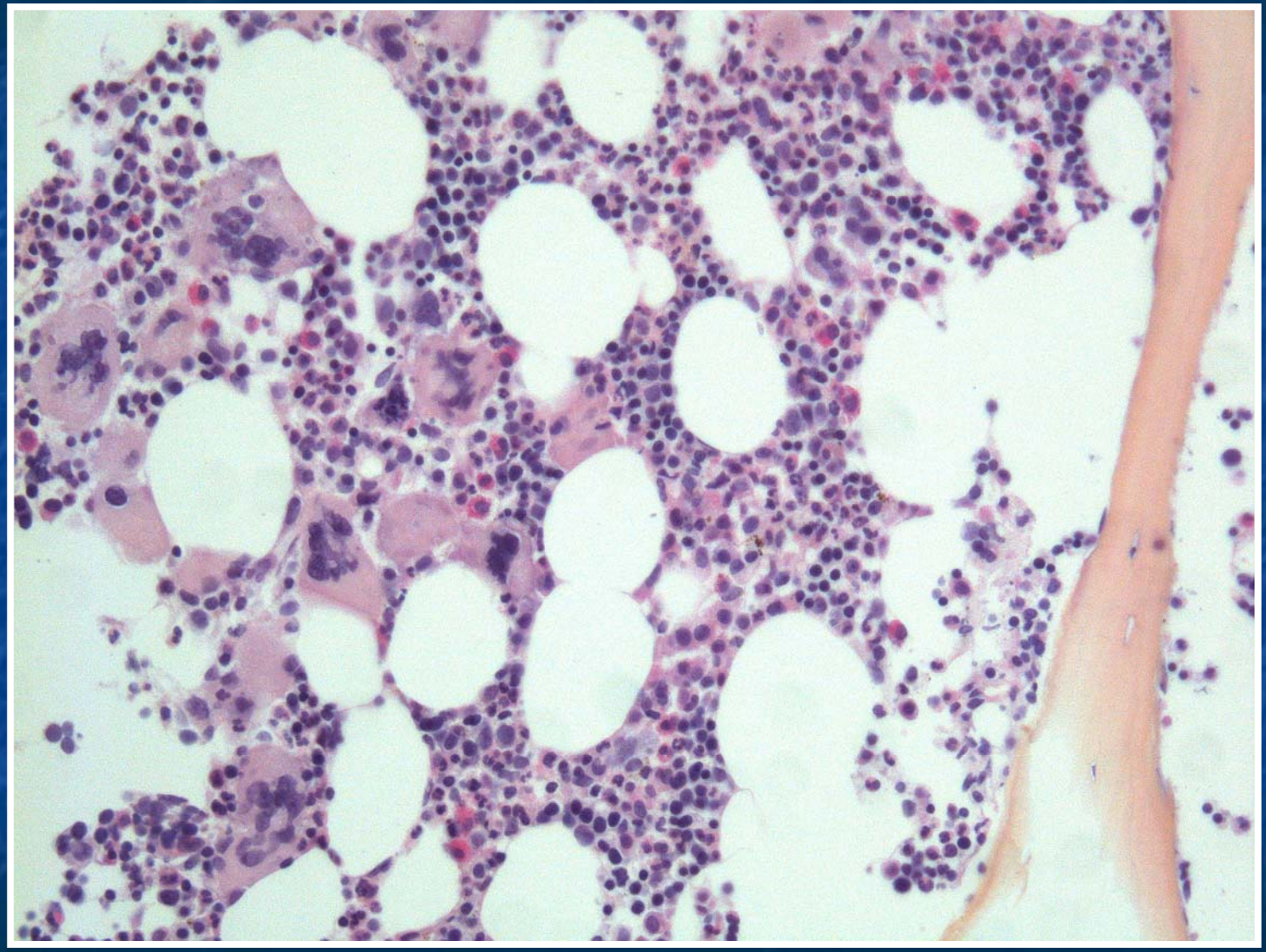


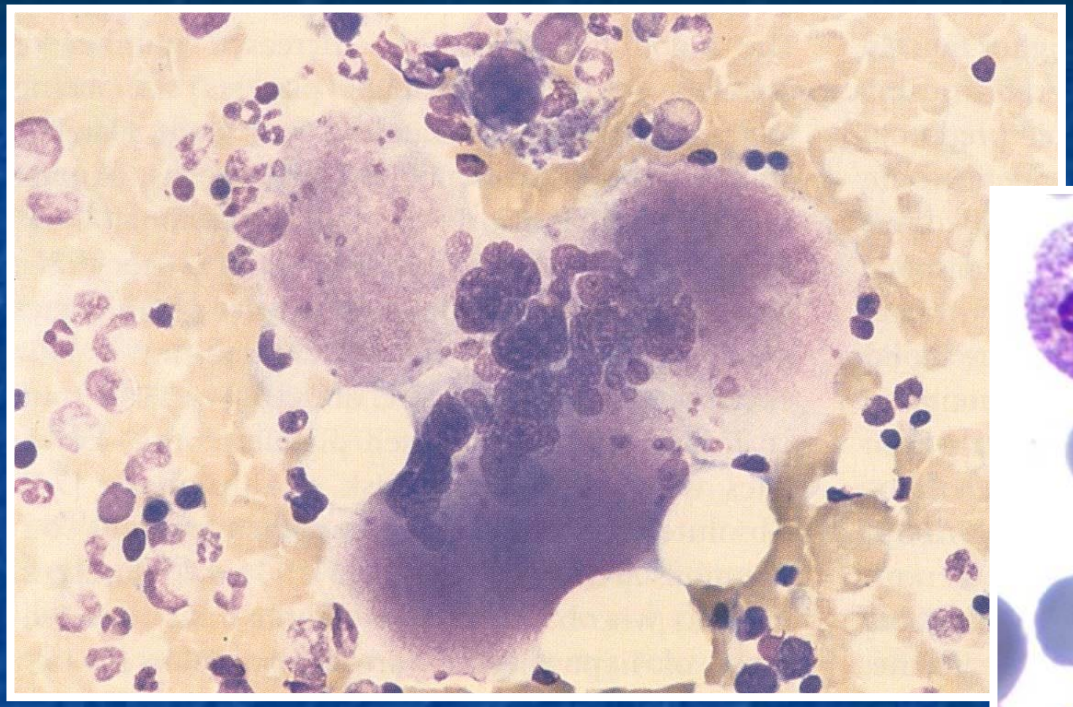
http://atlasgeneticsoncology.org/

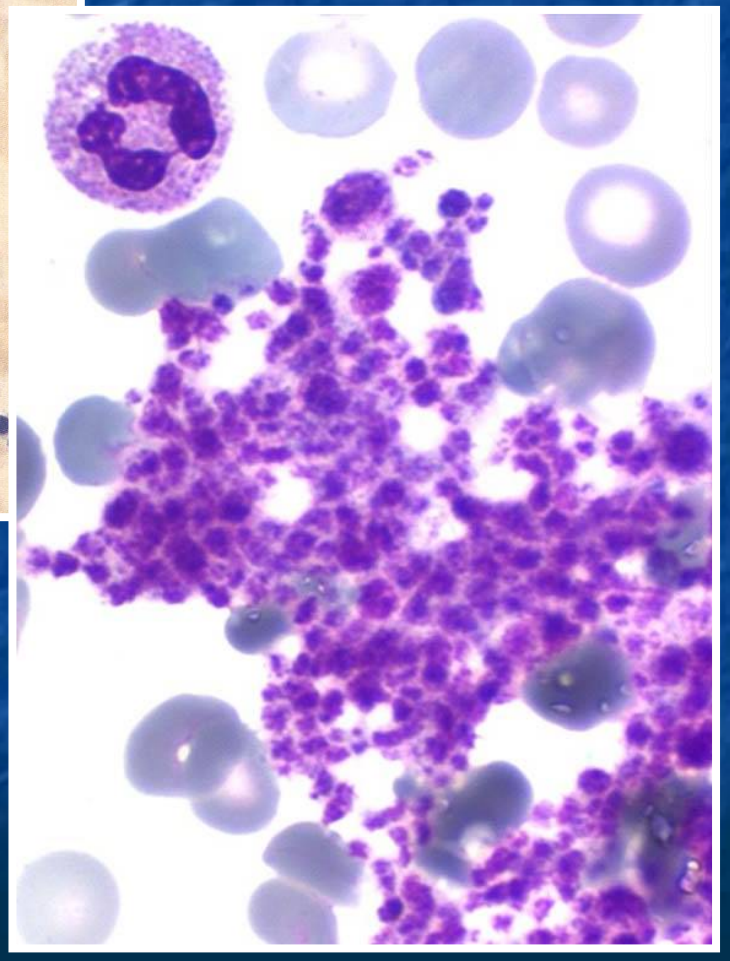
Thrombocytémie essentielle

- MO de richesse Normale ou peu augmentée
- Hyperplasie de la lignée mégacaryocytaire en clusters lâches. Mégacaryocytes non atypiques, souvent de grande taille voire géants
- Fibrose peu marquée
- Pas d'altération des autres lignées

Thrombocytémie essentielle







Trombocythémie essentielle

- Nombreux gènes impliqués dans MPN

  … mais rôle ds classification et diagnostic doit encore être établi

Table 1. Genes involved in the pathogenesis of MPN

Gene	Localization	Function	Comment	Disease (frequency)	References
JAK2	9p24	Tyrosine kinase, signaling	Gain of function	PV (95%-99%), ET (50%-70%), PMF (40%-50%)	6-9,11,12
MPL	1p34	Receptor, signaling	Gain of function	ET (4%), PMF (11%)	13-16
LNK	12q24	Adaptor, signal regulation	Loss of function	ET (< 5%), PMF (< 5%), JAK2 mutation-negative erythrocytosis (25%), post-MPN AML (13%)	17-19
CBL	11q23	Adaptor, E3 ubiquitin ligase, signal regulation	Dominant negative	PMF (6%), post-MPN AML	20,21
SOCS1	16p13.2	E3 ubiquitin ligase, signal regulation	Methylation	ET (14%-25%), PV (11%-13%), PMF (17%)	22,23
SOCS2	12q22	E3 ubiquitin ligase, signal regulation	Methylation	All MPN (28%)	24
SOCS3	17q25.3	E3 ubiquitin ligase, signal regulation	Methylation, mutation	ET (10%), PV (22%)	22,25-27
NRAS	1p13.2	GTPase, signaling	Gain of function	post-MPN AML (7%-13%)	21
NF1	17q11.2	RAS signaling regulation	Deletion	PMF (0%-6%), post ET/PV MF (14%)	28,29
TET2	4q24	DNA hydroxymethylation	Loss of function	PV (15%), ET (4%-11%), PMF (19%), post-MPN AML (26%)	30-33
ASXL1	20q11.21	Chromatin modifications	Loss of function	PV and ET (< 7%) PMF (19%-40%), post-MPN AML (19%)	34,35
EZH2	7q35	Chromatin methylation	Loss of function	PV (3%), PMF (13%)	36-38
IKZF1	7p12	Transcription factor, lymphopoiesis	Deletion	post-MPN AML (21%)	39
RUNX1	21q22.3	Transcription factor, hematopoiesis	Loss of function	post-MPN AML (37%)	21,40
RB	13q14	Cell cycle, apoptosis	Deletion	PMF (19%)	29
TP53	17p13.1	Cell cycle, apoptosis	Loss of function	post-MPN AML (20%)	21,41
IDH1	2q33.3	Metabolism	Neomorphic enzyme	PMF (2%), post-MPN AML (8%)	42
IDH2	15q26.1	Metabolism	Neomorphic enzyme	PMF (2%), post-MPN AML (18%)	42

AML indicates acute myeloid leukemia; ET, essential thrombocythemia; JAK2, Janus kinase 2; MPN, myeloproliferative neoplasms; PMF, primary myelofibrosis; and PV, polycythemia vera.

Vainchenker W et al, Blood2011

MDS

- Classification OMS 2008

Table 2
WHO classification of MDS [27].

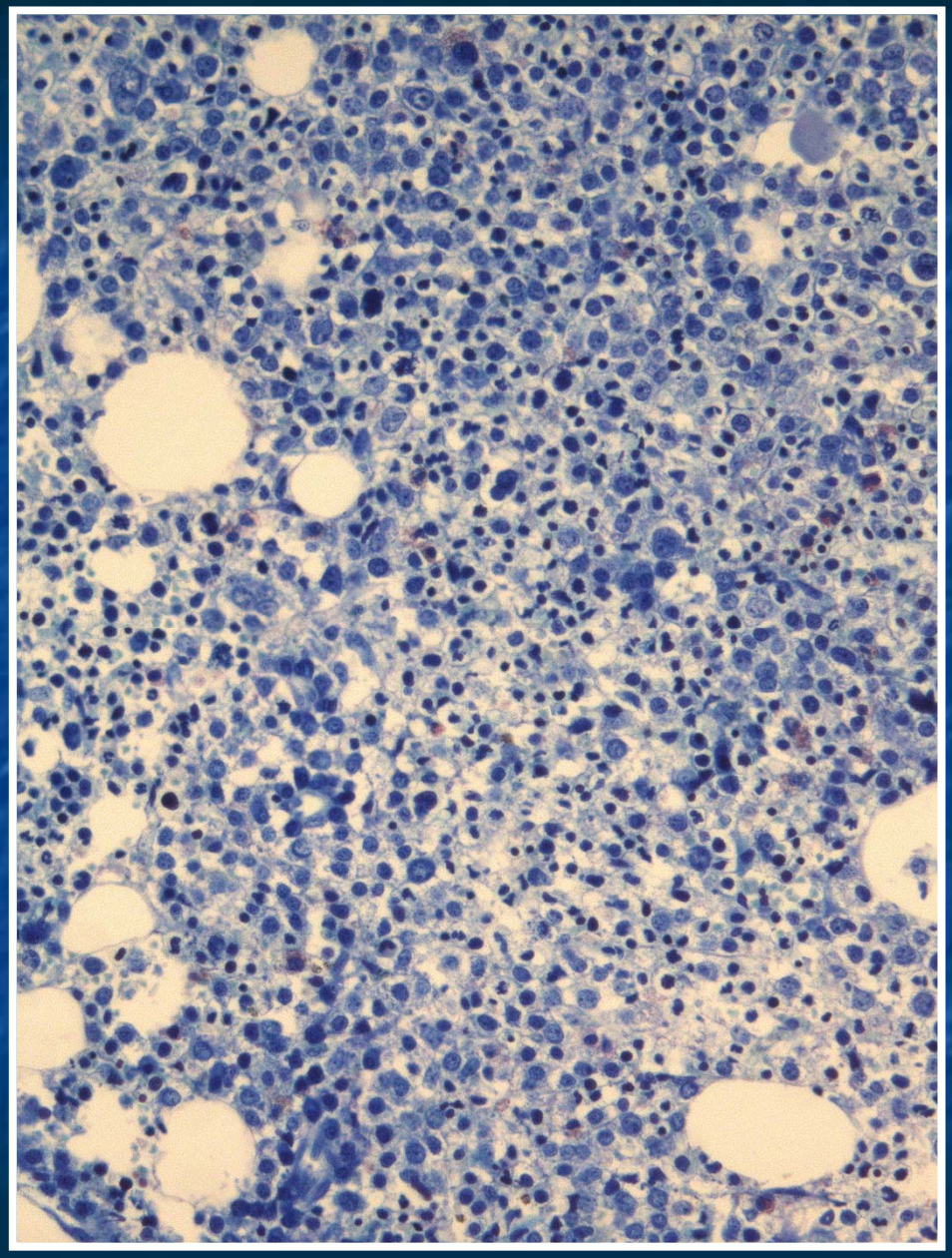
Vardiman J et al, *Leukemia Research* 2012

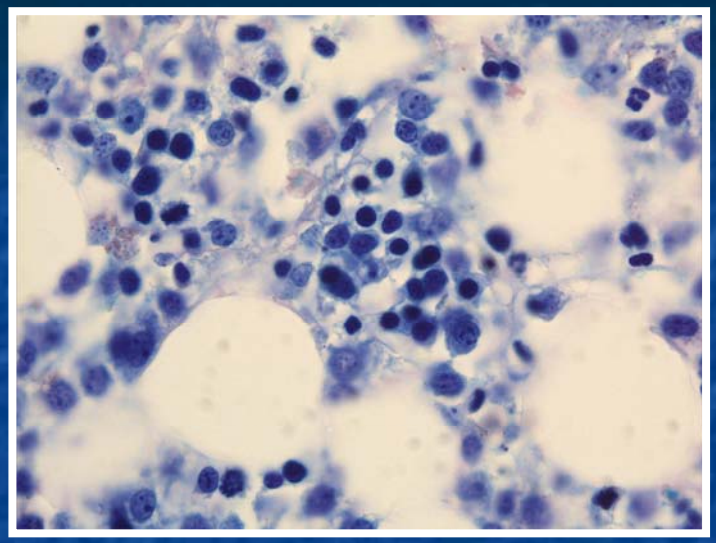
Disease entity	Blood findings	Bone marrow findings
Refractory cytopenias with unilineage dysplasia (RCUD): refractory anemia (RA), refractory neutropenia (RN), refractory thrombocytopenia (RT)	Unicytopenia or bicytopenia[a] No or rare blasts (<1%)[b]	Unilineage dysplasia: ≥10% of the cells in one myeloid lineage <5% blasts 15% of erythroid precursors are ring sideroblasts
Refractory anemia with ring sideroblasts (RARS)	Anemia No blasts	≥15% of the erythroid precursors are ring sideroblasts Dyserythropoiesis only <5% blasts
Refractory anemia with multilineage dysplasia (RCMD)	Cytopenia(s), no or rare blasts[b] No Auer rods $<1 \times 10^9$/L monocytes	Dysplasia in >10% of cells in 2 or more lineages <5% blasts in marrow No Auer rods $<1 \times 10^9$/L monocytes
Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenia(s) <5% blasts No Auer rods $<1 \times 10^9$/L monocytes	Unilineage or multilineage dysplasia 5–9% blasts No Auer rods
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenia(s) 5–19% blasts Auer rods ±[c] $<1 \times 10^9$/L monocytes	Unilineage or multilineage dysplasia 0–19% blasts Auer rods ±[c]
MDS, unclassifiable (MDS-U)	Cytopenias <1% blasts[b]	Unequivocal dysplasia in less than 10% of cells in one or more myeloid lines when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS <5% blasts
MDS associated with isolated del(5q)	Anemia Usually normal to elevated platelets No or rare blasts	Normal to increased megakaryocytes with hypolobated nuclei <5% blasts del(5q) is the sole cytogenetic abnormality No Auer rods

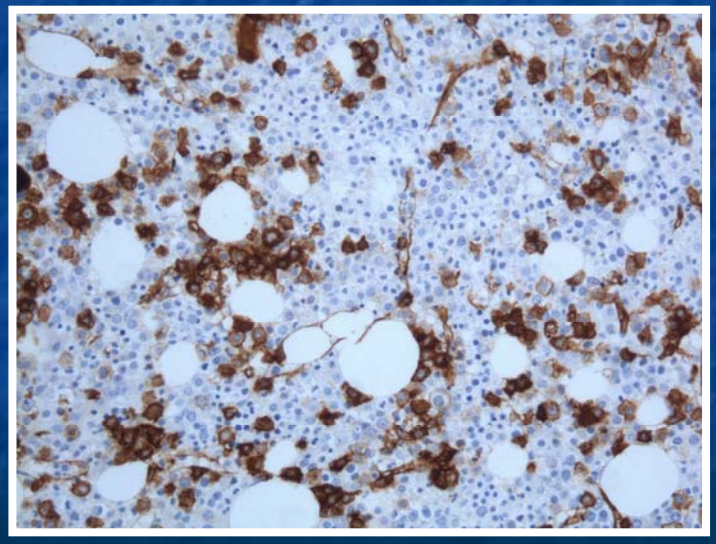
[a] Bicytopenia may be occasionally observed. Cases with pancytopenia should be classified as MDS-U.
[b] If marrow blast % is less than 5%, but there are 2–4% myeloblasts in blood, the diagnosis is RAEB-1. Cases of RCUD and RCMD with 1% blasts in blood are classified as MDS-U.
[c] Cases with Auer rods and <5% myeloblasts in the blood and <10% in the marrow should be classified as RAEB-2.

Syndromes myélodysplasiques

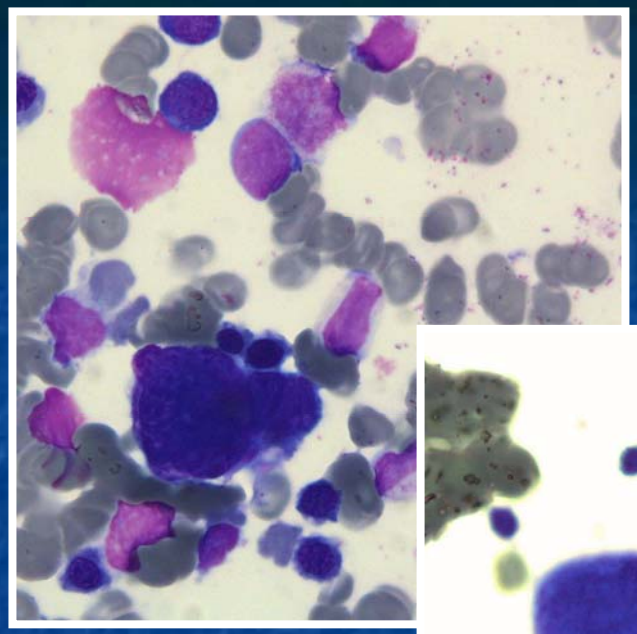
- Moelle hyperplasique avec anomalies de maturation dans les différentes lignées
  - Rouge : clusters au même stade, grands erythro basophiles en clusters
  - Granulo : altération des granulations
  - Mégacaryo : microméga…
- Exclure dysmyélopoièses réactionnelles
  - Carences (vit B12…)
  - Infections (VIH)
  - Chimiothérapie

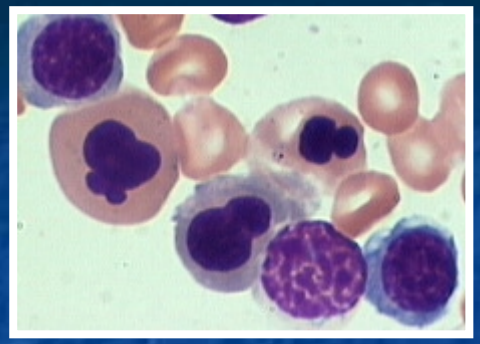


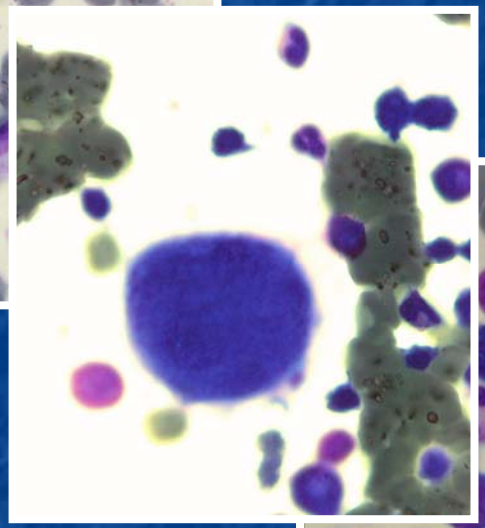


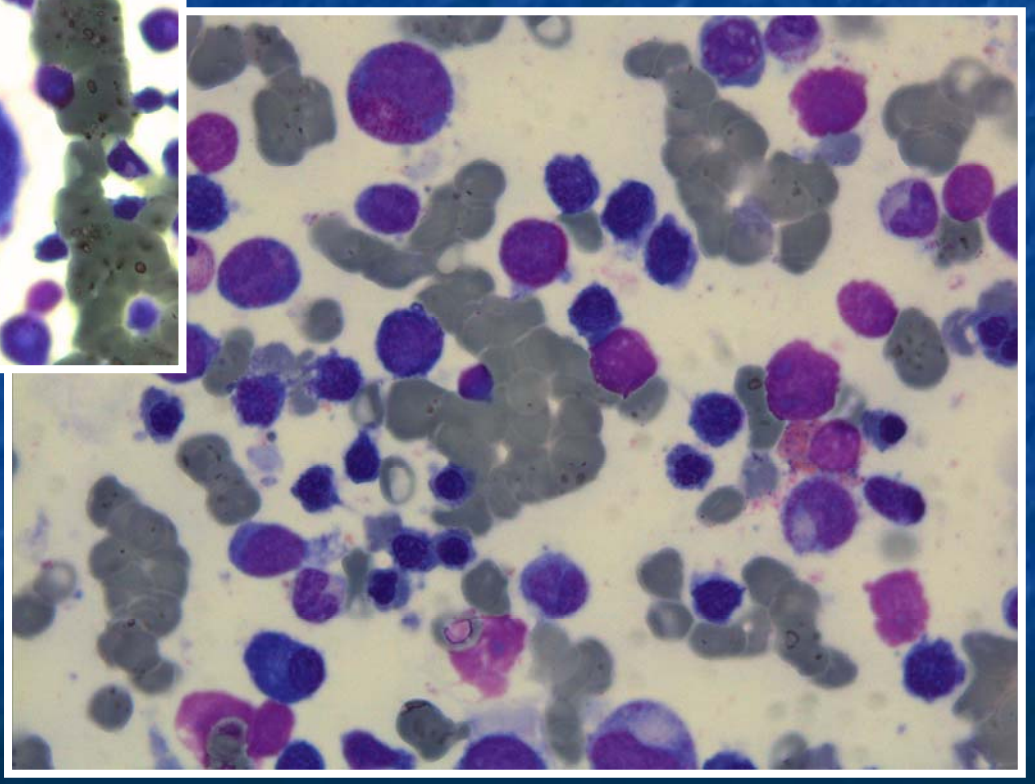


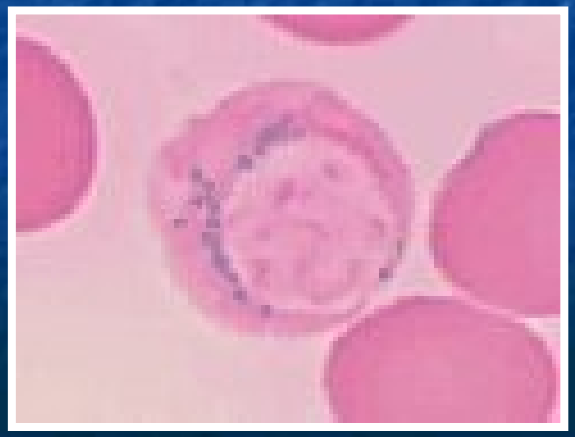
MDS avec excès de blastes





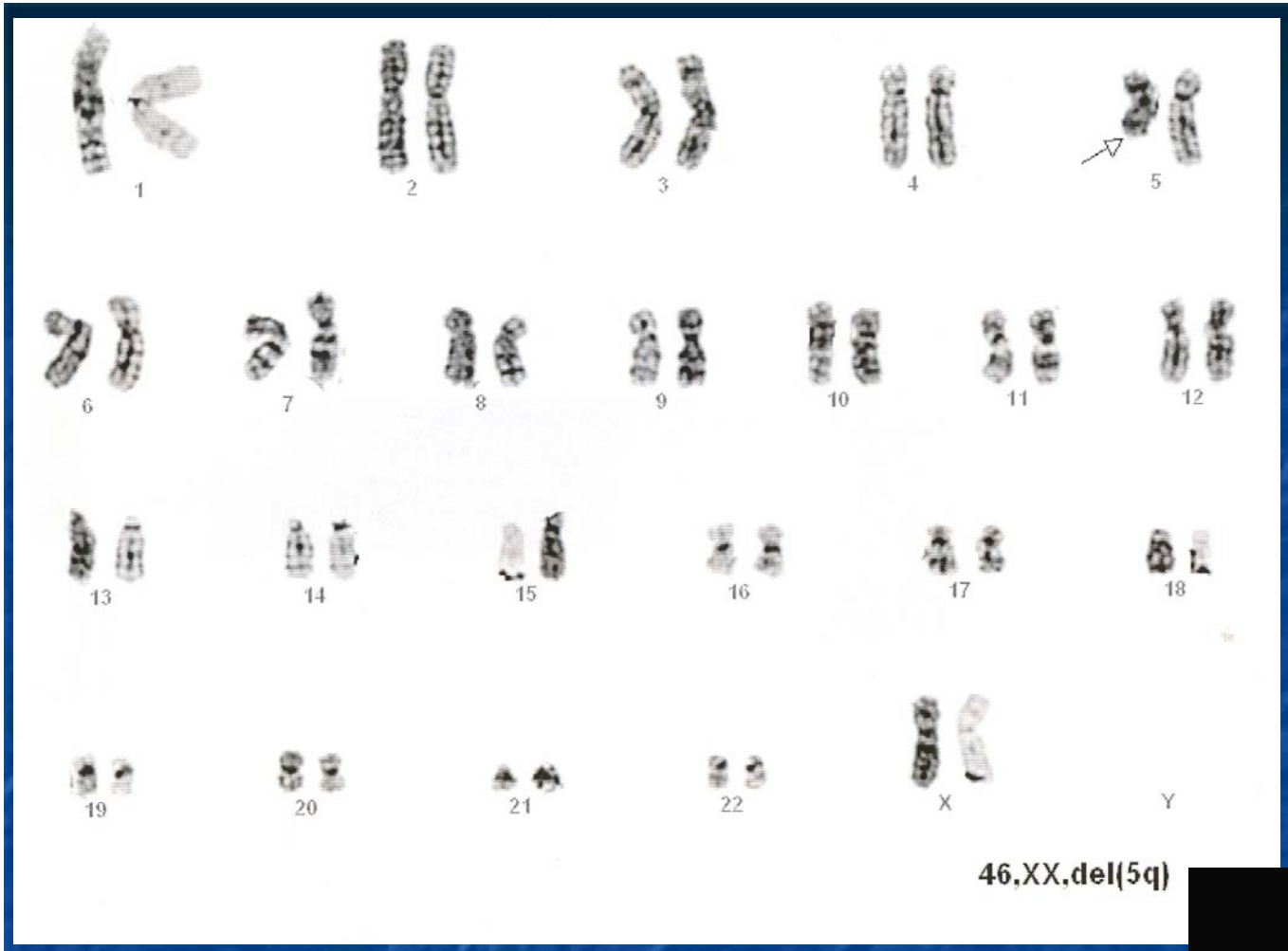






- Anomalies cytogénétiques :
  - 50% SMD
  - Prouvent nature clonale
  - Pas de spécificité absolue : la plupart peuvent aussi être vues dans MPN
  - Seule entité définie par cytogénétique = 5q- :
    - ✓ Femmes, >65 ans
    - ✓ PB : anémie, thrombocytose, <1% blastes
    - ✓ BM : <5% blastes, pas de bâtonnets d'Auer et

      MGK : svt nombreux, taille N ou<N, noyaux hypo- ou non-lobulés
    - ✓ Aucune autre anomalie chr
    - ✓ Pronostic favorable et faible incidence de LAM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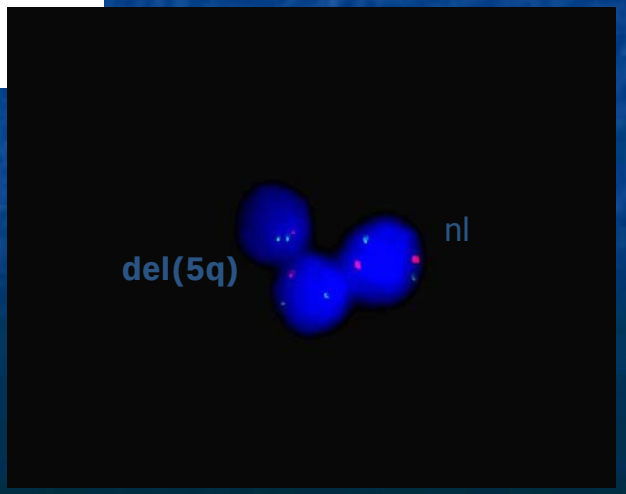
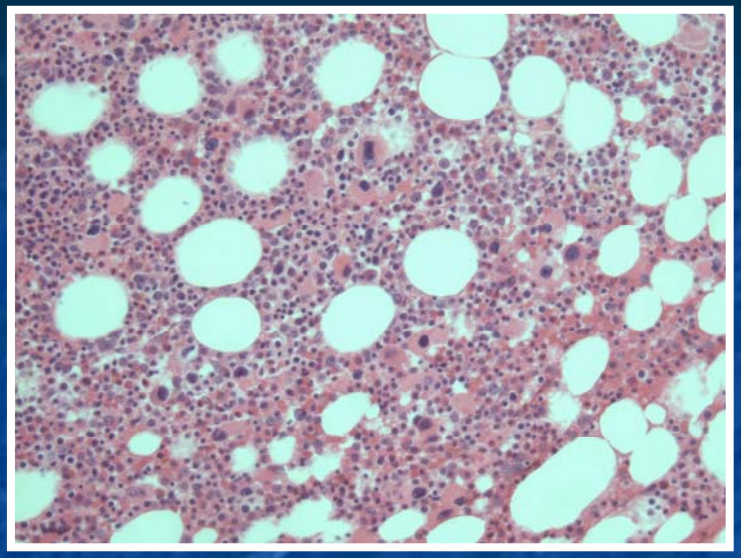
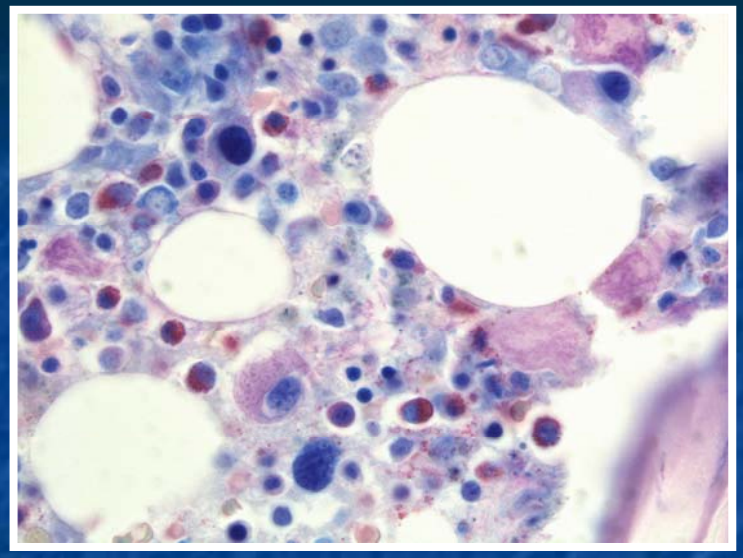
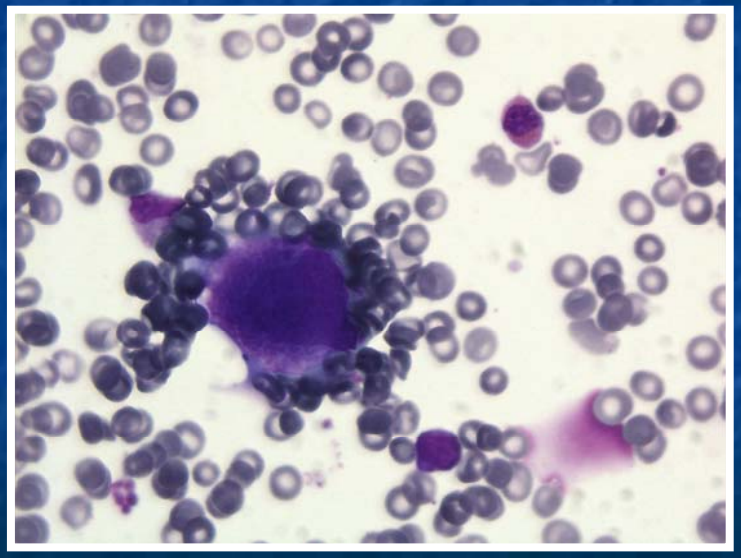

del(5q)
nl

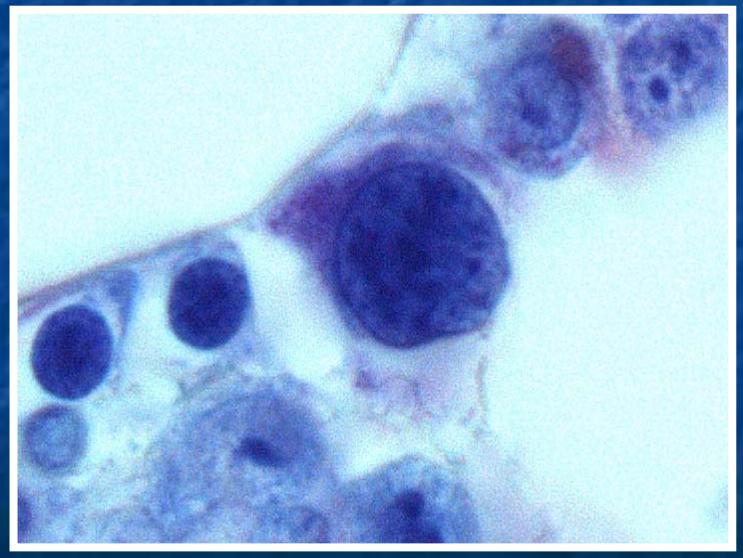
Caractéristiques morphologiques du syndrome 5q-

- Moelle normo-ou hypercellulaire
- Hypoplasie erythroïde est fréquente (tendance macrocytaire)
- Mégacaryocytes svt augmentés en nombre
- Aspect caractéristique des méga : svt de petite taille avec un noyau non segmenté ou hypolobulé
- Blastes médullaires<5%









Syndrome 5q-

- Certaines anomalies cytogénétiques

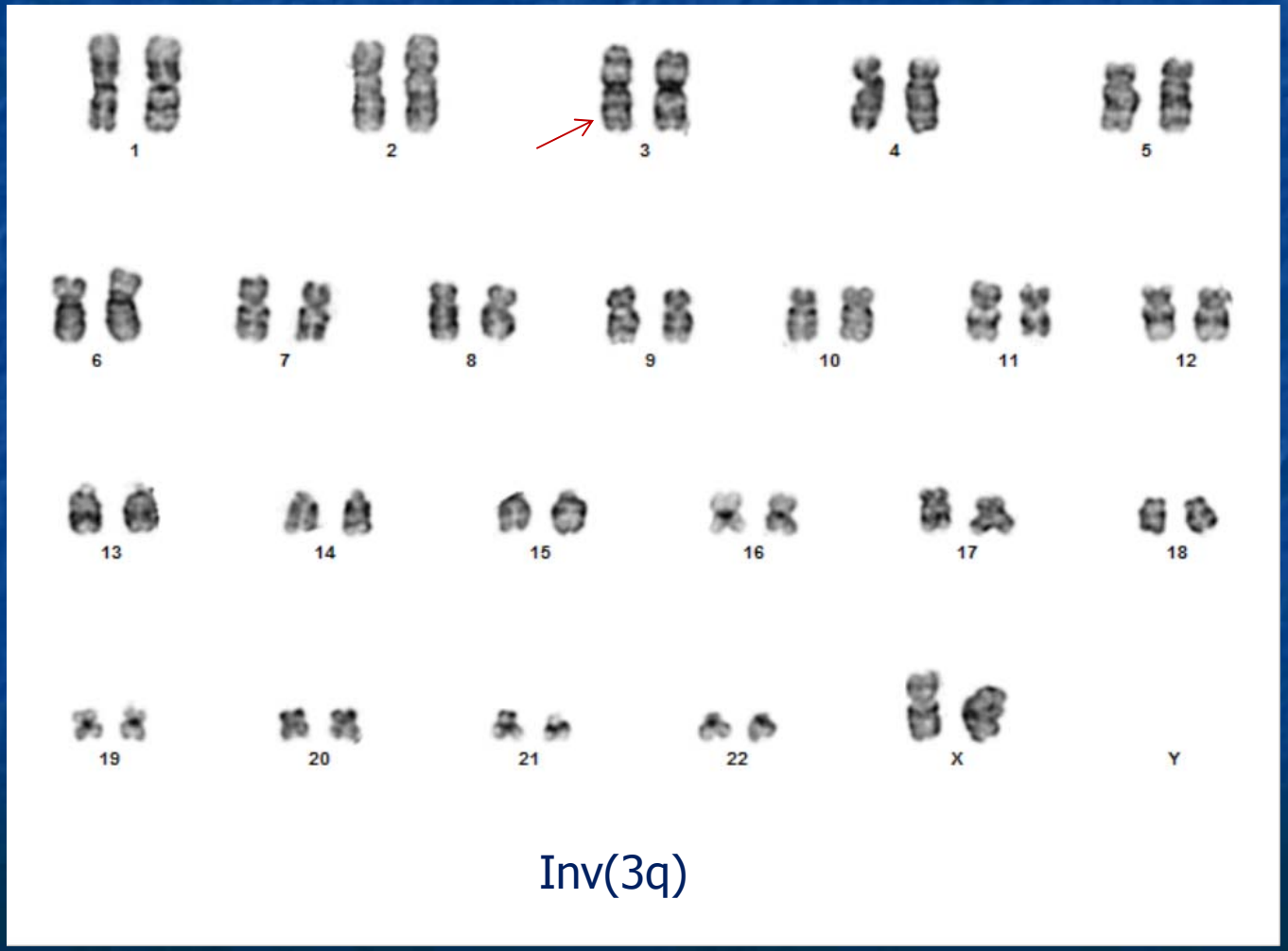
➔ diagnostic présomptif de SMD si caractéristiques cliniques et hématologiques compatibles mais que morphologie ne permet pas de conclure

Unbalanced abnormalities
−7 or del(7q)
−5 or del(5q)
i(17q) or t(17p)
−13 or del(13q)
del(11q)
del(12p) or t(12p)
del(9q)
idic(X)(q13)
Balanced abnormalities
t(11;16)(q23;p13.3)
t(3;21)(q26.2;q22.1)
t(1;3)(p36.3;q21.2)
t(2;11)(p21;q23)
inv(3)(q21q26.2)
t(6;9)(p23;q34)

Vardiman J et al, *Leukemia Research* 2012

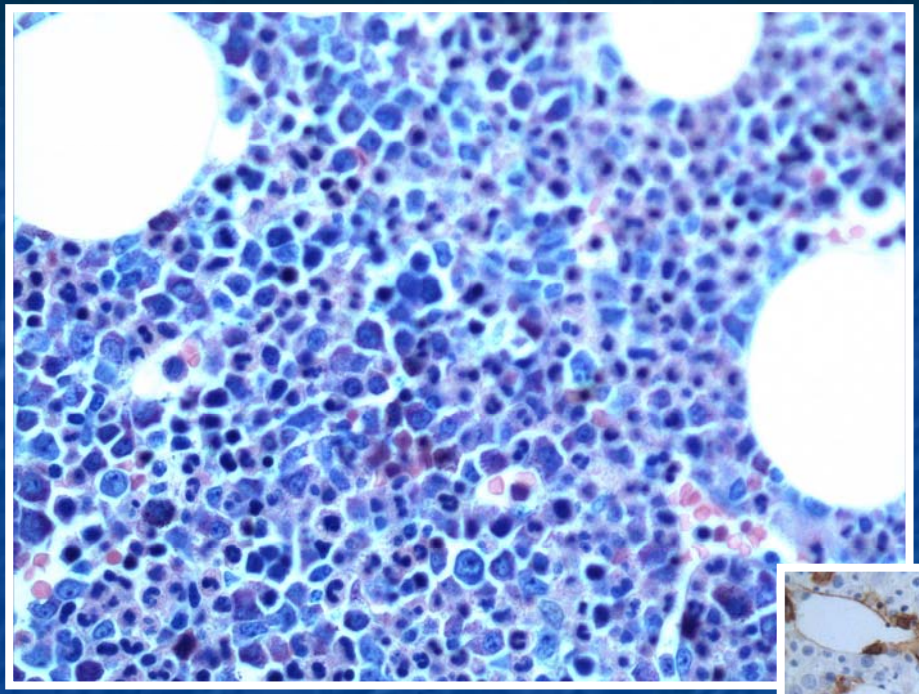
« réarrangement EVI1 » : inv(3q) ou t(3q;21q) ou t(1p;3q)

- PLT élevées, hyperplasie MGK qui sont en général dysplasiques (et dysplasie tri-lignée)
- diagnostic présomptif de SMD
- Mauvais pronostic



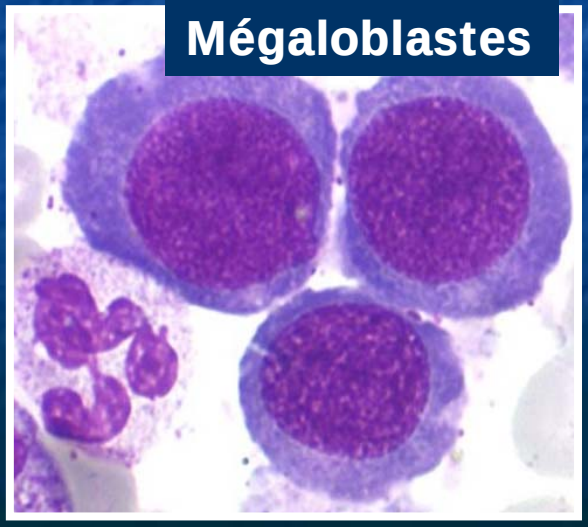
Inv(3q)

Moelle hypercellulaire

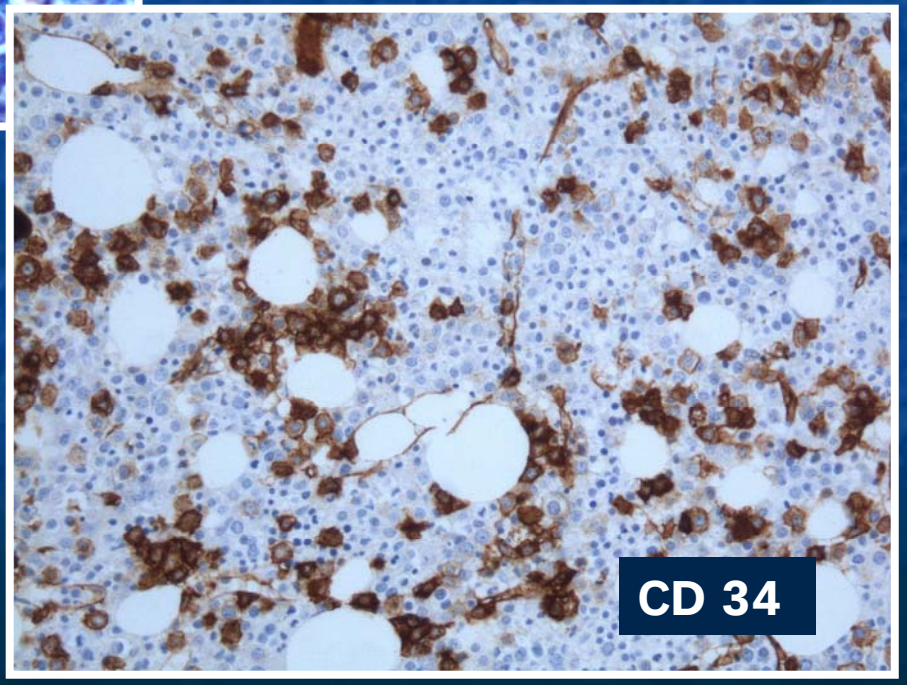


SMD EVI1+

Sidérobl en anneau



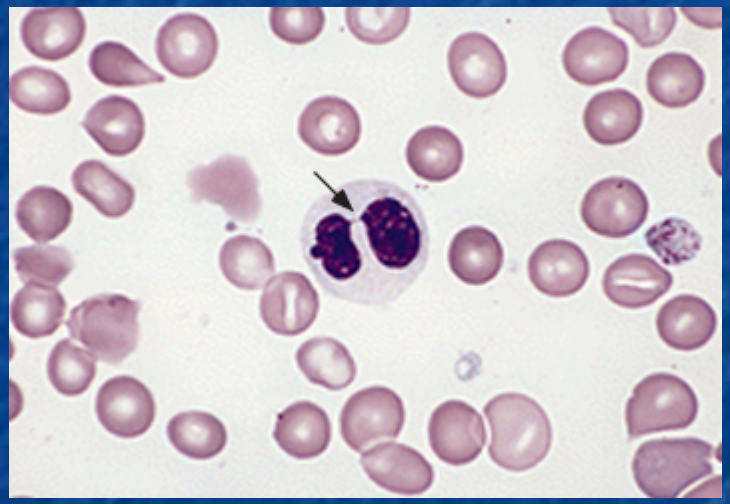
Mégaloblastes



CD 34

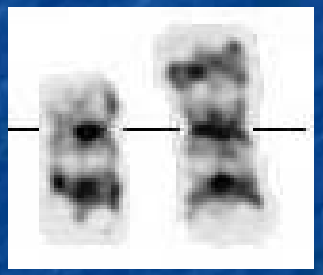
« 17p- » :

- Dysgranulopoïèse
- anomalies de type pseudo-Pelger-Huet
- neutrophiles (et parfois basos et éosinos) vacuolisés
- diagnostic présomptif de SMD
- Mauvais pronostic



UpToDate, de « *Brunning, RD, McKenna, RW. Tumors of the bone marrow. Atlas of tumor pathology (electronic fascicle), Third series, fascicle 9, 1994, Washington, DC. Armed Forces Institute of Pathology* »

i(17q)



http://atlasgeneticsoncology.org/

- Impact pronostique de la cytogénétique :
  5 groupes de pronostics différents -> IPSS-R

Table 2. MDS Cytogenetic Scoring System

Prognostic subgroups, % of patients	Cytogenetic abnormalities	Median survival,* y	Median AML evolution, 25%,* y	Hazard ratios OS/AML*	Hazard ratios OS/AML†
Very good (4%*/3%†)	−Y, del(11q)	5.4	NR	0.7/0.4	0.5/0.5
Good (72%*/66%†)	Normal, del(5q), del(12p), del(20q), double including del(5q)	4.8	9.4	1/1	1/1
Intermediate (13%*/19%†)	del(7q), +8, +19, i(17q), any other single or double independent clones	2.7	2.5	1.5/1.8	1.6/2.2
Poor (4%*/5%†)	−7, inv(3)/t(3q)/del(3q), double including −7/del(7q), complex: 3 abnormalities	1.5	1.7	2.3/2.3	2.6/3.4
Very poor (7%*/7%†)	Complex: > 3 abnormalities	0.7	0.7	3.8/3.6	4.2/4.9

OS indicates overall survival; and NR, not reached.
*Data from patients in this IWG-PM database, multivariate analysis (n = 7012).
†Data from Schanz et al[8] (n = 2754).

Greenberg PL et al, *Blood* 2012;120(12):2454-2465

IPSS-R

Table 3. IPSS-R prognostic score values

Prognostic variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very good	—	Good	—	Intermediate	Poor	Very poor
BM blast, %	≤ 2	—	> 2%- < 5%	—	5%-10%	> 10%	—
Hemoglobin	≥ 10	—	8- < 10	< 8	—	—	—
Platelets	≥ 100	50-< 100	< 50	—	—	—	—
ANC	≥ 0.8	< 0.8	—	—	—	—	—

— indicates not applicable.

Table 4. IPSS-R prognostic risk categories/scores

Risk category	Risk score
Very low	≤ 1.5
Low	> 1.5-3
Intermediate	> 3-4.5
High	> 4.5-6
Very high	> 6

Table 10. IPSS-R prognostic risk category: clinical outcomes of Medical University of Vienna patients (n = 200)

	Very low	Low	Intermediate	High	Very high
Patients, %	21	38	18	14	8
Survival					
All*	9.3	6.3	3.4	1.2	0.6
Hazard ratio	0.8	1	2.1	4.3	9.4
95% CI	0.4-1.5	0.7-1.5	1.3-3.5	2.4-7.7	4.3-20.8
AML transformation					
AML/25%*†	NR	NR	2.4	0.8	0.6
Hazard ratio	0	1	8.0	18.7	52.2
95% CI	0-∞	0.3-3.9	3.1-20.5	7.0-49.7	13.8-198.2

NR indicates not reached.
*Medians, years (95% CI; $P < .001$).
†Median time to 25% AML evolution (95% CI; $P < .001$).

Greenberg PL et al, *Blood* 2012;120(12):2454-2465

- Anomalies moléculaires :
  - // pronostic

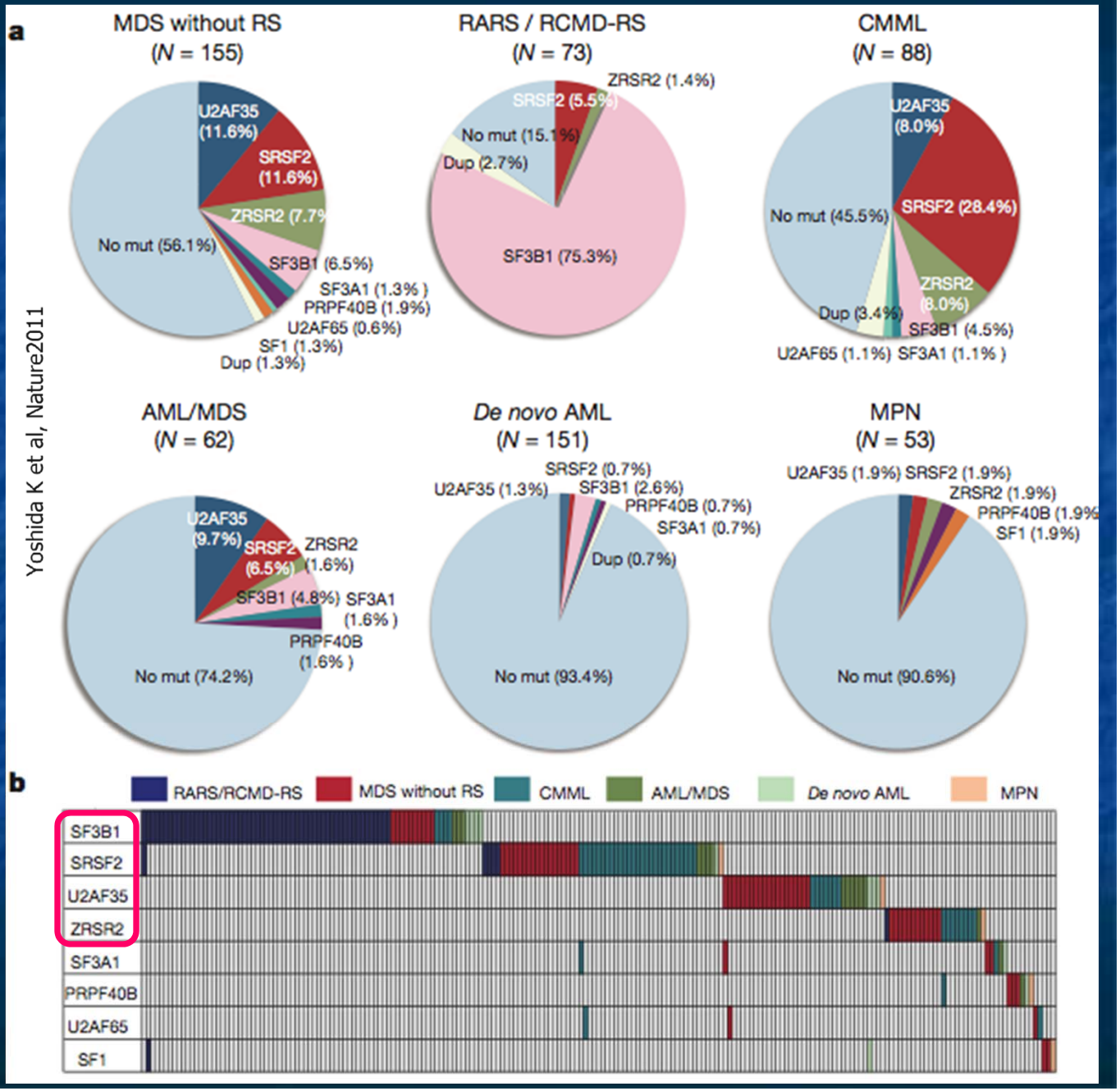
Bejar R et al, *NEJM* 2011

Table 2. Hazard Ratios for Death in a Multivariable Model.*

Risk Factor	Hazard Ratio (95% CI)	P Value
Age ≥55 yr vs. <55 yr	1.81 (1.20–2.73)	0.004
IPSS risk group		
Intermediate-1 vs. low	2.29 (1.69–3.11)	<0.001
Intermediate-2 vs. low	3.45 (2.42–4.91)	<0.001
High vs. low	5.85 (3.63–9.40)	<0.001
Mutational status		
TP53 mutation present vs. absent	2.48 (1.60–3.84)	<0.001
EZH2 mutation present vs. absent	2.13 (1.36–3.33)	<0.001
ETV6 mutation present vs. absent	2.04 (1.08–3.86)	0.03
RUNX1 mutation present vs. absent	1.47 (1.01–2.15)	0.047
ASXL1 mutation present vs. absent	1.38 (1.00–1.89)	0.049

* The model was generated from a stepwise Cox regression model that included the International Prognostic Scoring System (IPSS) risk category (based on the percentage of blasts in bone marrow, the karyotype, and the number of cytopenias [see Table 2 in the Supplementary Appendix]), age, sex, and mutation status for genes that were mutated in 1% or more of the 428 samples for which the IPSS classification was recalculated. Age was included in the analysis as a categorical variable on the basis of a best-split algorithm showing a significant difference in overall survival between patients less than 55 years of age and those 55 years of age or older (see Table 8 in the Supplementary Appendix).

// phénotype SMD : mutations du spliceosome



Yoshida K et al, Nature2011

MPN /MDS

- Ne pas se lancer seul dans ce type de diagnostic
- Prendre son temps
- Intégration indispensable des différentes données
- BOM = concertation multidisciplinaire

EN CONCLUSION

- MPN :
  - Ponction de moelle
  - Biopsie (non essentielle dans les maladies de Vaquez non compliquées)
  - FISH BCR-ABL BM ou PB (tube hépariné)
  - Recherche mutation V617F JAK2 BM ou PB (tube EDTA)
  - Caryotype BM si ? PMF ou si pas de nature clonale démontrée (tube hépariné)
- MDS :
  - Ponction de moelle
  - Biopsie
  - Caryotype BM (tube hépariné)
  - ? Biologie moléculaire (tube EDTA)

Merci

- A tous nos cliniciens
- A tous nos techniciens
- A nos collègues biologistes
- A mes collègues généticiens
- A mes collègues anatomopathologistes