XVIII CORSO DI AGGIORNAMENTO AIRTUM
PER OPERATORI DEI REGISTRI TUMORI
Monopoli (BA), 3-4-5 ottobre 2018

Markers dei Tumori Ematologici

4 Ottobre 2018

Giorgina Specchia
Ematologia con Trapianto

In Ematologia…….

- ✓ **Indagini Fondamentali per la Diagnosi !!!**

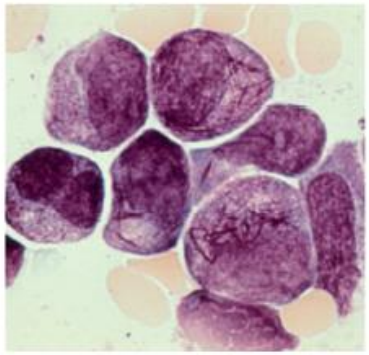
- ✓ Indagini Necessarie per lo score Prognostico

Appropriatezza Dx in Ematologia

- WHO 2008-2016
- Linee Guida/ELN
- PDTA/LEA

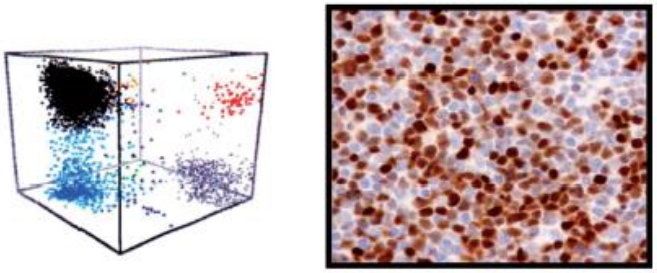
Multiple approach to the diagnosis of Haematological Neoplasms

Cytomorphology

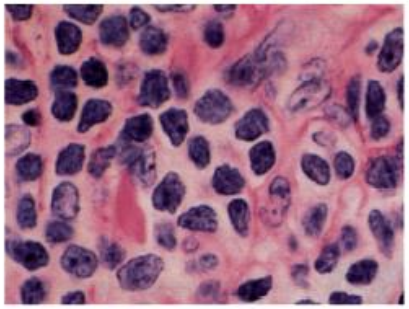


Cytogenetics

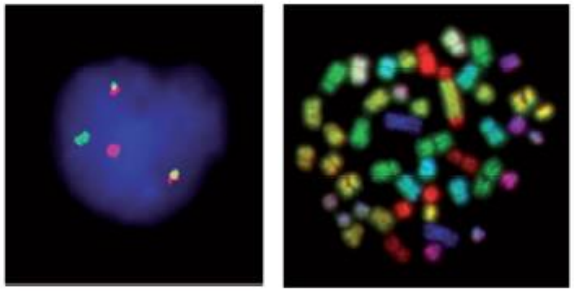
Immunophenotype



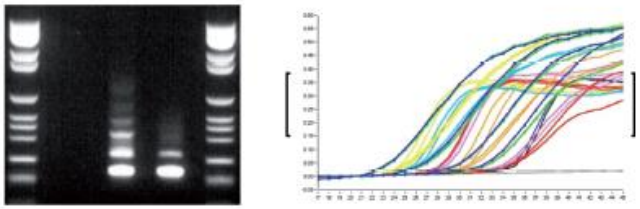
Histology



FISH

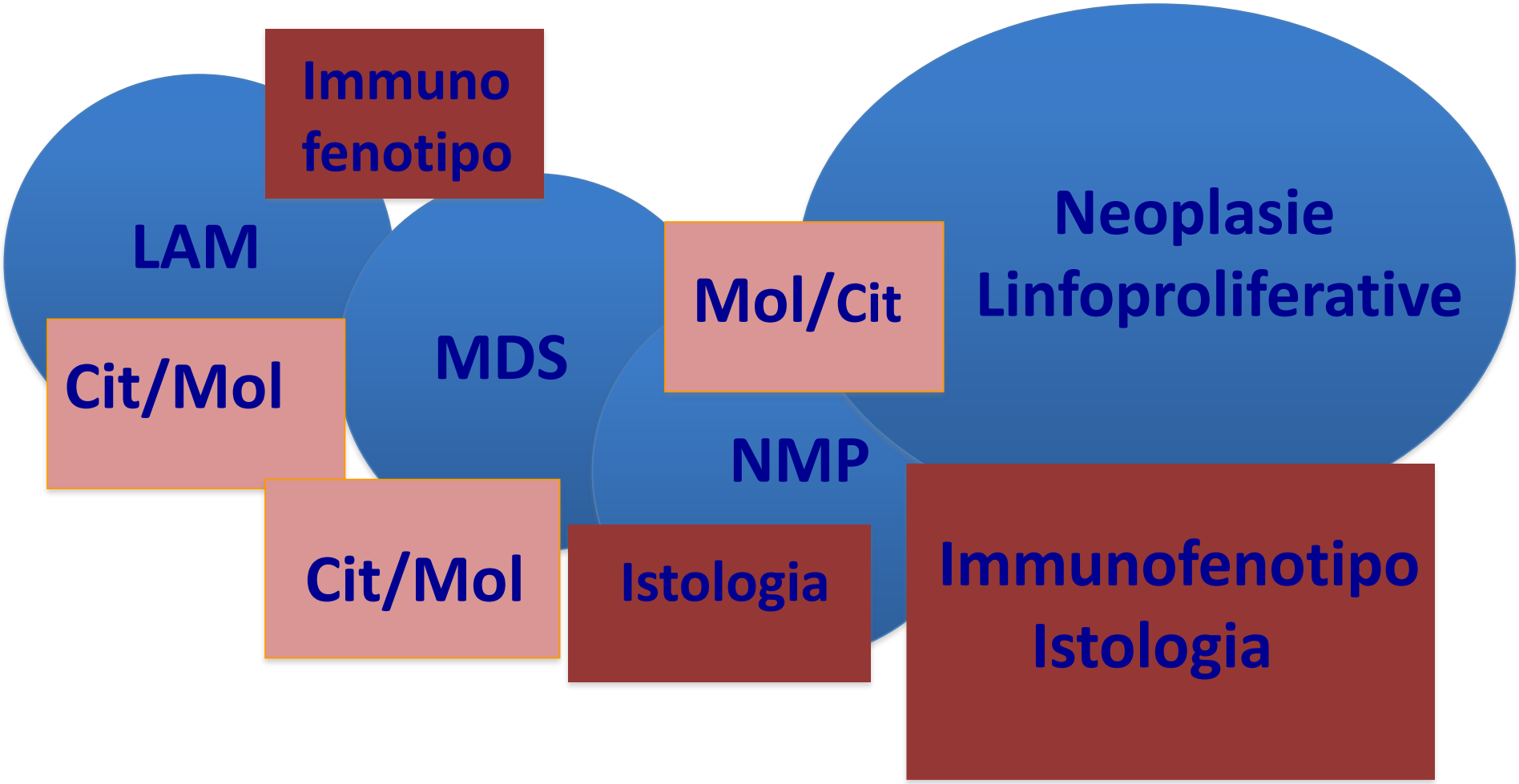


Molecular Genetics



Haferlach T, EHA 2015.

Criteri WHO nella DX delle Patologie Ematologiche



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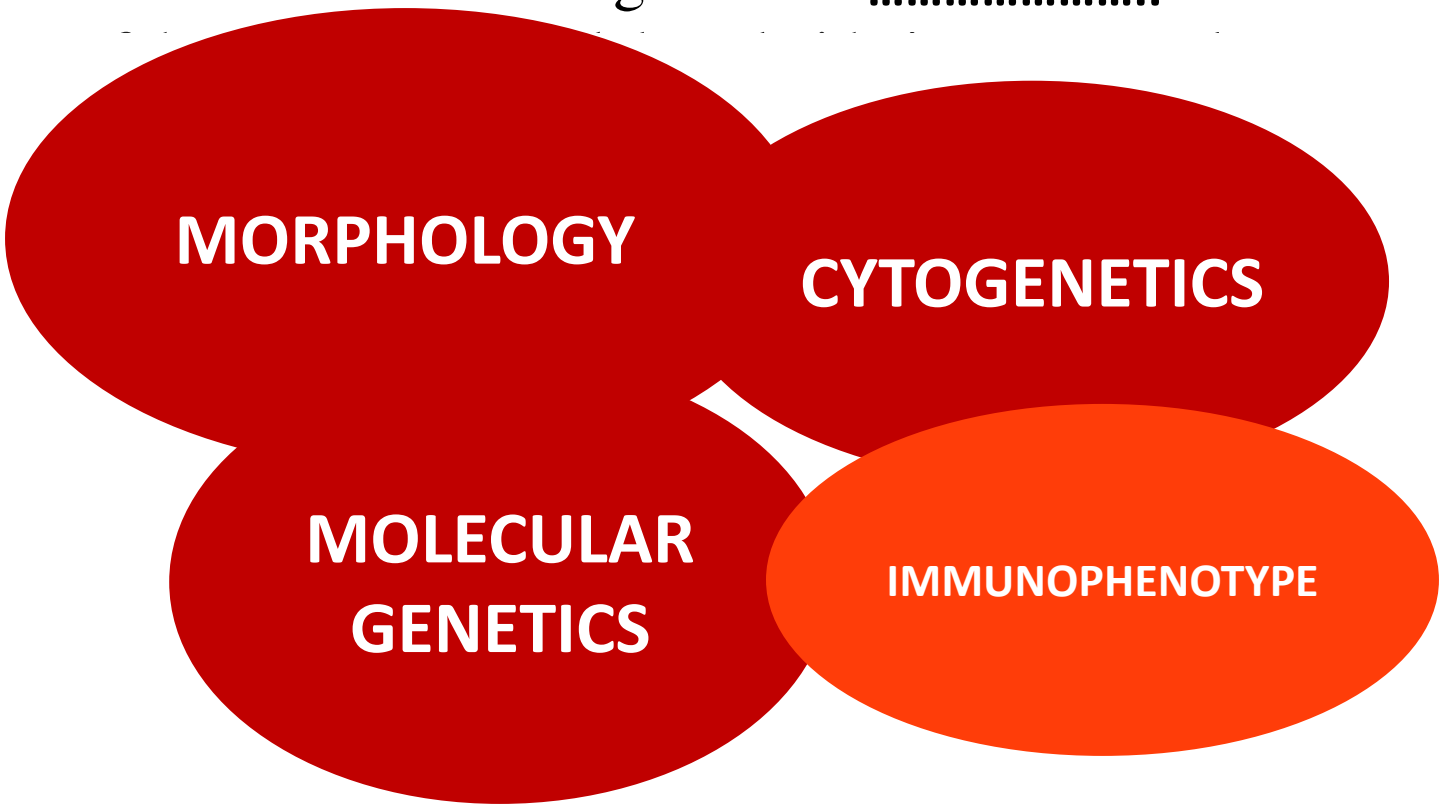
The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia

Daniel A. Arber, Attilio Orazi, Robert Hasserjian, Jürgen Thiele, Michael J. Borowitz, Michelle M. Le Beau, Clara D. Bloomfield, Mario Cazzola and James W. Vardiman

WHO 2016-2017

.....“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.“.....



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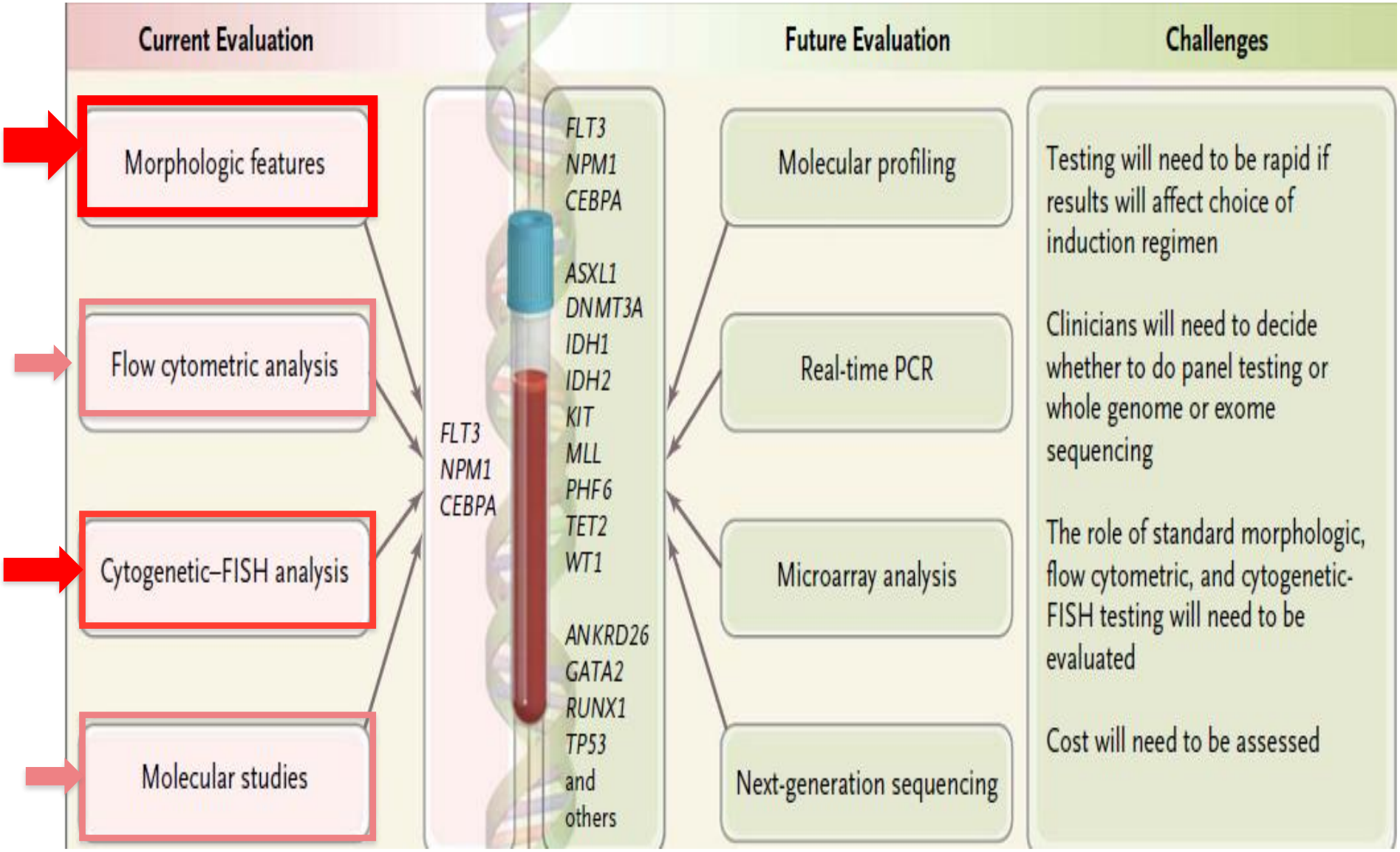
Acute myeloid leukemia (AML) and related neoplasms

- AML with recurrent genetic abnormalities
  - AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11*
  - APL with *PML-RARA*
  - AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A*
  - AML with t(6;9)(p23;q34.1);*DEK-NUP214*
  - AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
  - AML (megakaryoblastic) with t(1;22)(p13.3;q13.3);*RBM15-MKL1*
  - *Provisional entity: AML with BCR-ABL1*
  - AML with mutated *NPM1*
  - AML with biallelic mutations of *CEBPA*
  - *Provisional entity: AML with mutated RUNX1*
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms

Diagnostica nelle LAM



ELN 2010/2013

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AML, NOS	
AML with minimal differentiation	M0
AML without maturation	M1
AML with maturation	M2
Acute myelomonocytic leukemia	M4
Acute monoblastic/monocytic leukemia	M5
Pure erythroid leukemia	M6
Acute megakaryoblastic leukemia	M7
Acute basophilic leukemia	
Acute panmyelosis with myelofibrosis	

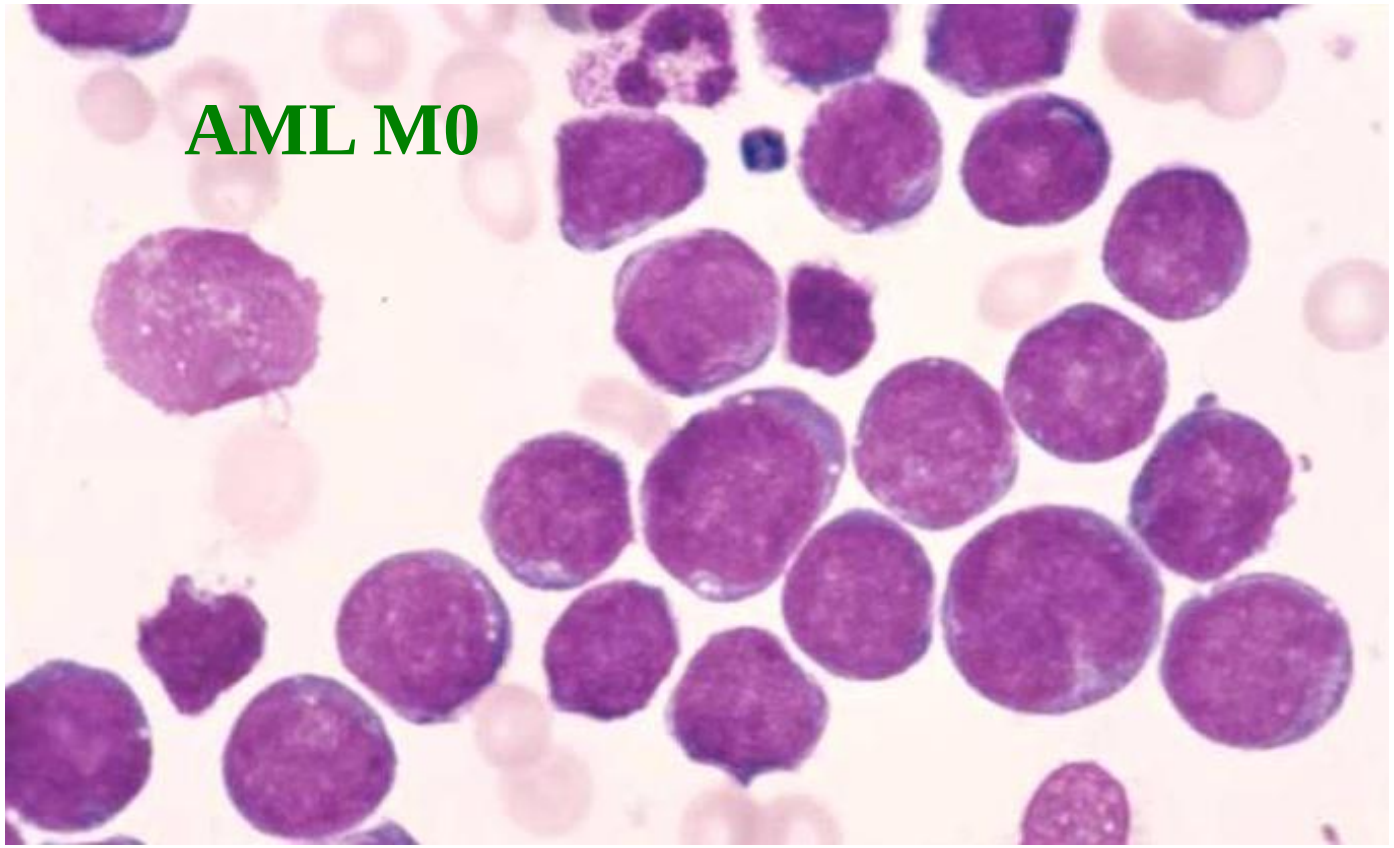
AML M0

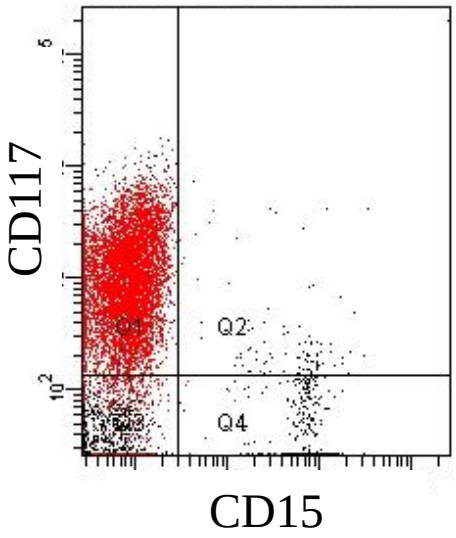
CD34+

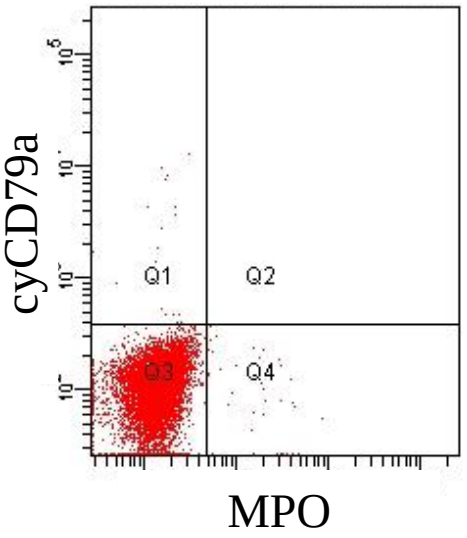
CD117+

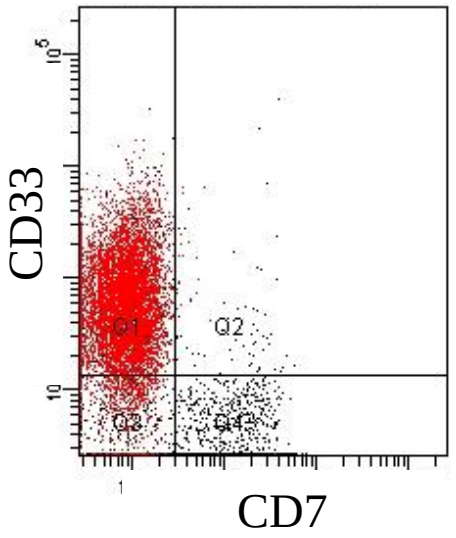
CD33+

MPO -









AML, NOS

- Myeloid sarcoma
- Myeloid proliferations related to Down syndrome
  - Transient abnormal myelopoiesis
  - Myeloid leukemia associated with Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm

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Acute leukemias of ambiguous lineage

- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); *BCR-ABL1*
- MPAL with t(v;11q23.3); *KMT2A* rearranged
- MPAL, B/myeloid, NOS
- MPAL, T/myeloid, NOS

Criteria for lineage assignment for a diagnosis of MPAL

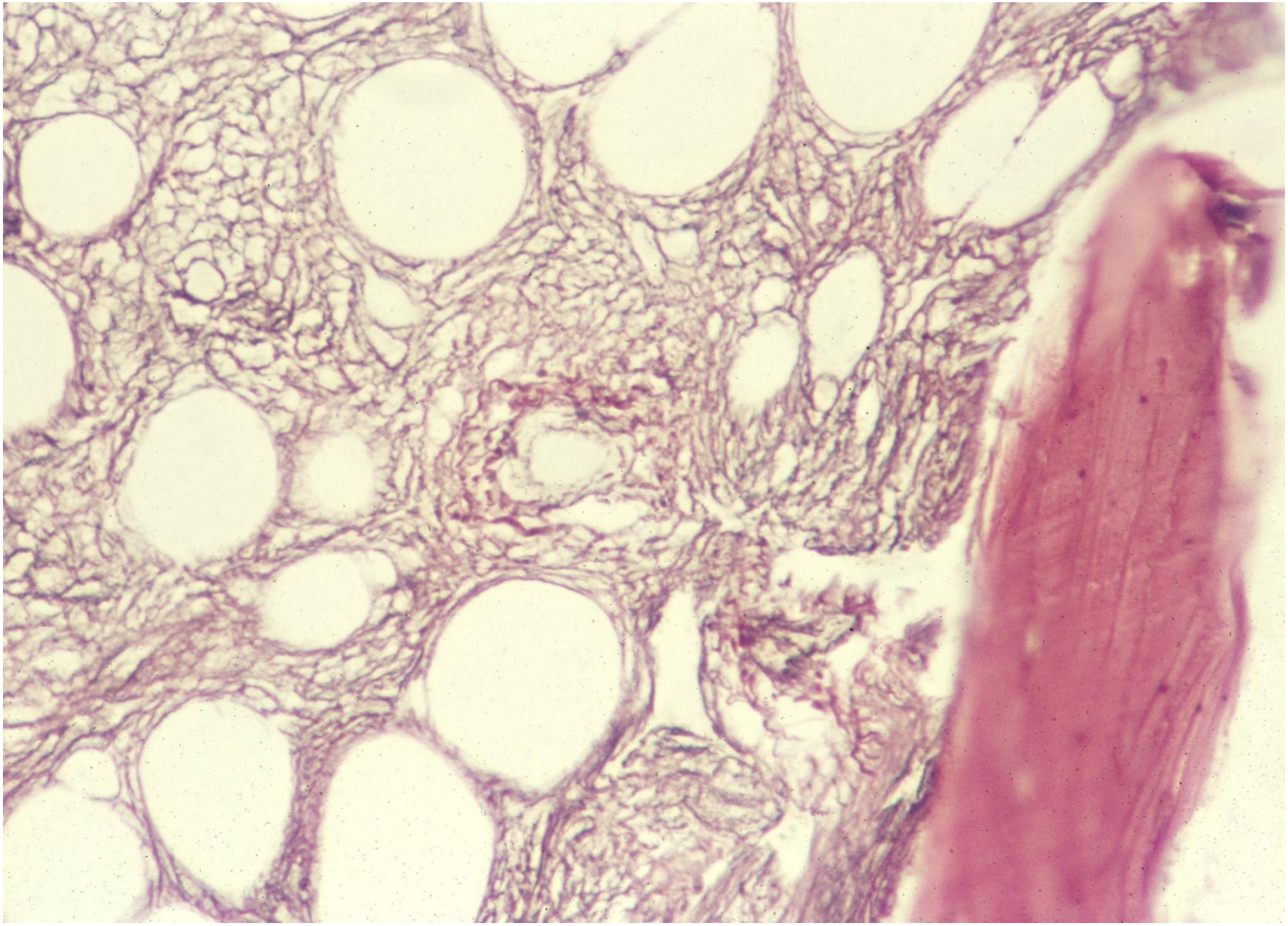
Lineage assignment criteria
Myeloid lineage
MPO* (flow cytometry, immunohistochemistry, or cytochemistry)
or
Monocytic differentiation (at least 2 of the following: nonspecific esterase cytochemistry, CD11c, CD14, CD64, lysozyme)
T-lineage
Strong† cytoplasmic CD3 (with antibodies to CD3 ε chain)
or
Surface CD3
B-lineage
Strong† CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10
or
Weak CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10

Criticità Diagnostiche per le Leucemie Acute

- Agoaspirato midollo **ipocellulare** : SMD, Mielofibrosi….
- Morfologia atipica : **Carcinosi Midollare** (?)…..

BOM

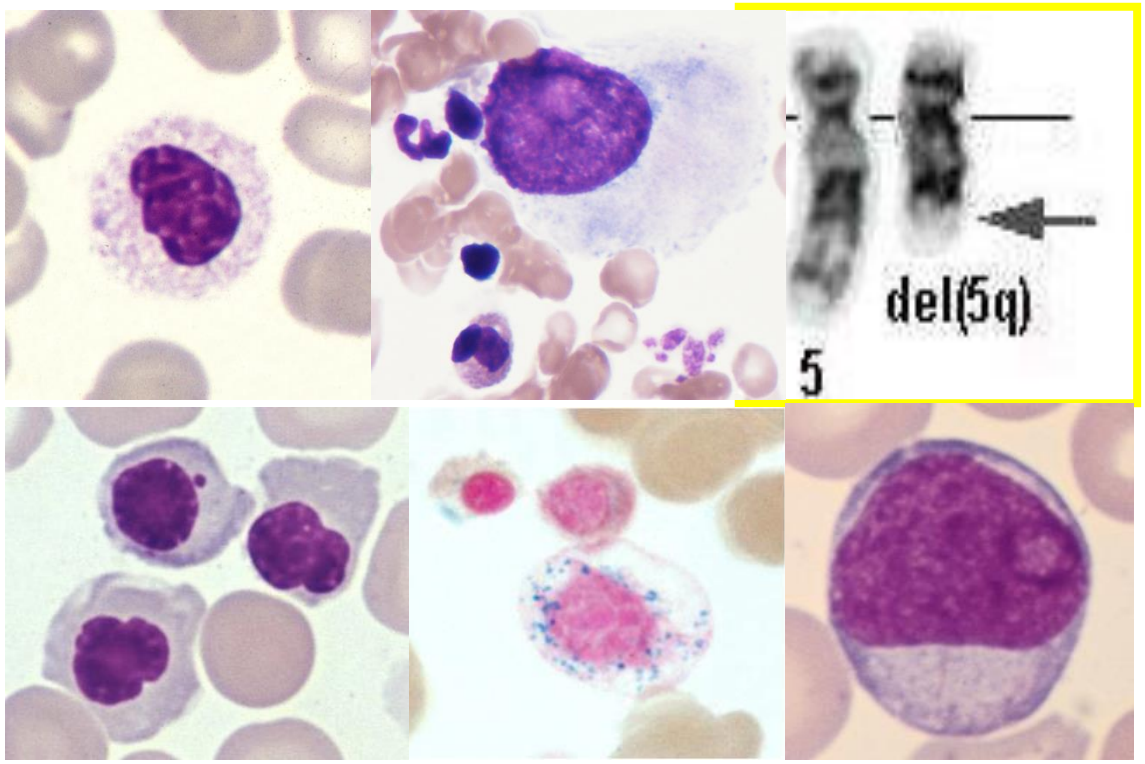
(Conta Blasti, **Immunoistochimica**, Fibrosi …….)



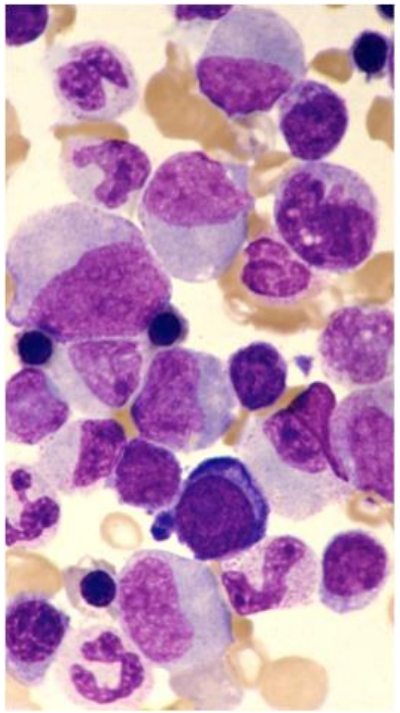
SINDROMI MIELODISPLASTICHE

Myelodysplastic syndromes / Neoplasms

Clonal haematopoietic stem cell diseases characterized by cytopenia(s), dysplasia, **ineffective haematopoiesis**, increased risk of AML development



Progression to AML
(25-30%)

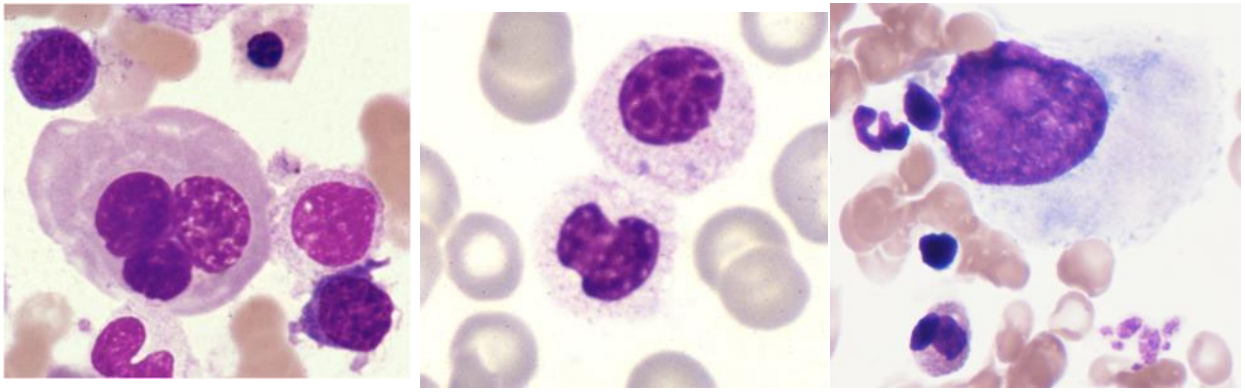


Morphologic and cytogenetic findings

Current diagnosis of MDS 2018

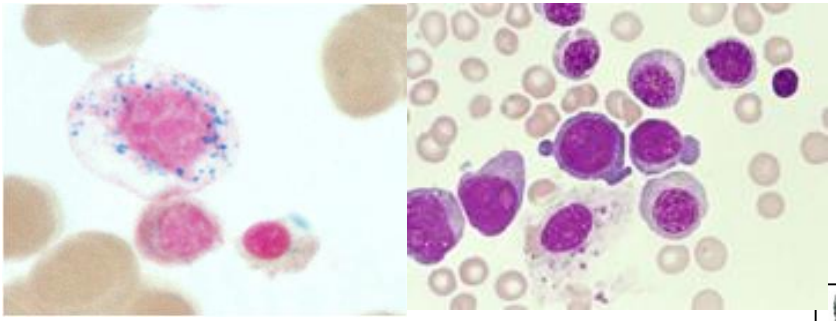
Peripheral Cytopenia

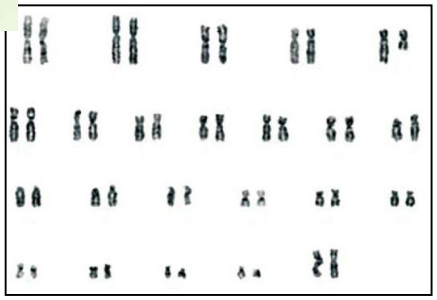
Dysplasia



Sideroblasts

Blasts





Clonal cytogenetic abnormalities Gene Mutations : SF3B1, ASXL1, TP53……..

Curr Hematol Malig Rep (2012) 7:310–320

Dysplasia Has A Differential Diagnosis: Distinguishing Genuine Myelodysplastic Syndromes (MDS) From Mimics, Imitators, Copycats and Impostors

David P. Steensma

Minimal Diagnostic Criteria for MDS as Proposed by 2006 Vienna Workshop [12•]

Both "prerequisite criteria" are necessary to make the diagnosis of MDS:

1) Marked cytopenia (i.e., hemoglobin <11 g/dL, absolute neutrophil count $<1.5\times10^9$/L, or platelets $<100\times10^9$/L) in at least one lineage lasting for ≥6 months, unless cytogenetic studies reveal MDS,

2) Exclusion of another clonal or non-clonal hematopoietic disease or non-hematopoietic disease

At least **one of three "decisive criteria"** is also required (but see below):

1) Morphologic dysplasia in at least 10 % of all cells in one or more of the major cell lineages in the bone marrow aspirate,

2) A typical MDS-associated cytogenetic abnormality (e.g., del(5q), monosomy 7)

3) Marrow blast cell proportion 5–19 %.

There are also **two "co-criteria"** for patients who meet both "prerequisite" criteria but none of the "decisive" criteria; at least one must be met:

1) Abnormal marrow immunophenotype by flow cytometry that is compatible with a diagnosis of MDS according to European Leukemia Network criteria

2) Evidence of a monoclonal cell population based on either a human androgen receptor assay, gene chip analysis, or mutation analysis.

MDS: Differential Diagnosis

Other Neoplasms :MDS/MPN…..

Congenital Syndromes:Fanconi Anemia…

Immune Disorders : SAA,ITP,PRA……

Nutritional Deficiencies :B12, Folate…..

Reactive Conditions or Infections: HIV,

Alcohol,Drugs…….

Diagnosi Differenziale

- ✓ Anemia Aplastica-EPN
- ✓ Disordini Nutrizionali (Anemia Megaloblastica)
- ✓ Alterazioni citomorfologiche da Alcolismo
- ✓ Infezioni virali (HBV, HCV, CMV,Parvovirus B19,HIV, etc)
- ✓ Sostanze tossiche (antibiotici,chemioterapici, piombo, benzene)
- ✓ Anemia dell'anziano non altrimenti definita
- ✓ Epatopatie
- ✓ Patologie Autoimmuni
- ✓ ……

PERCORSO DIAGNOSTICO……..

- Anamnesi !!
- Emocromo-Reticolociti
- Es. morfologico s. periferico
- Es. morfologico s. midollare con Perls
- Citogenetica/FISH
- Biopsia Osteomidollare*
- s-EPO
- Assetto marziale
- HLA*
- ……

Per la Diagnosi di SMD

- **E' indispensabile** rilevare una displasia in > 10% delle cellule della linea mieloide /eritroide/megacariocitaria
- **E' necessario** contare i blasti nel SP e MI
- **E' fondamentale** effettuare l'analisi cariotipica (normale però nel 40-50% circa dei casi !!)
- **E' importante** ,soprattutto nei casi senza evidenza di anomalie cromosomiche e con displasia modesta monitorare il paziente (SMDI ? o altro ?)

BLOOD, 19 MAY 2016 • VOLUME 127, NUMBER 20

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]

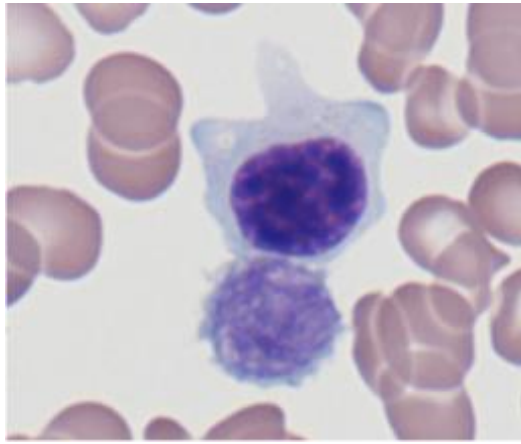
Myelodysplastic syndromes (MDS)

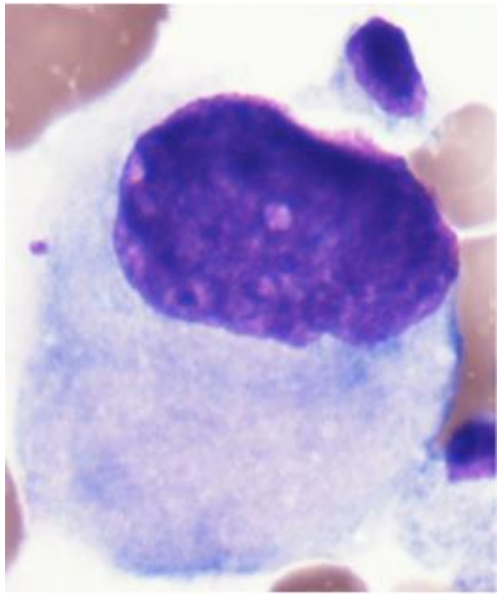
- MDS with single lineage dysplasia
- MDS with ring sideroblasts (MDS-RS)
  - MDS-RS and single lineage dysplasia
  - MDS-RS and multilineage dysplasia
- MDS with multilineage dysplasia
- MDS with excess blasts
- MDS with isolated del(5q)
- MDS, unclassifiable
- *Provisional entity: Refractory cytopenia of childhood*

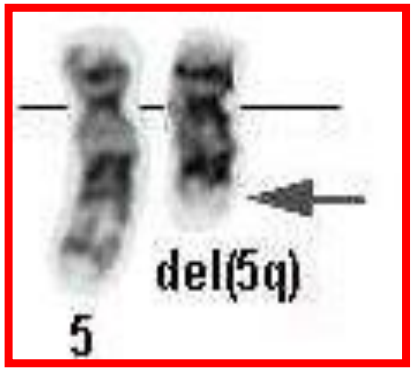
Myeloid neoplasms with germ line predisposition

Sindrome 5q-

- 5q- unica anomalia
- M/F 1:4
- Anemia Macrocitica
- Megacariociti mononucleati
- Ipoplasia eritroide……
- Decorso clinico favorevole
- Rara trasformazione in Leucemia Acuta
- Risposta alla terapia con lenalidomide







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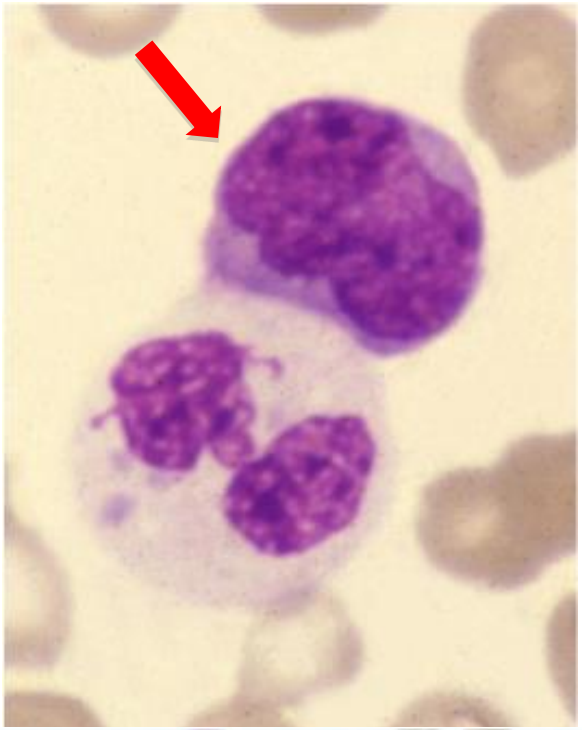
Peripheral blood and BM findings of MDS

Name	Dysplastic lineages	Cytopenias*	Ring sideroblasts as % of marrow erythroid elements	BM and PB blasts	Cytogenetics by conventional karyotype analysis
MDS with excess blasts (MDS-EB)					
MDS-EB-1	0-3	1-3	None or any	BM 5%-9% or PB 2%-4%, no Auer rods	Any
MDS-EB-2	0-3	1-3	None or any	BM 10%-19% or PB 5%-19% or Auer rods	Any
MDS, unclassifiable (MDS-U)					
with 1% blood blasts	1-3	1-3	None or any	BM <5%, PB = 1%,‡ no Auer rods	Any
with single lineage dysplasia and pancytopenia	1	3	None or any	BM <5%, PB <1%, no Auer rods	Any
based on defining cytogenetic abnormality	0	1-3	<15%§	BM <5%, PB <1%, no Auer rods	MDS-defining abnormality
Refractory cytopenia of childhood	1-3	1-3	None	BM <5%, PB <2%	Any

SMD con eccesso di blasti

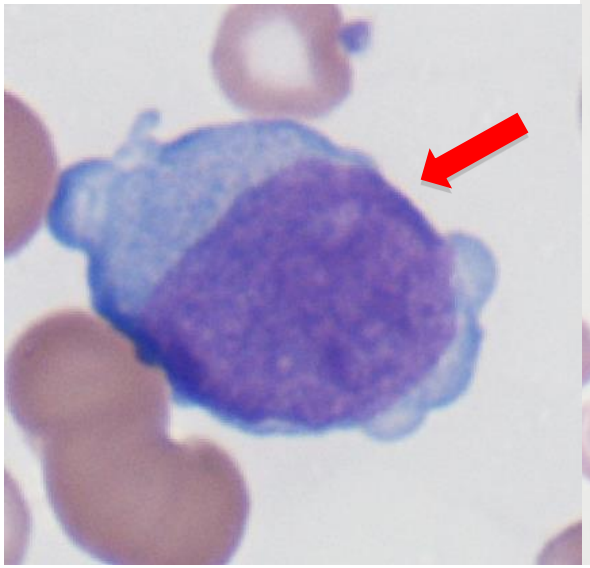
SP

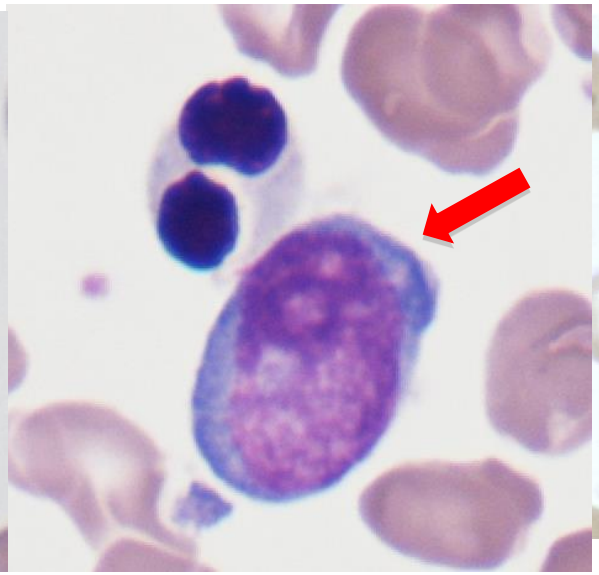


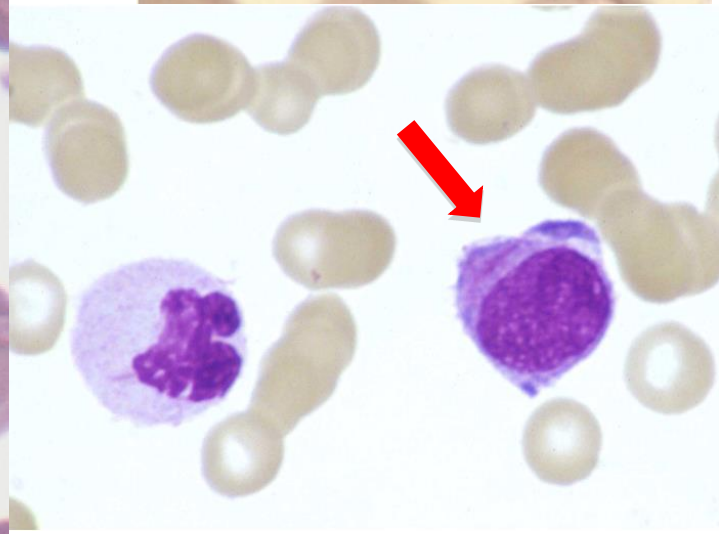
✓Anisopoichilocitosi con macrocitosi

✓ Disgranulopoiesi

✓Blasts <5% (AREB-1)
5-19% (AREB-2)







SMD con eccesso di Blasti

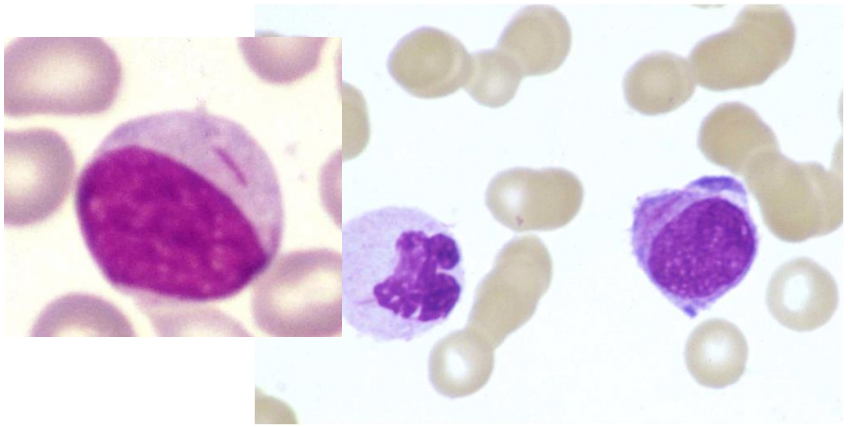
MI

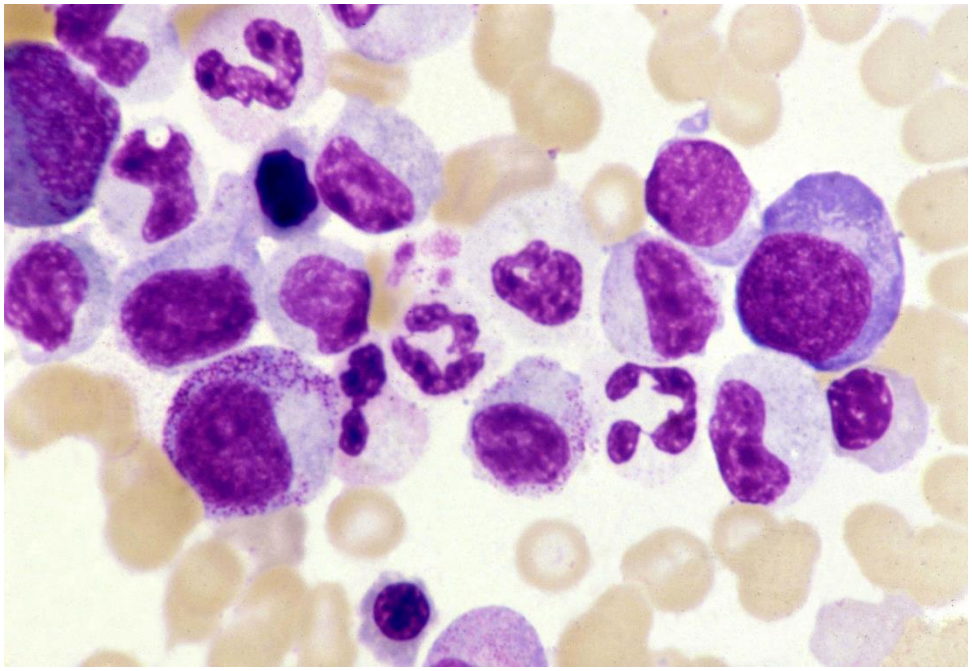
✓ Disgranulopoiesi, Diseritropoiesi, Dismegacariocitopoiesi

✓BOM: Ipercellulare o ipocellulare in 10-15%; localizzazione abnorme di precursori immaturi (ALIP)

✓**Blasti 5-9% (EB-1)**
10-19% (EB-2)

✓o **Blasti con corpi di Auer (EB-2)**





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MDS-U

	Dysplastic lineages	Cytopenias	Bone marrow and peripheral blood blasts
MDS, unclassifiable (MDS-U)			
• with 1% blood blasts	1-3	1-3	BM <5%, PB=1%[3], no Auer rods
• with single lineage dysplasia and pancytopenia	1	3	BM <5%, PB <1%, no Auer rods
• based on defining cytogenetic abnormality	0	1-3	BM <5%, PB <1%, no Auer rods

Cytopenias defined as **haemoglobin <10 g/dL, platelet count <100 x 109/L, and absolute neutrophil count <1.8 x 109/**L

Criticità Diagnostiche per le SMD

- Agoaspirato midollo ipocellulare : Aplasia midollare ,LAM

↓

BOM

(conta Blasti ,Fibrosi ,Immunoistochimica….)

- Diagnosi Differenziale con Displasia associata ad altre patologie (Infezioni,M.Autoimmuni,Deficit di B12….)

↓

Altre indagini…….

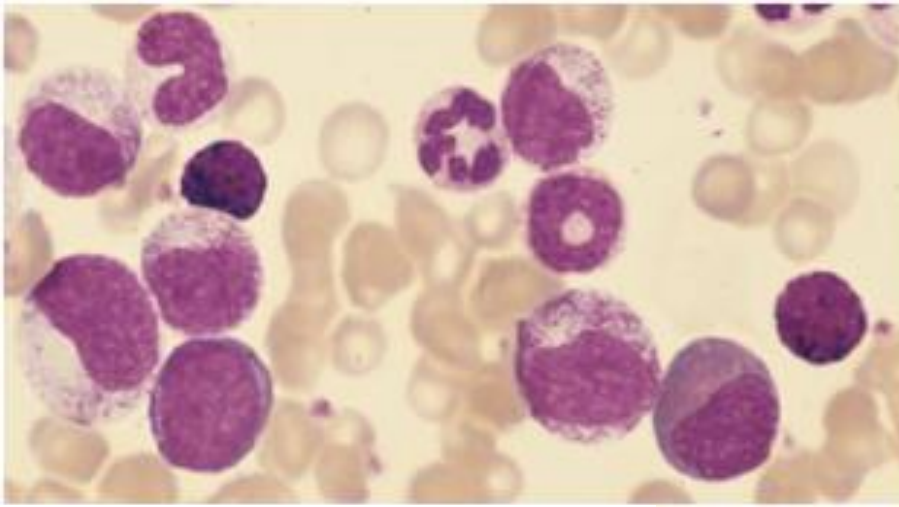
Rivalutazione nel tempo…….

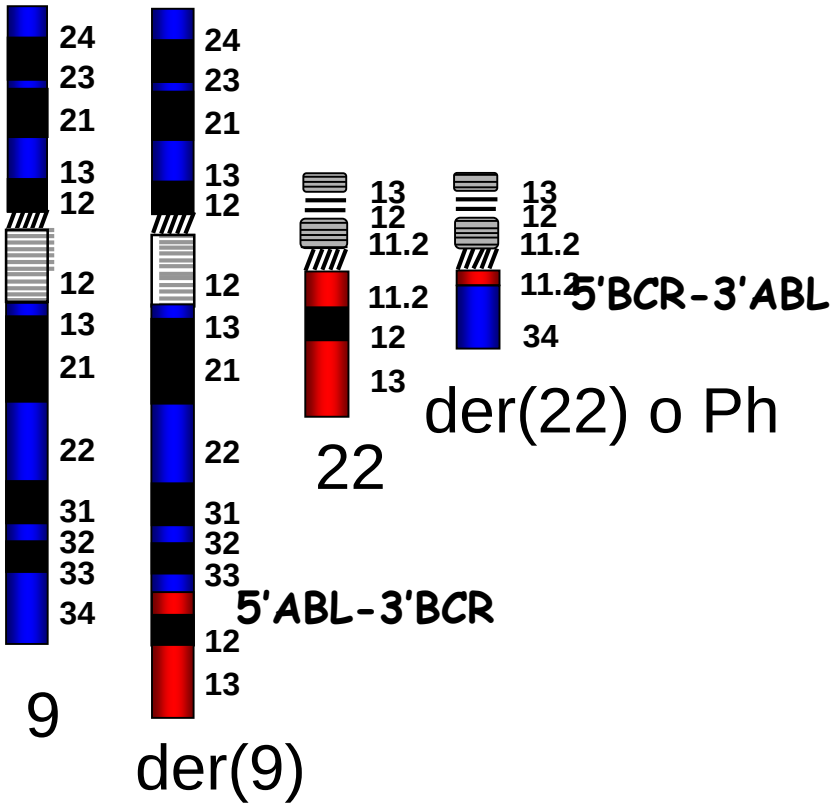
Per la diagnosi e Classificazione delle NMPcr

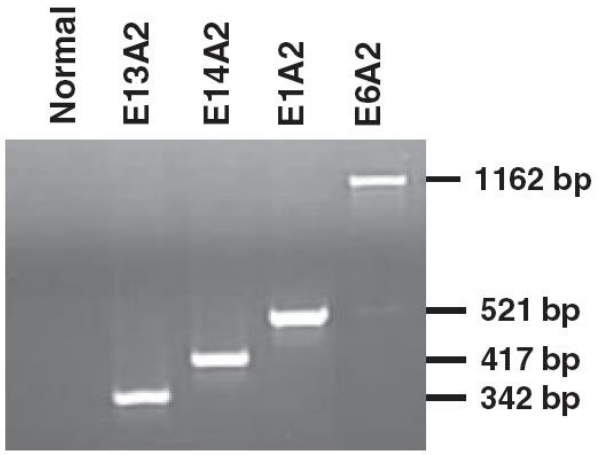
- ✓ Caratteristiche cliniche (Splenomegalia,Trombosi..)
- ✓Caratteristiche Istologiche /Citologiche **(BOM*)**
- ✓Anomalie molecolari (JAK2,MPL..) **(Mut.JAK2..)**
- ✓Altri Tests (**Hb, Hct,WBC, Plts , MorfologiaPe EPO**,Citogenetica/ FISH,Rx….)

La diagnosi della LMC

Morfologia Pe +
Morfologia MI (Asp)

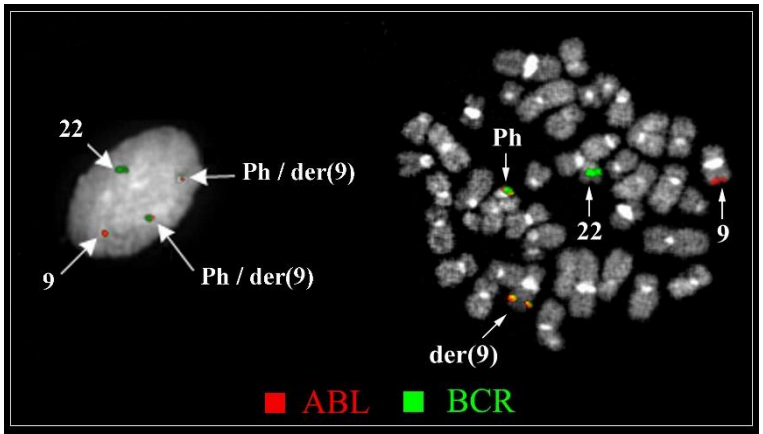






RT-PCR

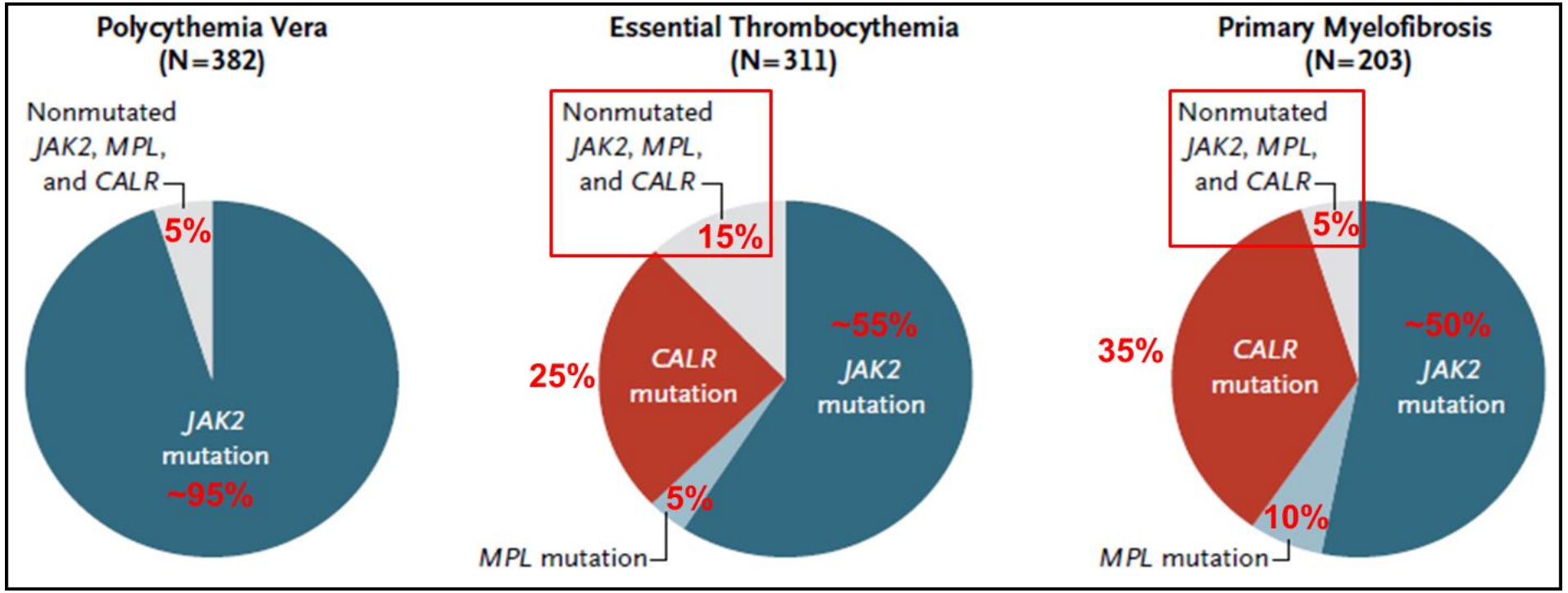
BCR-ABL



e/o **FISH**

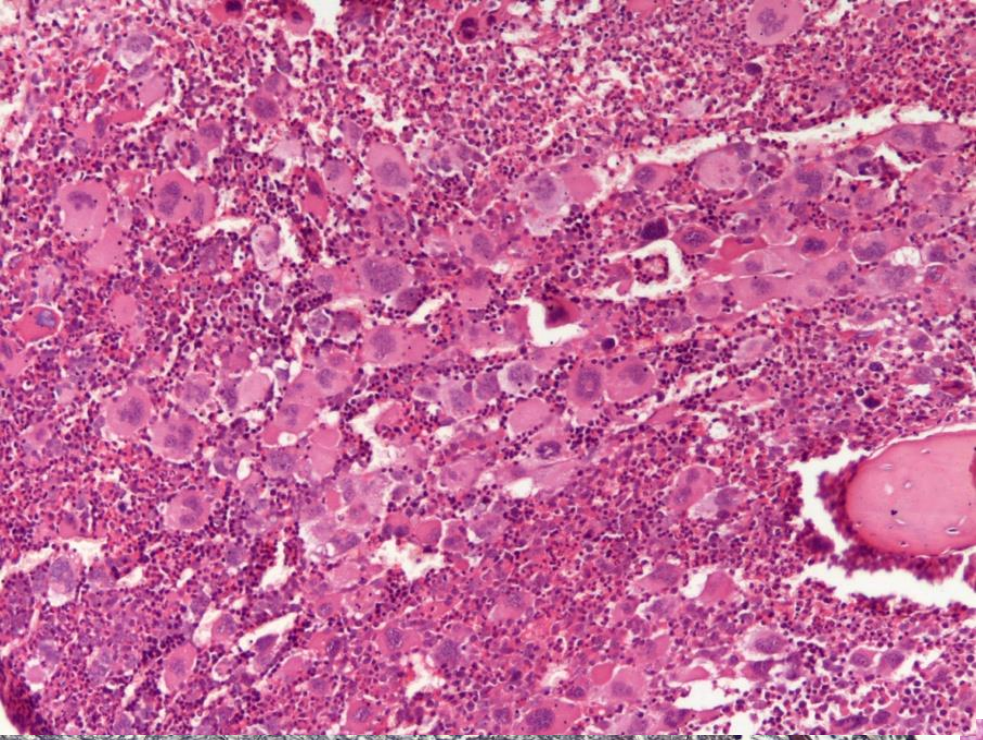
Citogenetica Convenzionale
t(9;22)(q34;q11.2)

Novel Marker in MPN: Mutations of *CALR*



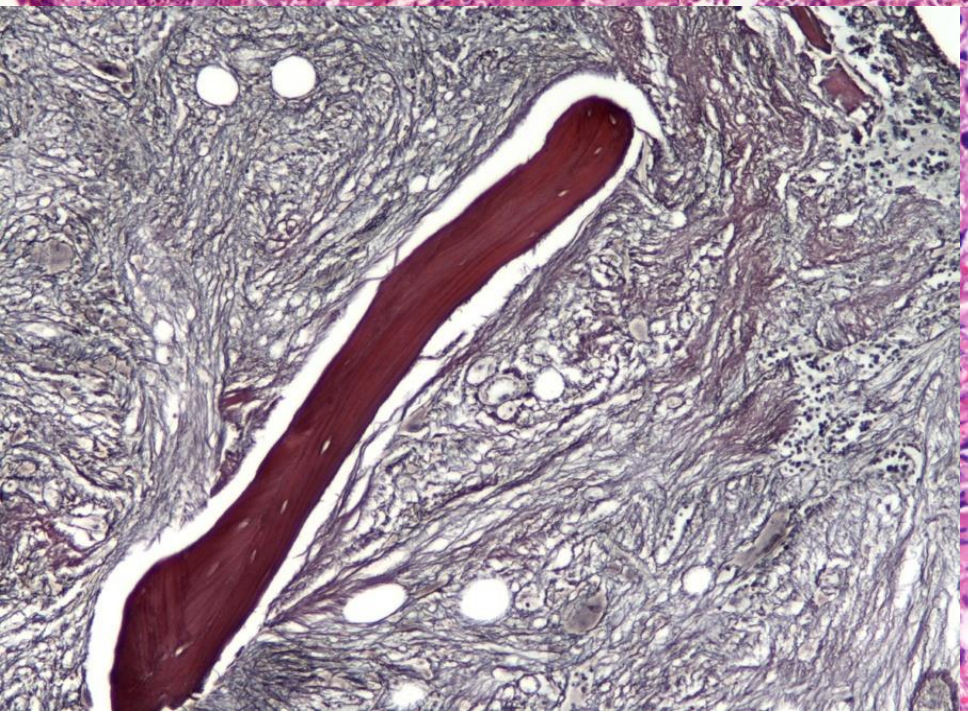
Klampfl et al., N Engl J Med 2013

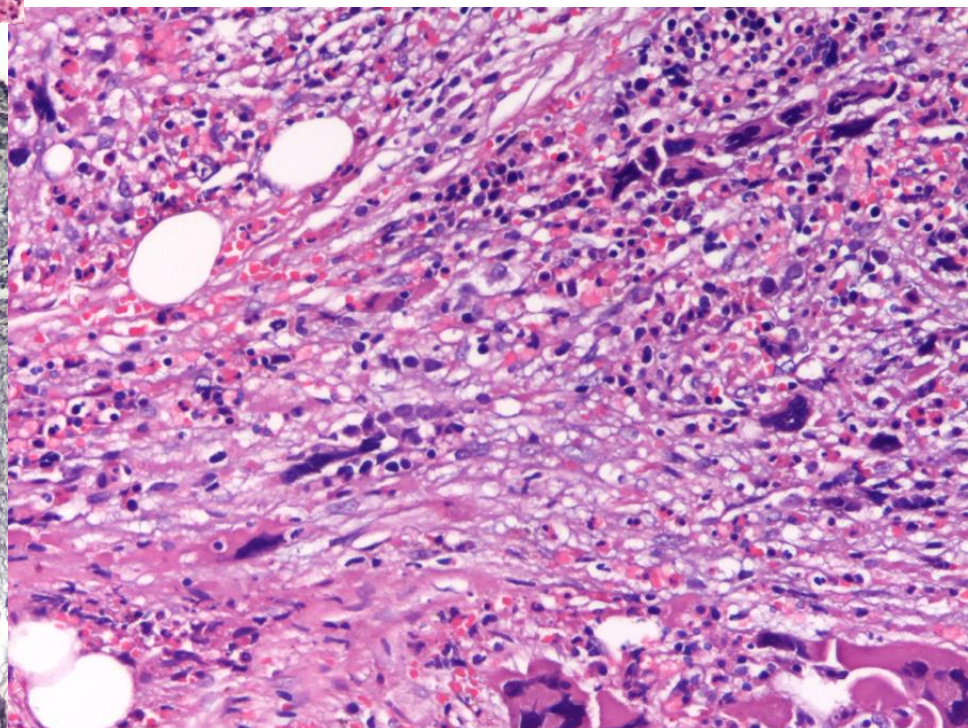
Screening for *JAK2*^{mut} + *CALR*^{mut} + *MPL*^{mut} → Clonality in ~95% of MF



Megakaryocytes

- Anisocytosis(small-large)
- Abnormal nuclear-cytoplasmic ratio
- Abnormal chromatin clumping
- Hyperchromatic nuclei,plump lobulation “bulbous,cloud-like,balloon-shaped,bare”



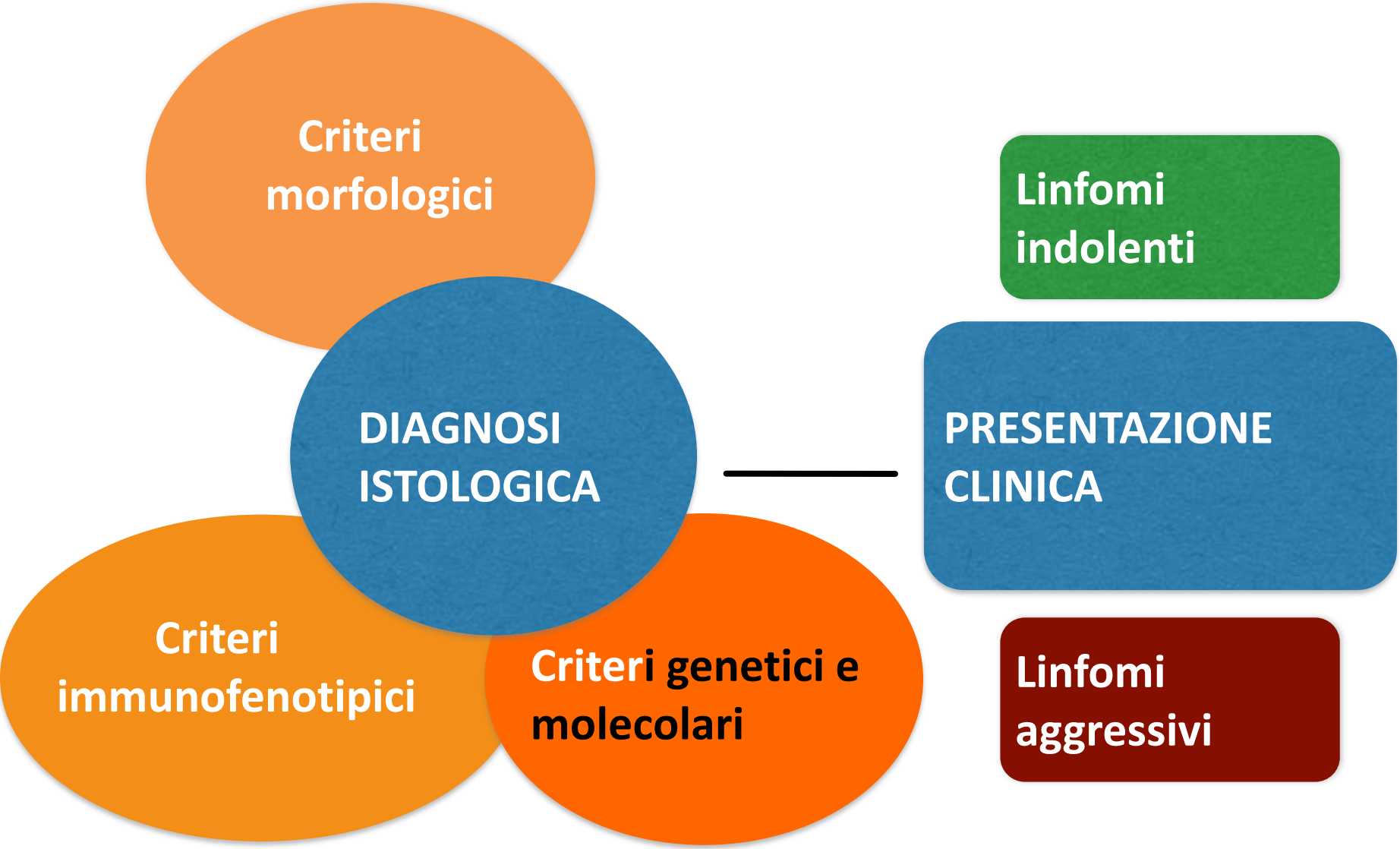


Criticità Diagnostiche per le NMP Ph-

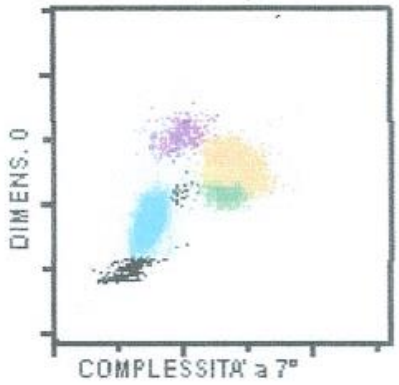
- Casi con Eritrocitosi ,Trombocitosi “reattive”
- Casi con Fibrosi Midollare sec. ad altre Patologie
- Casi senza evidenza di alterazioni molecolari !!

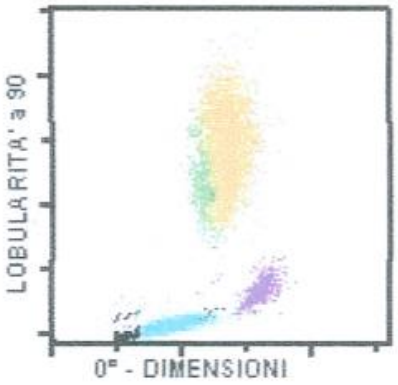
Altre indagini…….
Rivalutazione nel tempo…….

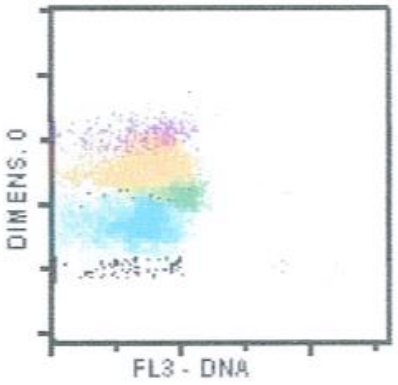
Neoplasie Linfoproliferative / Linfomi



Esame Ematologico



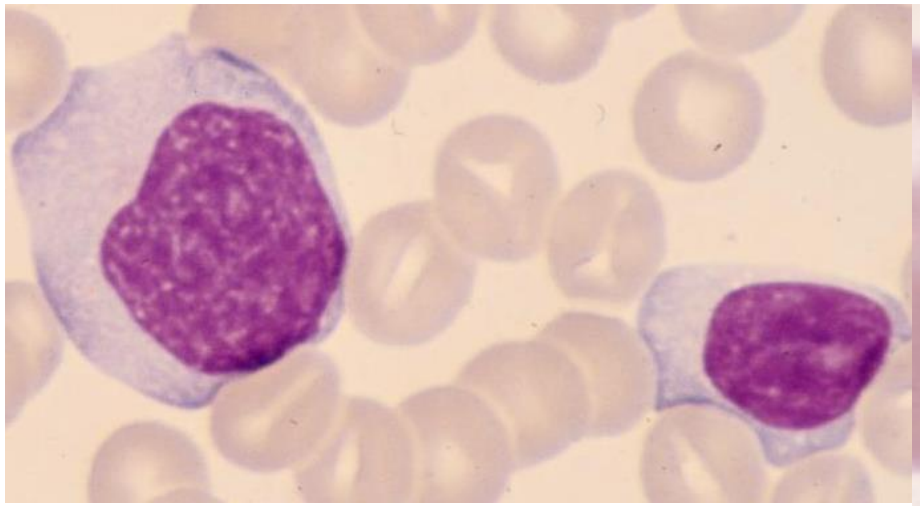


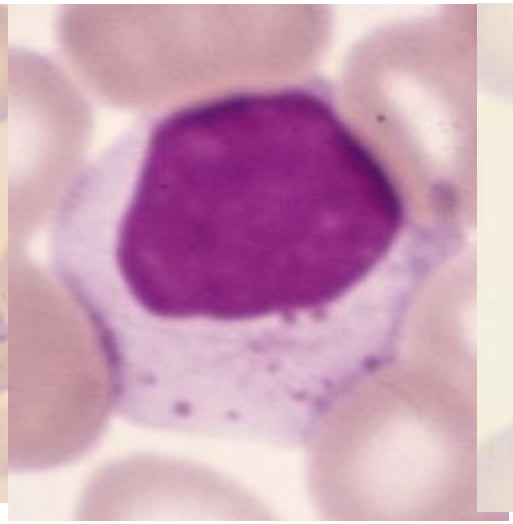


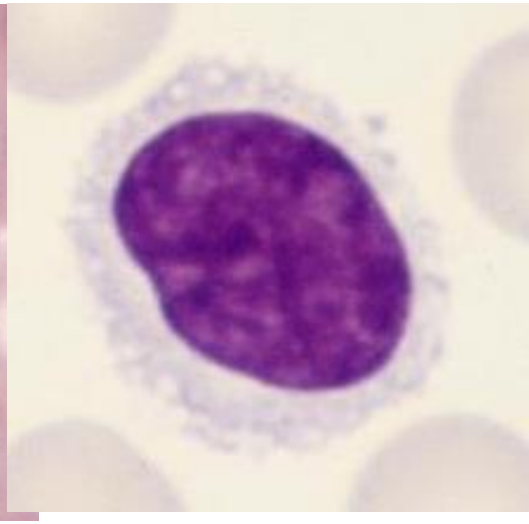
WBC

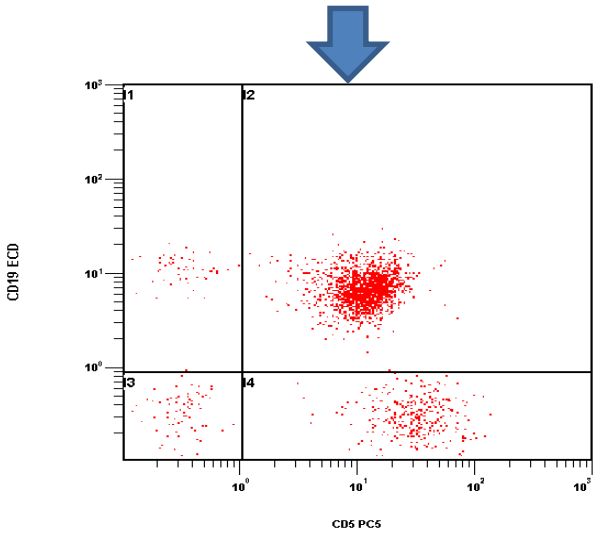
WBC	14.0	*10e3/ul*
Neu #	7.28	*10e3/ul*
Linfo #	4.58	*10e3/ul*
Mono #	1.09	*10e3/ul*
Eos #	1.03	*10e3/ul*
Baso #	.067	*10e3/ul*

WVF	.996	
Neu %	51.9	%
Linfo %	32.6	%
Mono %	7.73	%
Eos %	7.32	%
Baso %	.478	%









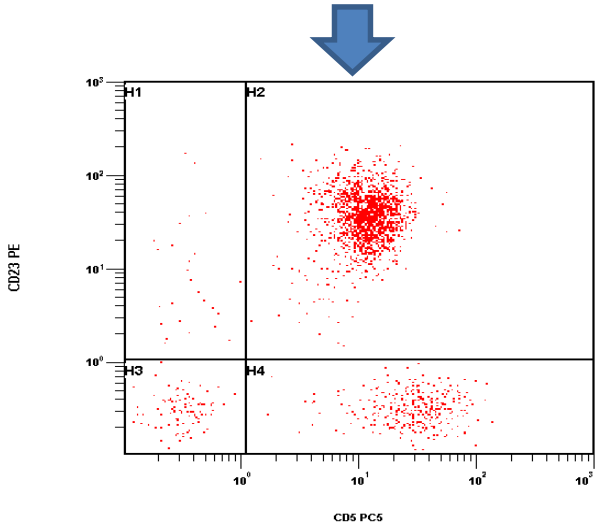


Tabella 1. Marcatori di membrana nelle diverse sindromi linfoproliferative

	CD5	*CD19/CD20*	*FMC7*	*CD23*	*CD25*	*CD38*	*CD10*	*CD43*	*sIg*
LLC	+	+	-/+	++	-	-/+	-	+	+/-
LPL	-	+	++	-/+	-	-	-	+/-	++
HCL	-	+	+	++	++	-/+	-	+	++
LM	+	+	+	-	-	-	-	+	+
LF	-	+	+	-	-	-/+	++	-	+

Abbreviazioni. LLC: Leucemia Linfatica Cronica; LPL: Leucemia Prolinfocitica; HCL: Leucemia a Cellule Capellute; LM: Linfoma Mantellare; LF: Linfoma Follicolare

Diagnosis of CLL
NCI, iwCLL and ESMO guidelines

Variable	NCI[1]	iwCLL[2]	ESMO[3]
Diagnosis			
Lymphocytes (x 10^9/L)	> 5 Markers: CD19, CD20, CD23, CD5	≥ 5 Markers: CD5, CD19, CD20, CD23 surface immunoglobulin, CD79b	≥ 5 Markers: CD5, CD19, CD20, CD23 surface immunoglobulin, CD79b
Prolymphocytes (%)	≤ 55	≤ 55	Not stated
Duration of lymphocytosis	None defined	≥ 3 months	≥ 3 months
Bone marrow lymphocytes (%)	≥ 30	> 30	Bone marrow biopsy not needed for diagnosis
Staging	Modified Rai, correlate with Binet	Modified Rai or Binet	Modified Rai or Binet

Graphical Elaboration from text data

2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

Mature B-cell neoplasms	
Chronic lymphocytic leukemia/small lymphocytic lymphoma	**CLL**
Monoclonal B-cell lymphocytosis*	
B-cell prolymphocytic leukemia	**PLL**
Splenic marginal zone lymphoma	**SML**
Hairy cell leukemia	
Splenic B-cell lymphoma/leukemia, unclassifiable	
Splenic diffuse red pulp small B-cell lymphoma	
Hairy cell leukemia-variant	**HCL**
Lymphoplasmacytic lymphoma	
Waldenström macroglobulinemia	**MW**
Monoclonal gammopathy of undetermined significance (MGUS), IgM*	
μ heavy-chain disease	
γ heavy-chain disease	
α heavy-chain disease	
Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*	
Plasma cell myeloma	
Solitary plasmacytoma of bone	
Extraosseous plasmacytoma	
Monoclonal immunoglobulin deposition diseases	

MGUS/ Plasma Cell Myeloma

WHO Classification of Lymphoid Neoplasms

Precursor B –cell Neoplasms

Precursor B-lymphoblastic leukemia/lymphoma

(precursor B-ALL)

Precursor T-cell and NK-cell Neoplasms

Precursor T-lymphoblastic lymphoma/leukemia

(precursor T-ALL)

Review Series

BLOOD, 19 MAY 2016 • VOLUME 127, NUMBER 20

THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

The 2016 revision of the World Health Organization classification of lymphoid neoplasms

Steven H. Swerdlow,[1] Elias Campo,[2] Stefano A. Pileri,[3] Nancy Lee Harris,[4] Harald Stein,[5] Reiner Siebert,[6] Ranjana Advani,[7] Michele Ghielmini,[8] Gilles A. Salles,[9] Andrew D. Zelenetz,[10] and Elaine S. Jaffe[11]

Primary DLBCL of the central nervous system (CNS)
Primary cutaneous DLBCL, leg type
EBV$^+$ DLBCL, NOS*
*EBV$^+$ mucocutaneous ulcer**
DLBCL associated with chronic inflammation
Lymphomatoid granulomatosis
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
ALK$^+$ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
*HHV8$^+$ DLBCL, NOS**
Burkitt lymphoma
*Burkitt-like lymphoma with 11q aberration**
High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
High-grade B-cell lymphoma, NOS*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

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Hodgkin lymphoma
Nodular lymphocyte predominant Hodgkin lymphoma
Classical Hodgkin lymphoma
Nodular sclerosis classical Hodgkin lymphoma
Lymphocyte-rich classical Hodgkin lymphoma
Mixed cellularity classical Hodgkin lymphoma
Lymphocyte-depleted classical Hodgkin lymphoma

Criticità Diagnostiche in Ematologia

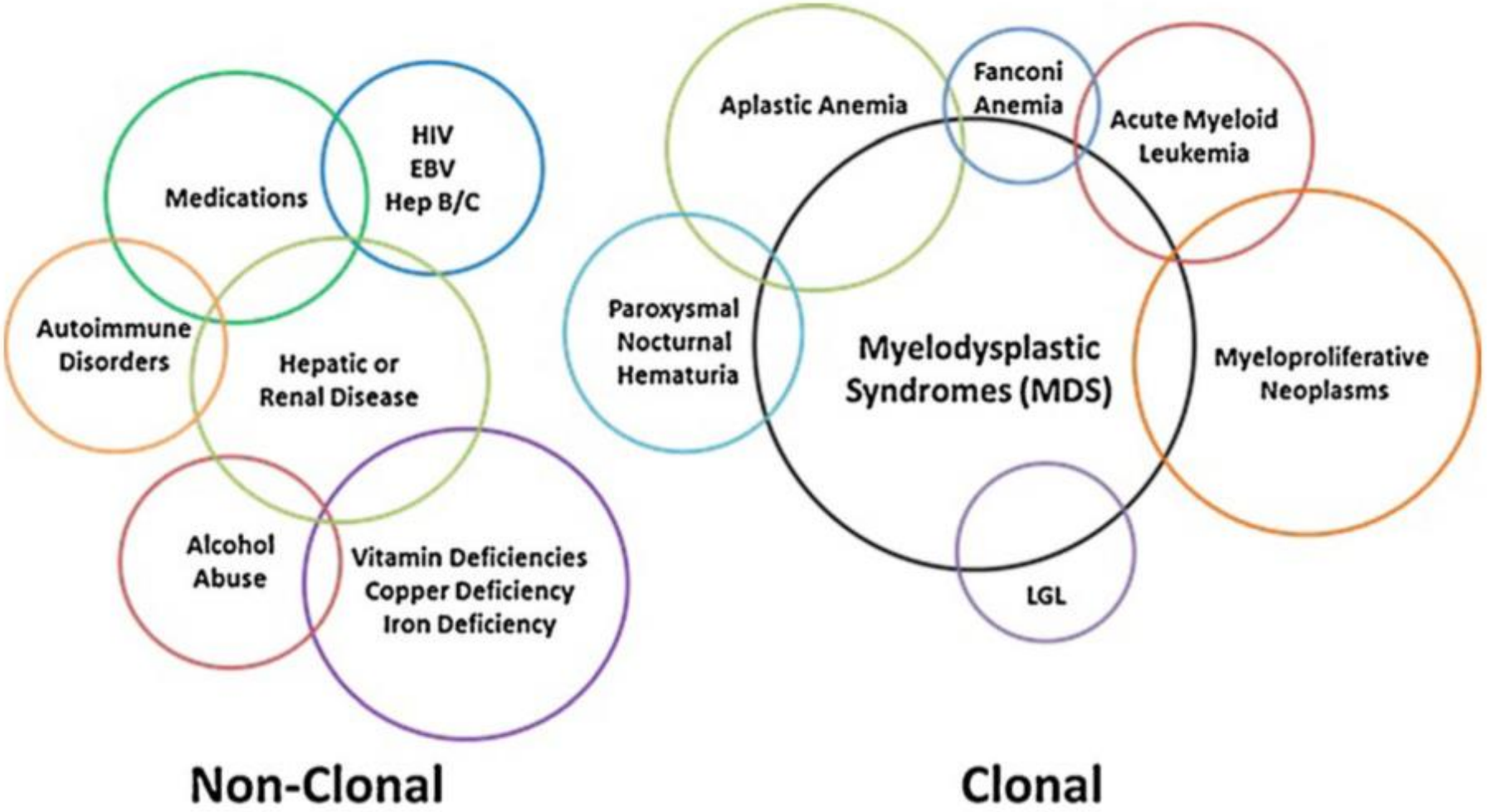
- **Citopenie ……**SMD
- **Eosinofilie……**LECr
- **Monocitosi**……SMD, LMMcr
- **Piastrinosi/Eritrocitosi**……TE,LMC,MF
- **Linfocitosi**…… LLC , Linfomi
- **MGUS**……Mieloma Multiplo

Esempi

Criticità Diagnostiche in Ematologia

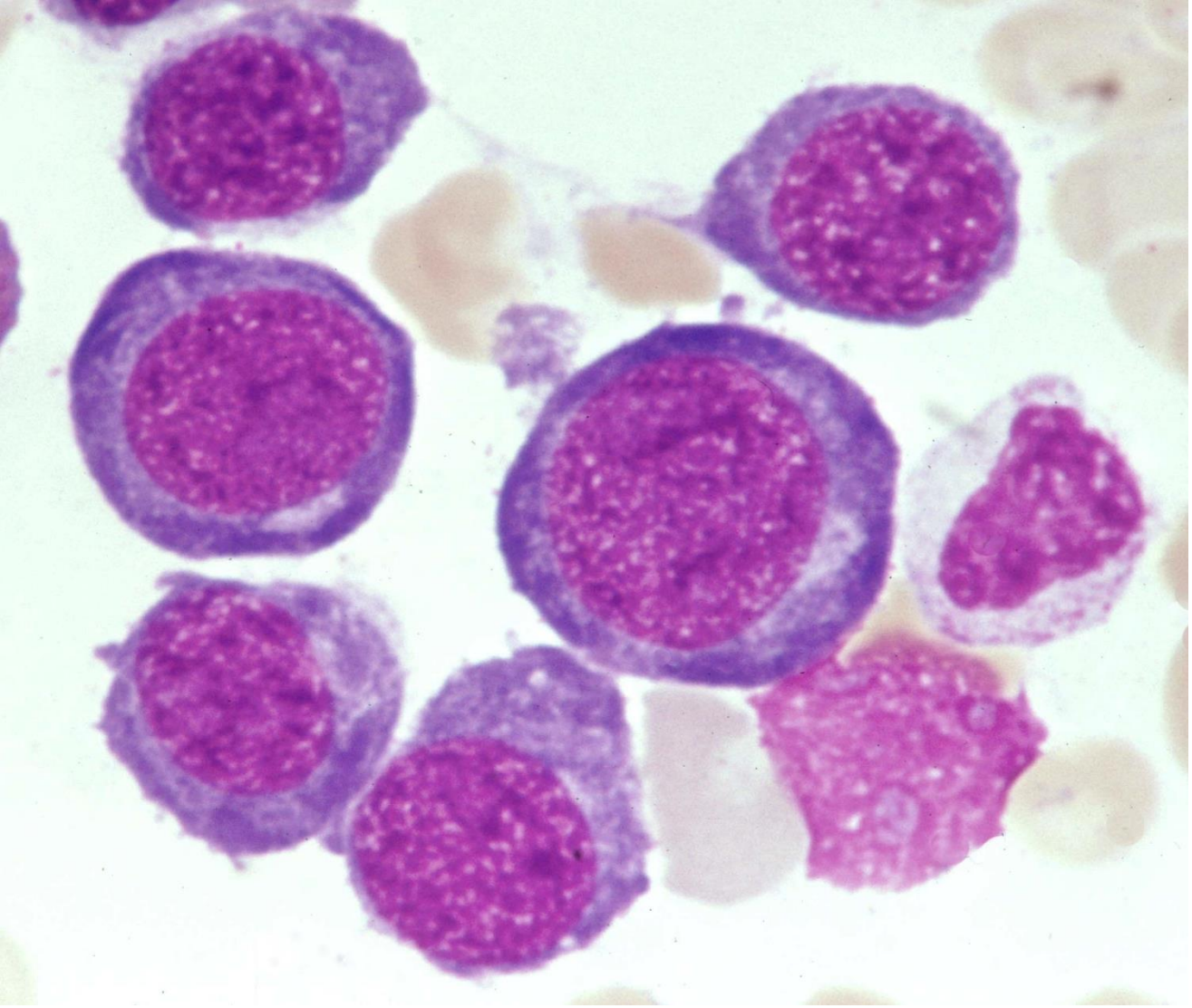
- **Citopenie ……**SMD
- **Eosinofilie……**LECr
- **Monocitosi**……SMD, LMMcr
- **Piastrinosi/Eritrocitosi**……TE,LMC,MF
- **Linfocitosi**…… LLC , Linfomi
- **MGUS**……Mieloma Multiplo

CYTOPENIAS



….the diagnosis of **Cytopenias** remains a **core task** of hematology clinical practice……..

Bejar, Curr Hematol Malig Rep 2015



C .A. 41 y

Hb 4.5gr/dl
ANC 1.5×10^9/L
Plt 90×10^9 /L

Cobalamin 150 pg/ml

Anemia Megaloblastica

Caso Clinico

- Paz. 55aa
- Febbre
- Disfagia,Disfonia ,Tosse
- Tumefazioni corde vocali
- Rx Torace ndp
- E+P Monocitosi
- Episodio Influenzale : Tp Ab/Prd
- Apiressia 7 gg
- Febbricola /Disfagia
- E+P **Monocitosi***
- ……

Per la Dx in Ematologia…….