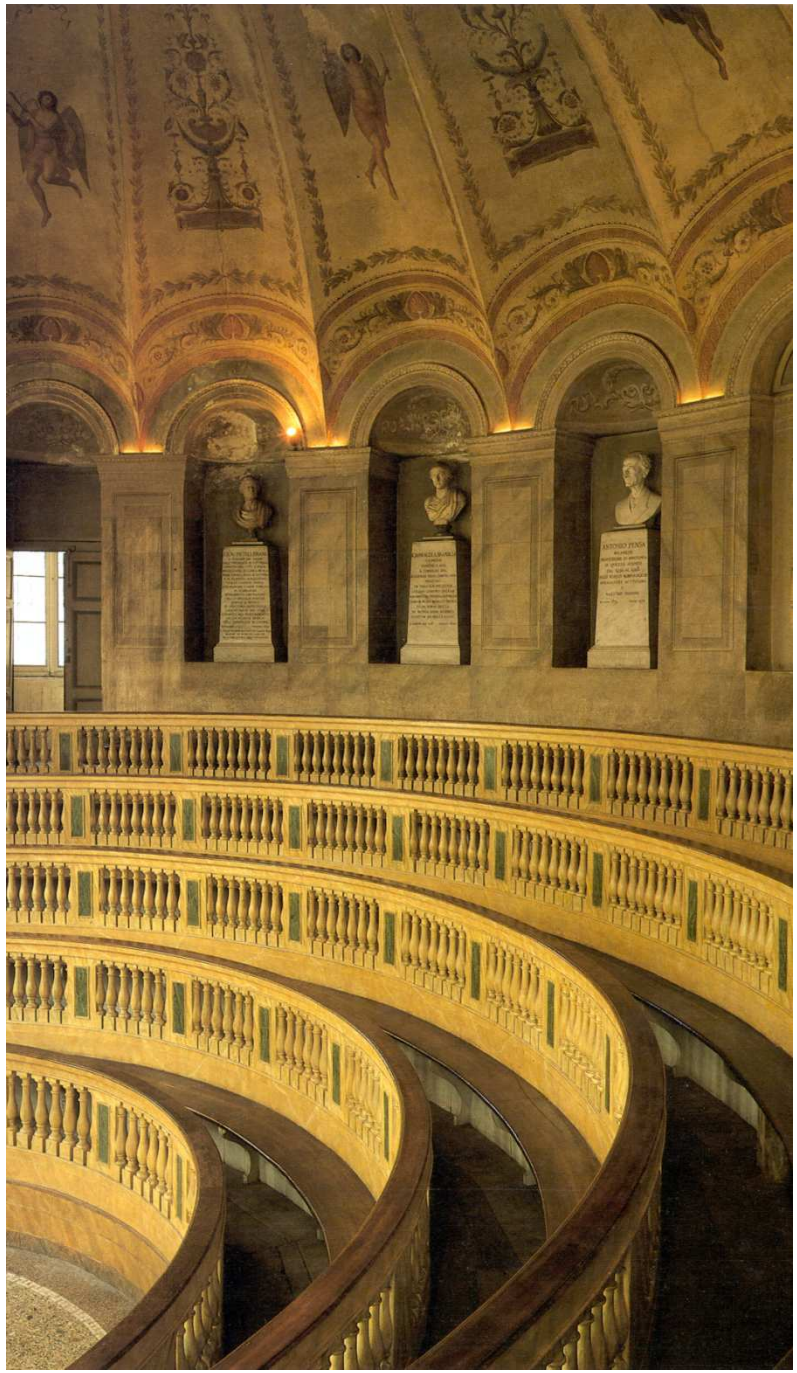

Sindromi Mielodisplastiche: il percorso diagnostico

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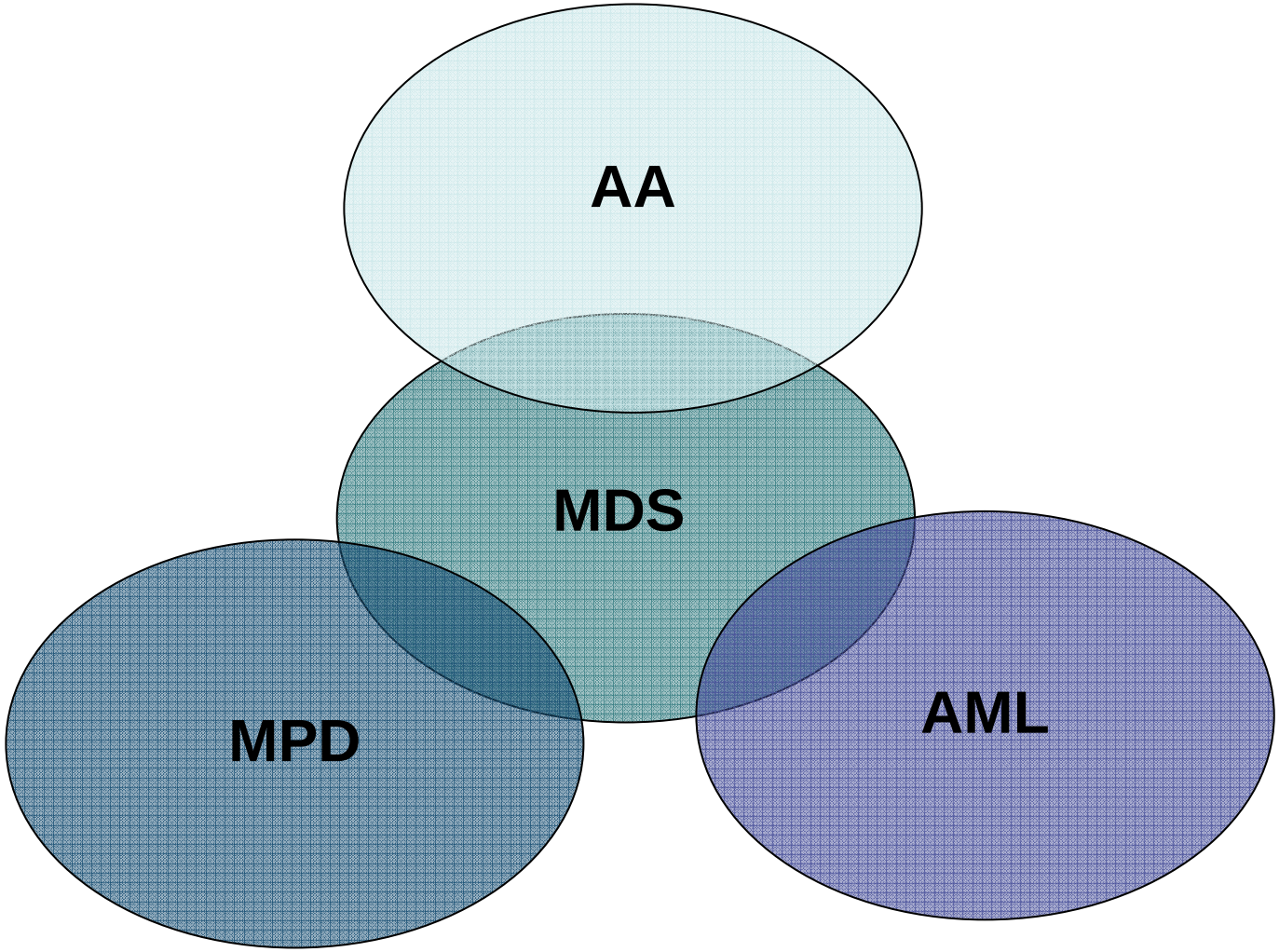
Myeloid Disorders



Myelodysplastic syndromes:
Clinical features at diagnosis

Pavia MDS Cohort

- Anemia (90%);
  - MCV 80-100 fL (55%),
  - MCV >100 fL (40%);
- Neutropenia (50%);
- Thrombocytopenia (40%).

- Splenomegaly: 12%

Blood tests

- WBC, full differential count, Hb, Plt, red blood cell indices (MCV), reticulocyte count, peripheral blood smear;
- ALAT, ASAT, ALP, Albumin, S-protein electrophoresis;
- S-folic acid, cobalamin;
- iron, TIBC, ferritin;
- LDH, bilirubin, haptoglobin, Coombs test;
- Uric acid, Creatinine, S-erythropoietin;
- Thyroid function tests;
- Anti-HIV, *anti-HCV*, anti-Parvovirus B19 (hypoplastic MDS), CMV-test.

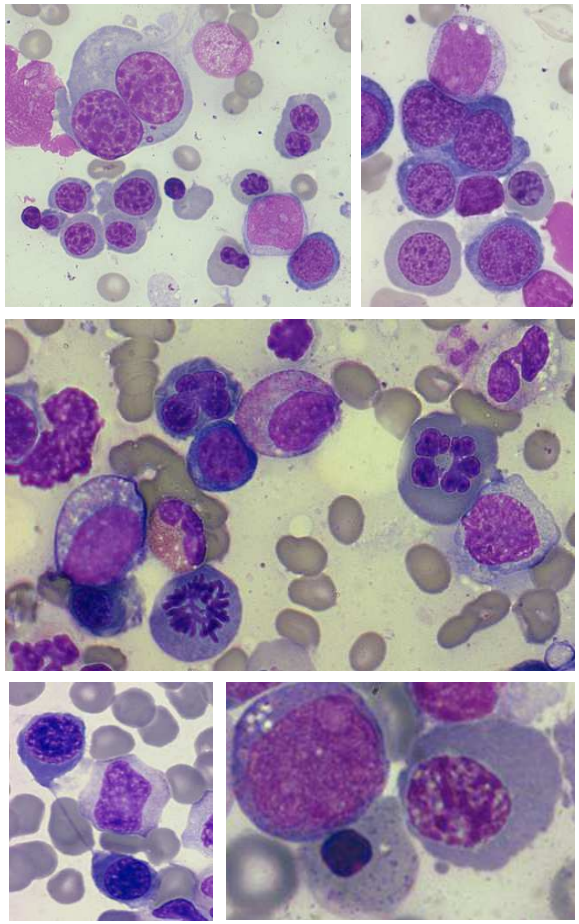
Bone marrow analysis

- Bone marrow aspirate + biopsy;
- Bone marrow slides staining should include May-Grünwald-Giemsa / equivalent and iron staining;
- At least 400 marrow cells (100 erythroblasts), 20 megakaryocytes should be evaluated;
- For significant dysplasia, dysplastic features should be present in at least 10% of the nucleated cells in the lineage in consideration.

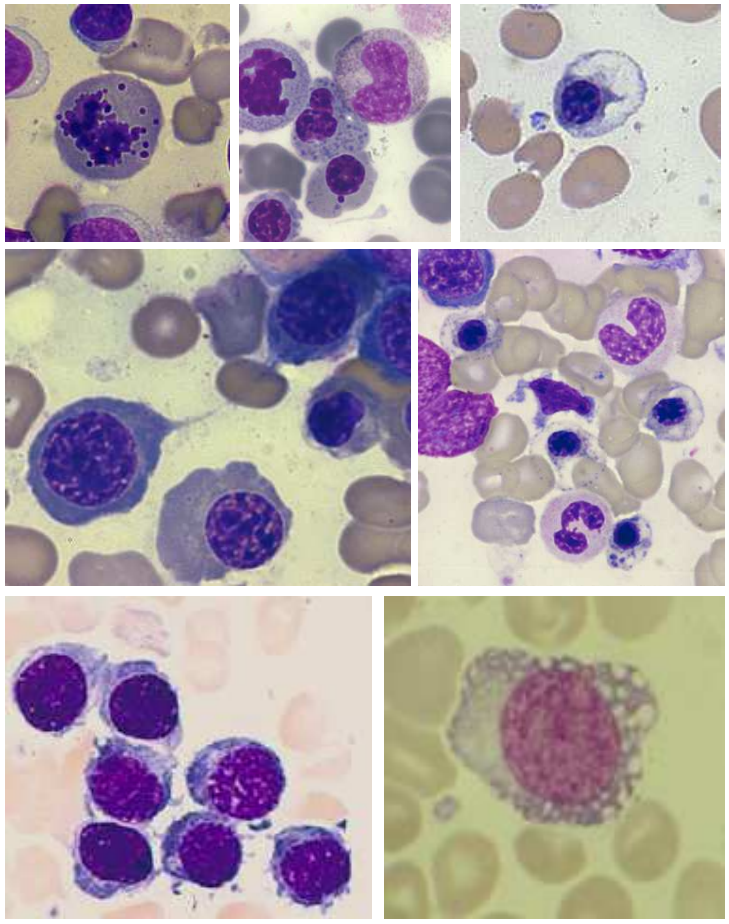
- A cytogenetic analysis of bone marrow aspirate should be done in all cases, at least 25 metaphases, whenever possible.

Bone marrow dyserythropoietic changes in MDS

Nuclear abnormalities
- Megaloblastosis
- Pycnosis
- Irregular nuclear edges
- Multinuclearity
- Nuclear bridging

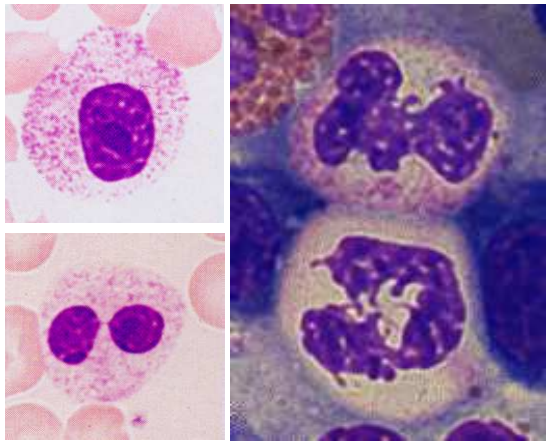


Cytoplasmic abnormalities
- Inclusions
- Cytoplasmic bridging
- Incomplete hemoglobinization
- Fringed cytoplasm
- Vacuolization

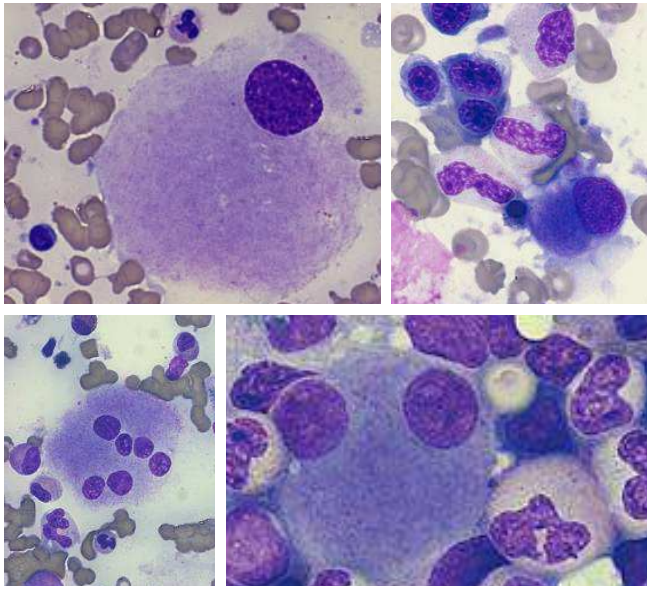


Bone marrow dysmyelopoietic changes in MDS

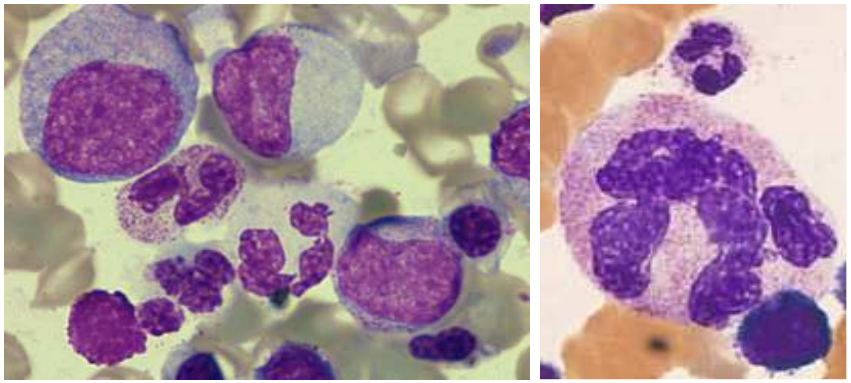
Myeloid nuclear abnormalities
- Bizzare nuclear shape
- Pelgeroid nuclei



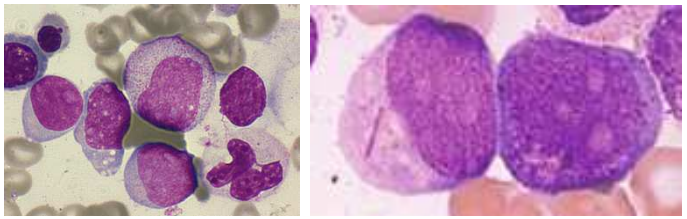
Megakaryocitic abnormalities
- Large monolobar forms
- Small binucleated elements
- Dispersed nuclei
- Micromegakaryocytes
- Degranulation



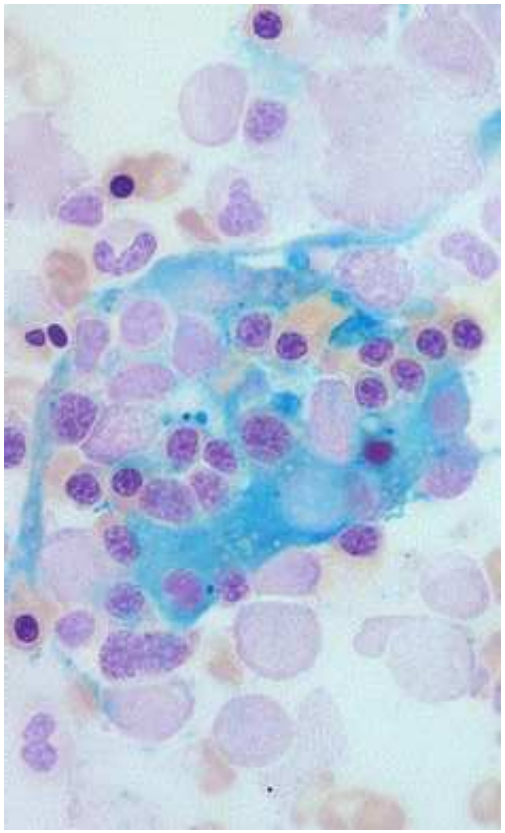
Myeloid cytoplasmic abnormalities
- Hypo- degranulation
- Anysocitosis

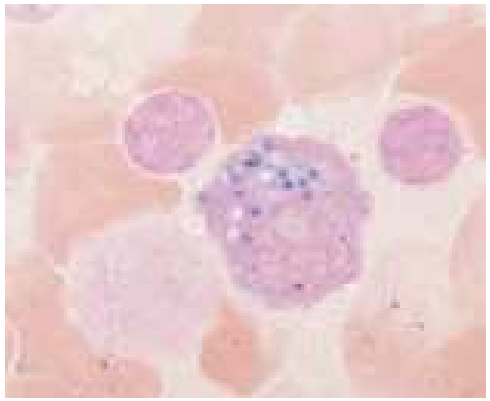


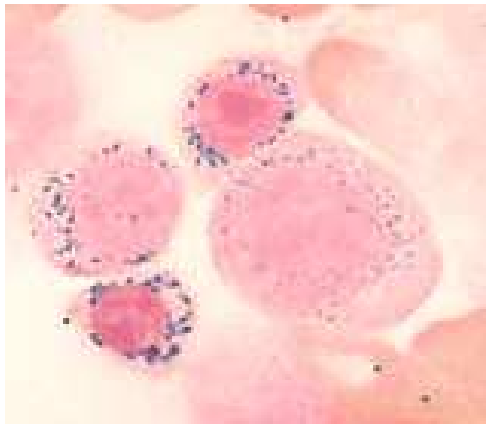
BM blasts and Auer rods



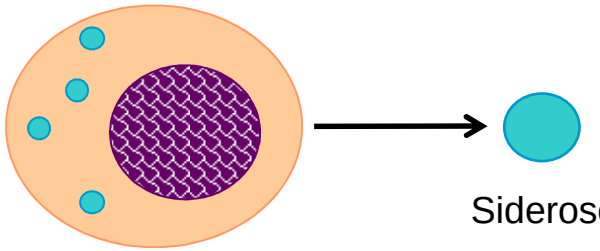
Bone marrow iron staining:
Prussian Blue reaction



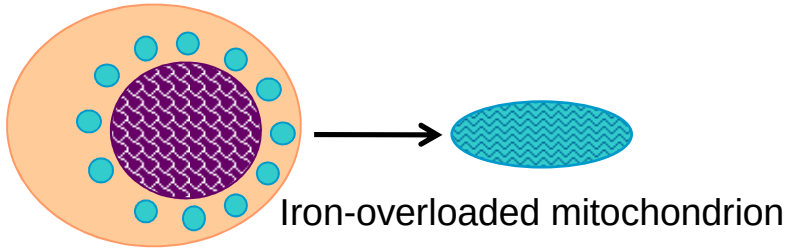




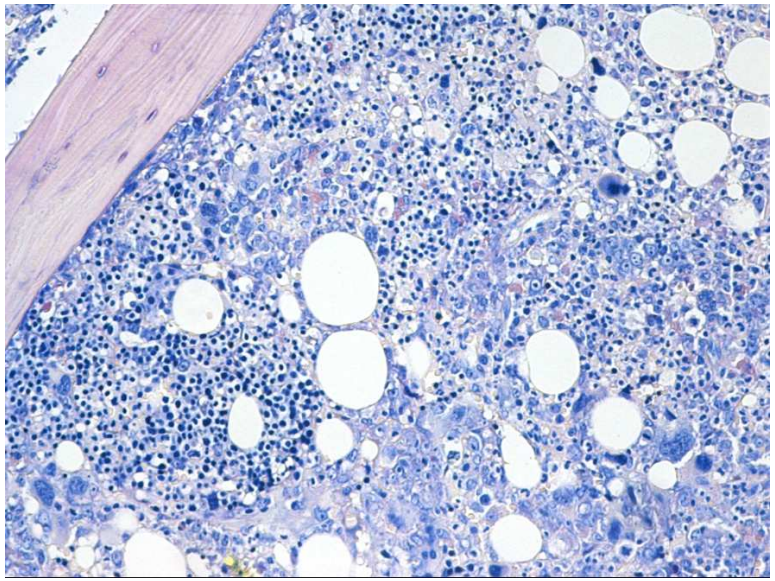
Ferritinic sideroblast

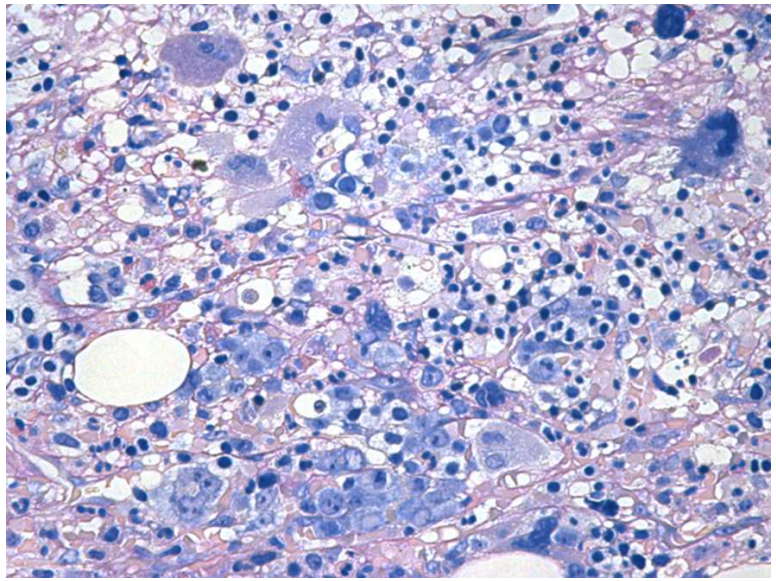


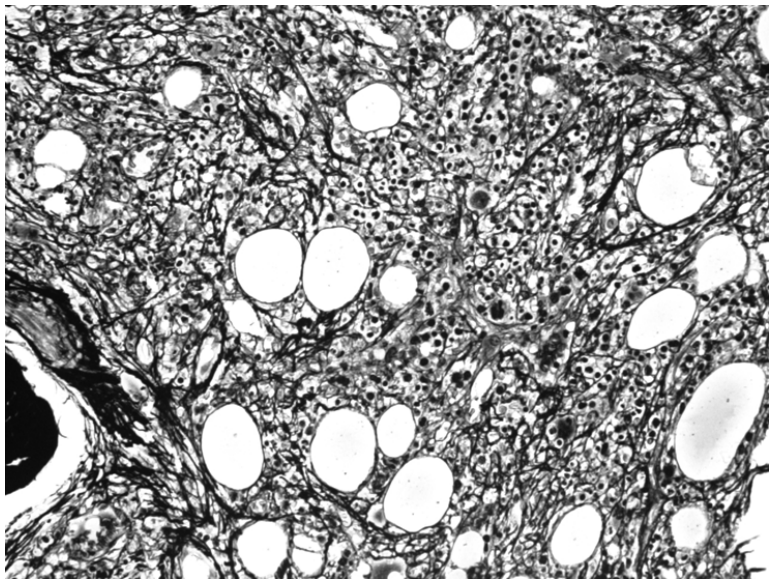
Ring sideroblast

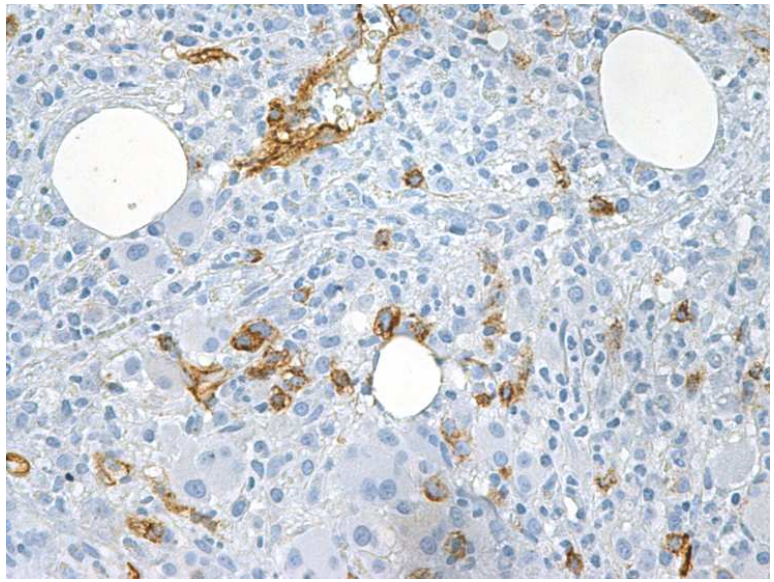


Bone marrow trephine biopsy in MDS

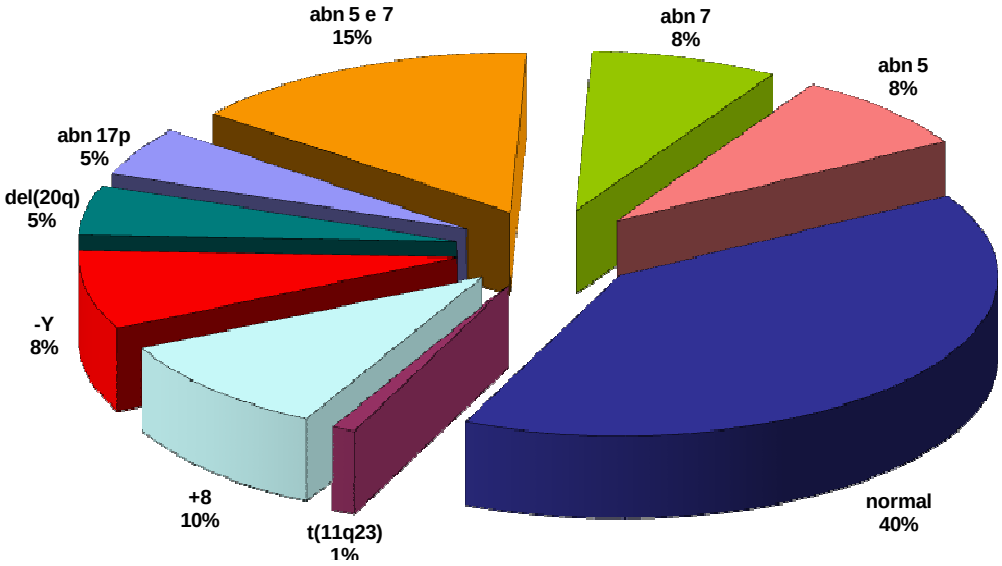


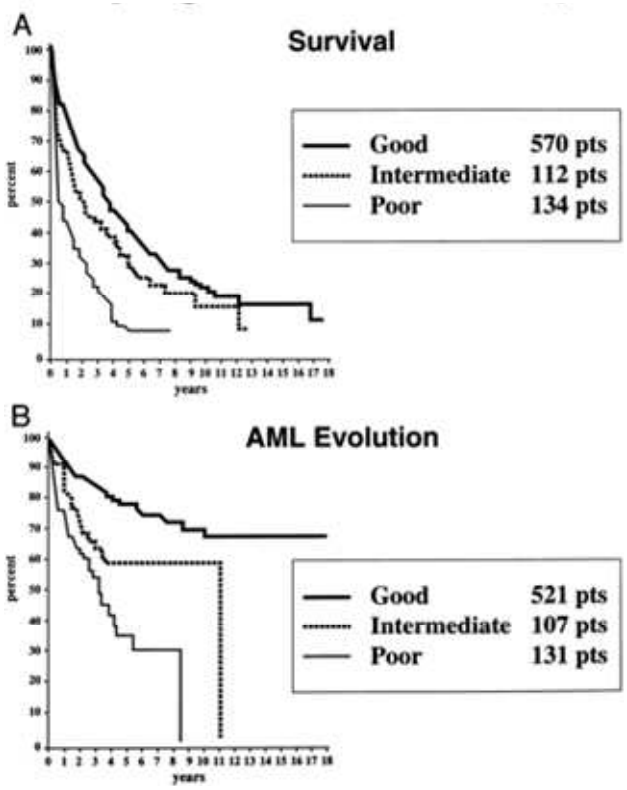






Cytogenetic abnormalities in primary MDS:
Incidence and prognostic value





IPSS

WHO classification of Myelodysplastic Syndromes

Disease	Blood findings	Bone marrow findings
Refractory cytopenia with unilineage dysplasia (RCUD): (refractory anemia [RA]; refractory neutropenia [RN]; refractory thrombocytopenia [RT])	Unicytopenia or bicytopenia* No or rare blasts (<1%)	Unilineage dysplasia: 10% of the cells in one myeloid lineage, <5% blasts, <15% of erythroid precursors are ring sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia, no blasts.	Erythroid dysplasia only, < 5% blasts, ≥15% ringed sideroblasts.
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s), no or rare blasts (<1%), no Auer roads, $<1x10^9$/L monocytes.	Dysplasia in 10% of the cells in 2 myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes), <5% blasts in marrow No Auer rods, ±15% ring sideroblasts
Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenia(s), <5% blasts, no Auer roads, $<1x\ 10^9$/L monocytes.	Unilineage or multilineage dysplasia, 5-9% blasts, no Auer roads.
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenia(s), 5-19% blasts, Auer roads ±, $<1x10^9$/L monocytes.	Unilineage or multilineage dysplasia, 10-19% blasts, Auer roads ±.
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenias, <1% blasts, no Auer roads.	Unequivocal dysplasia in <10% of cells in one or more myeloid lineages when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS, <5% blasts
MDS associated with isolated del(5q)	Anemia, normal or increased platelet count, no or rare blasts (<1%)	Normal to increased megakaryocytes with hypolobated nuclei, <5% blasts, no Auer roads, isolated del(5q)

Blood 2008;114:937-51

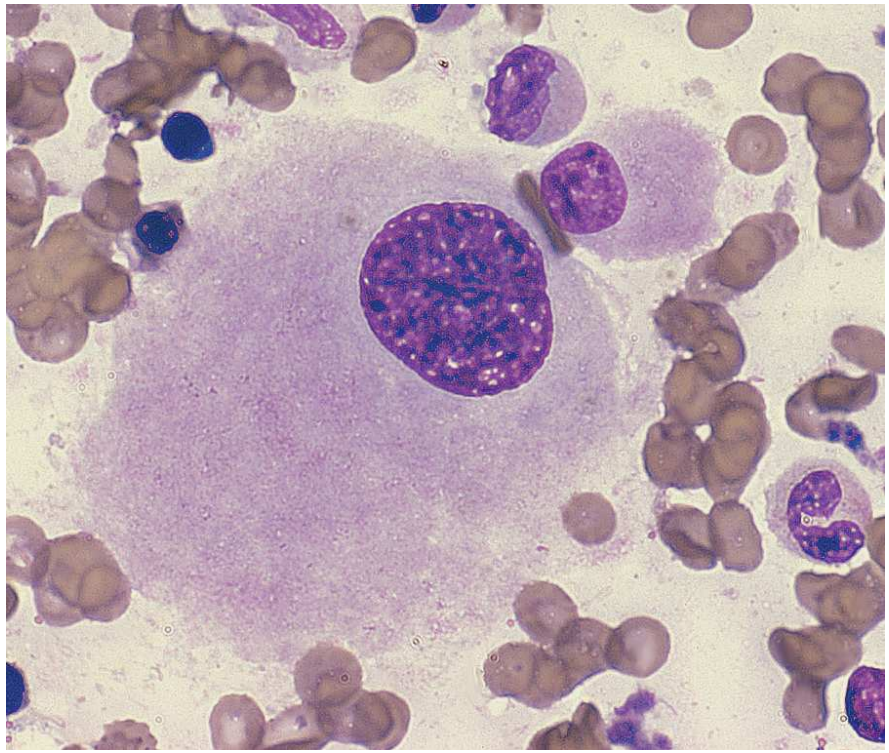
WHO classification of Acute Myeloid Leukemia

- **Acute myeloid leukemia with recurrent genetic abnormalities**
  - AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
  - APL with t(15;17)(q22;q12); *PML-RARA*
  - AML with t(9;11)(p22;q23); *MLLT3-MLL*
  - AML with t(6;9)(p23;q34); *DEK-NUP214*
  - AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
  - AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
- **Acute myeloid leukemia with myelodysplasia-related changes**
- **Therapy-related myeloid neoplasms**
- **Acute myeloid leukemia, not otherwise specified**

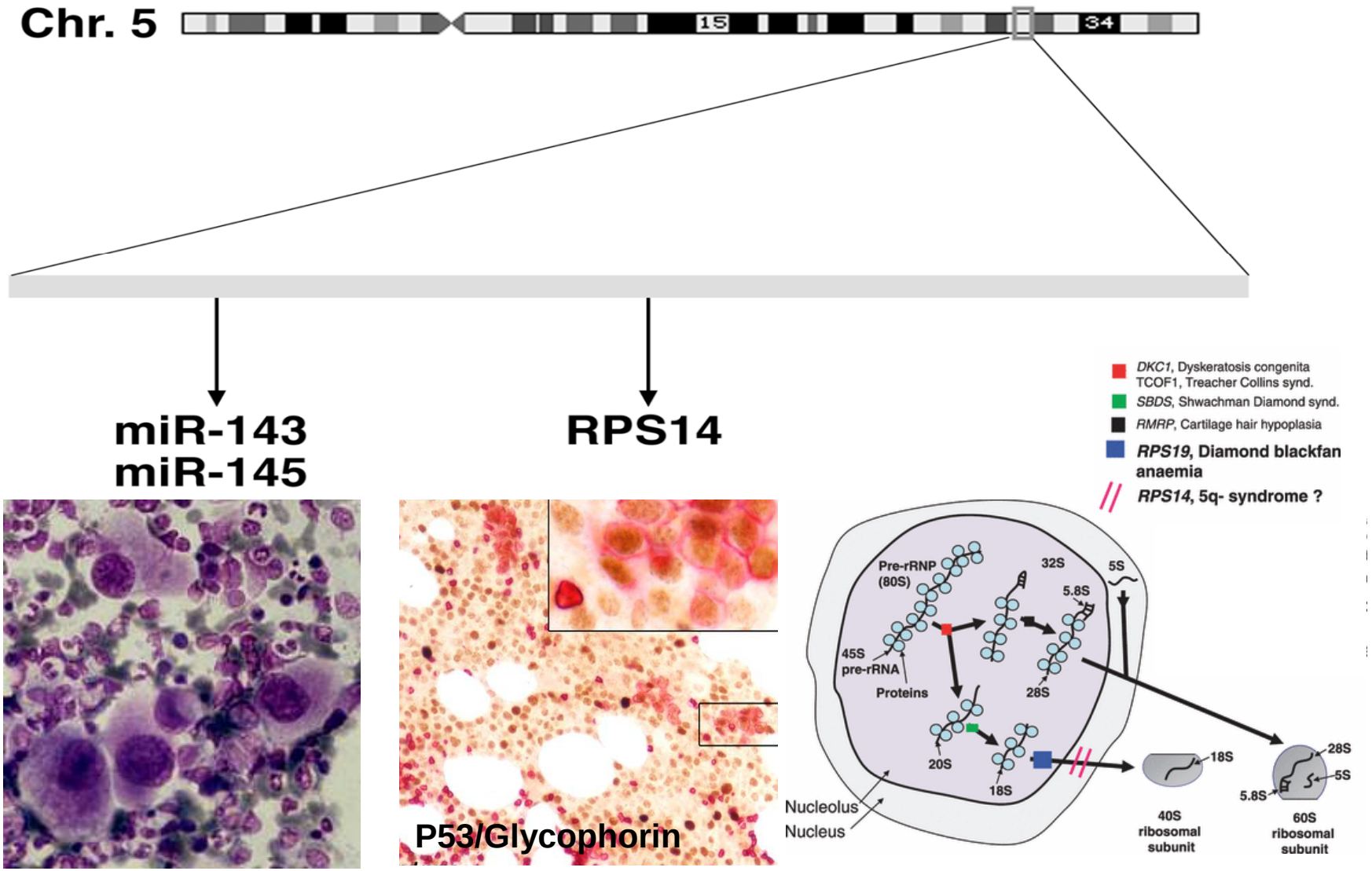
Distinct haematological disorder with deletion of long arm of No. 5 chromosome

van Den Berghe, *Nature*, **251**, 437-438 (1974)

- Female preponderance
- 5q- sole karyotypic abnormality
- Macrocytic anemia (MCV>100 fL)
- High platelet count
- Increased megakaryocytes with monolobulated nuclei
- Prolonged survival



Insights into the molecular basis of the 5q- syndrome



Nature 2008;451:335-9; Br J Haematol 2008;144:157-68; Blood 2010;115:2721-2723

The WHO classification of the myeloid neoplasms: *Myelodysplastic syndromes (MDS)*

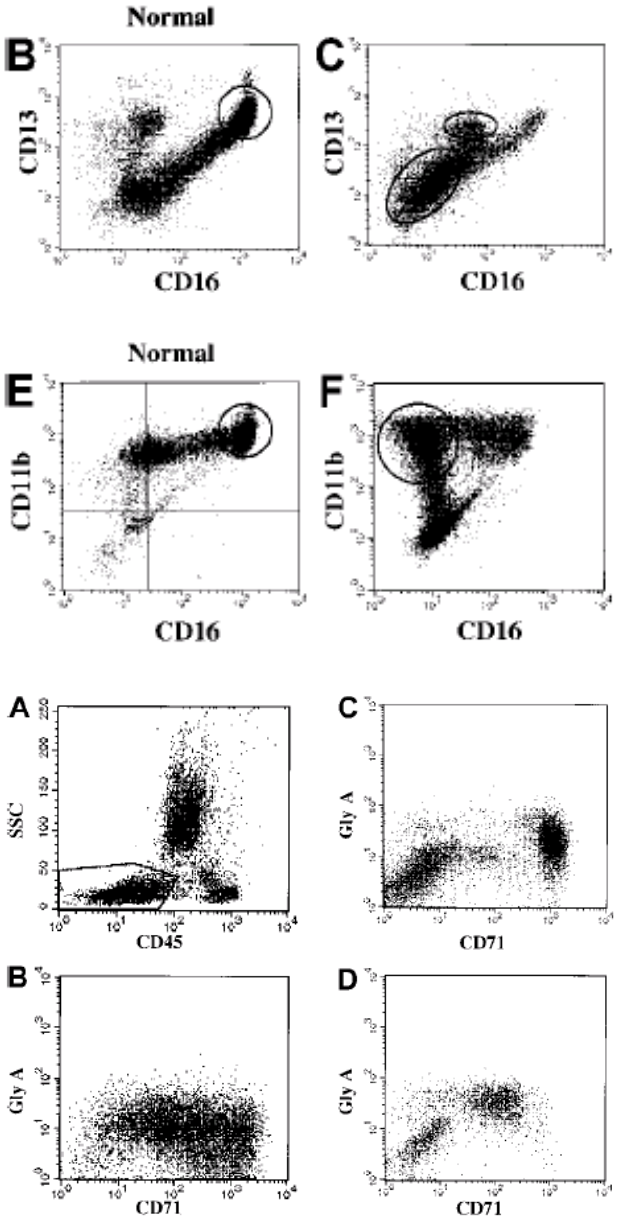
«**Until more reliable markers of erythroid dysplasia are widely available, the category of RA will likely continue to include some cases that are nonclonal erythroid disorders.** In addition, occasional patients may present with cytopenias affecting more than one cell lineage and have multilineage dysplasia but not at the 10% level required for a diagnosis of RCMD. If blasts are fewer than 5% in the bone marrow, such cases are difficult to classify or even to recognize as MDS with confidence. In cases like these a presumptive diagnosis of RCMD might be appropriate. However, in such cases as well as **for cases suspected to be RA, if there is no evidence of clonality by genetic studies, the WHO recommends observation for 6 months prior to making a diagnosis of MDS**».

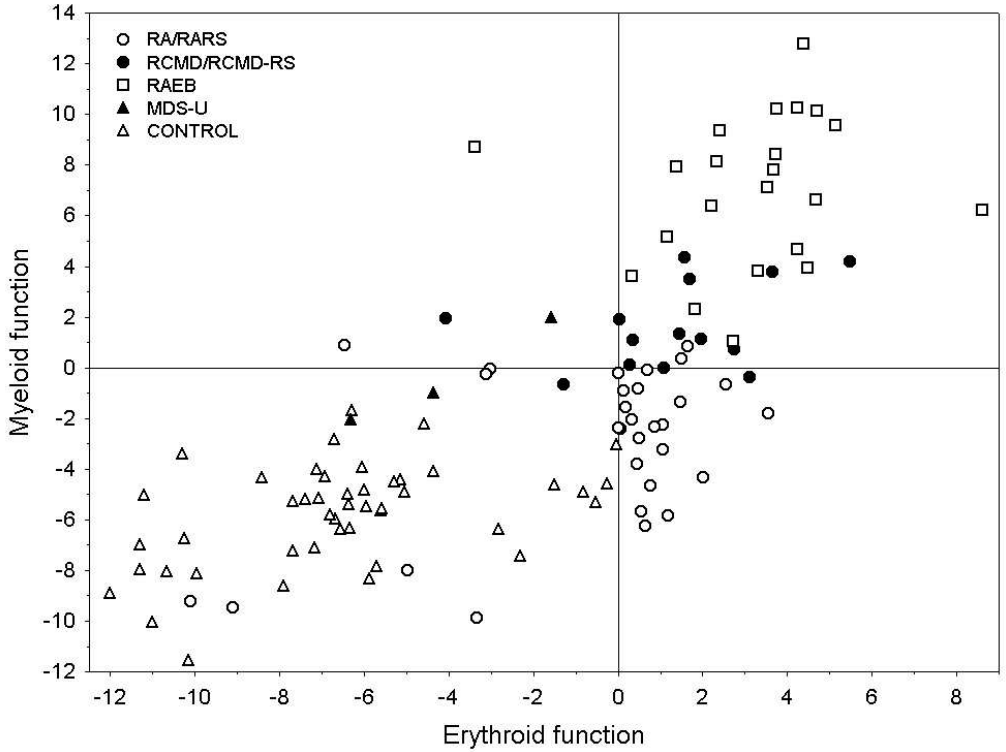
Blood 2002;100:2292-2322

Flow Cytometry Immunophenotyping in MDS

Myeloid abnormalities

Erythroid abnormalities





Blood. 2001;98:979-987; Leukemia 2005 ;19:776-83

Flow cytometry in MDS: open questions

- No single immunophenotypic parameter proved able to discriminate accurately between MDS and other conditions.
- Recognition of abnormal patterns requires an expert operator. Quantitative immunophenotypic analysis (MFI) would be more objective.
- Inter-laboratory repeatability and reproducibility are a critical issue towards the standardization of a flow cytometry approach.

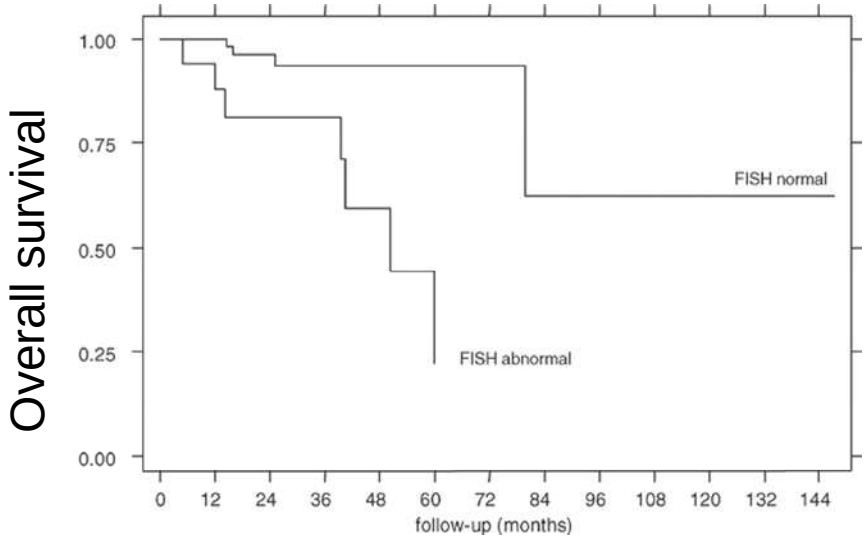
FISH in MDS with a normal chromosome pattern on conventional cytogenetics

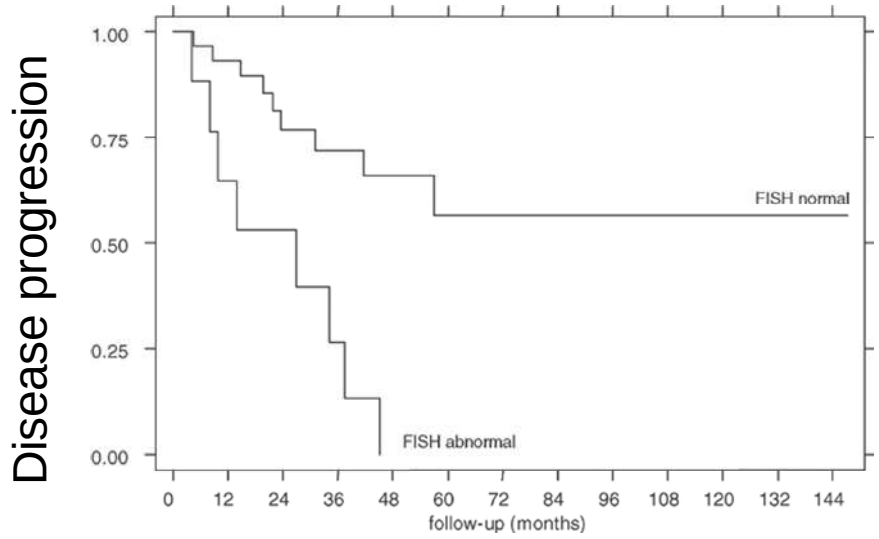
Methods

Probes: 5q31, 7q31, 11q13.3, 12p13, 13q14, 17p13.1, 20q12.

Results

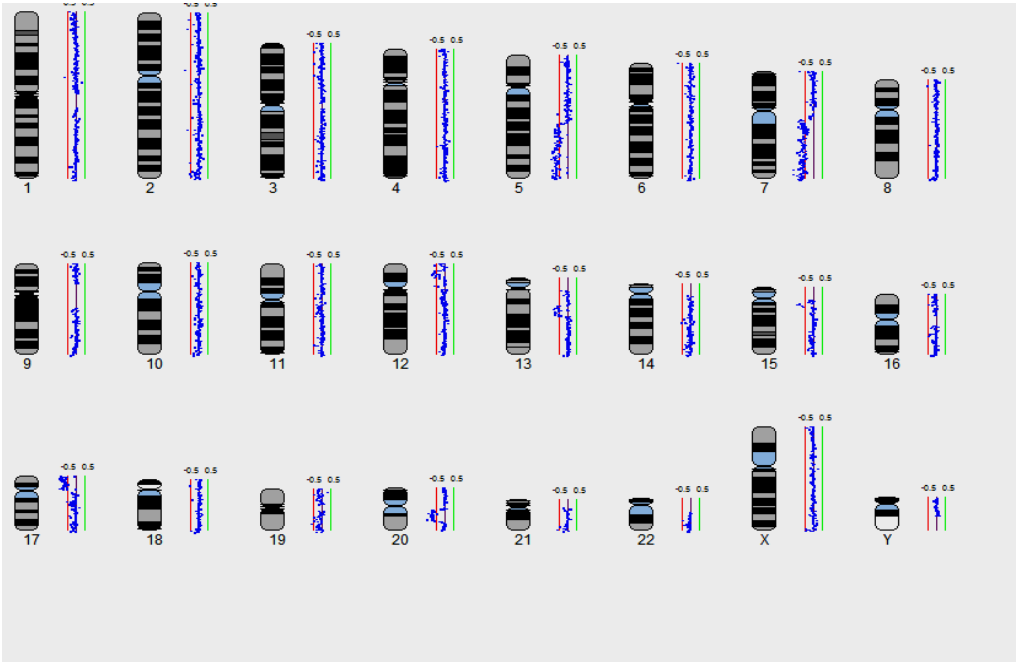
Clonal abnormalities in 12% of the RA and 21% of the RAEB patients (0% IPSS low, 12% int-1, 41% int-2 risk).



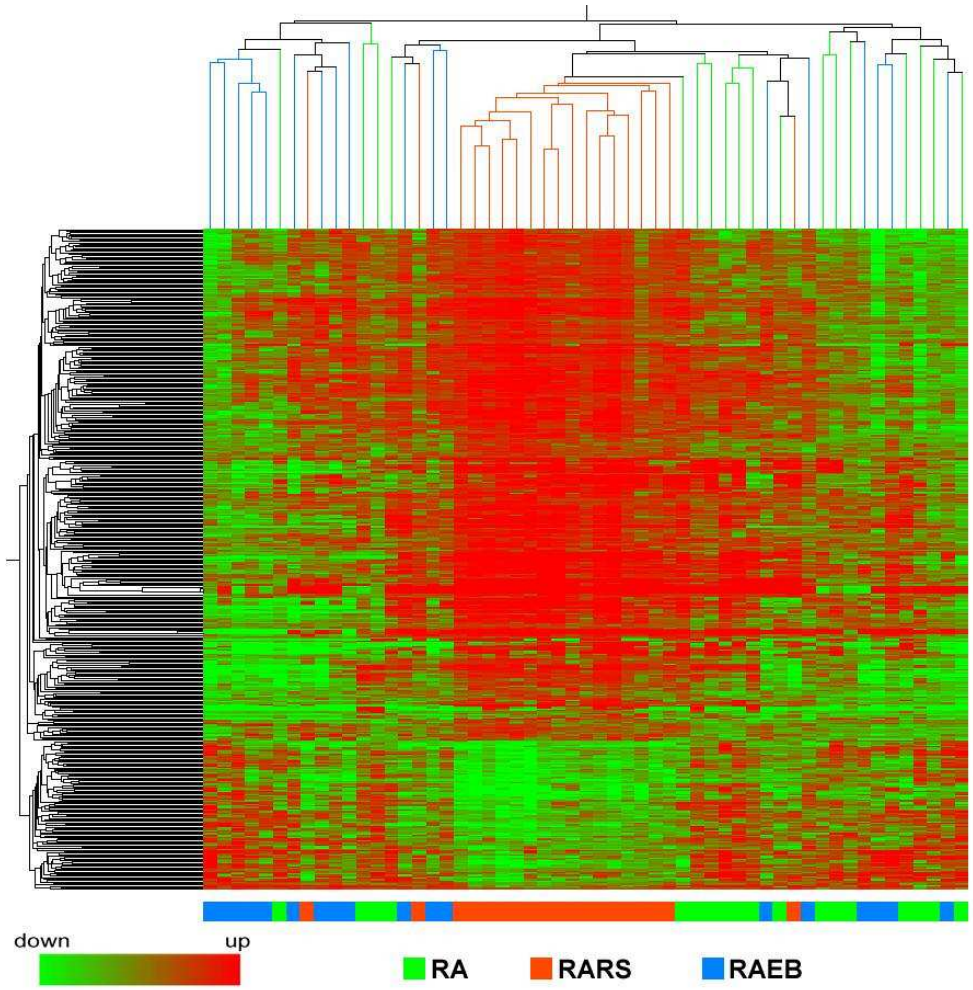


Leukemia, 2003;17:2107-12

CGH Microarray



Gene expression profiling of CD34+ cells in myelodysplastic syndromes



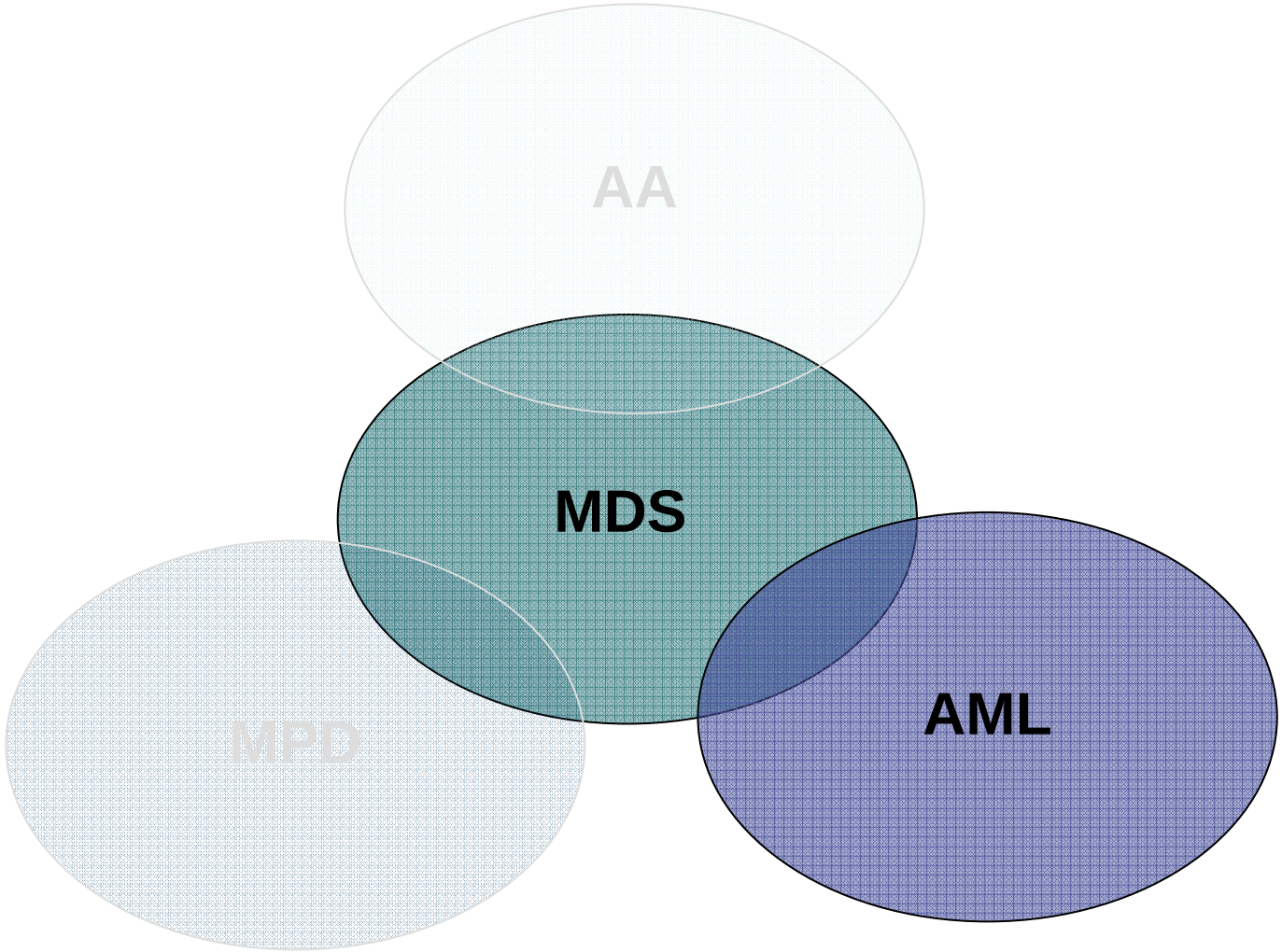
Blood 2006;108:337-45.

Genes implicated in the pathogenesis of MDS

Gene	% mutations	(Putative) Gene Function	Chromosomal location
TET2	25%	Epigenetic DNA modification	4q24
RPS14	15%	Ribosomal protein, Protein Translation	5q32
CTNNA1	15%	E-cadherin anchoring to to the cytoskeletal actin	5q31
Mir145/146a	15%	Expression of targets (TIRAP, TRAF6)	5q33
ASXL1	10%	Gene transcription	20q11.21
N-RAS	10%	Signal transduction	1p13.2
P53	5-10%	Apoptosis, DNA repair, cell cycle regulation	17p13.1
RUNX1/AML1	5-10%	Transcription factor	21q22.3
NPM1	5%	Nuclear export ribosome, p53 function, transcription	5q35
JAK2	5%	Tyrosine kinase, signal transduction	9p24
FLT3	2-5%	Growth factor receptor	13q12
C/EBPalpha	1-4%	Transcription factor, myelopoiesis	19q13.1
EVI-1	2%	Transcription factor	3q26
CBL	1-2%	E3 ubiquitin ligase, signal transduction	11q23.3

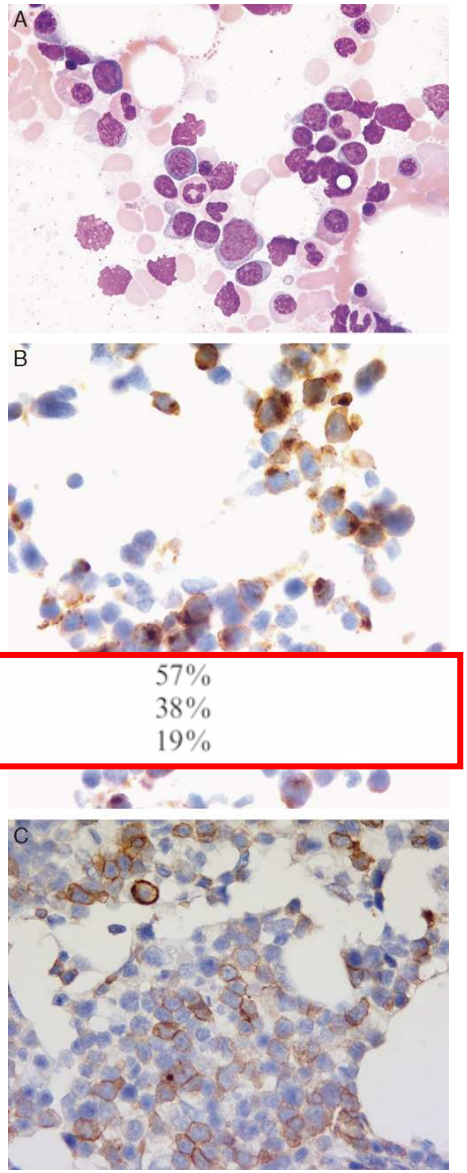
EHA Educ Progr 2010

Myeloid Disorders



Acute Myeloid Leukemia/Myelodysplastic Syndromes

- MDS with more than 20-30% BM blasts (RAEBt according to FAB classification) assimilated to AML
- AML with multilineage dysplasia (de novo, following MDS, following MDS/MPD)
- AML and MDS, therapy-related



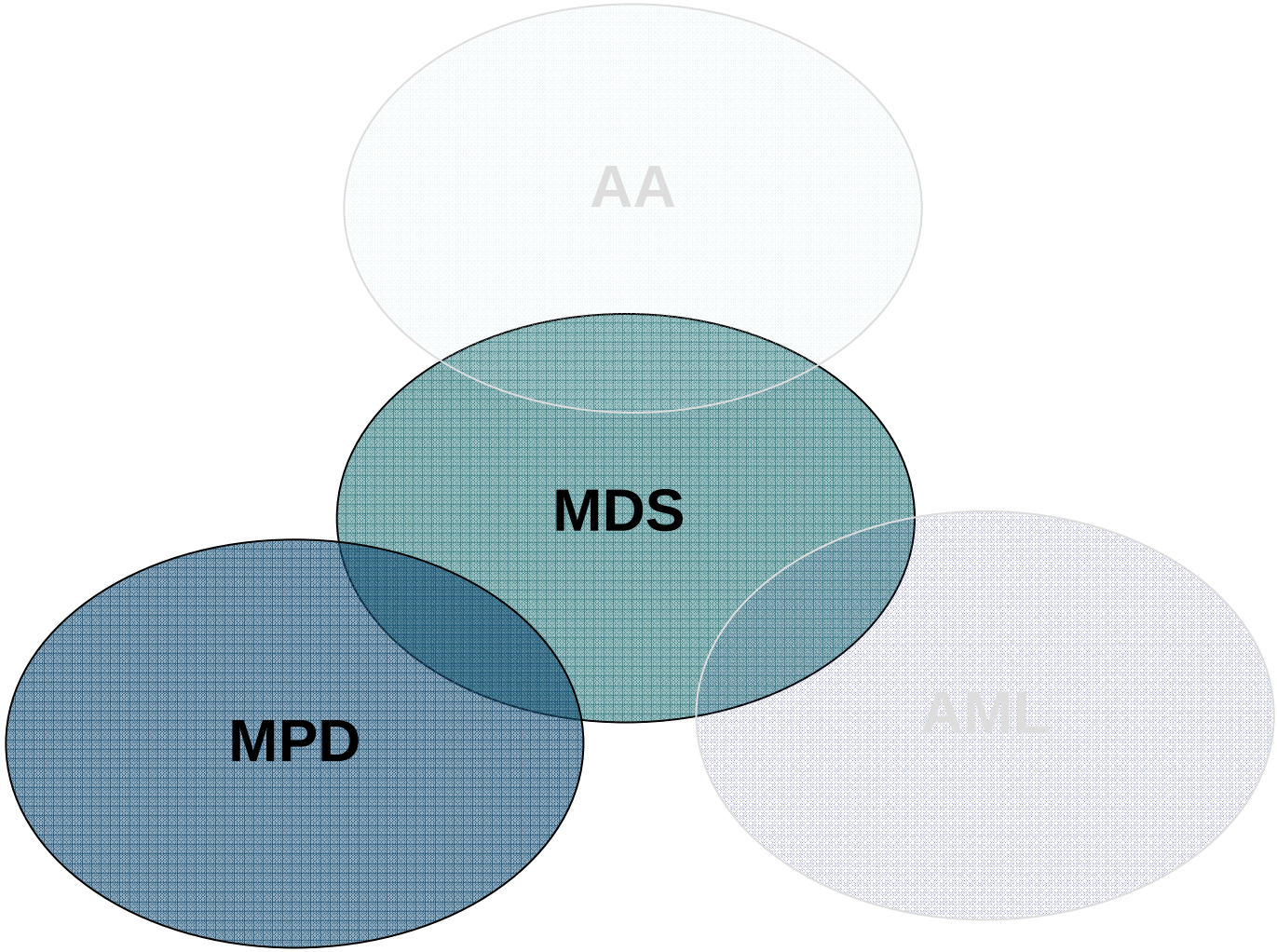
Comparison of myeloblast count by BMA and IHC

% of cases with discrepant BCs: initial vs. retrospective review	31%
% with higher BC on retrospective review*	80%
% of cases with discordant BCs: BMA vs. BMTP differential	28%
% with higher BC by BMTP differential	77%
% of cases with discordant BCs: BMA or BMTP differential vs. IHCS with CD34	57%
% with significantly lower BC by IHCS†	38%
% with significantly higher BC by IHCS, resulting in different final diagnosis‡	19%
% with significantly lower BC by IHCS§	44%
% with significantly higher BC by IHCS, resulting in different final diagnosis	19%
% of Discordant BCs: IHCS with CD34 in BM clot vs. BM core biopsy sections	19%
% with stronger intensity/higher percentage of cells staining in the BMCB sections	83%
% of Discordant BCs: IHCS with CD117 in BM clot vs. BM core biopsy sections	40%
% with stronger intensity/higher percentage of cells staining in the BMCB sections	33%

*10 different final diagnoses: 1, changing from refractory anemia with excess blasts (RAEB)-1 to RAEB-2; 1, from CMML-1 to CMML-2; 4, from RAEB-2 to AML; 3, from RAEB-1 to AML; and 1, from CMML-1 with eosinophilia to AML.
†Primarily due to CD34-negativity/variable reactivity of the blasts by flow cytometry.
‡5 different final diagnoses: all RAEB-2s by initial review changed to AMLs by IHCS with CD34 and CD117.
§Largely unexplained.

Appl Immunohistochem Mol Morphol. 2007;15:154-9

Myeloid Disorders

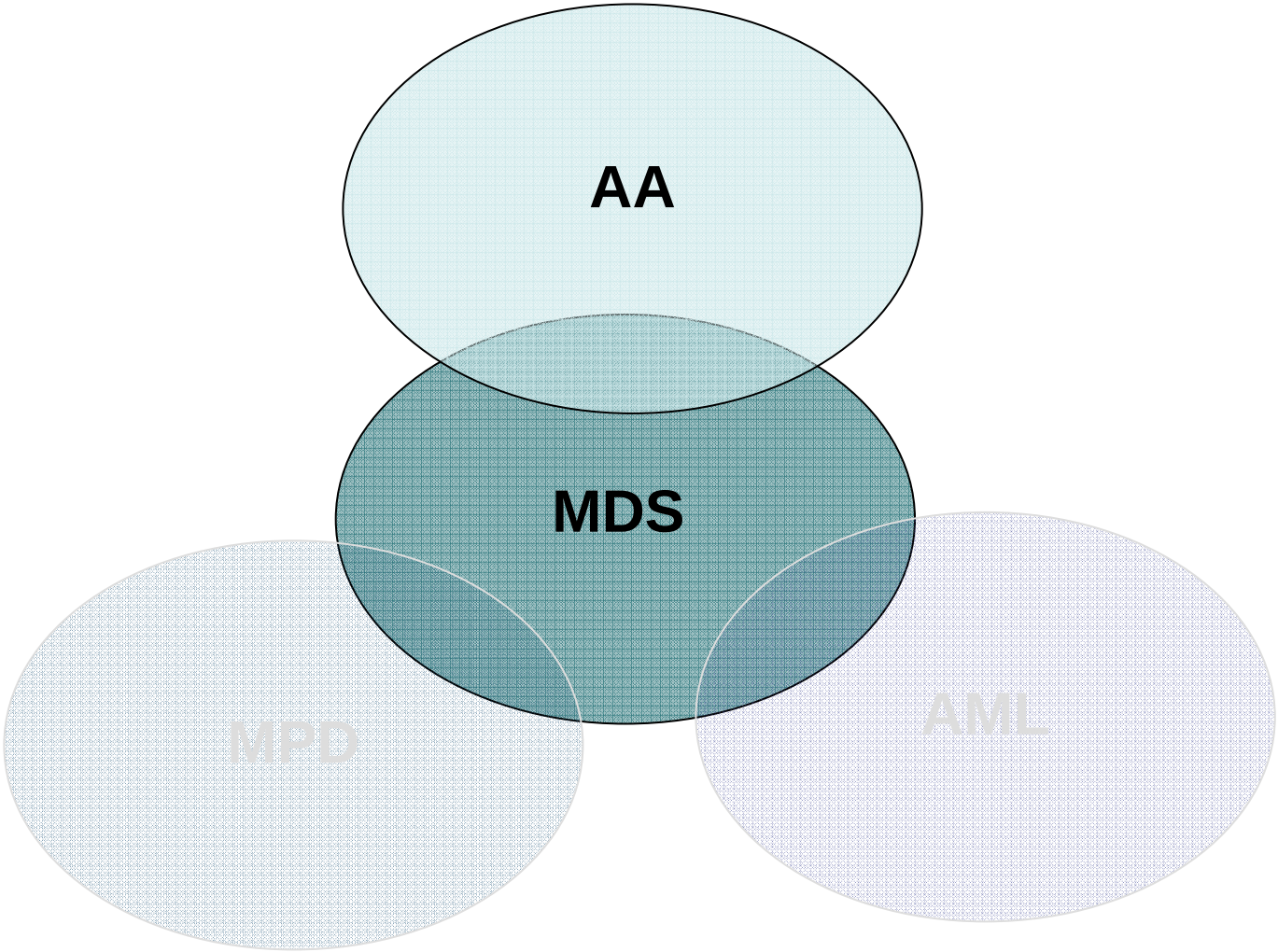


Myelodysplastic/Myeloproliferative Disorders

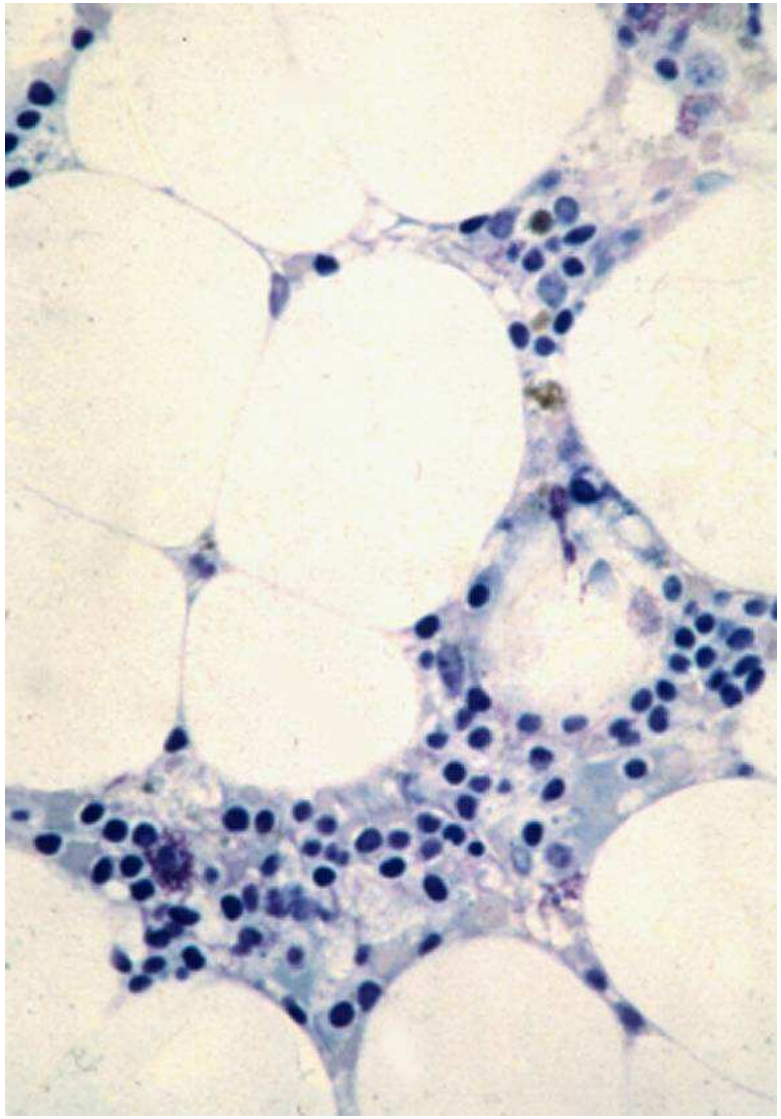
- Chronic myelomonocytic leukemia
- Atypical chronic myeloid leukemia
- Juvenile myelomonocytic leukemia
- Myelodysplastic/myeloproliferative disease, unclassifiable

Blood 2002, 100: 2292-2302

Myeloid Disorders



Hypoplastic MDS



- 10-20% of MDS patients have hypocellular marrow.
- Cytogenetic abnormalities are more objective evidence of MDS, but are also found in a subset of otherwise typical AA.
- The differential diagnosis between MDS and AA often relies on "soft" histologic criteria.
- Both AA and MDS are associated with expansion of PNH clones.

Leukemia 2008;22:544–550; Haematologica. 2009;94:264-8

ELN European LeukemiaNet