Burning issues regarding AML & MDS classification: origin (*de novo & secondary*), genetics, grey zones

Jordi Esteve, Hospital Clínic of Barcelona, Spain

Data Management Education sessions

EBMT meeting-2016, Valencia, 5th April

Education session on AML & MDS: outline

- AML & MDS: definition, evolution
- Classification (WHO, FAB)
- Relevance of genetics in AML & MDS
- Grey zones & confounding concepts
- Capturing complexity in MED-A & MED-B forms
- Q & As

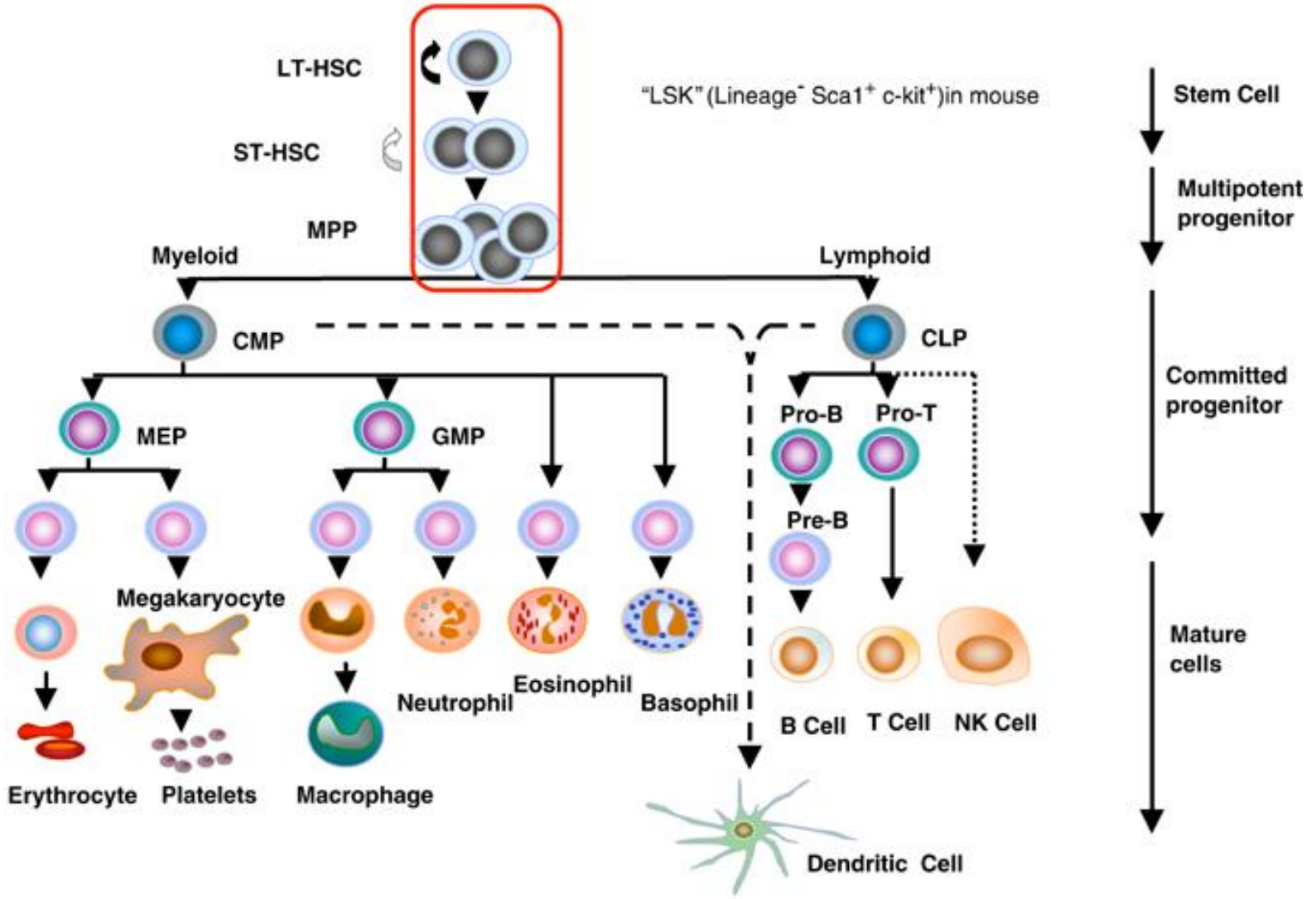
Acute myeloid leukemia (AML): WHO 2008 definition

- Clonal expansion of myeloid blasts in bone marrow (BM), peripheral blood (PB) or other tissue

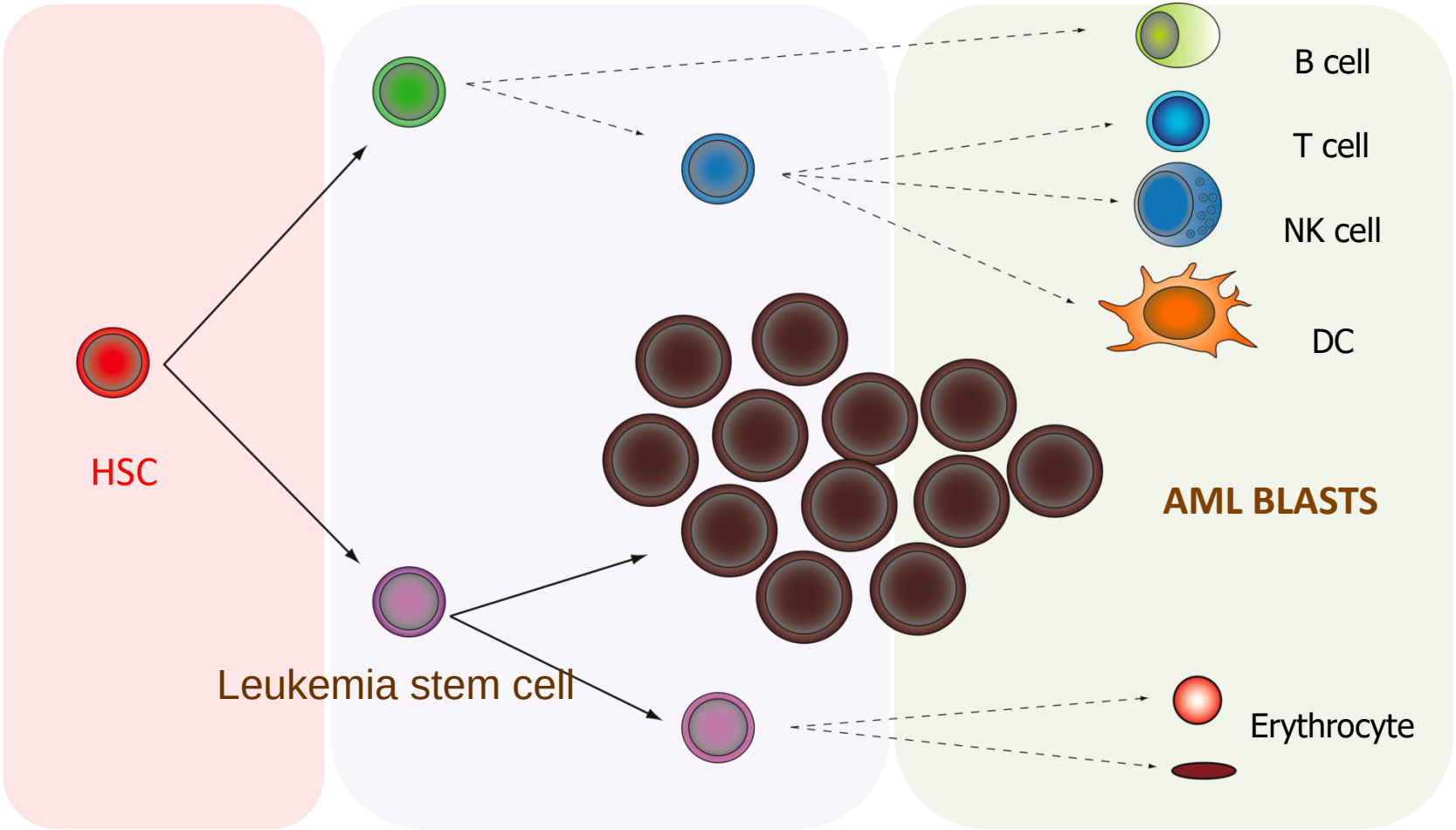
- Minimum threshold of blast cells for AML diagnosis (BM):
  - 20% blasts (WHO, 2008)
  - Any blast count in cases with RUNX1/RUNX1T1, CBFb/MYH11 or PML/RARA rearrangement

Proliferation + differentiation block/maturation arrest

Normal hematopoiesis

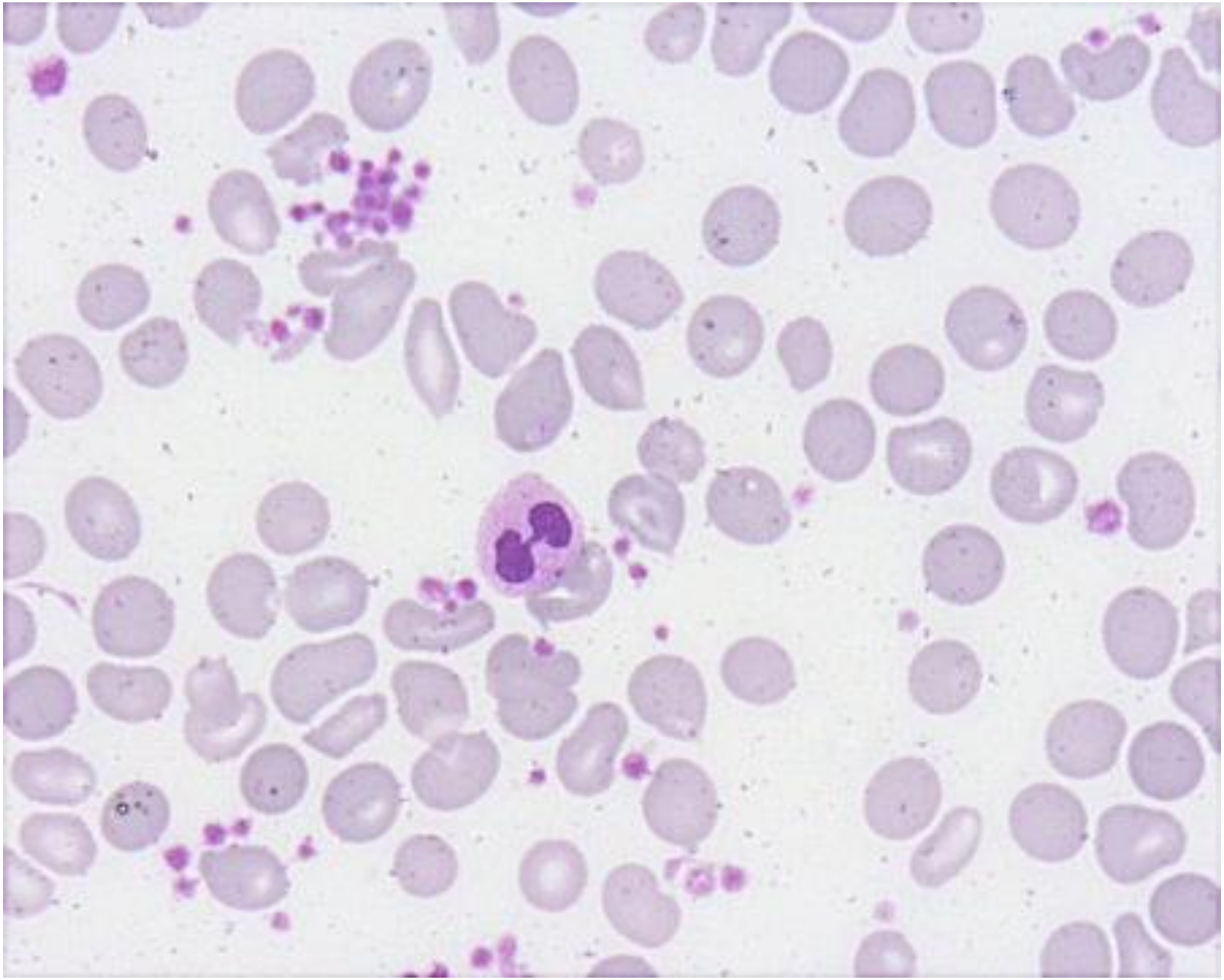


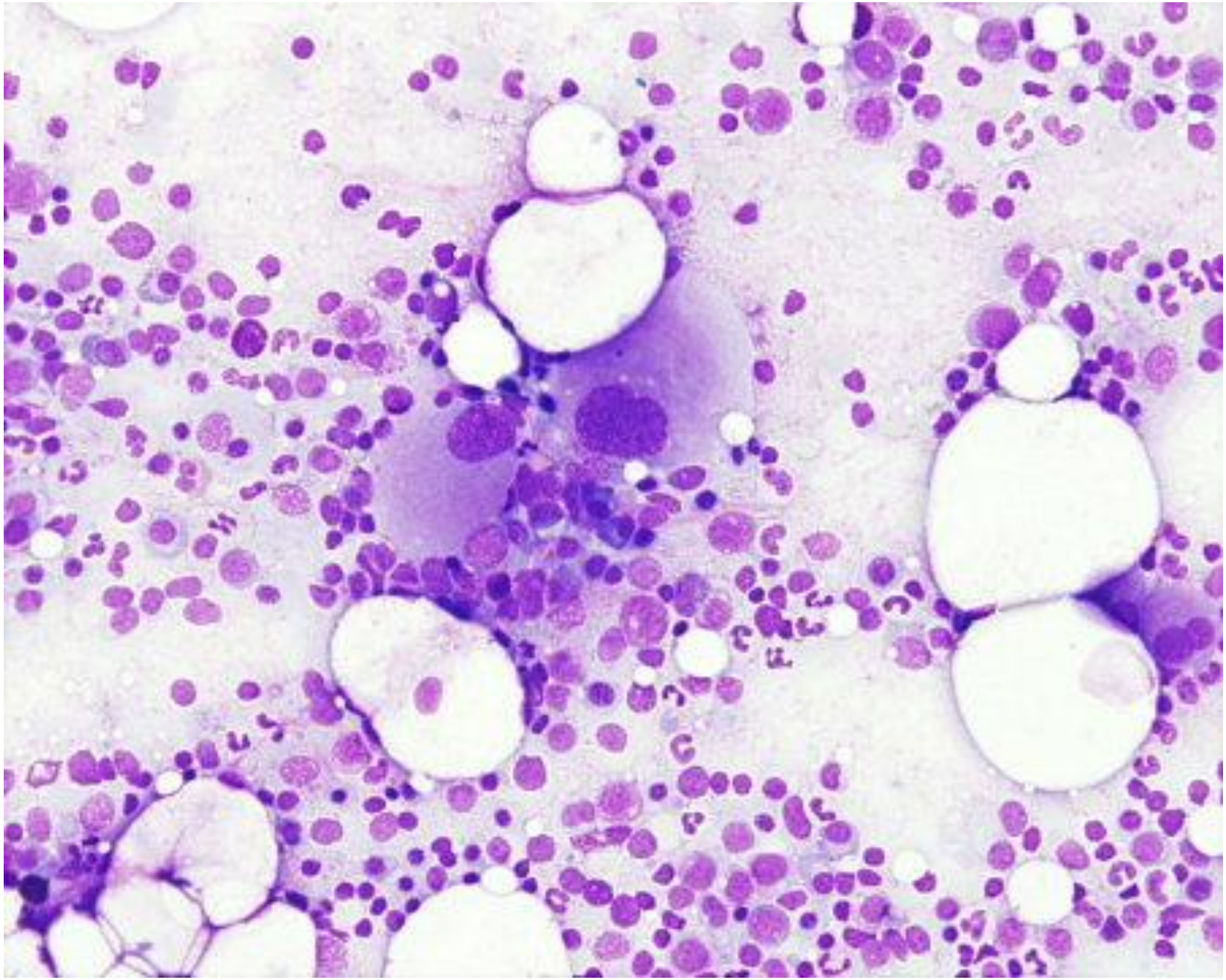
Hematopoiesis in AML patients

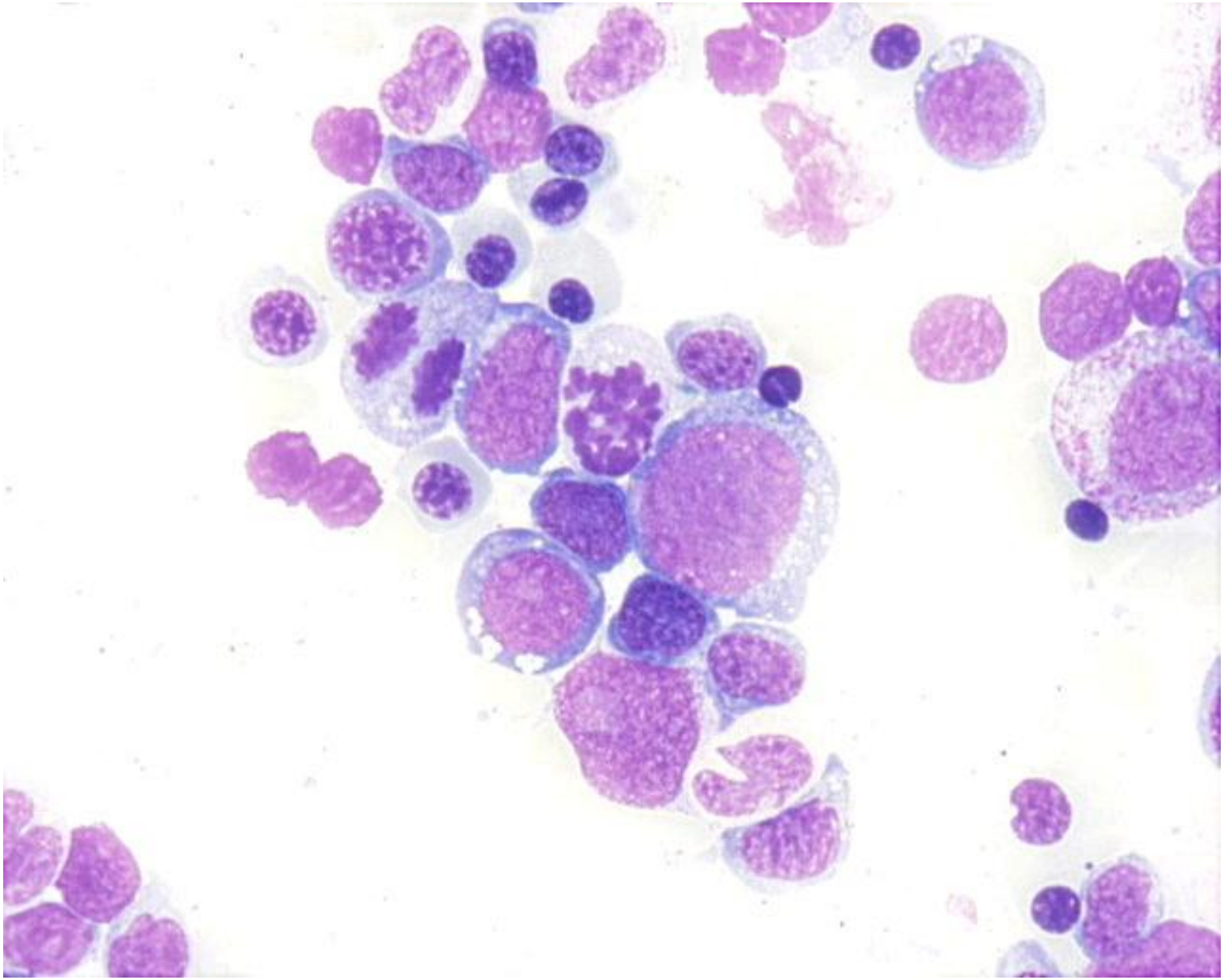


Myelodysplastic syndromes (MDS): concept

- Heterogeneous disorder
- Clonal neoplasm
- Abnormal (dysplastic) hematopoietic precursors
- Ineffective hematopoiesis – cytopenia & malfunctioning
- <20% of blasts but…
- Risk of transformation to AML



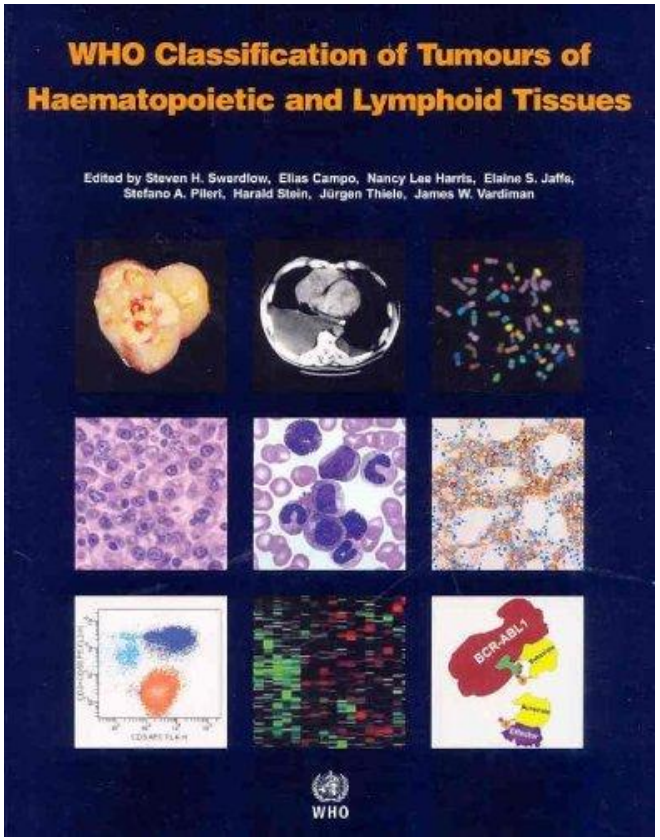




AML vs. MDS: differential characteristics

	Blood counts	BM blasts	BM cellularity	Morphology	Genetics
MDS	Cytopenias	<20%	Increased, with maturation	Dysplasia in 1 or more lineages	Characteristic genetic lesions
AML	↑ WBC (blasts) or leucopenia Cytopenias	≥20%	Increased, w/o maturation	Absent or present (AML with MRC)	Characteristic genetic lesions, although in some pts with MDS

2008 WHO CLASSIFICATION OF AML



I. AML with recurrent cytogenetic abnormalities

- AML with t(8;21)(q22;q22)/RUNX1-RUNXT1
- AML with inv(16) or t(16;16)(p13;q22)/CBFβ-MYH11
- Acute promyelocytic leukemia [t(15;17) & PML-RAR-α]
- AML with t(9;11)(p22;q23)/AF9(MLLT3)-MLL
- AML with t(6;9)(p23;q34)/DEK-CAN(NUP214)
- AML with inv(3) or t(3;3)(q21;q26)/RPN1-EVI1
- Megakaryoblastic AML with t(1;22)(p13;q13)/RBM15-MKL1
- AML with mutated NPM
- AML with normal karyotype and CEBPA mutation

II. AML with myelodysplasia-related changes

III. Therapy-related AML/MDS

IV. AML, not otherwise specified

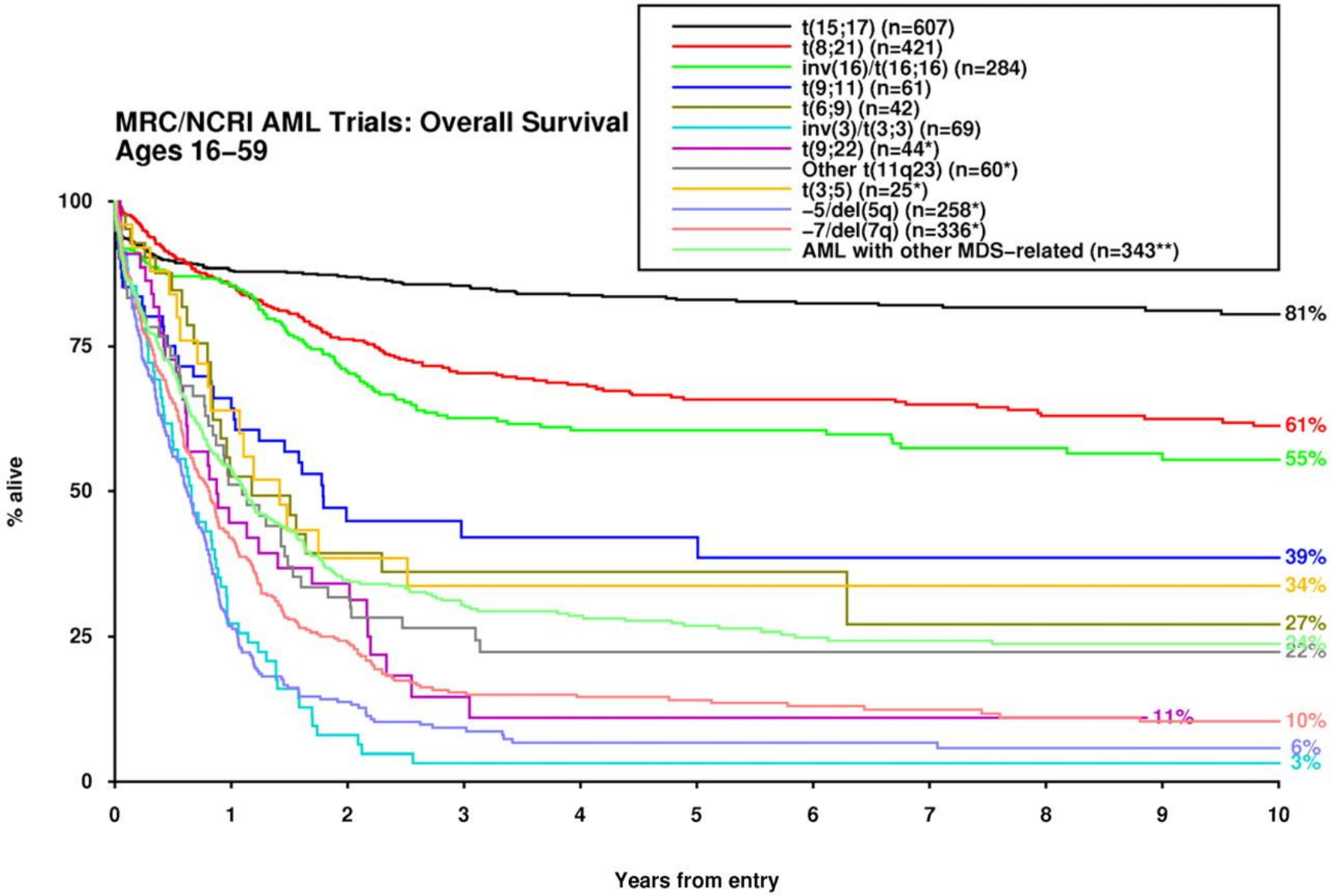
- AML without differentiation
- AML minimally differentiated
- AML with differentiation
- AML myelomonocytic
- AML monoblastic and monocytic
- Acute erythroid leukemia
- Acute megakaryoblastic leukemia

V. Myeloid sarcoma

VI. Myeloid proliferations related to Down syndrome

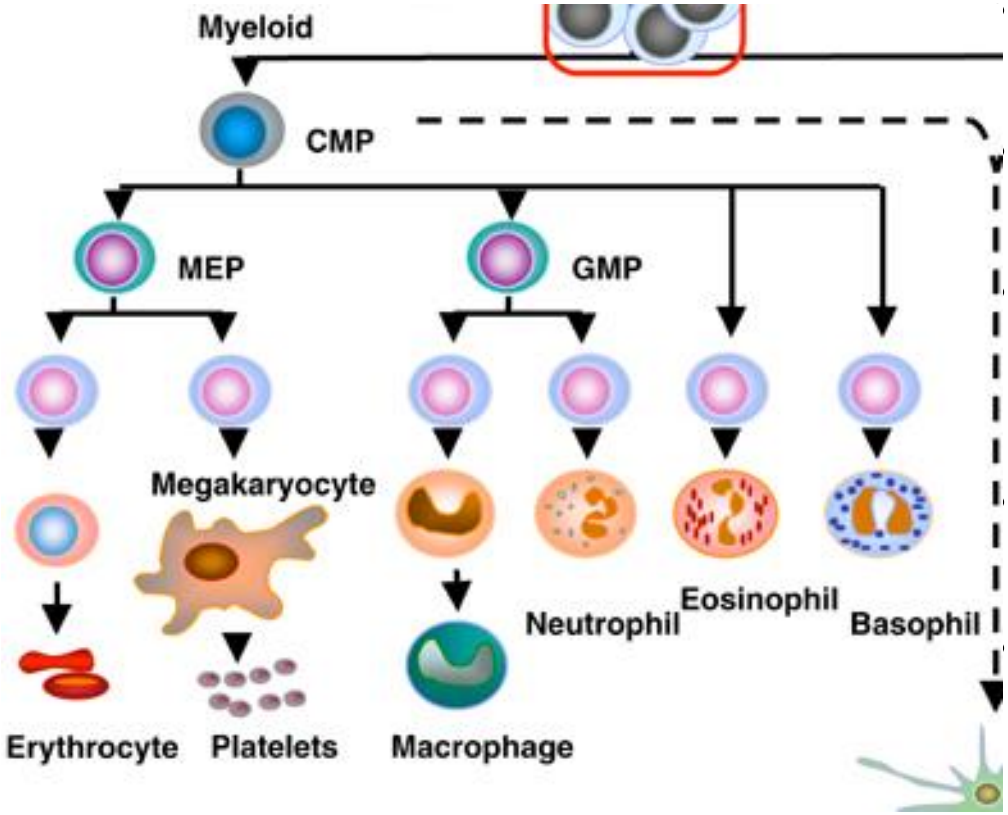
VII. Blastic plasmacytoid dendritic cell neoplasms

Impact of cytogenetic entities recognized in 2008 WHO classification on survival



Grimwade D, et al. Blood 2010

AML classification before 2001 (FAB)



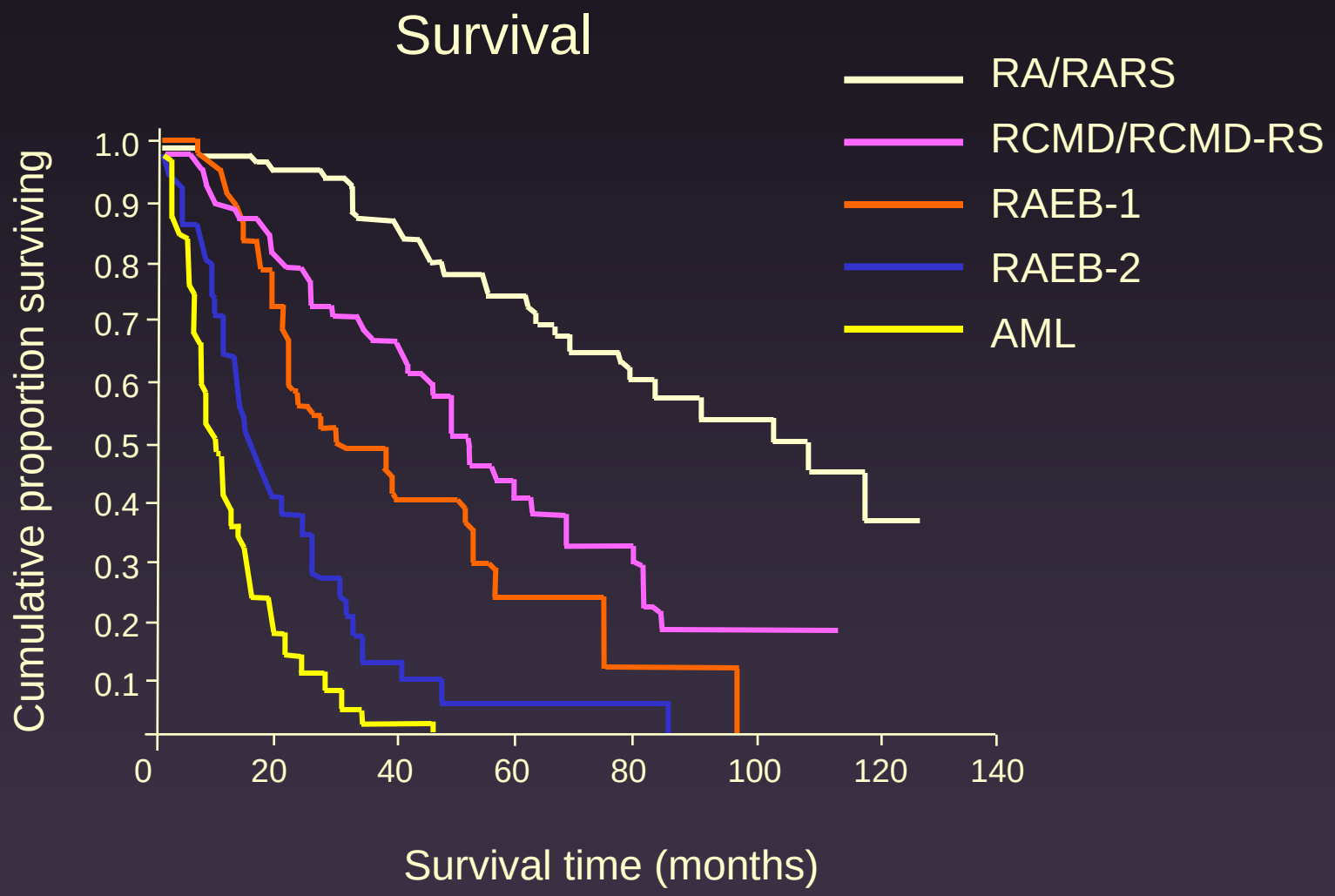
- M0 Myeloid without differentiation
- M1 Myeloid minimally differentiated
- M2 Myeloid with differentiation
  - t(8;21), RUNX1-RUNX1T1
- M3 Promyelocitic
  - t(15;17), PML/RARA
- M4 Myelomonocitic
  - Eosinophilia, Inv(16) or t(16;16), CBFB/MYH11
- M5 Monoblastic/monocytic
- M6 Erythroid
- M7 Megakaryoblastic

WHO 2008

Classification for Myelodysplastic syndromes

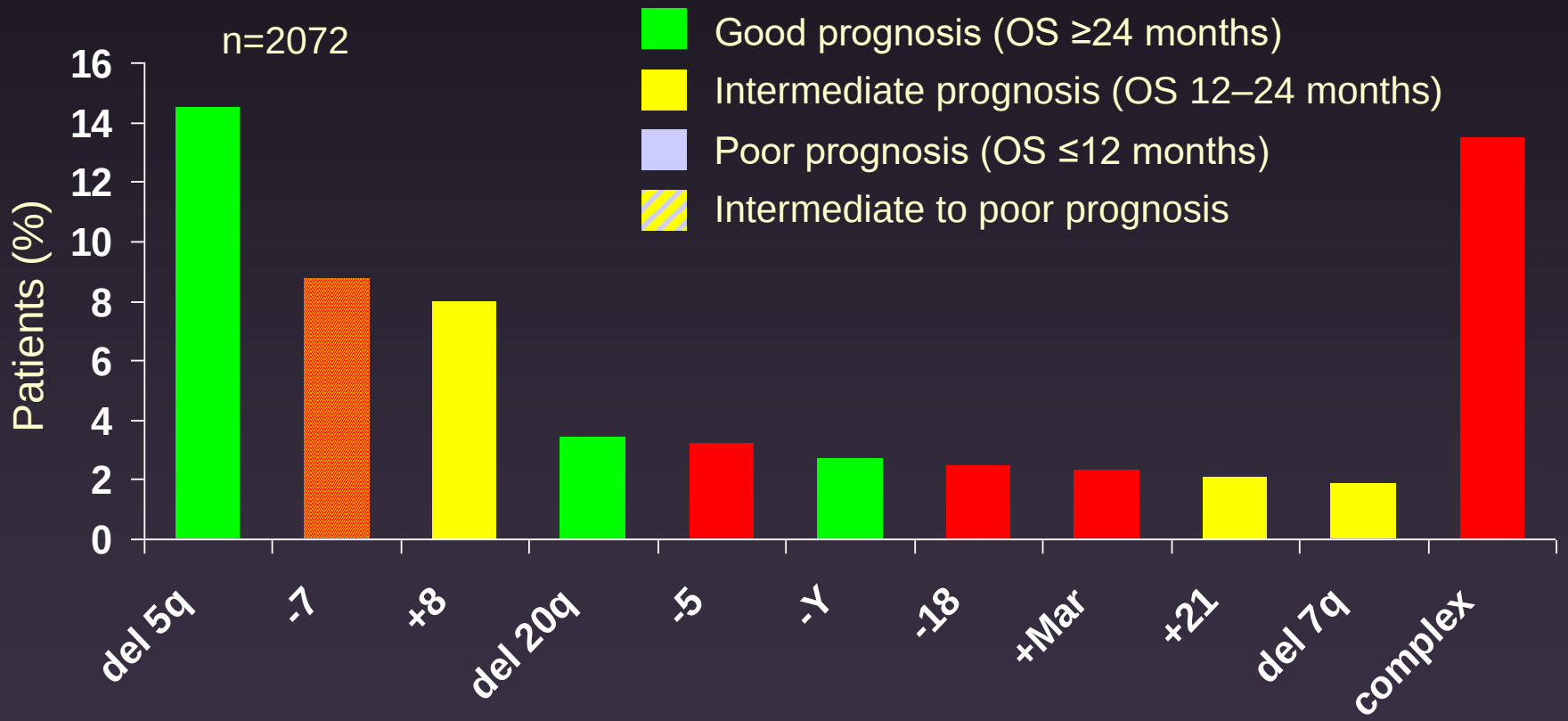
- Refractory cytopenia with unilineage dysplasia (RCUD)
  Refractory anaemia (RA)
  Refractory neutropenia (RN)
  Refractory thrombocytopenia (RT)
- Refractory anaemia with ring sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia (RCMD)
- Refractory anaemia with excess blasts (RAEB I & II)
  - RAEB-1: 5-9% bone marrow blasts
  - RAEB-2: 10-19% BM blasts
- Myelodysplastic syndrome with isolated del (5q)
- Myelodysplastic syndrome unclassifiable (MDS,U)
- *Childhood myelodysplastic syndrome*

WHO Classification



From Malcovati L *et al*. *J Clin Oncol* 2005;23:7594–7603

Most-Frequent Chromosome Abnormalities in Patients with MDS



OS = overall survival.

Haase D *et al*. *Blood* 2005;106:232a

When classifications capture reality: SF3B1 mutation was the underlying mutation In Refractory Anemia with Ring Sideroblats (RARS)

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Somatic *SF3B1* Mutation in Myelodysplasia with Ring Sideroblasts

E. Papaemmanuil, M. Cazzola, J. Boultwood, L. Malcovati, P. Vyas, D. Bowen, A. Pellagatti, J.S. Wainscoat, E. Hellstrom-Lindberg, C. Gambacorti-Passerini, A.L. Godfrey, I. Rapado, A. Cvejic, R. Rance, C. McGee, P. Ellis, L.J. Mudie, P.J. Stephens, S. McLaren, C.E. Massie, P.S. Tarpey, I. Varela, S. Nik-Zainal, H.R. Davies, A. Shlien, D. Jones, K. Raine, J. Hinton, A.P. Butler, J.W. Teague, E.J. Baxter, J. Score, A. Galli, M.G. Della Porta, E. Travaglino, M. Groves, S. Tauro, N.C. Munshi, K.C. Anderson, A. El-Naggar, A. Fischer, V. Mustonen, A.J. Warren, N.C.P. Cross, A.R. Green, P.A. Futreal, M.R. Stratton, and P.J. Campbell for the Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium

N Engl J Med 2011 october 13; 365: 1384-95

AML origin: primary vs. secondary

De novo AML: arising in a patient w/o previous antecedent of hematologic neoplasia or exposition to leukemogenic agents (tAML)

De novo AML

Myelodysplastic syndrome (MDS)

Myeloproliferative neoplasm (MPN)

Secondary AML

Therapy-related AML (tAML) & other myeloid neoplasms: cytotoxic agents

Alkylating agents

- Melphalan, cyclophosphamide, nitrogen mustard, chlorambucil, busulfan, carboplatin, cisplatin, dacarbazine, procarbazine, carmustine, mitomycin C, thiotepa, lomustine, etc.

Ionizing radiation therapy

- Large fields including active bone marrow

Topoisomerase II inhibitors

- Etoposide, teniposide, doxorubicin, daunorubicin, mitoxantrone, amsacrine, actinomycin
- *Topoisomerase II inhibitors may also be associated with therapy-related lymphoblastic leukaemia

Others

- Antimetabolites: thiopurines, mycophenolate, fludarabine
- Antitubulin agents (usually in combination with other agents): vincristine, vinblastine, vindesine, paclitaxel, docetaxel

WHO Classification of Tumours of Hematopoetic and Lymphoid Tissues, 2008

AML with myelodysplasia-related changes: diagnostic criteria

≥20% blasts (BM or PB)

+

1. Prior history of MDS or MDS/MPN (secondary AML) *and/or*
2. MDS-related cytogenetic abnormalities (de novo or secondary AML) *and/or*
3. Multilineage dysplasia (≥50% of the cells in at least 2 BM myeloid lineage) (de novo or secondary)

Diagnosis of AML/MDS: integration of multiple tools

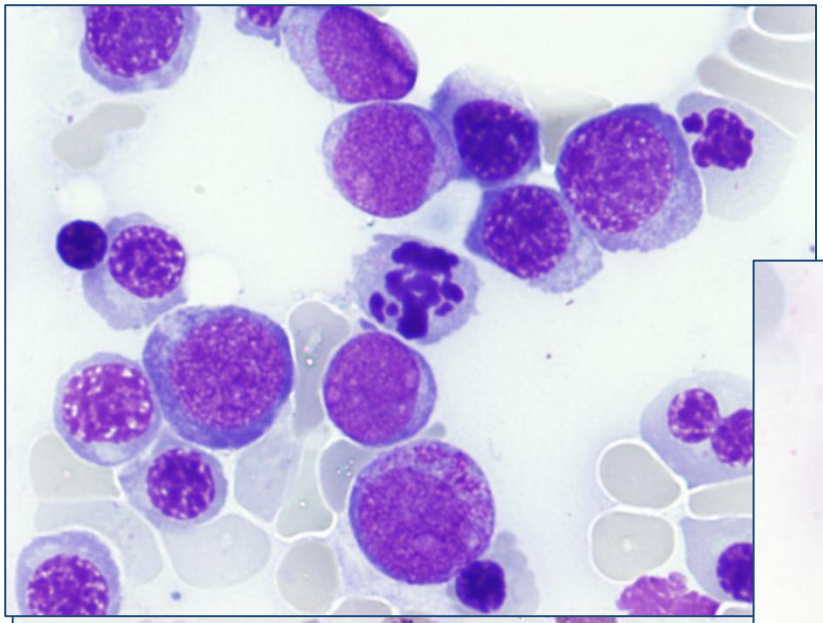
- Clinical history
- Cytology: PB slide, BM (BM aspirate ± BM biopsy)
- Cytochemistry: MPO & Sudan Black B (myeloid); α-naphthyl acetate & α-naphthyl butyrate (monocytic)
- Inmunophenotype: multiparameter flow cytometry – based
- Cytogenetics
  - Conventional cytogenetics
  - FISH; other (CGH, SNParray,...)
- Molecular biology
  - Fusion transcripts (RT-PCR): PML/RAR-α, AML1/ETO, CBF-β/MYH11, MLL/..., BCR/ABL, DEK/CAN,...
  - Gene mutations: FLT3-ITD, CEBPalfa, NPM1, …

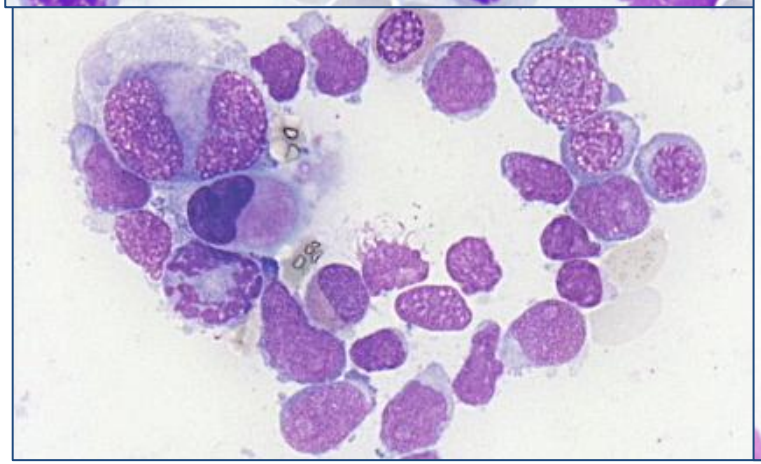
Diagnosis of AML with myelodysplasia-related changes : dysplasia assessment

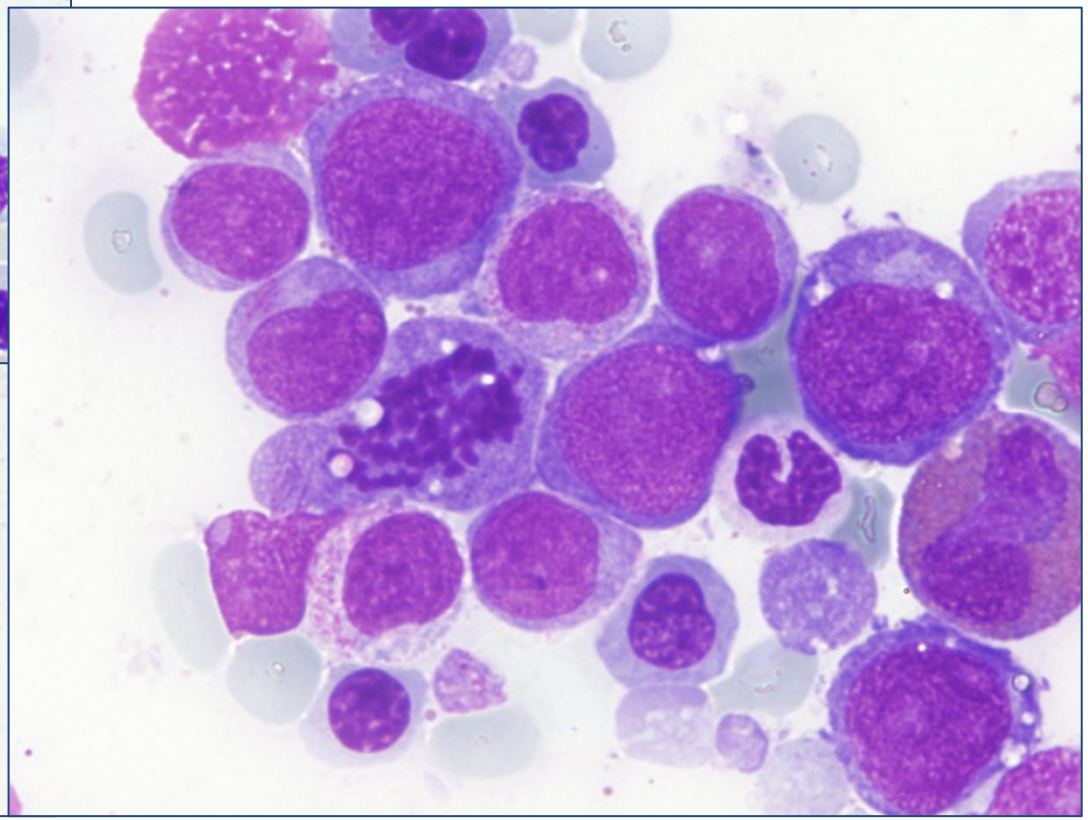
- Dysgranulopoiesis: 25-100 neutrophils
  - hypogranular cytoplasm, hyposegmented nuclei or bizarrely segmented nuclei, cytoplasmic vacuoles
  - MPO deficiency (50%, 20 cells)
- Dyserytropoiesis: at least 25 mature erythroblasts
  - megaloblastosis, karyorhexis & nuclear irregularity, fragmentation or multinucleation
  - ring sideroblasts, PAS positivity
- Dysmegakaryopoiesis: at least 6 megakaryocytes
  - micromegakaryocytes, normal sized or large megakaryocytes with non-lobulated or multiple nuclei

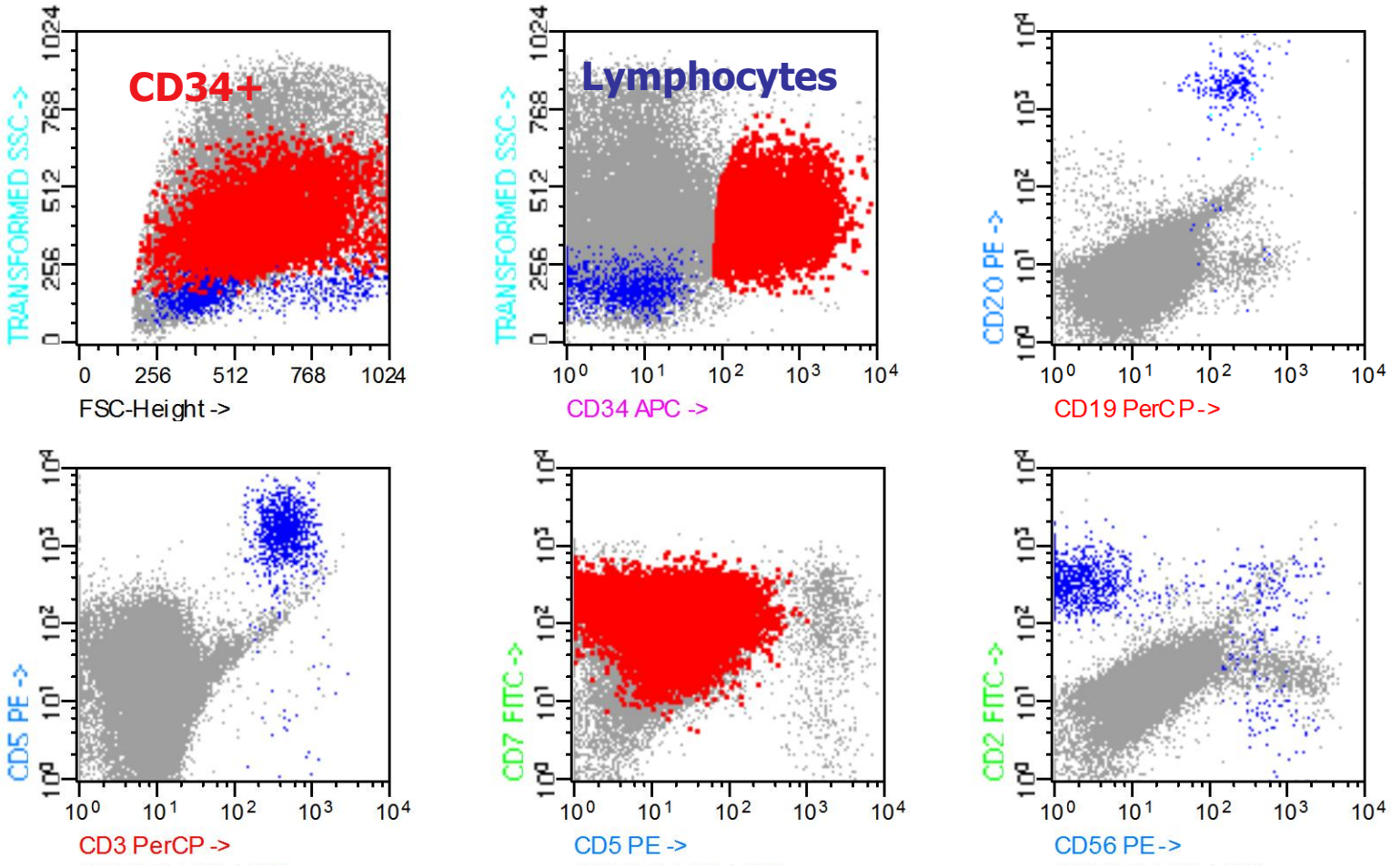
**Goasguen et al. Leukemia 1992, WHO classification IARC Press 2008*

Evaluation of dysplasia





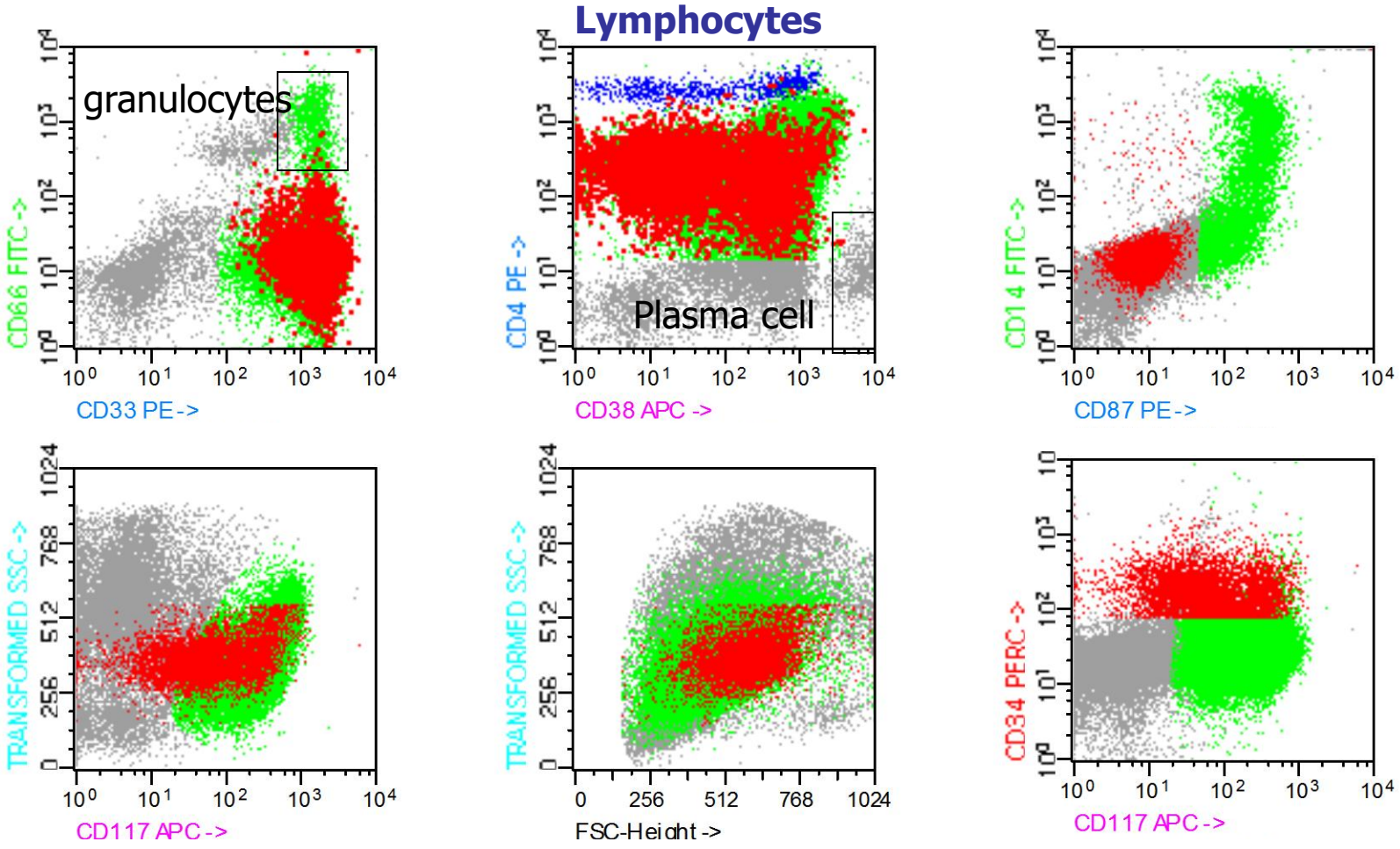




CD34+ (partial), CD56-

B-cell markers (CD19, CD10, CD20): negative

T-cell markers (CD2, CD3) negative CD7++ and CD5 (partial)



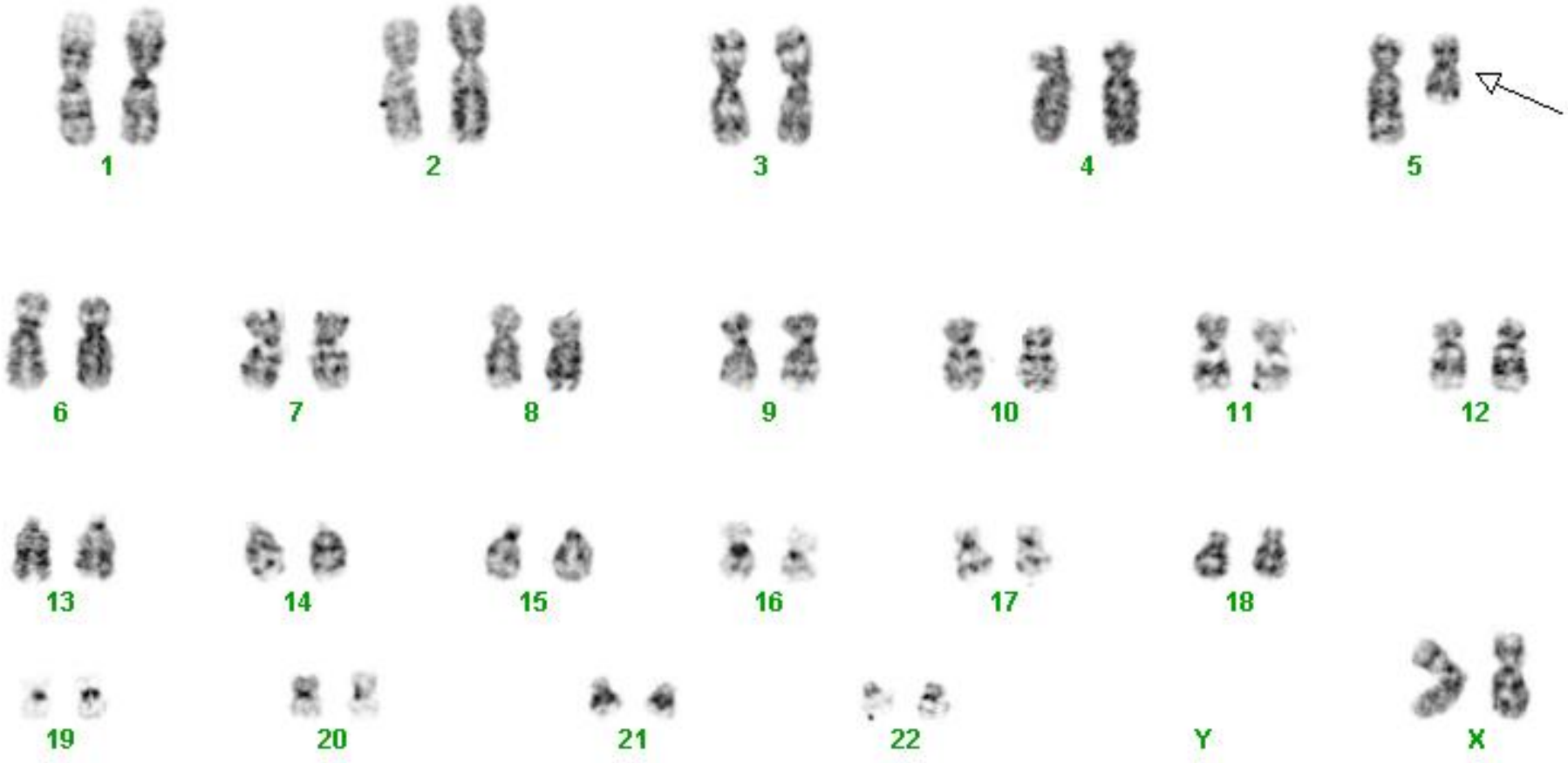
HLA-DR+

Myeloid markers: CD117+ MPO+ (weak) CD13+(het) CD33++(hom)

Monocytic markers: CD4+ CD36 (partial) CD64+ lysozime+ CD11+ (weak)

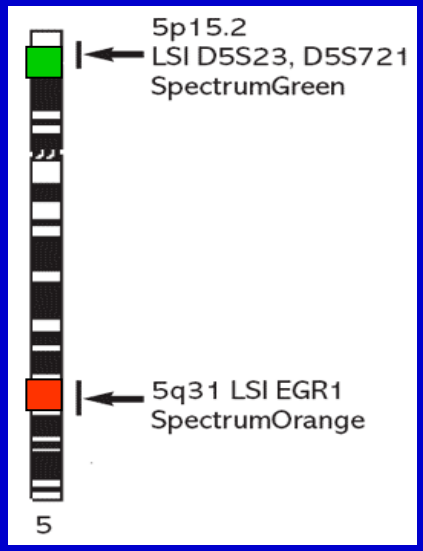
CD66- CD65- NG2- CD14- CD87-

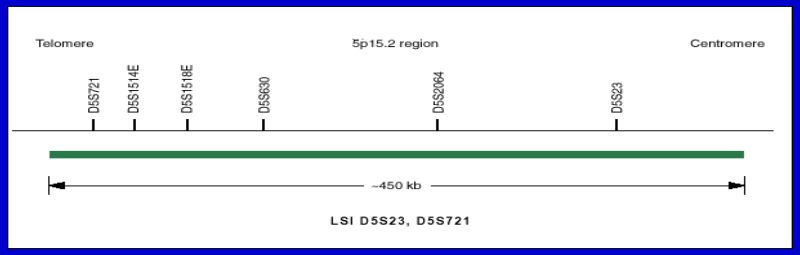
Conventional cytogentics

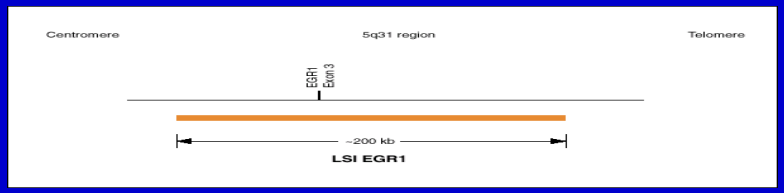


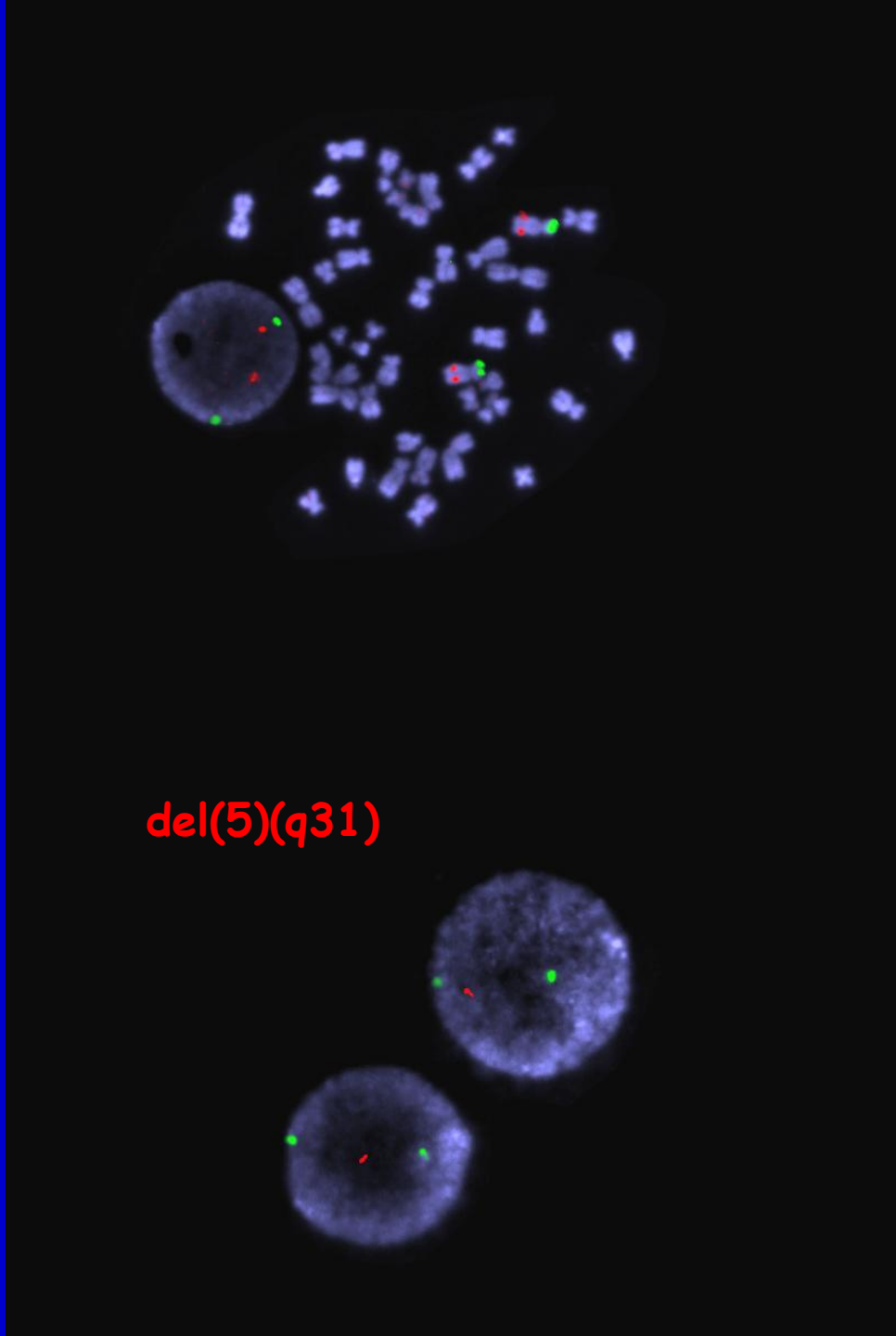
46, XX, del (5)(q22q33)

FISH in MDS

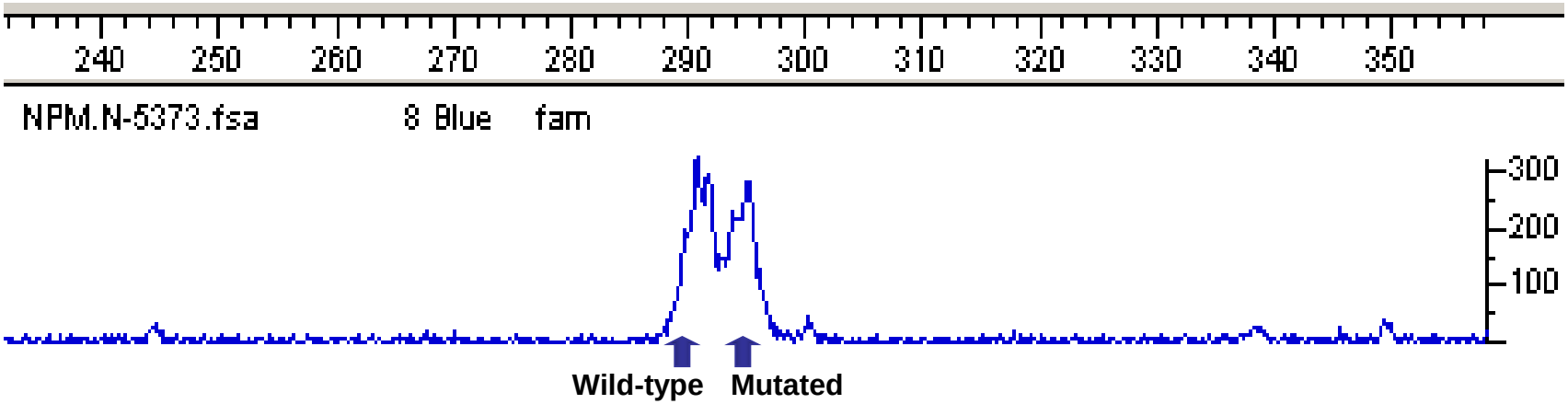

5p15.2
LSI D5S23, D5S721
SpectrumGreen
5q31 LSI EGR1
SpectrumOrange
5


Telomere
5p15.2 region
Centromere
D5S721
D5S1514E
D5S1518E
D5S630
D5S2064
D5S23
~450 kb
LSI D5S23, D5S721


Centromere
5q31 region
Telomere
EGR1
Exon 3
~200 kb
LSI EGR1

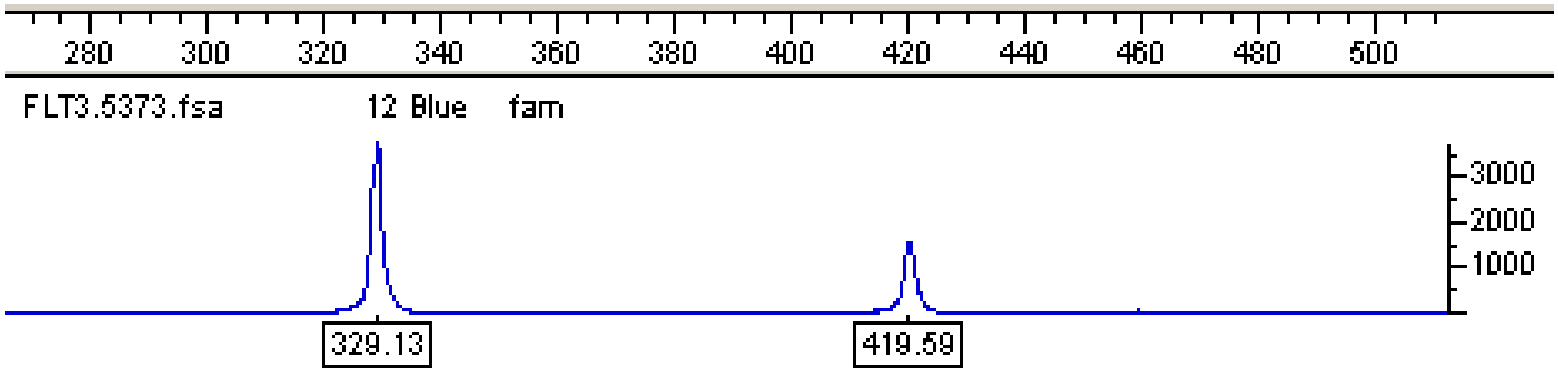

del(5)(q31)

RT-PCR of Exon 12 of Nucleophosmin (*NPM1*)



Heterozygous mutation (4-bp insertion)

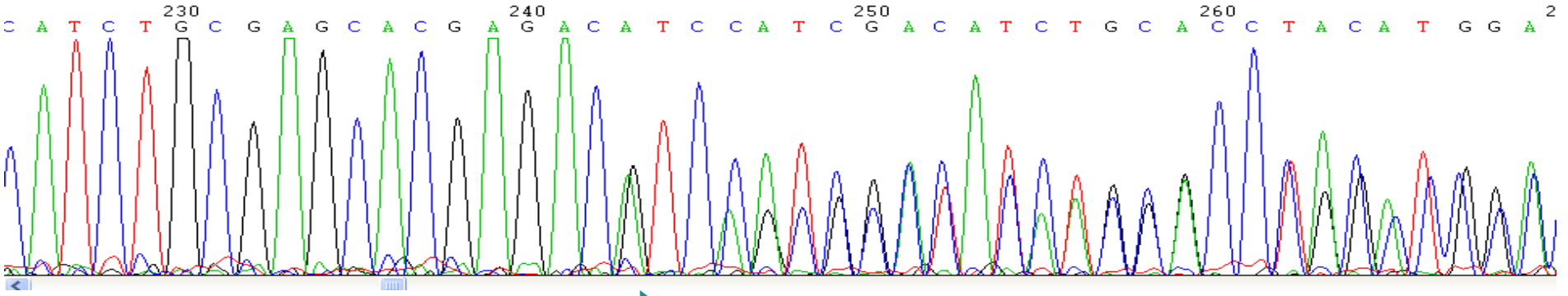
PCR of exons 11-12 of FLT3 gene (screening of internal tandem duplication, ITD)



Presence of a FLT3-ITD mutation of 90 bp and a ratio mutant allele/wt 0.45

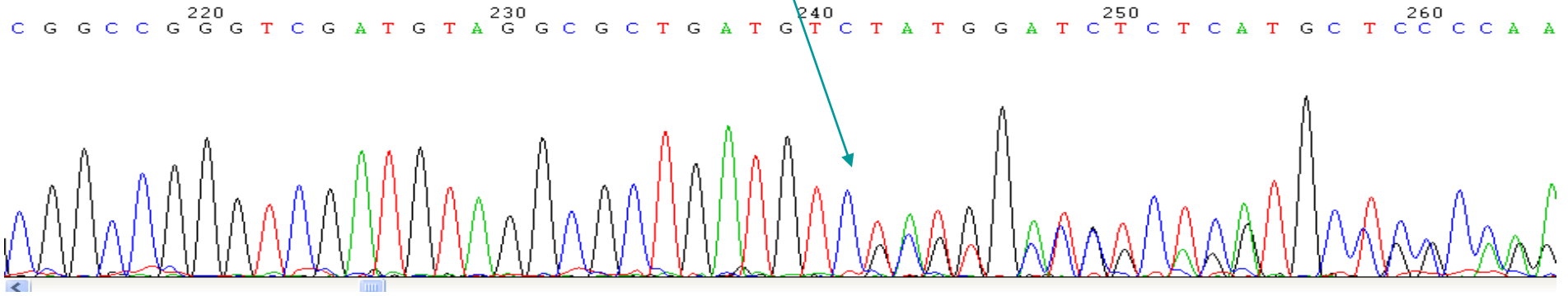
CEBPA **mutation**

FORWARD SEQUENCE

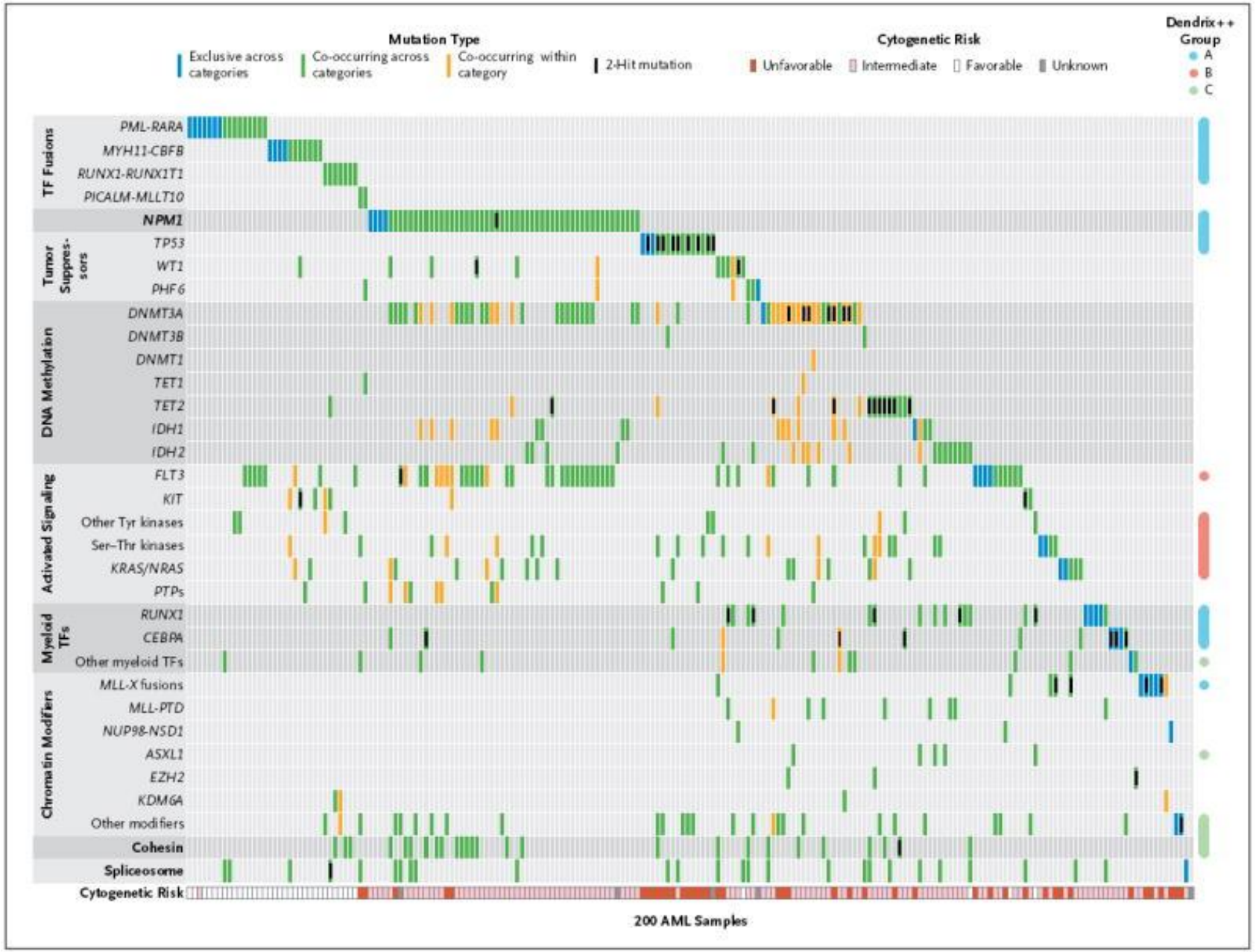


beginning of sequence deletion (13pb)

REVERSE SEQUENCE

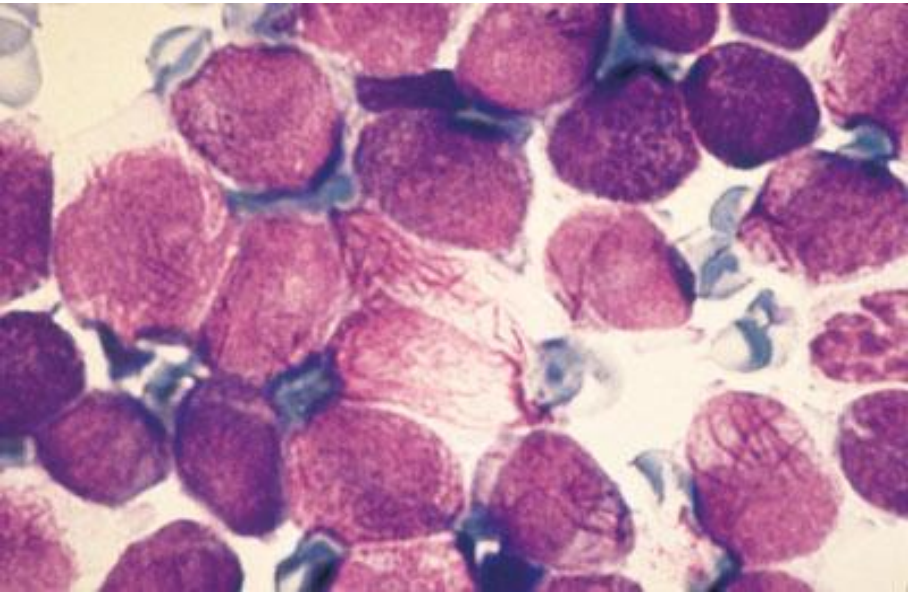


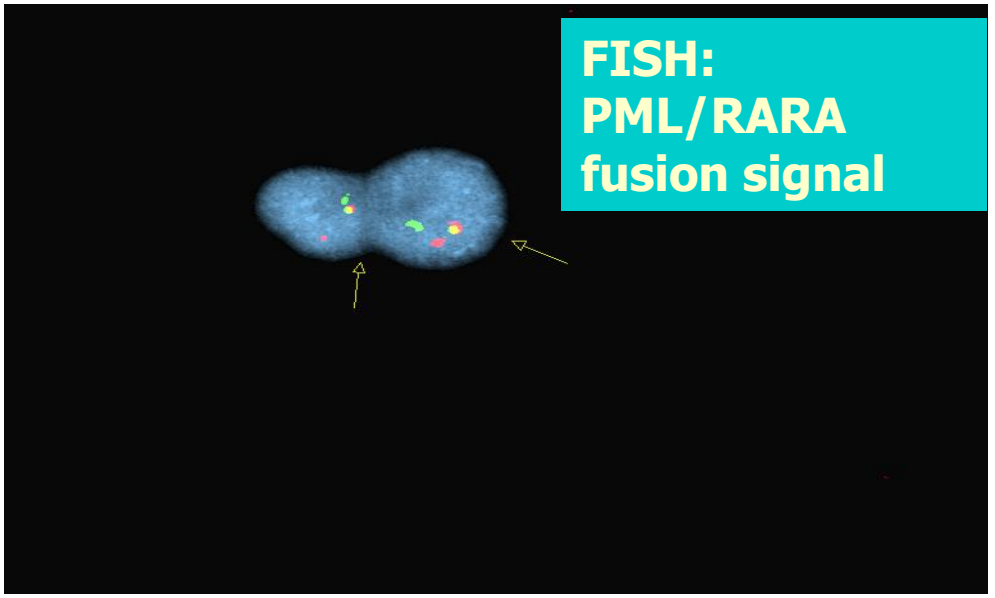
AML mutations: distribution into categories of related genes

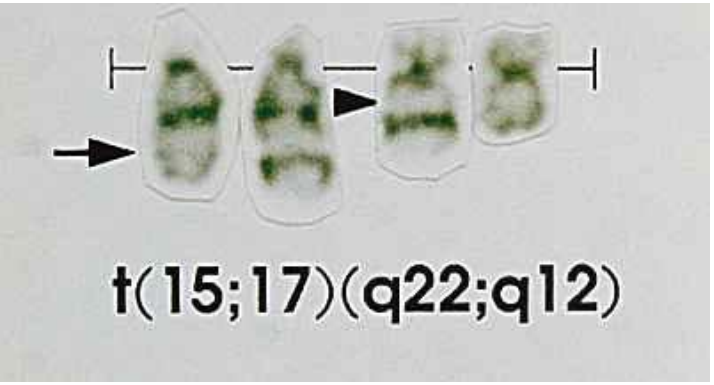


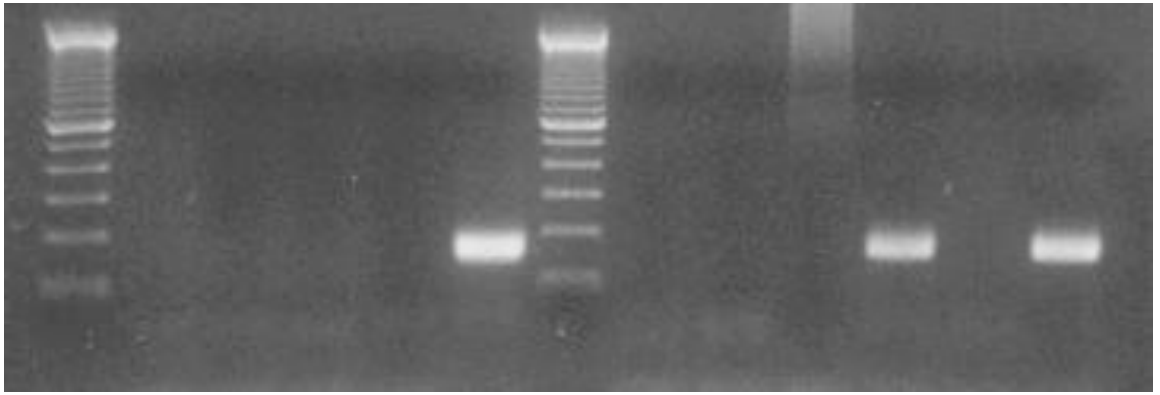
The Cancer Genome Atlas Research Network, NEJM 2013

Acute promyelocytic leukemia (APL): an example of integrated diagnosis









RT-PCR PML/RAR-alpha

Education session on AML & MDS: conclusions

- AML & MDS: clearly differentiated entities (clinical presentation, hematological picture, genetics)
- … although AML represents an evolutive phase of MDS in some patients
- AML & MDS are heterogenous diseases defined by complex, distinct genetic background: moving towards molecularly-defined classification
- Importance/difficulties of translating reality/biological complexity to pre-defined forms (MED-A & MED-B)

Questions?