LEUCEMIE ACUTE

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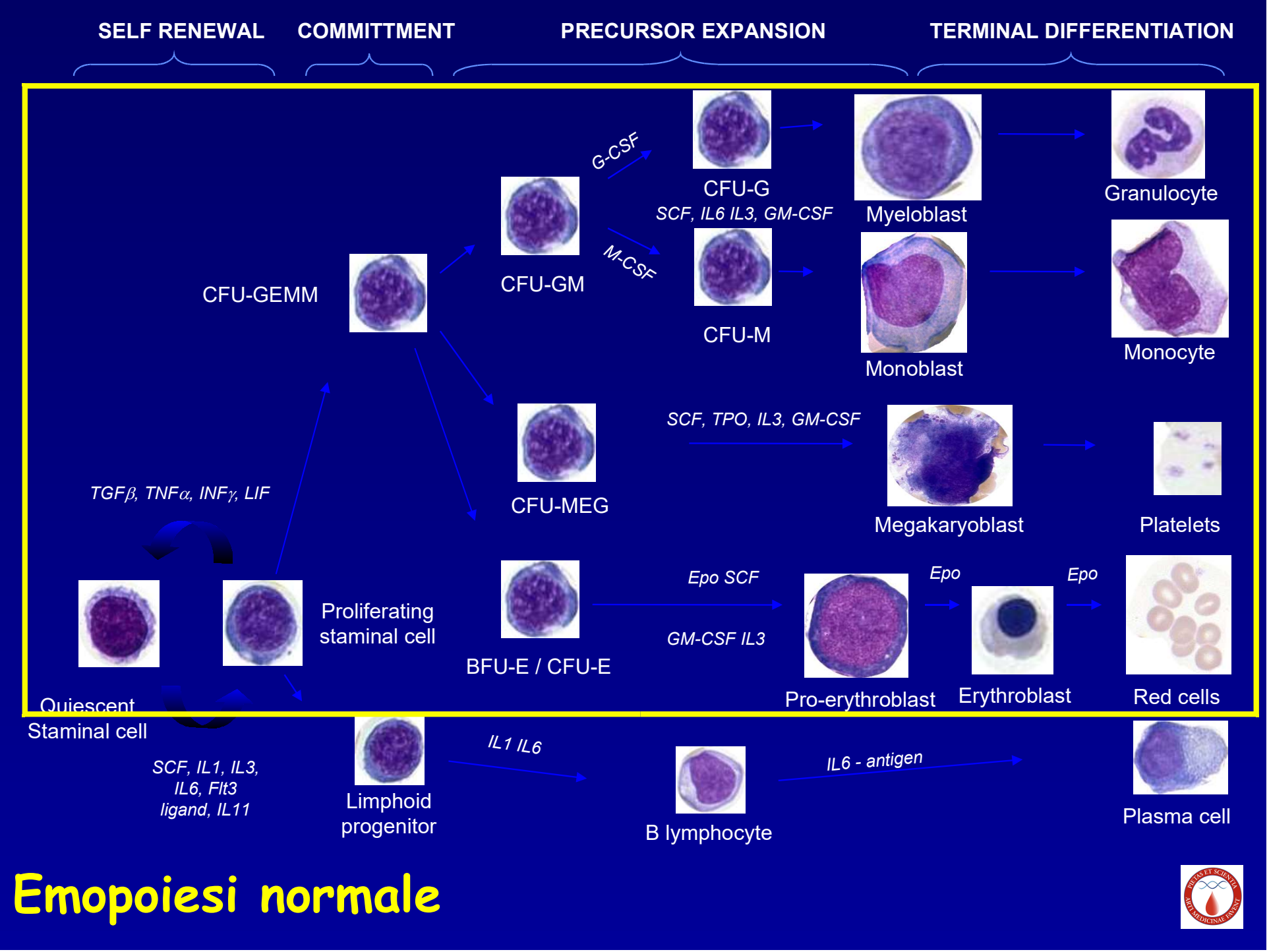
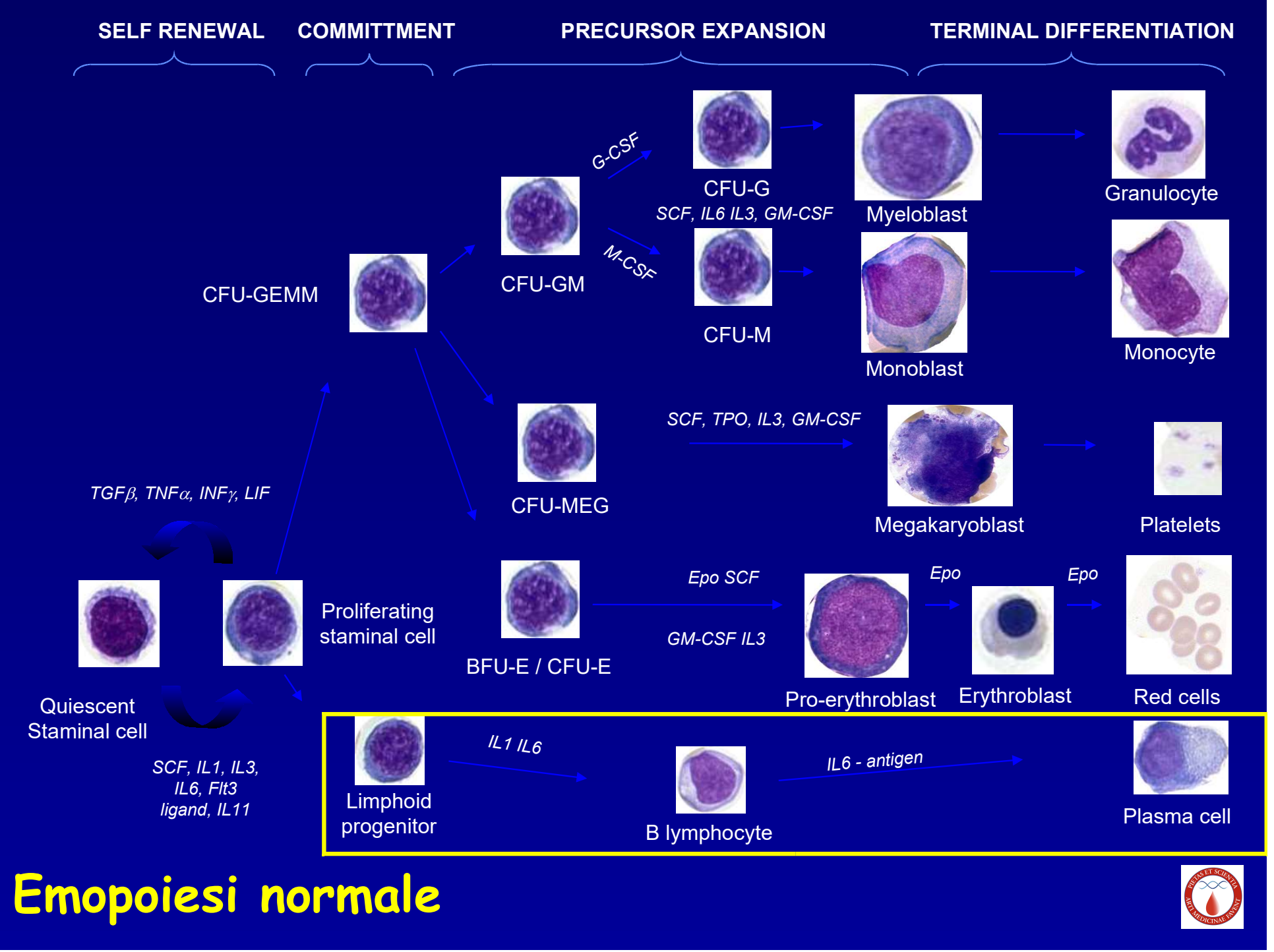


Emopoiesi normale



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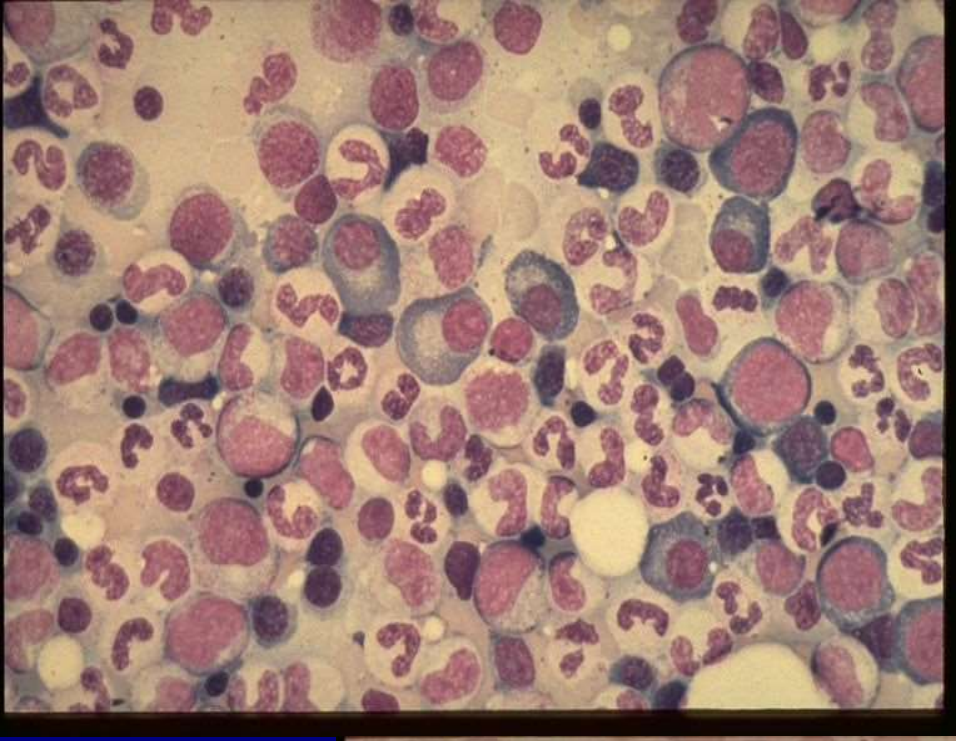
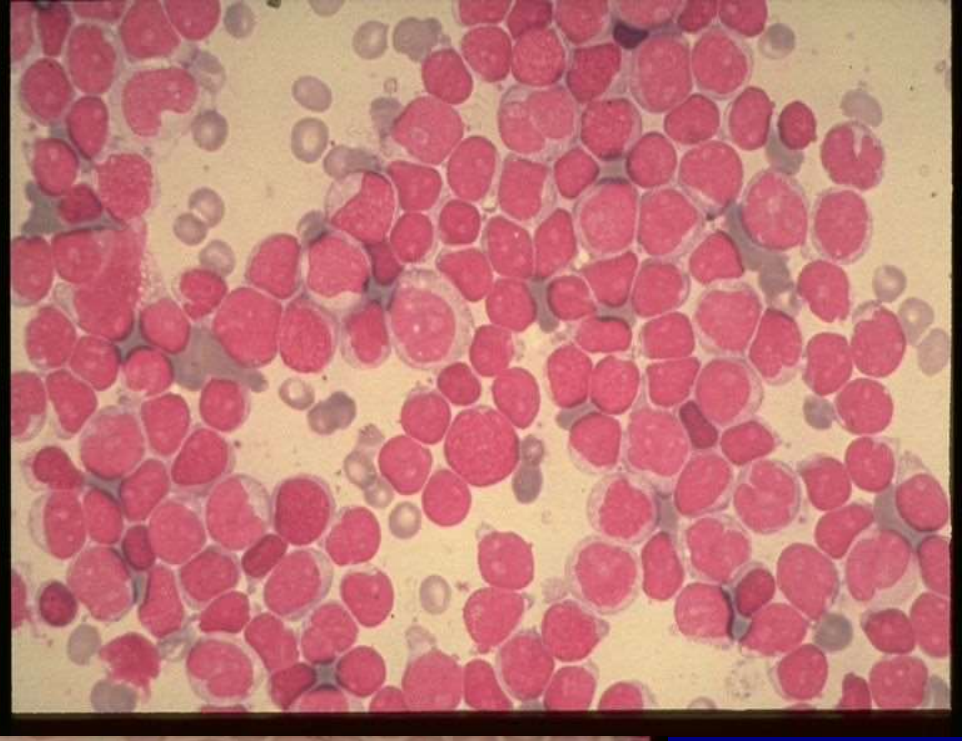
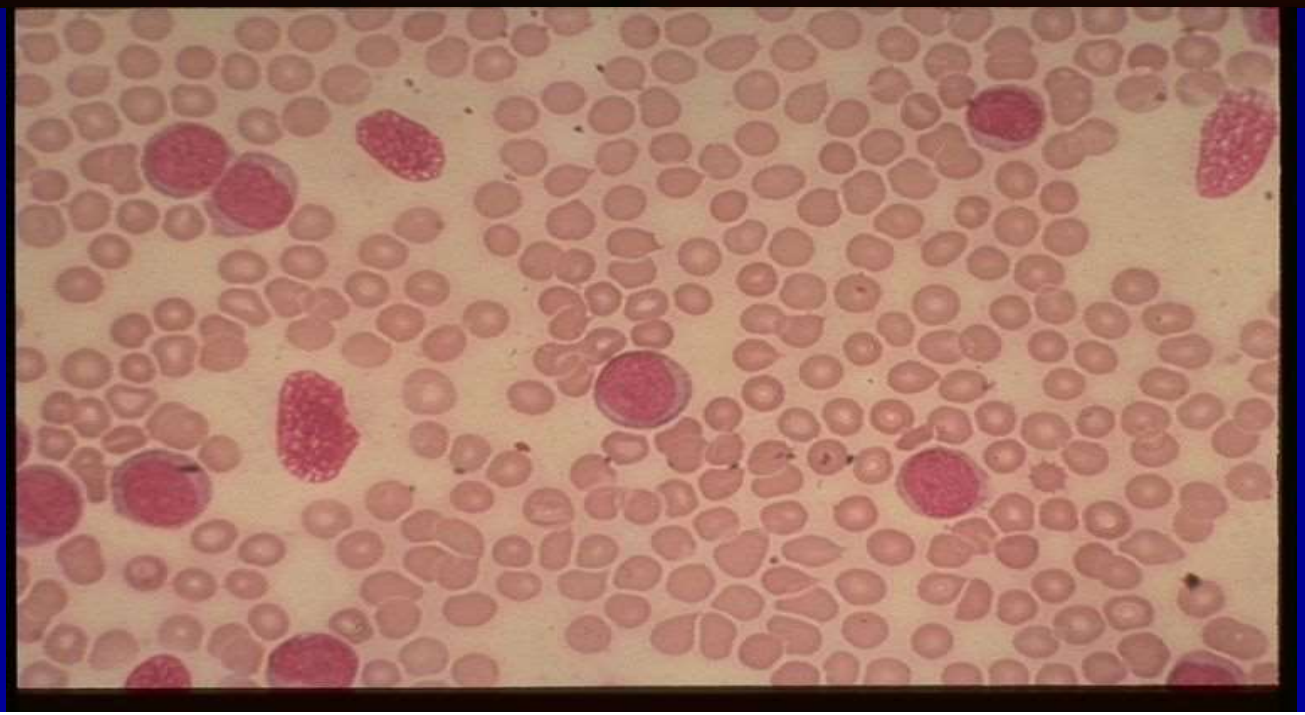
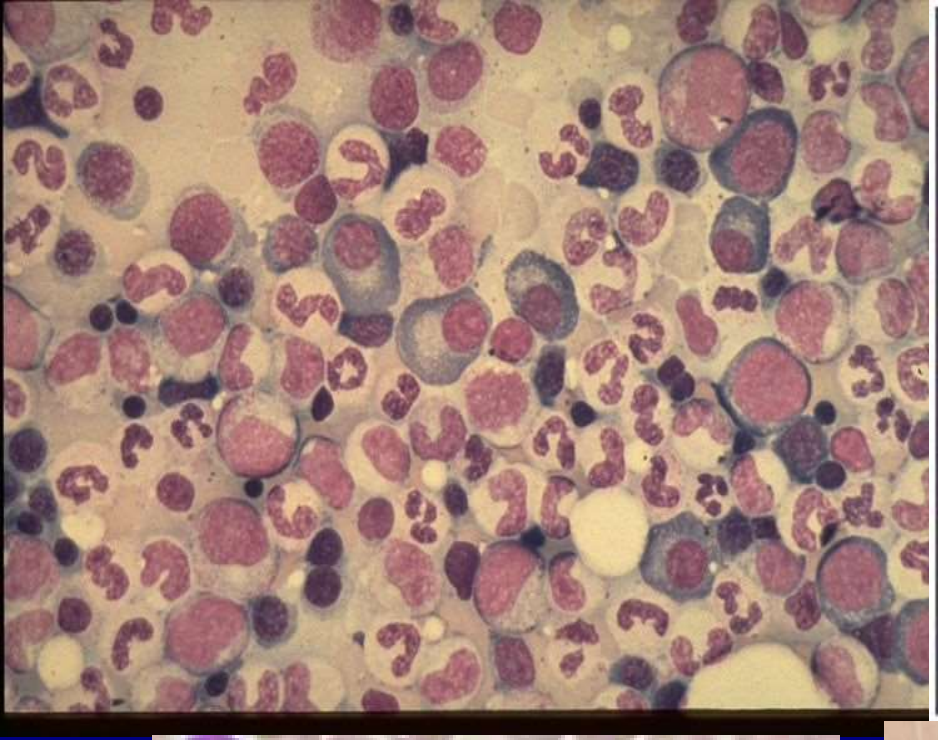
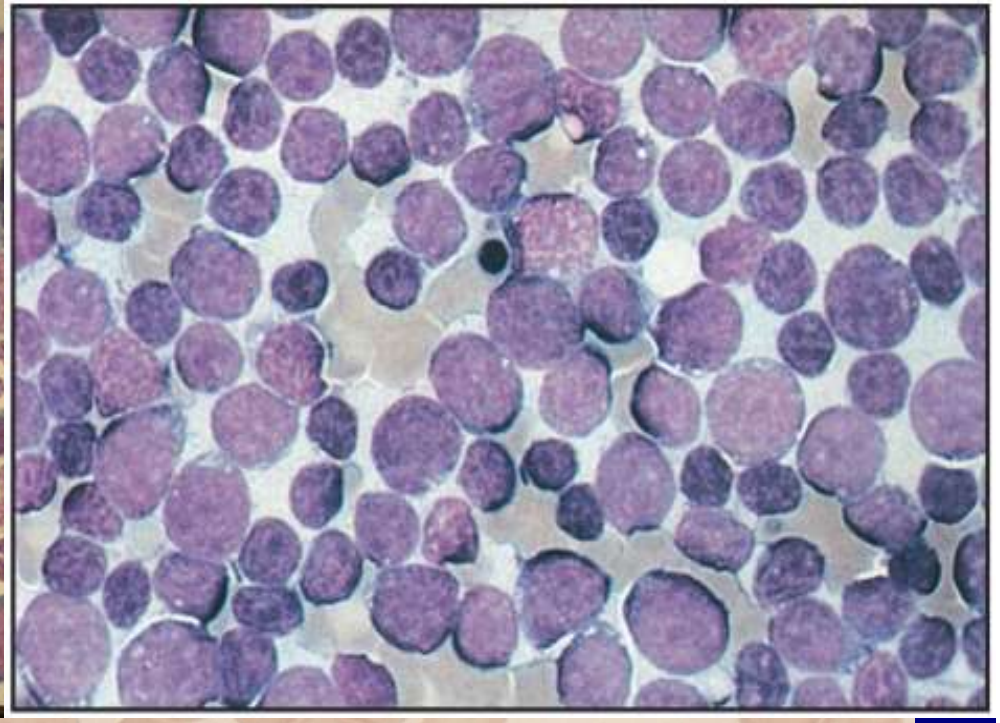
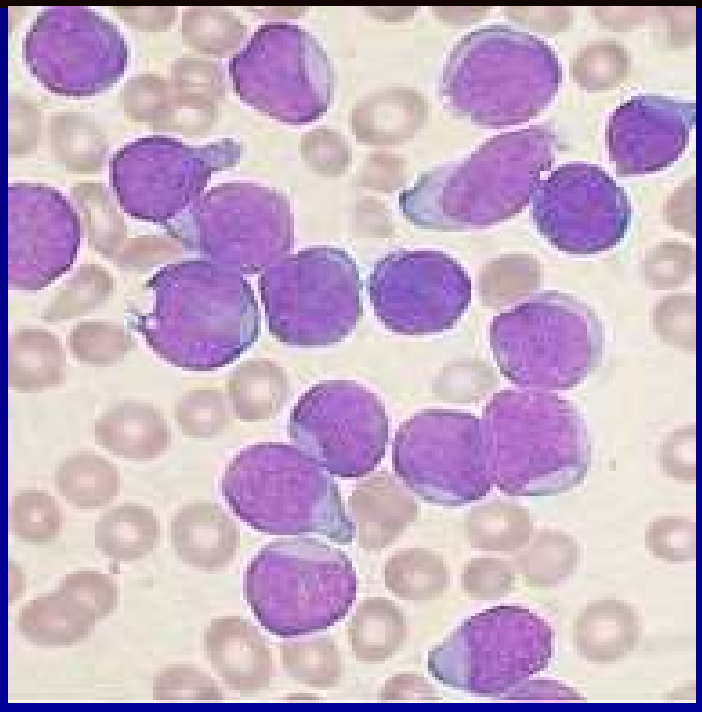


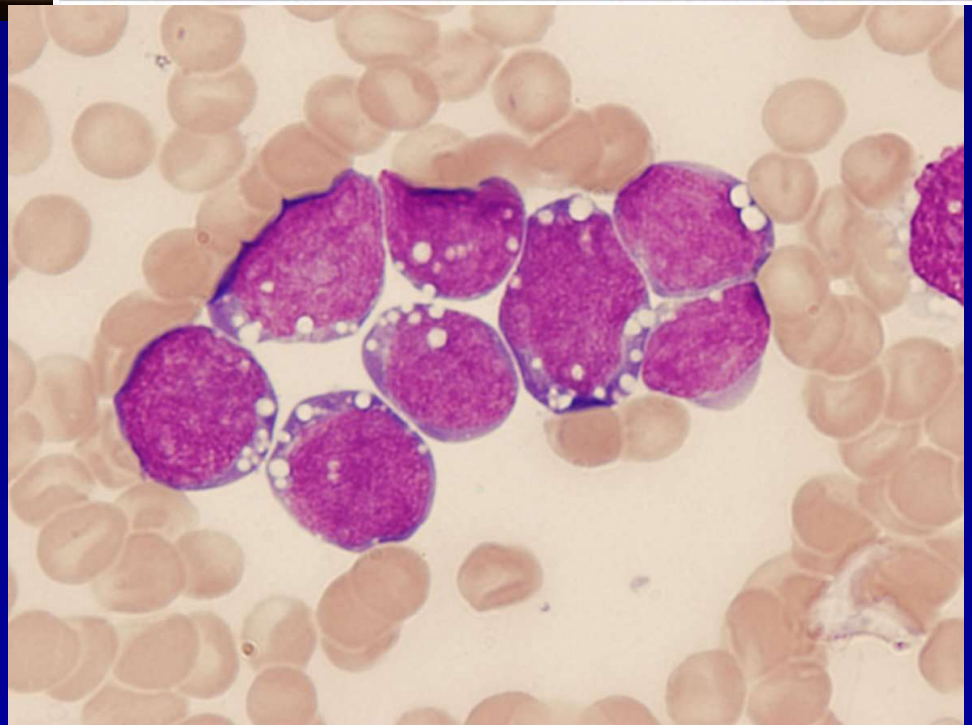




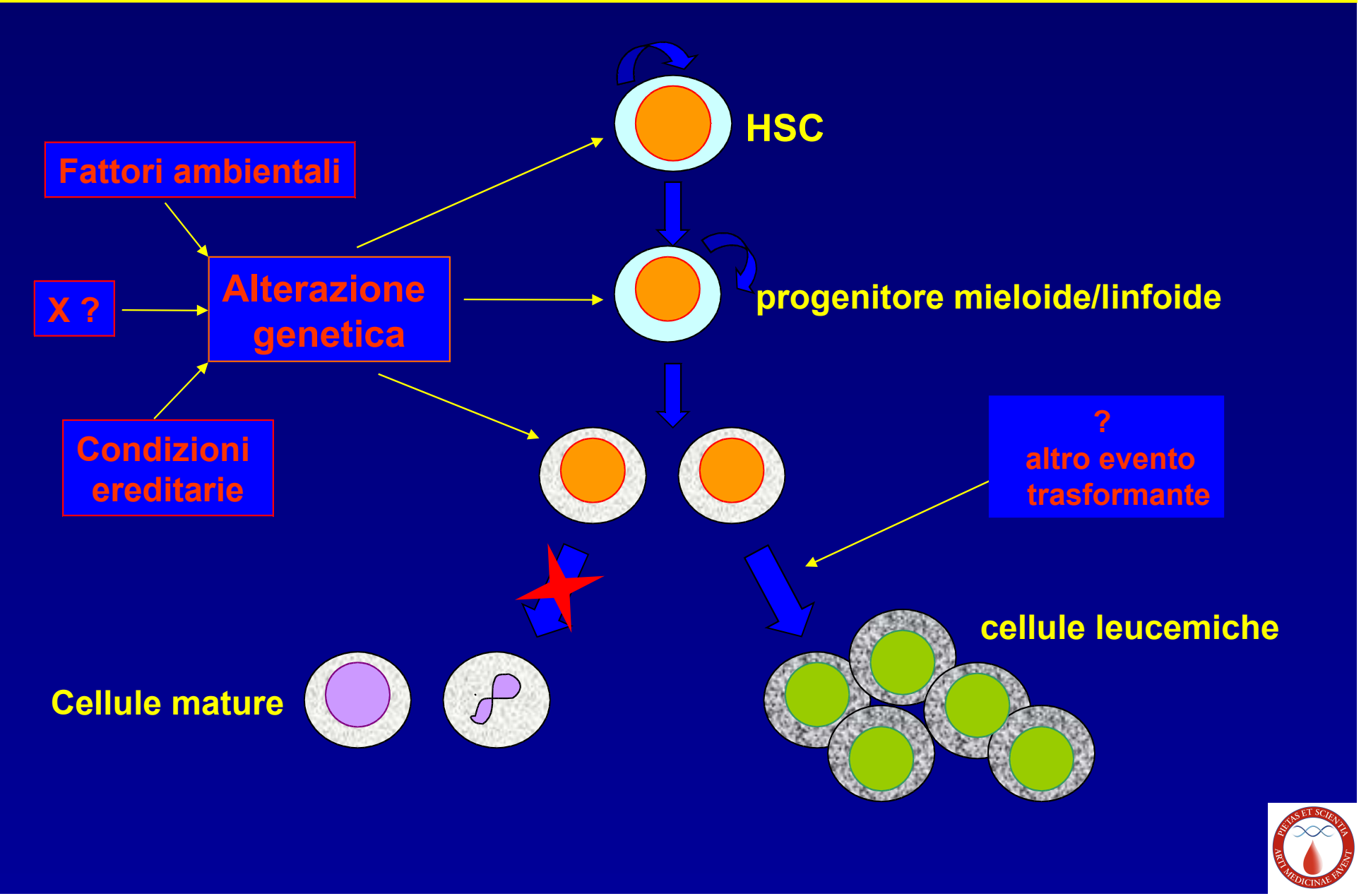




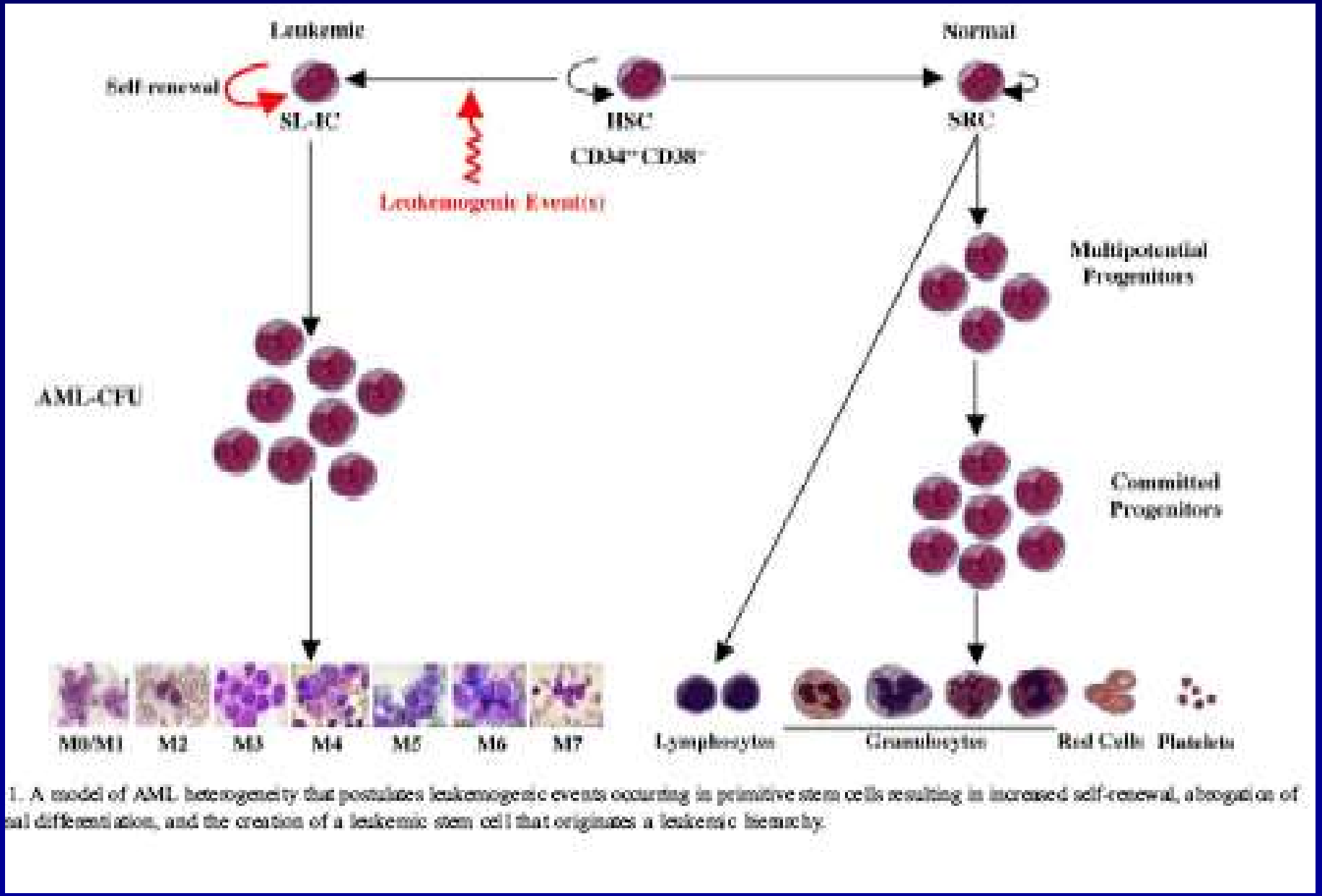




EZIOPATOGENESI DELLE LEUCEMIE ACUTE



LEUCEMOGNESI



1. A model of AML heterogeneity that postulates leukemogenic events occurring in primitive stem cells resulting in increased self-renewal, abrogation of al differentiation, and the creation of a leukemic stem cell that originates a leukemic hierarchy.

FATTORI AMBIENTALI

Agenti fisici →
- **Radiazioni ionizzanti**
- **Sorgenti elettriche/magnetiche**

Agenti chimici →
- **Benzene** *(sigarette, industrie inquinamento atmosferico)*
- **Altri composti aromatici**
- **Ossido di etilene**
- **Fenossi-erbicidi**
- **Uretano, nitrosamine** *(sigarette)*
- **Chemioterapia** *(es. alchilanti)*

Agenti virali →
- **HTLV-1**
- **EBV**

CONDIZIONI EREDITARIE

Sindromi ereditarie →

- Sindrome Down (trisomia 21)
- Sindromi con deficit del "DNA-repair" (S. Bloom, Anemia di Fanconi)
- Sindromi da immunodeficienza (S. Wiskott Aldrich)

Alto rischio familiare di LA →

- Monosomia del 7 (SMD)
- Mutazione AML-1

EPIDEMIOLOGIA DELLE LEUCEMIE ACUTE

Incidenza: 3,5 casi/100000 abitanti/anno

LAM

- Ogni età
- Età mediana: 60-65
- Esposizione professionale o iatrogena a Rx, benzene, chemioterapici

LAL

- 80% delle LA per età < 15 aa
- 20% delle LA nell'adulto
- Agenti ambientali (?)
- eziologia virale (EBV, HTLV-1)

LEUCEMIE ACUTE

FISIOPATOLOGIA

1) INSUFFICIENTE E DIFETTIVA PRODUZIONE DI LEUCOCITI (infezioni); ERITROCITI (anemie) E PIASTRINE (emorragie);

2) INFILTRAZIONE DI TESSUTI E ORGANI NON EMOPOIETICI DA PARTE DELLE CELLULE BLASTICHE (organomegalia; danno funzionale)

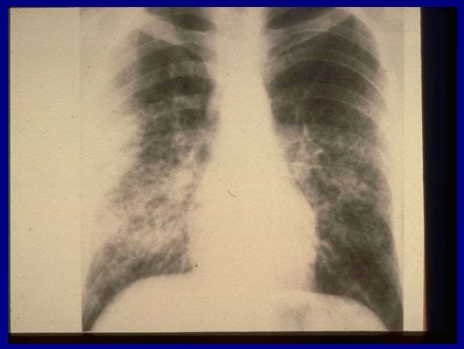
3) LIBERAZIONE DI CITOCHINE (febbre, algie, calo ponderale, sudorazioni)

Leucemia Acuta

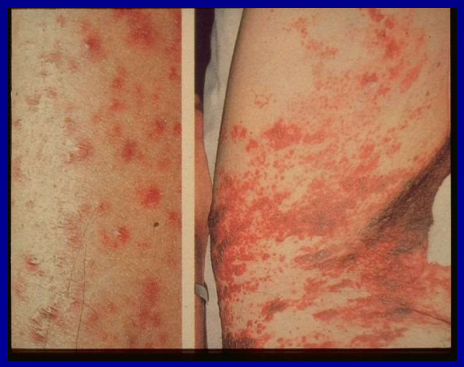
Quadro Clinico - Obiettivo

- Anemia

- Infezioni



- S. Emorragica



Laboratorio

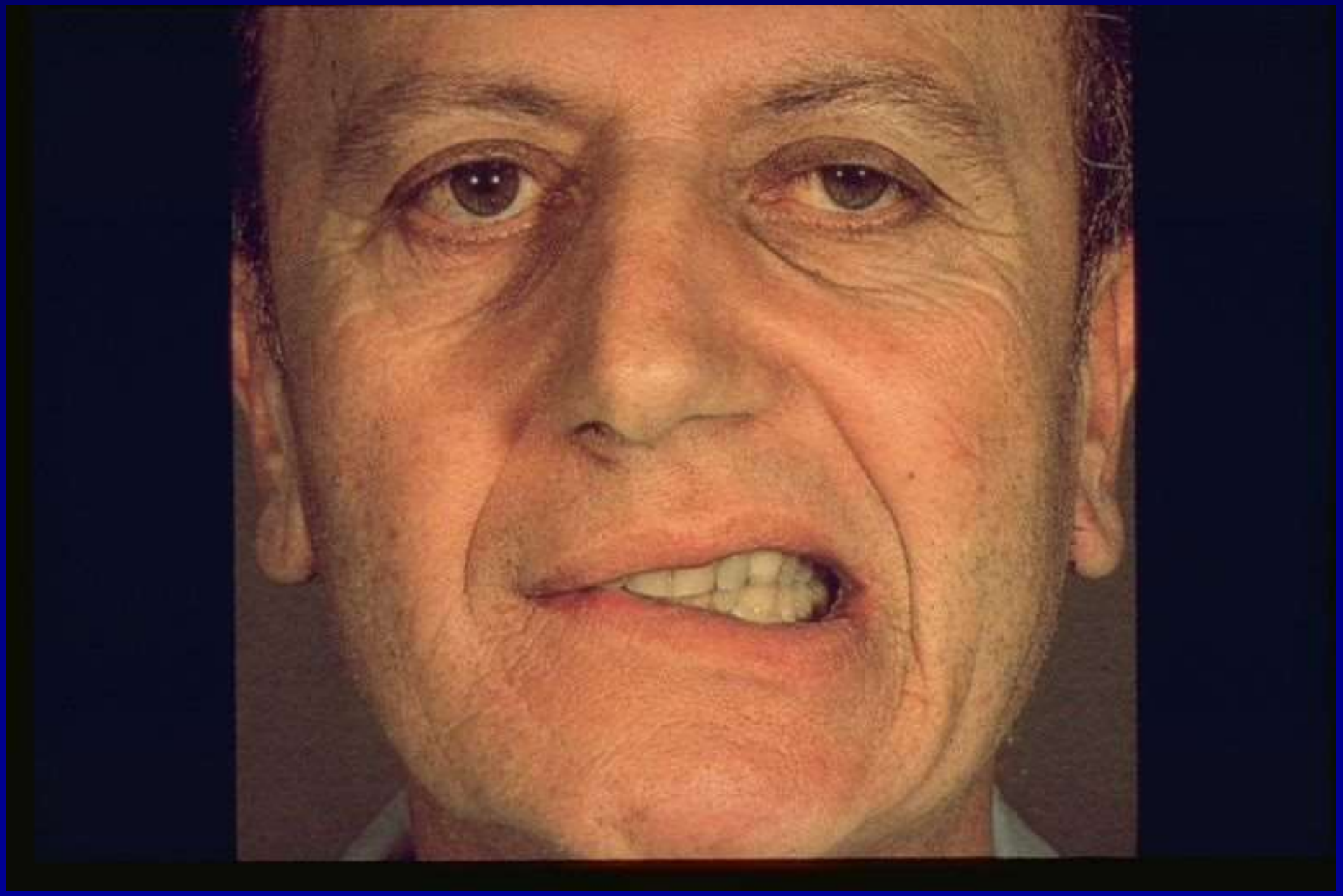
Hb < 12.5 g/dl uomo
Hb < 11.5 g/dl donna

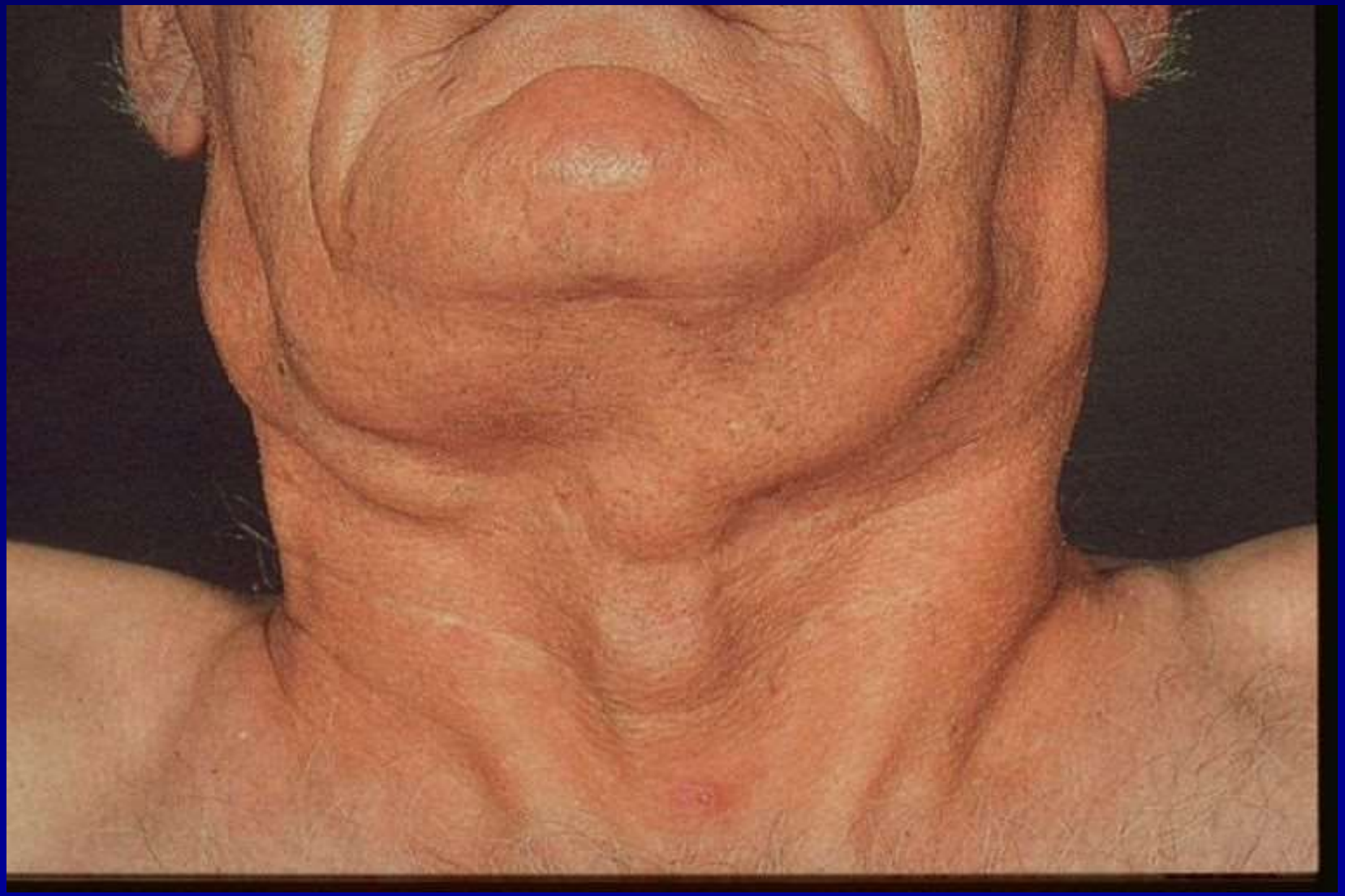
Neutropenia
< 1000/mm3

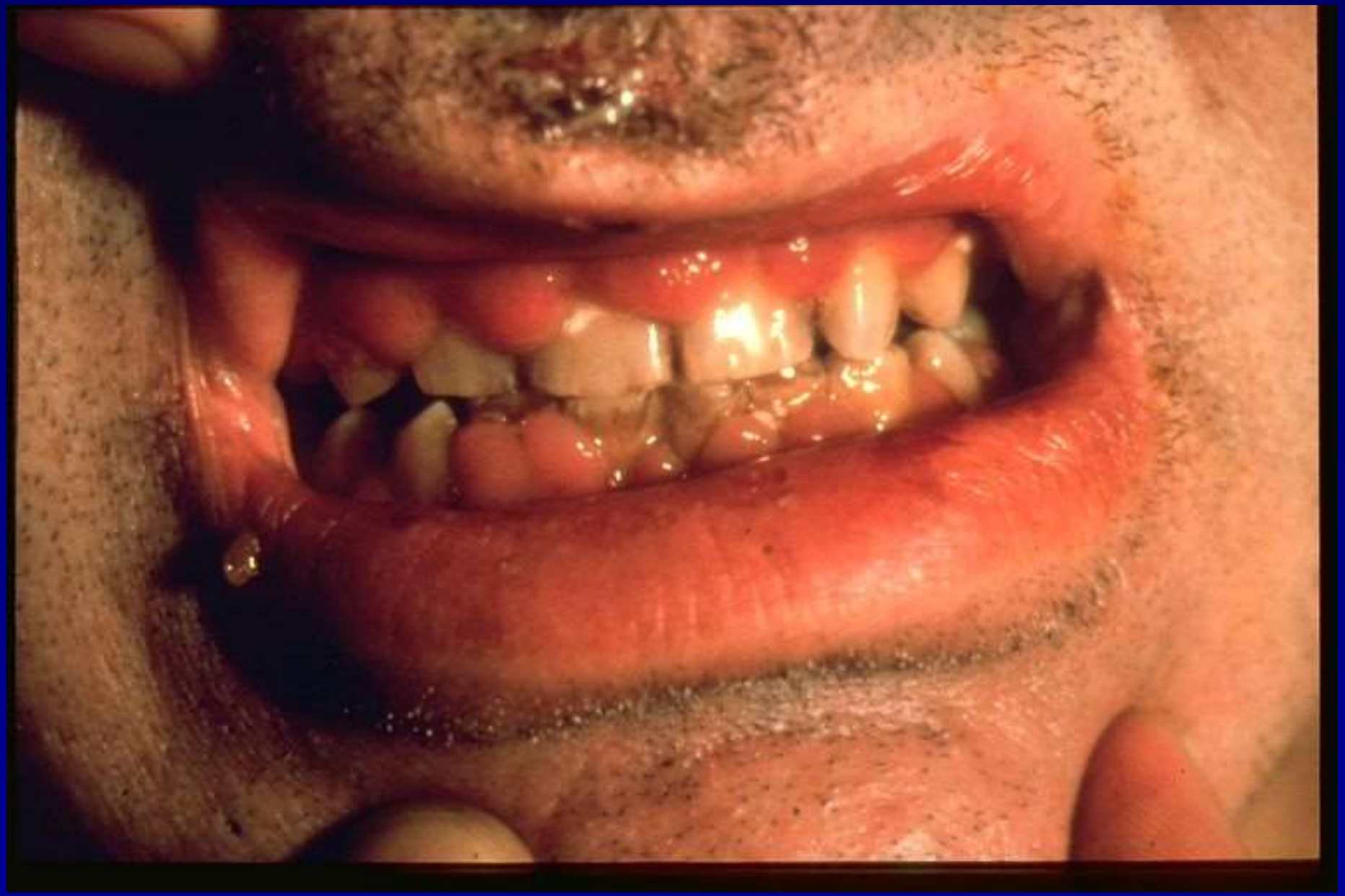
Blastosi Leucemica

PLT < 150 x 10^9/L

(CID)







SINTOMI E SEGNI CLINICI DI ESORDIO IN 466 CASI DI LAM «DE NOVO» O PRIMARIE

Astenia	93 (%)
Cardiopalmo, dispnea	55 (%)
Febbre	62 (%)
Febbricola	25 (%)
Emorragie	61 (%)
Dolori ossei o muscolari	23 (%)
Sudorazioni profuse	8 (%)
Splenomegalia	18 (%)
Epatomegalia	11 (%)
Linfoadenomegalia	9 (%)
Ipertrofia gengivale	11 (%)
Interessamento cutaneo	5 (%)
Sintomi e segni neurologici	1 (%)

LEUCEMIE ACUTE DIAGNOSI

SANGUE PERIFERICO

ASPIRATO MIDOLLARE

IMMUNOFENOTIPO

CARIOTIPO

BIOLOGIA MOLECOLARE

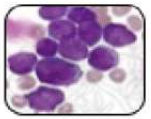
Misura della fluorescenza: utilità

- Le cellule possono essere identificate grazie alle loro molecole di superficie e relativo specifico marker (CD, "cluster di differenziazione")
- Anticorpi monoclonali per specifici antigeni CD possono essere utilizzati per identificare il tipo di cellula
- L'anticorpo monoclonale può essere coniugato con un Fluorocromo (FITC, PE, Tandem ECD, PC5) e si può eseguire un test di immunofluorescenza diretta

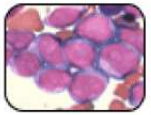
Analisi citofluorimetrica nelle leucemie acute: immunofenotipo

- **Diagnosi** delle leucemie acute
- **Classificazione** delle leucemie acute
- **Monitoraggio** malattia minima residua (sensibilità 10^{-3} – 10^{-4})

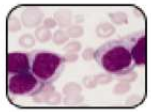
FAB CLASSIFICATION



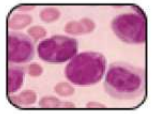
M0: Undifferentiated acute myeloblastic leukemia (5%)



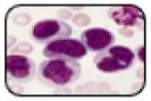
M1: Greater number of myeloblasts with <10% granulocytic differentiation.



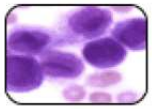
M2: Myeloblasts in great number with granulocytic differentiation >10% , NSE <20%.



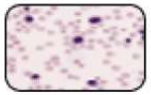
M3: Promyelocytes that are hyper granular with many Auer rods on CAE or Wright-stain and variant form cells with reniform nuclei, multilobed or bibbed, primeval cells with multiple Auer rods or relative scarcity of Hypergranular promyelocytes.



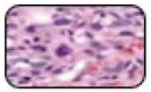
M4: >20% but <80% NSE-butyrate positivity in Monocytic cells



M5: Monocytic cells with >80% NSE positivity. (a) Monocytic differentiated (b) Monocytic, differentiated.

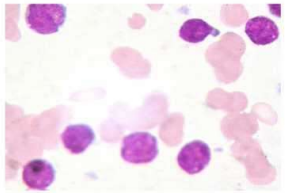


M6: >30% myeloblasts with more than 50% erythroblasts eliminating the erythroid cells.



M7: Acute megakaryoblastic leukemia <5%

FAB classification of lymphoblastic leukaemia



L1 Lymphoblastic leukaemia with homogeneous structure

Frequency:

Between 25% and 30% of cases in adults, and 85% of cases in children.

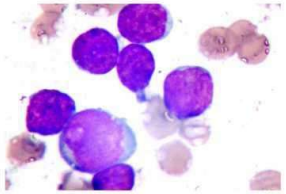
Morphology:

Blasts are homogeneous, nucleus is regular, chromatin is homogeneous, small or no nucleoli, scanty cytoplasm, and mild to moderate basophilia.

Immunophenotype

B:	T:
• CD19	•CD3
•CD22	•CD7
•CD79a	•CD5
•CD10	•CD2
•CD20	•CD4

•Cytoplasmic or superficial immunoglobulin



L2 Lymphoblastic leukaemia with varied structure

Frequency:

Accounts for 70% of cases in adults, and 14% in children.

Morphology:

Nucleus is irregular, heterogeneous chromatin structure, large nucleoli.

Immunophenotype

B:	T:
•CD19	•CD3
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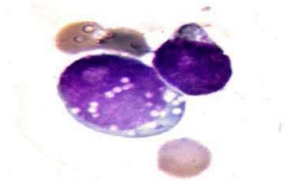

L3 Burkitt's leukaemia

Frequency:

Rare subtype, accounting for less than 1% to 2% of cases.

Morphology:

Large blasts, prominent nucleoli, stippled homogeneous chromatin structure, abundant cytoplasm, abundant cytoplasmic vacuolation (bubble type) covering the nucleus.

Immunophenotype

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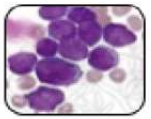
Rev Med Hosp Gen Mex. 2016;79:107–13

FAB CLASSIFICATION



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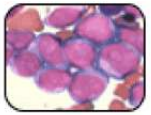

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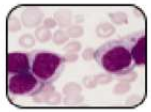

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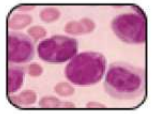

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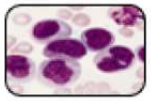

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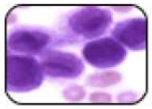

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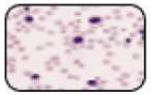

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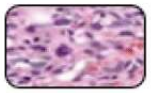

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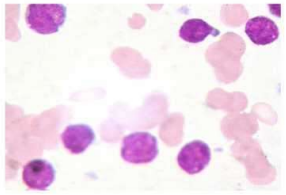
Molecular classification

FAB classification of lymphoblastic leukaemia



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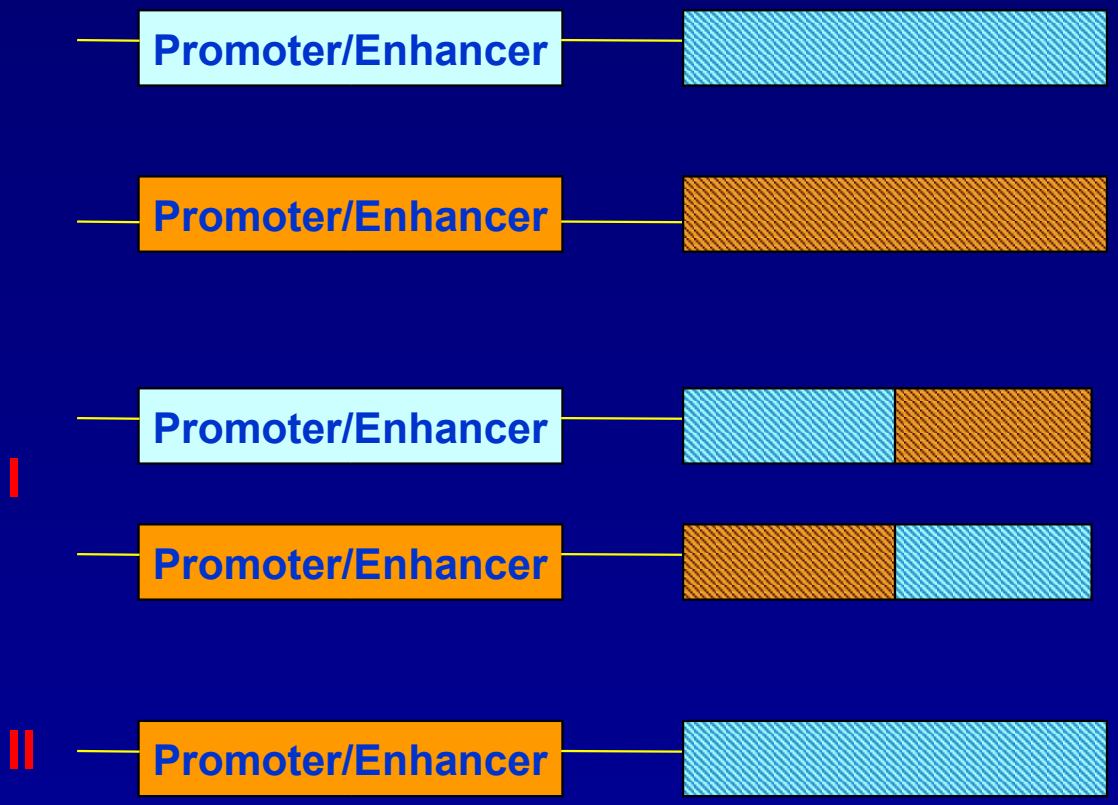
Rev Med Hosp Gen Mex. 2016;79:107–13

LEUCEMIE ACUTE: ALTERAZIONI GENETICHE

TRASLOCAZIONI CROMOSOMICHE



WHO classification (2016)

Acute myeloid leukaemia and related precursor neoplasms

- Acute myeloid leukaemia with recurrent genetic abnormalities
  - Acute myeloid leukaemia with balanced translocations/inversions
    - 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
    - Acute promyelocytic leukaemia with PML-RARA
    - AML with t(9;11)(p21.3;q23.3); KMT2A-MLLT3
    - 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;q13.1); RBM15-MKL1
    - AML with BCR-ABL1
  - AML with gene mutations
    - AML with mutated NPM1
    - AML with biallelic mutation of CEBPA
    - AML with mutated RUNX1

WHO classification (2016)

Acute myeloid leukaemia and related precursor neoplasms

- Acute myeloid leukaemia with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- Acute myeloid leukaemia, not otherwise specified
  - Acute myeloid leukaemia with minimal differentiation
  - Acute myeloid leukaemia without maturation
  - Acute myeloid leukaemia with maturation
  - Acute myelomonocytic leukaemia
  - Acute monoblastic and monocytic leukaemia
  - Pure erythroid leukaemia
  - Acute megakaryoblastic leukaemia
  - Acute basophilic leukaemia
  - Acute panmyelosis with myelofibrosis
- Myeloid sarcoma
- Myeloid proliferations associated with Down syndrome
  - Transient abnormal myelopoiesis associated with Down syndrome
  - Myeloid leukaemia associated with Down syndrome

WHO classification (2016)

Precursor lymphoid neoplasms

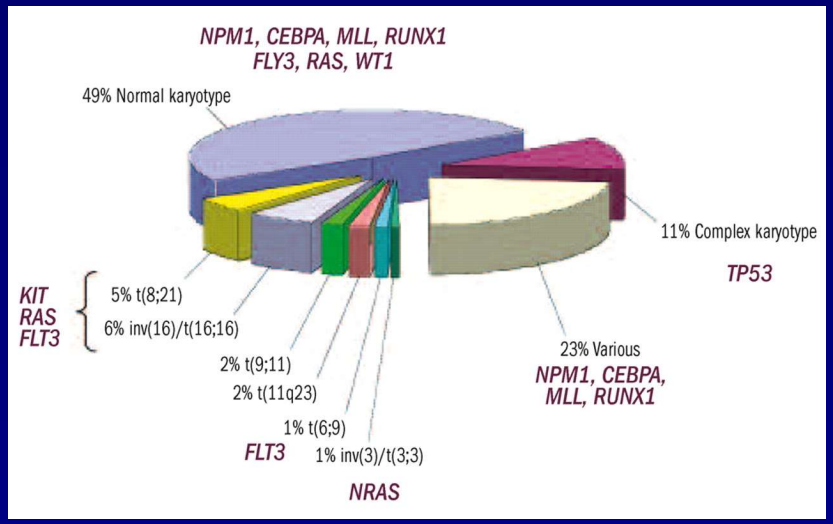
- B-lymphoblastic leukaemia/lymphoma, not otherwise specified
- B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities
  - B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34.1 ;q11.2); BCR-ABL1
  - B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3); KMT24-rearranged
  - B-lymphoblastic leukaemia/lymphoma with t(12;21)(p13.2;q22.1); ETV6-RUNX1
  - B-lymphoblastic leukaemia/lymphoma with hyperdiploidy
  - B-lymphoblastic leukaemia/lymphoma with hypodiploidy
  - B-lymphoblastic leukaemia/lymphoma with t(5;14)(q31.1;q32.1); IGH/IL3
  - B-lymphoblastic leukaemia/lymphoma with t(1 ;19)(q23;p13.3); TCF3-PBX1
  - B-lymphoblastic leukaemia/lymphoma, BCR-ABL1-like
  - B-lymphoblastic leukaemia/lymphoma with iAMP21
- T-lymphoblastic leukaemia/lymphoma
  - Early T-cell precursor lymphoblastic leukaemia
- NK-lymphoblastic leukaemia/lymphoma

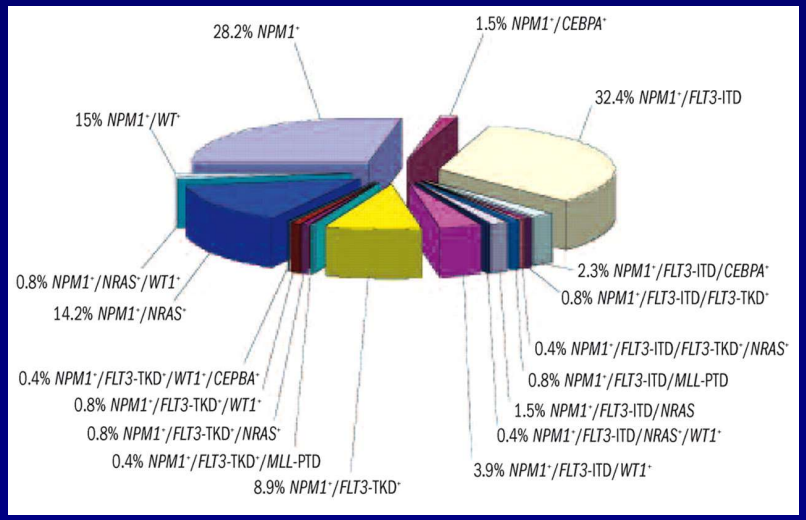
WHO classification (2016)

Acute leukaemias of ambiguous lineage

- Acute undifferentiated leukaemia
  - Mixed-phenotype AL with t(9;22)(q34.1;q11.2); BCR-ABL1
  - Mixed-phenotype AL with t(v;11q23.3); KMT2A-rearranged
  - Mixed-phenotype acute leukaemia, B/myeloid, NOS
  - Mixed-phenotype acute leukaemia, T/myeloid, NOS
  - Mixed-phenotype acute leukaemia, NOS, rare types
- Acute leukaemias of ambiguous lineage, NOS

Cytogenetics subgroups and genomic alterations - AML



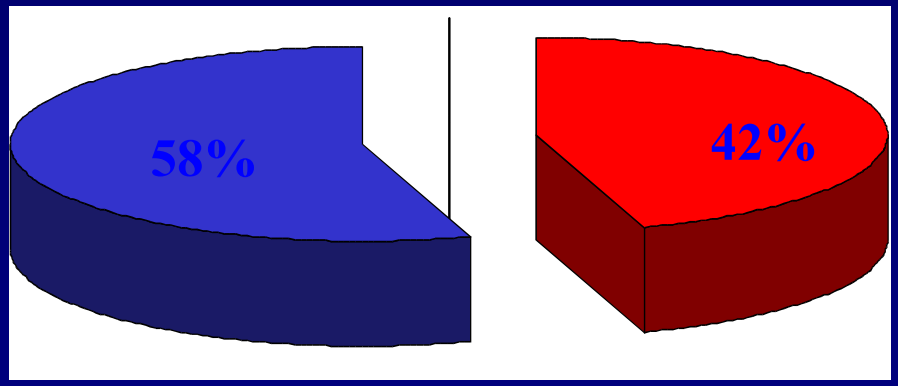


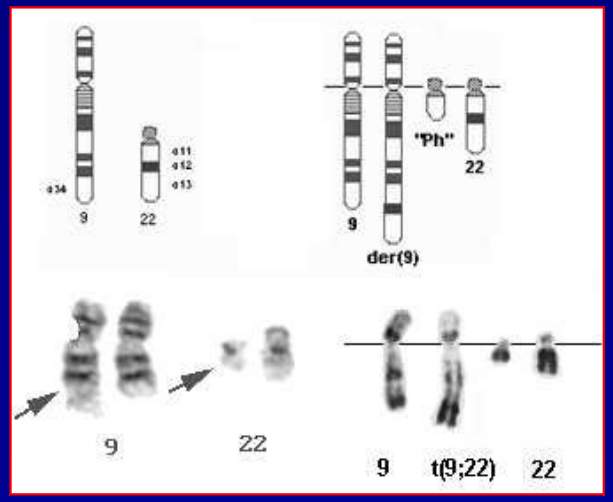
Dohner, Haematologica 2008

LAM - Citogenetica

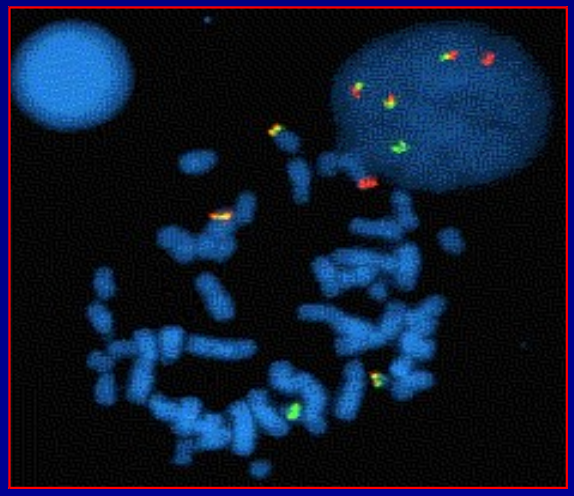
1612 casi LAM diagnosi

Grimwade, Blood 1998

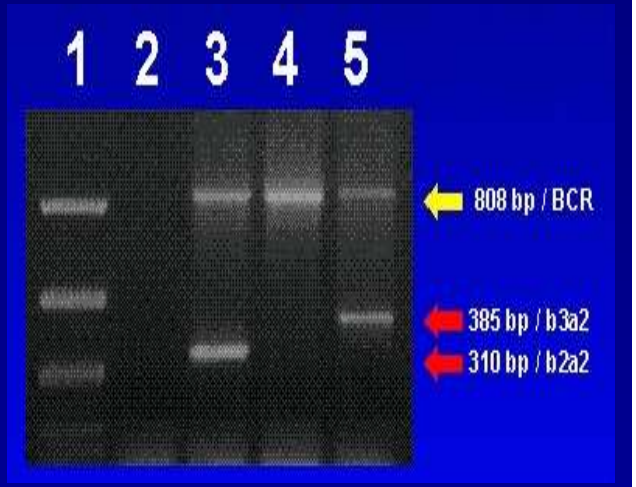




Citogenetica Convenzionale



Citogenetica Molecolare (FISH)

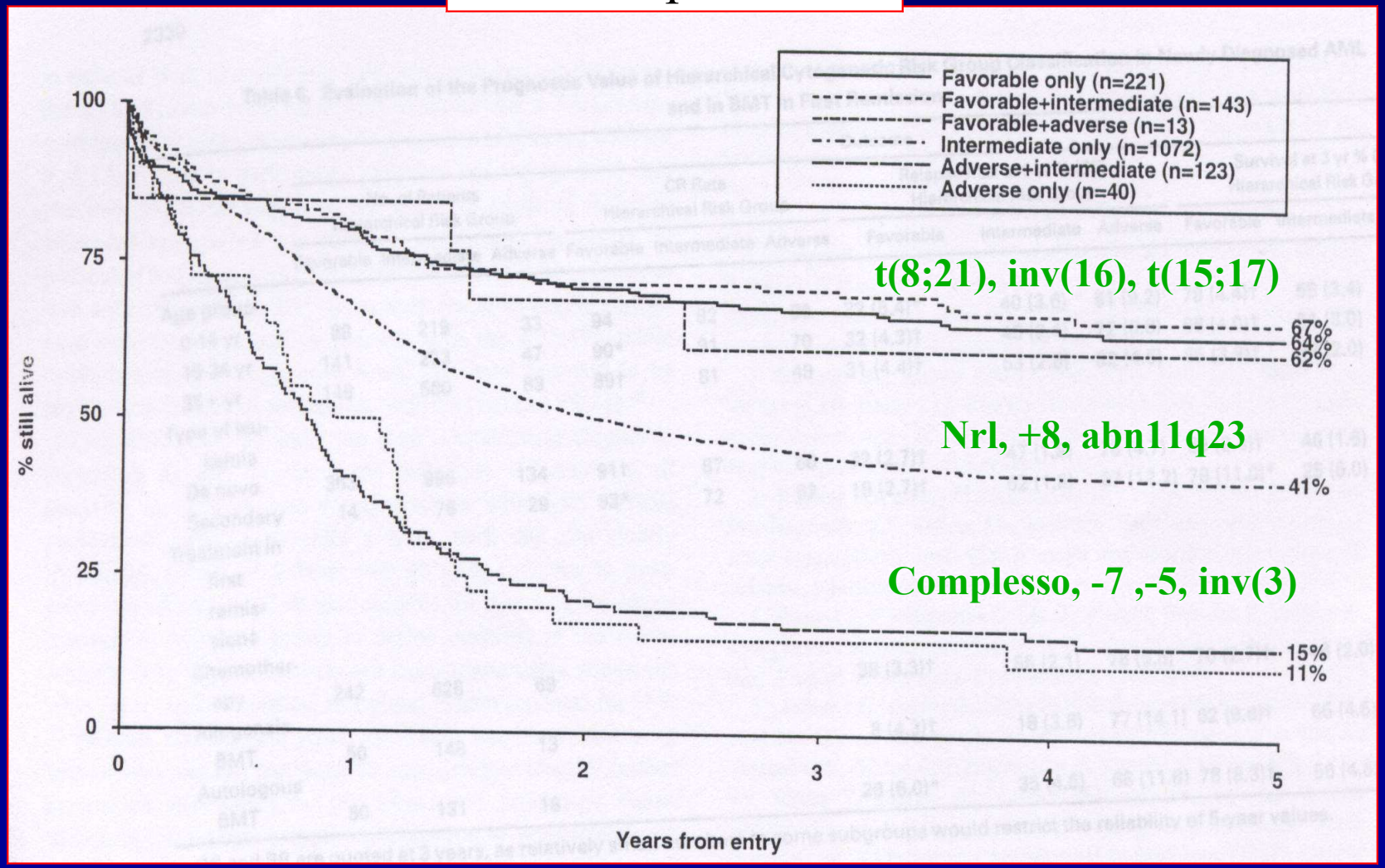


Biologia Molecolare

I parametri biologici e la prognosi delle LA

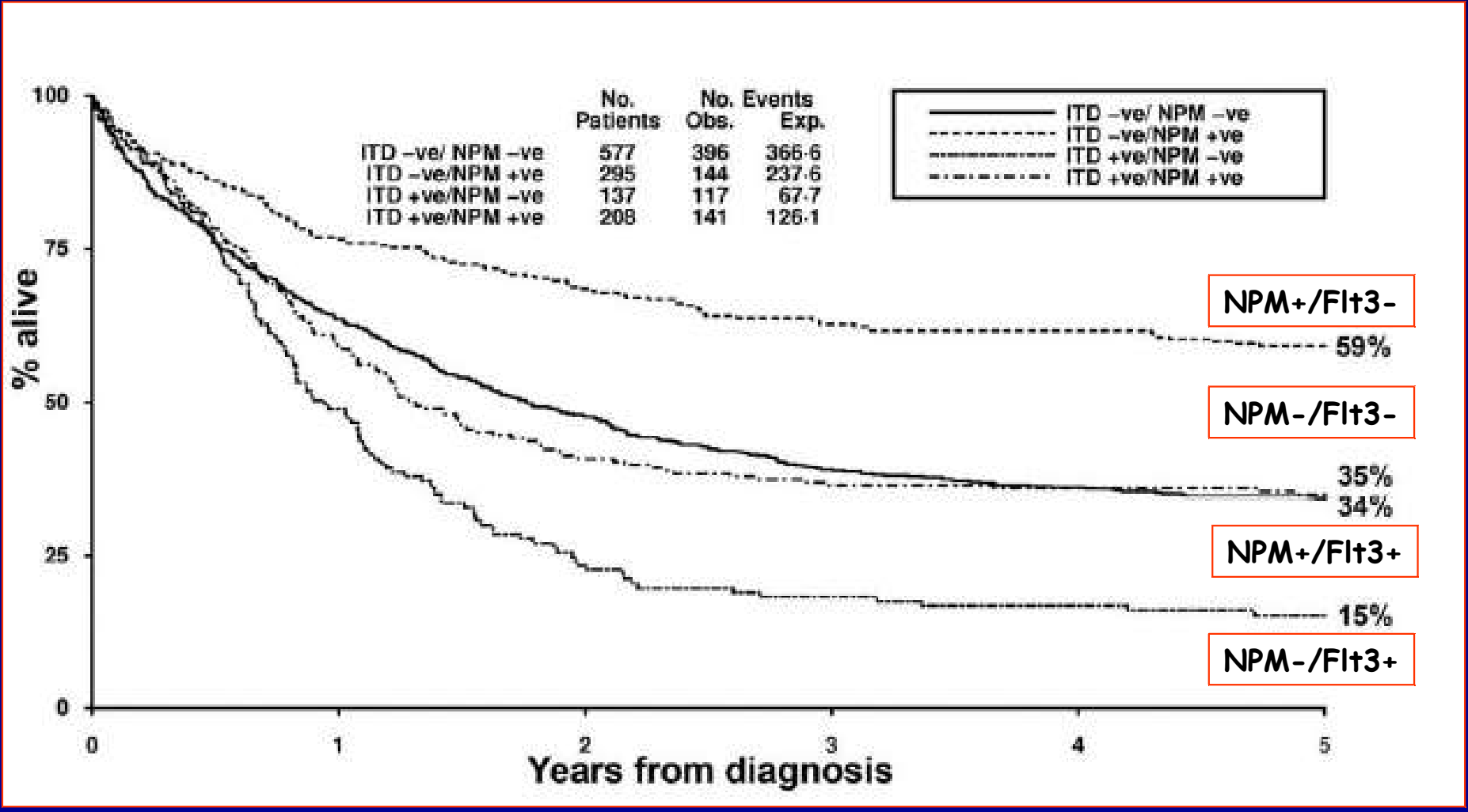
OS 1612 pz < 55 aa

Grimwade et al. Blood 1998



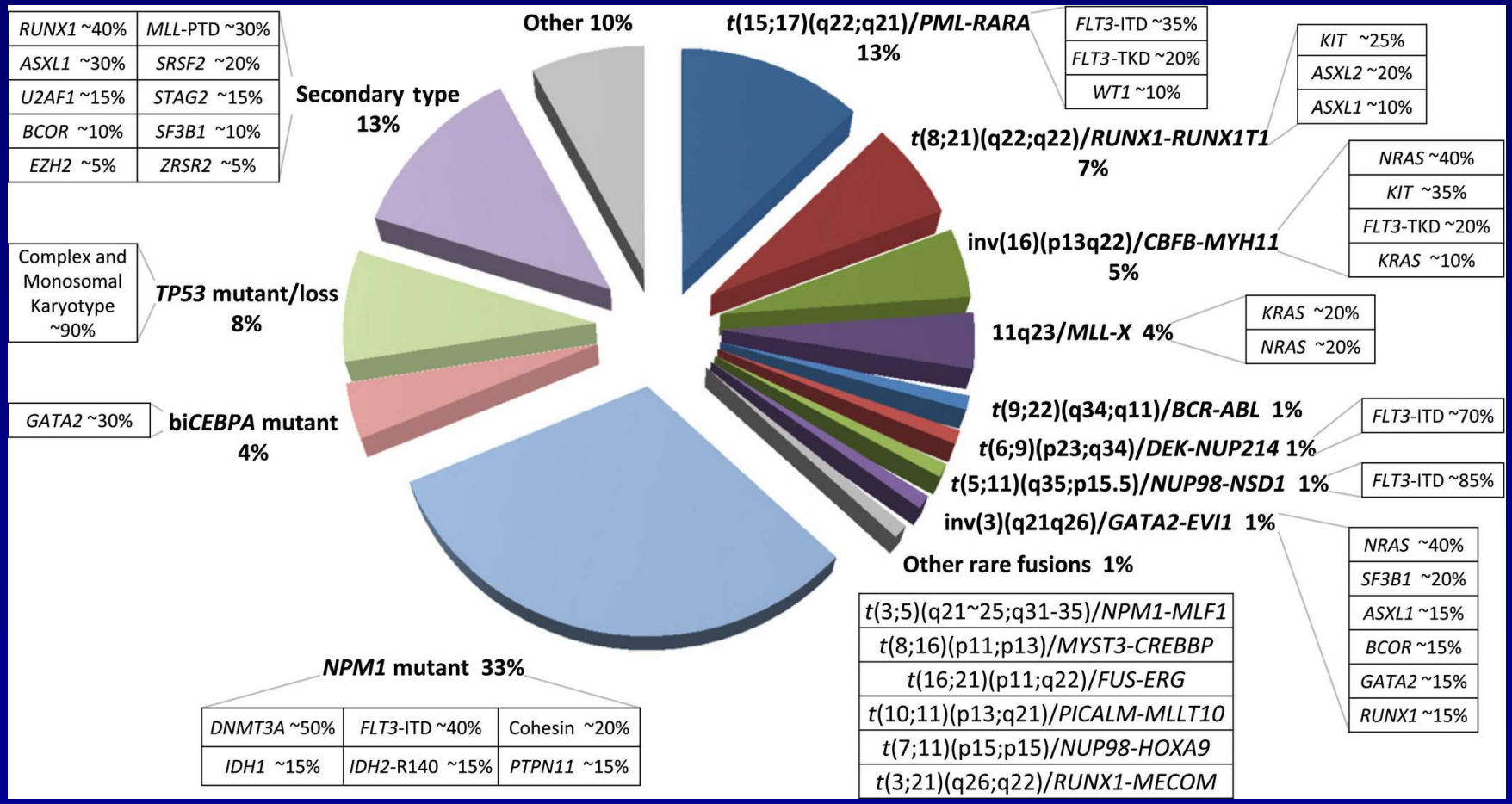
Cy-NPM e Flt3-ITD (3)

OS



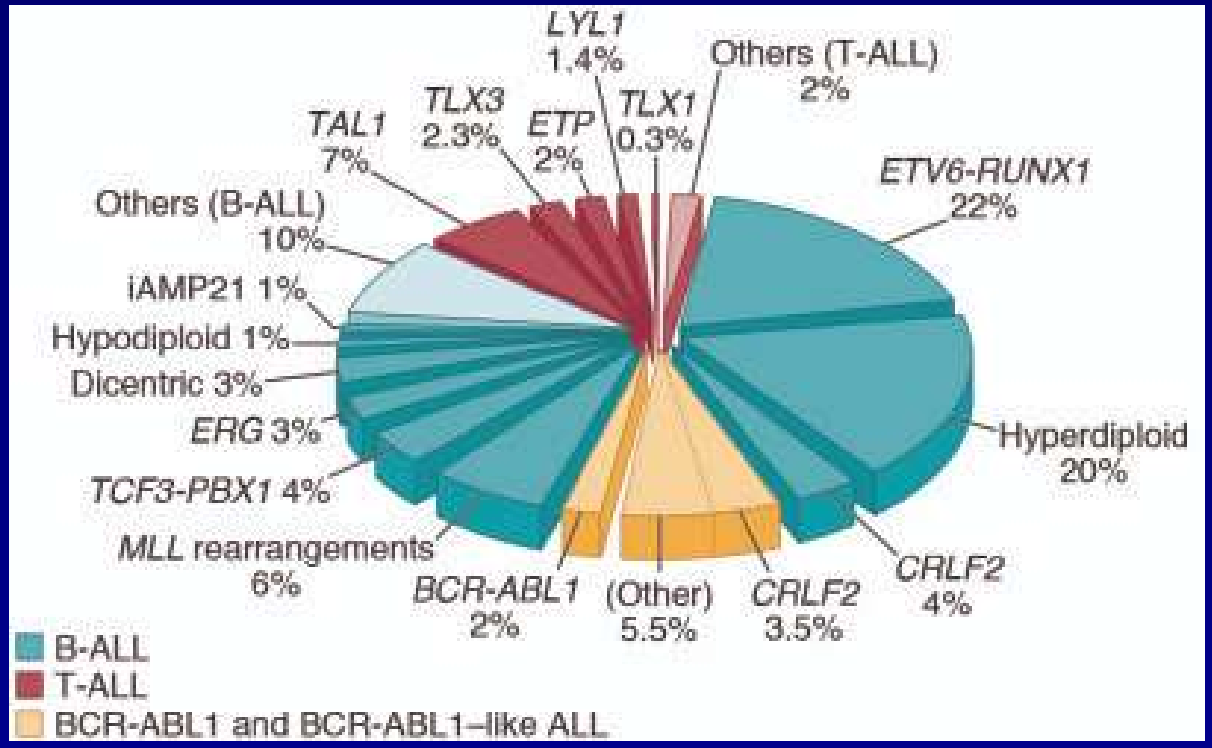
Gale R, Blood 2008 (MRC)

Genomic alterations by NGS - AML

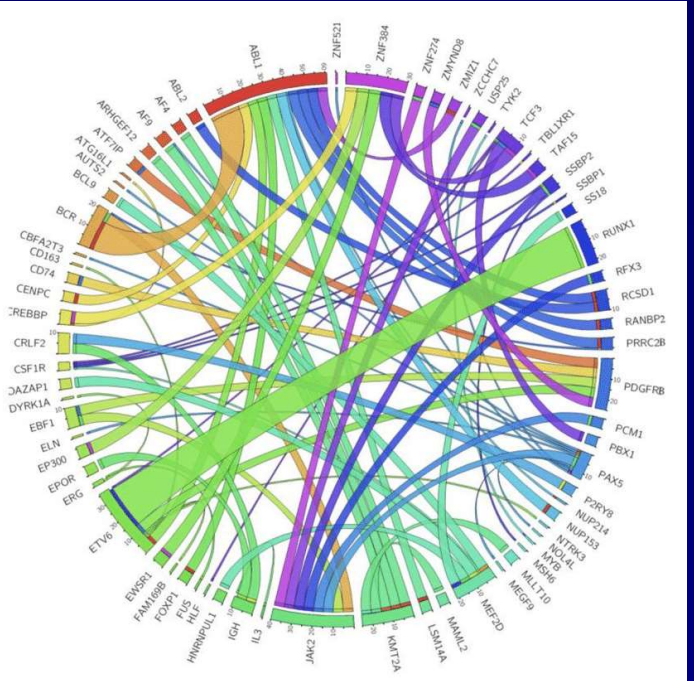


Medinger, BJH 2017

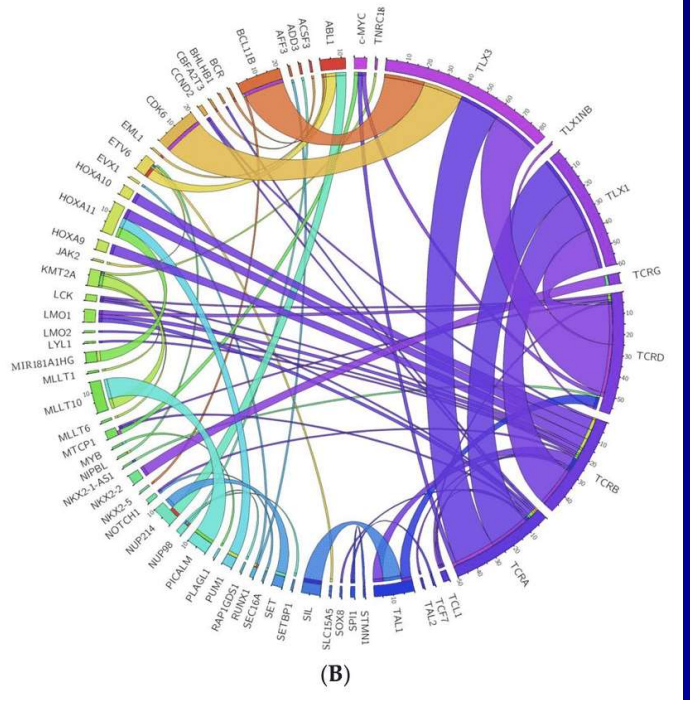
Genomic alterations - ALL



Mullighan, JCI 2012



(A)



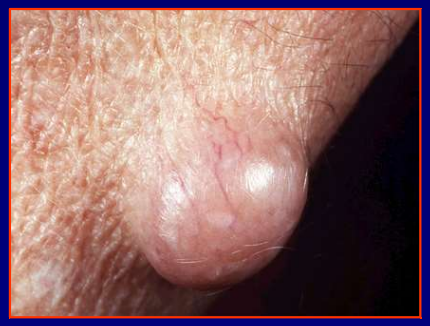
(B)

Genomic alterations by NGS - ALL

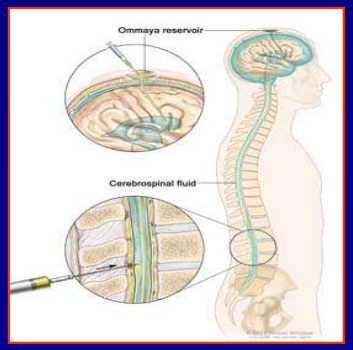
Schema della terapia delle leucemie acute e della sua azione sulla proliferazione cellulare leucemica

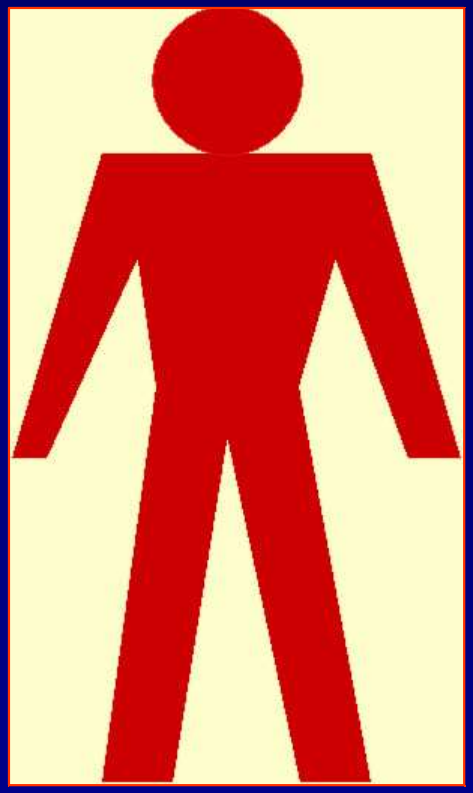
I "vecchi" (Storici) fattori prognostici clinici

Localizzazioni cutanee

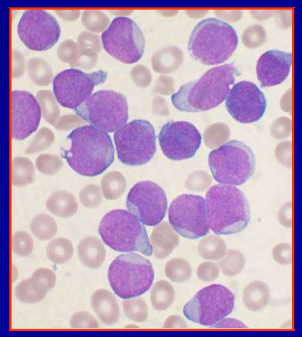


Localizzazioni SNC

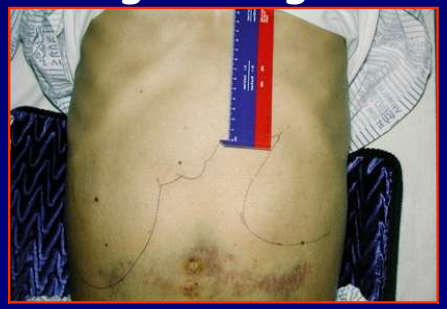




Leucocitosi



Organomegalia



- Età
- Chemioresistenza
- LAMs
- FAB M0, M6, M7
- LAM bifenotipiche
- ↑ LDH

LEUCEMIE ACUTE TERAPIA : DI SUPPORTO

INFEZIONI

- Igiene
- Isolamento
- Profilassi anti- batterica e micotica
- Terapia antibiotica ed antimicotica
- Fattori di crescita

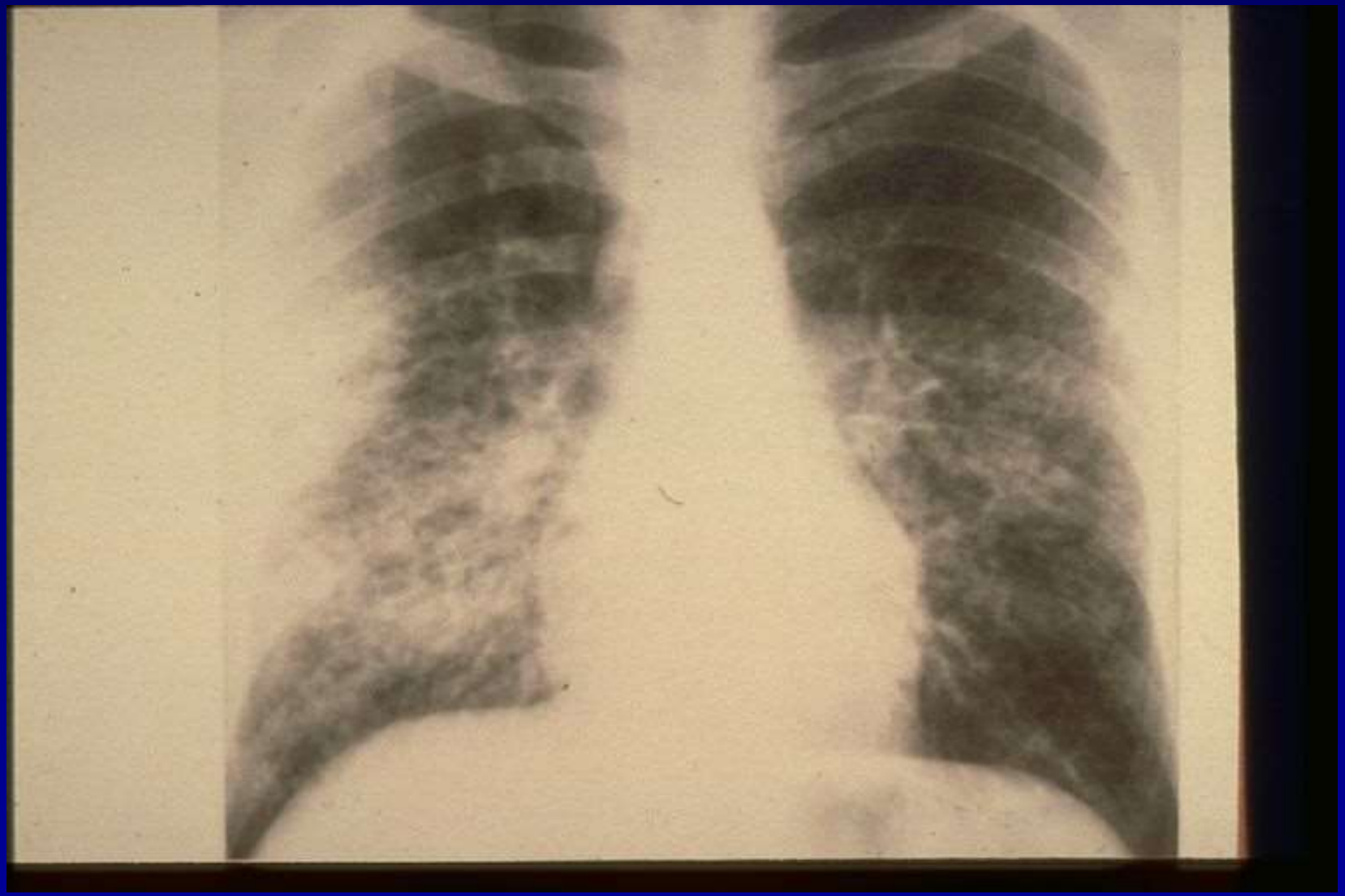
LEUCEMIE ACUTE TERAPIA : DI SUPPORTO

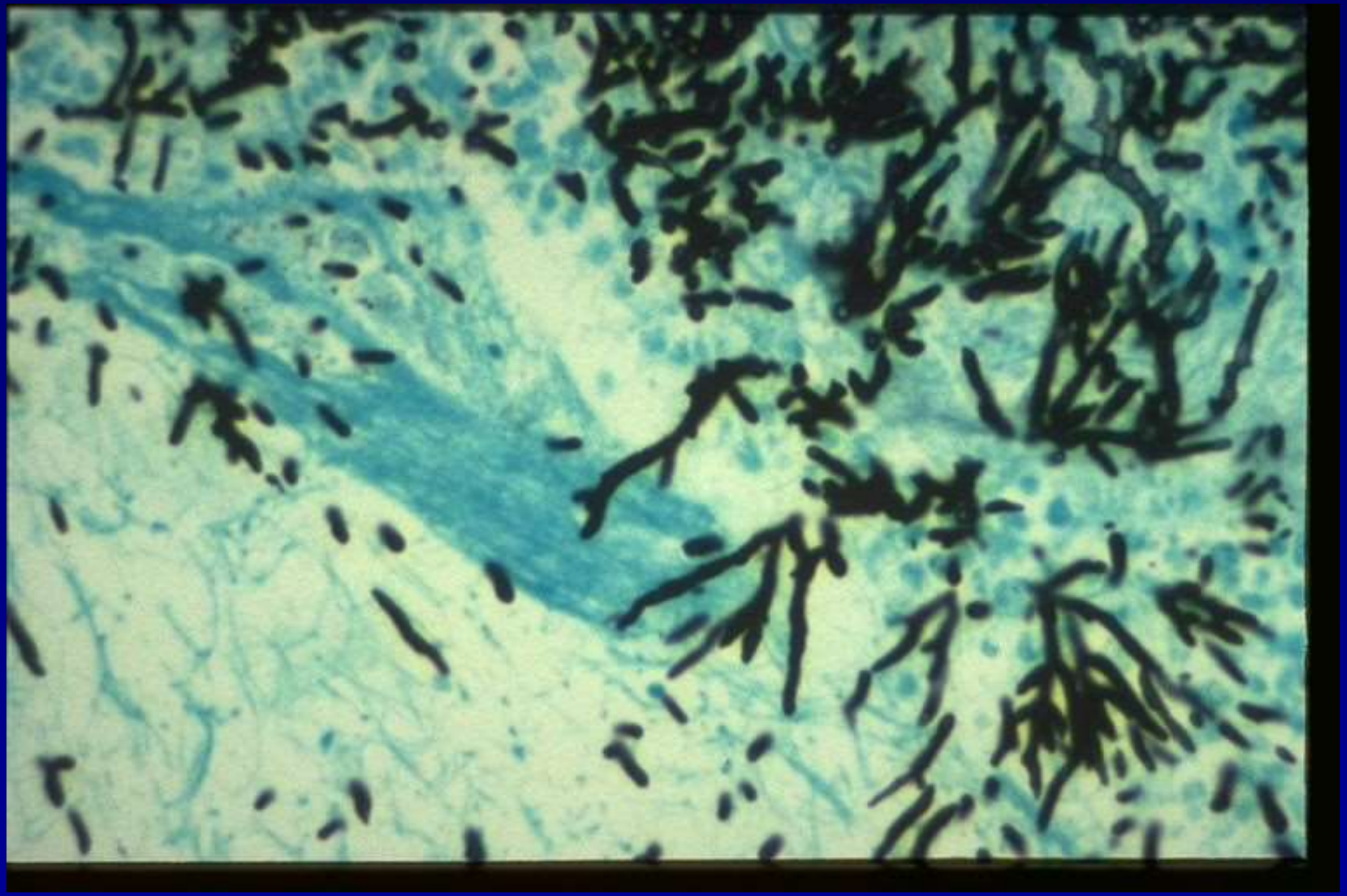
ANEMIA:

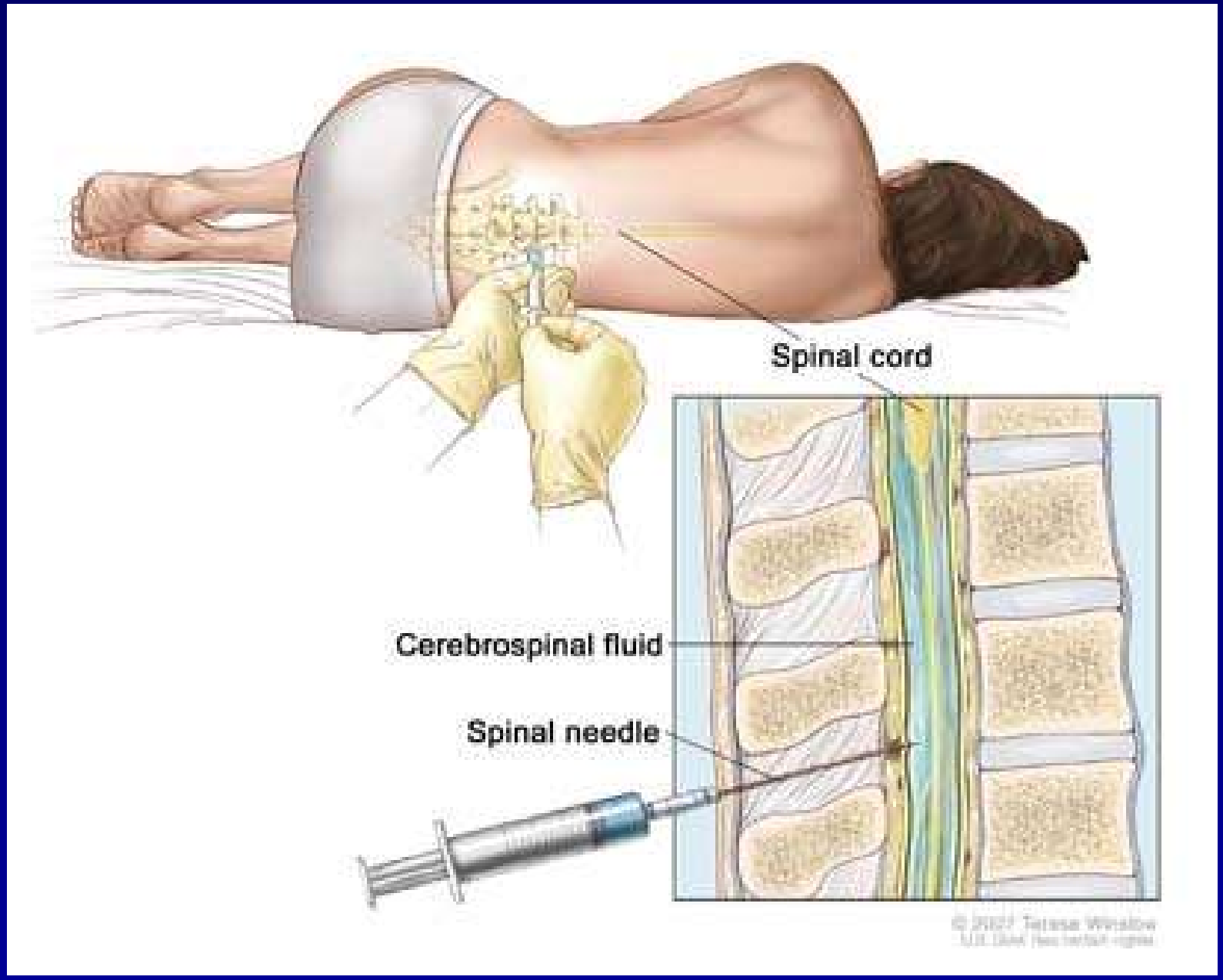
TRASFUSIONE DI UNITA' DI GLOBULI ROSSI CONCENTRATI.
Hb >9g/dl

PIASTRINOPENIA:

TRASFUSIONE DI PIASTRINE. PLT>20.000/mmc (generalmente 2-3 aferesi settimana)






Spinal cord
Cerebrospinal fluid
Spinal needle

LEUCEMIE ACUTE : I RISULTATI

LAM

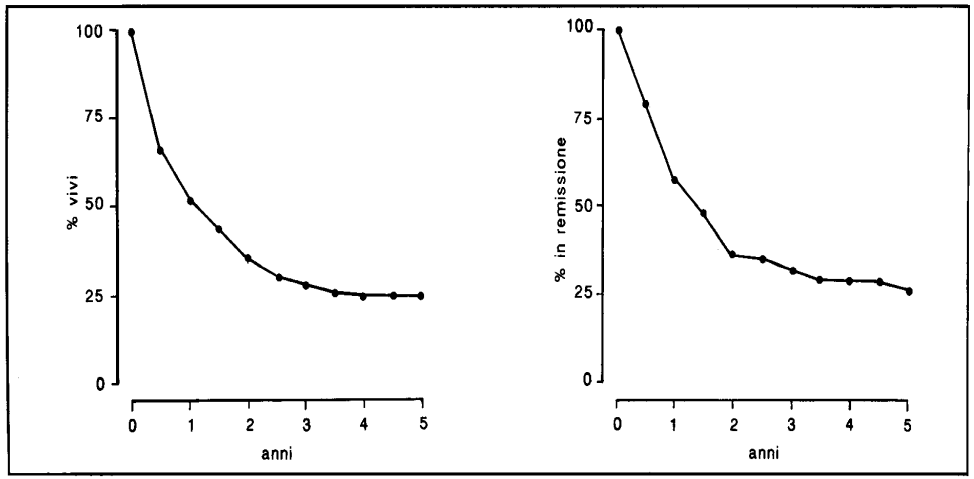
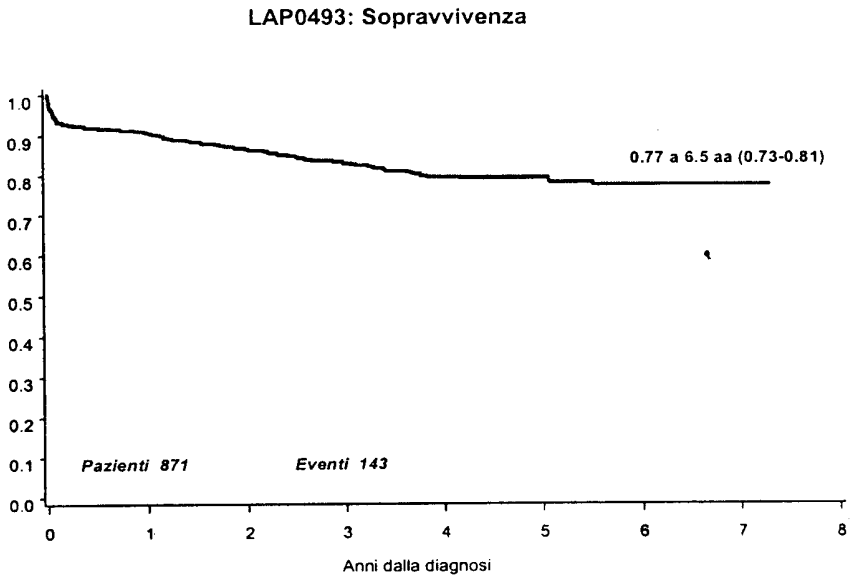
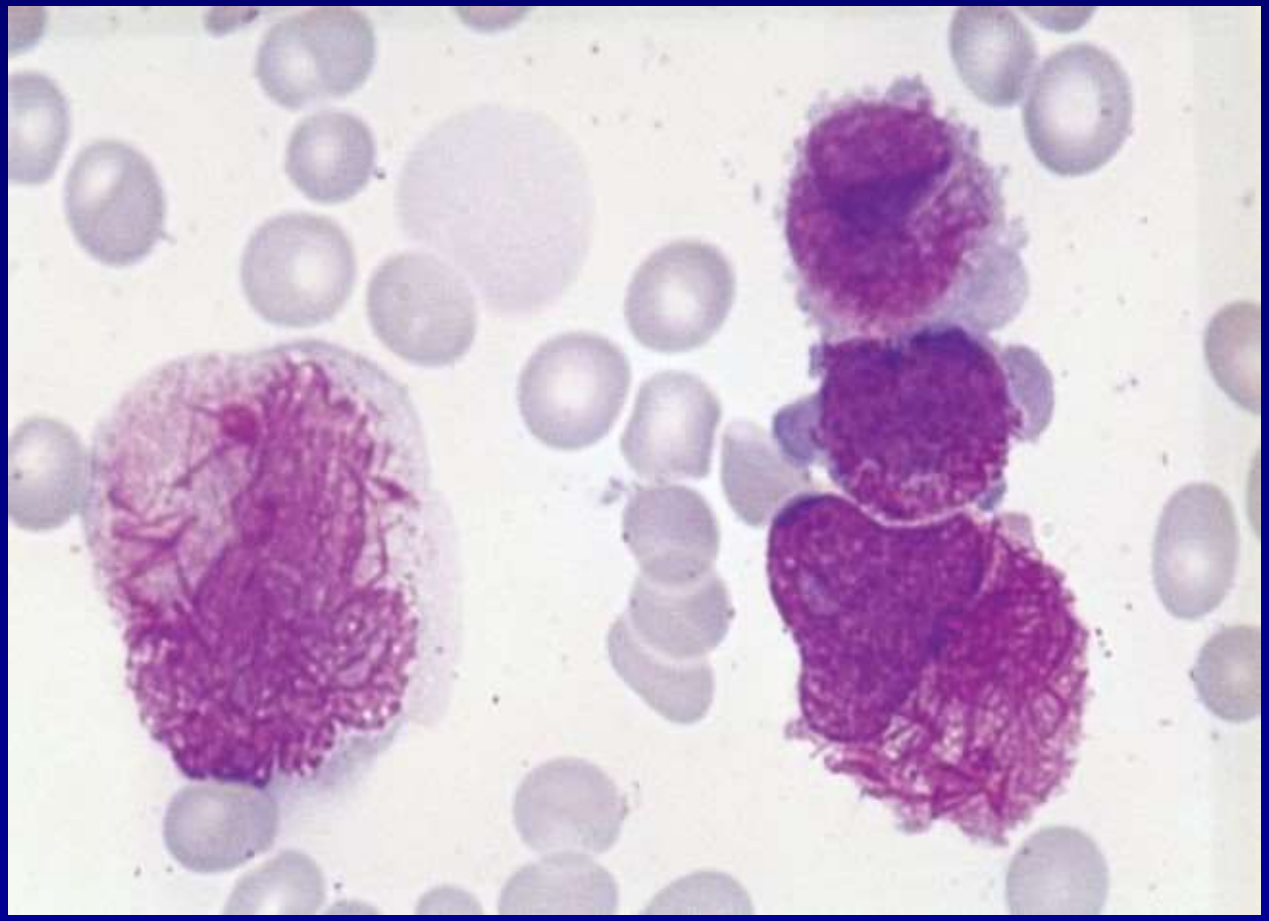


Figura 3.9 - Curve attuariali di durata della vita (a sinistra) e di durata della prima remissione (a destra) di 466 casi di LAM. Le due curve sono simili e poi diventano praticamente identiche perché i pazienti che restano in remissione possono diventare lunghi sopravviventi. Dopo 5 anni le ricadute sono ancora possibili, ma rare.

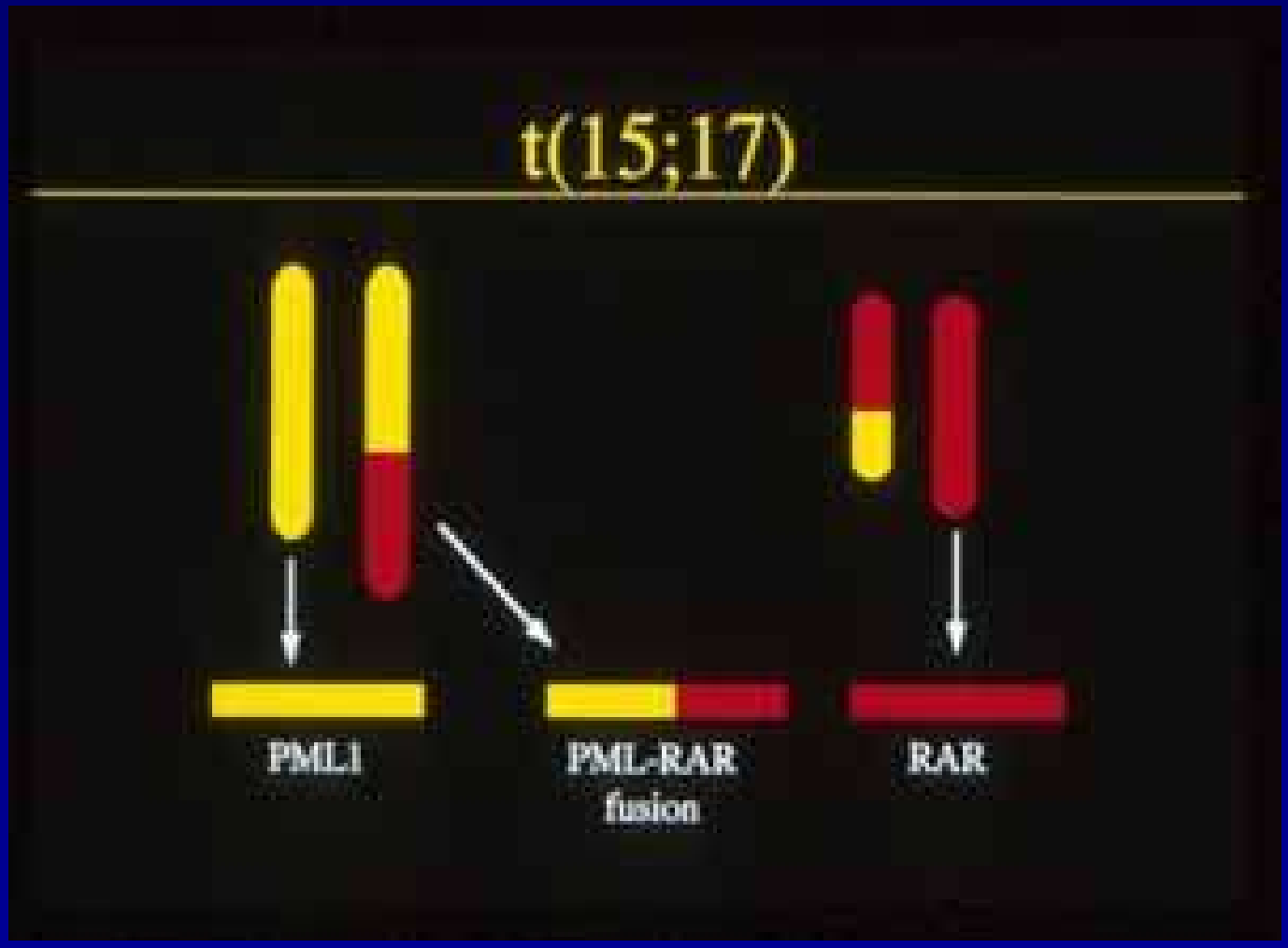
LAM-M3

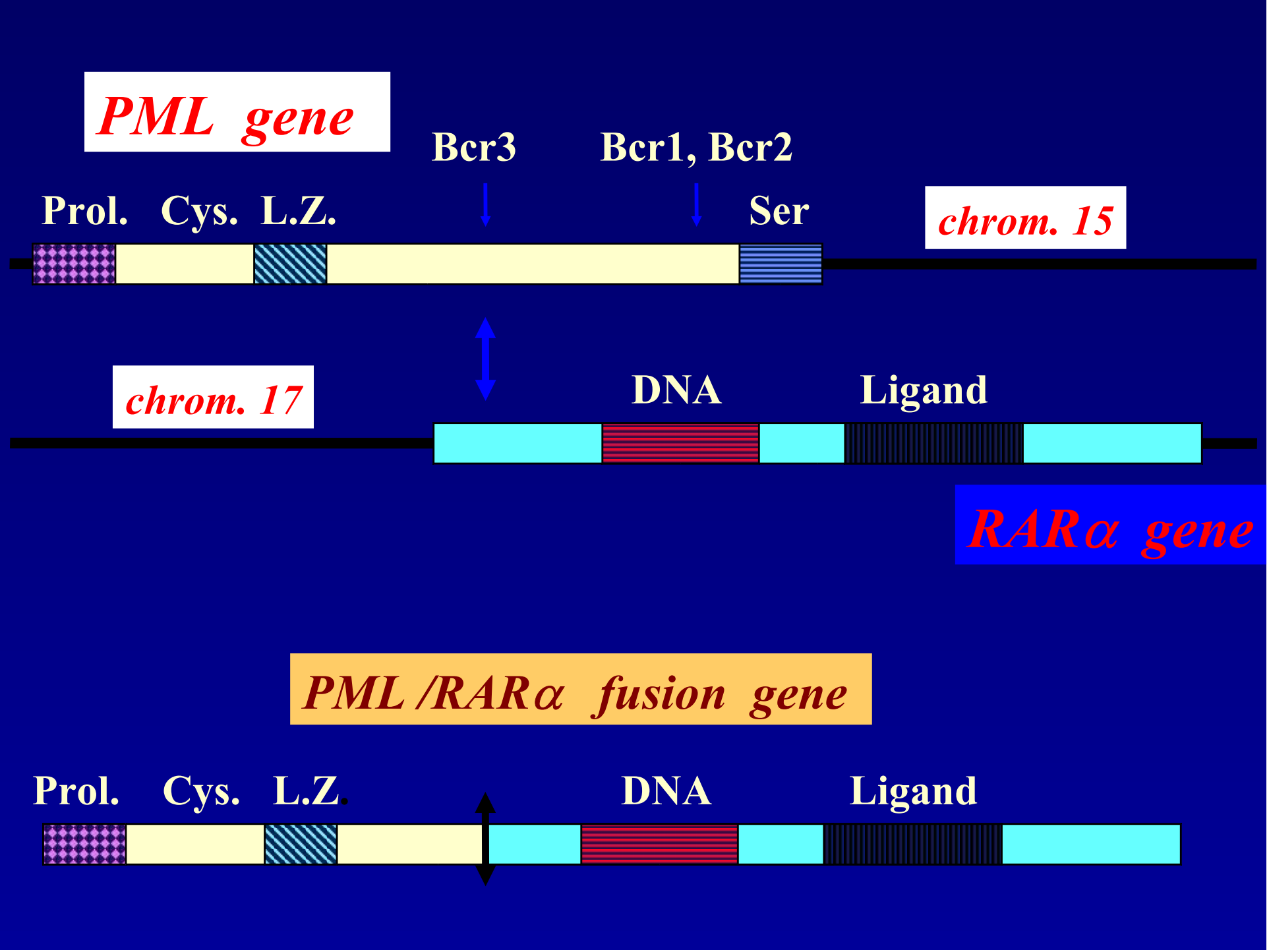


LAM M3

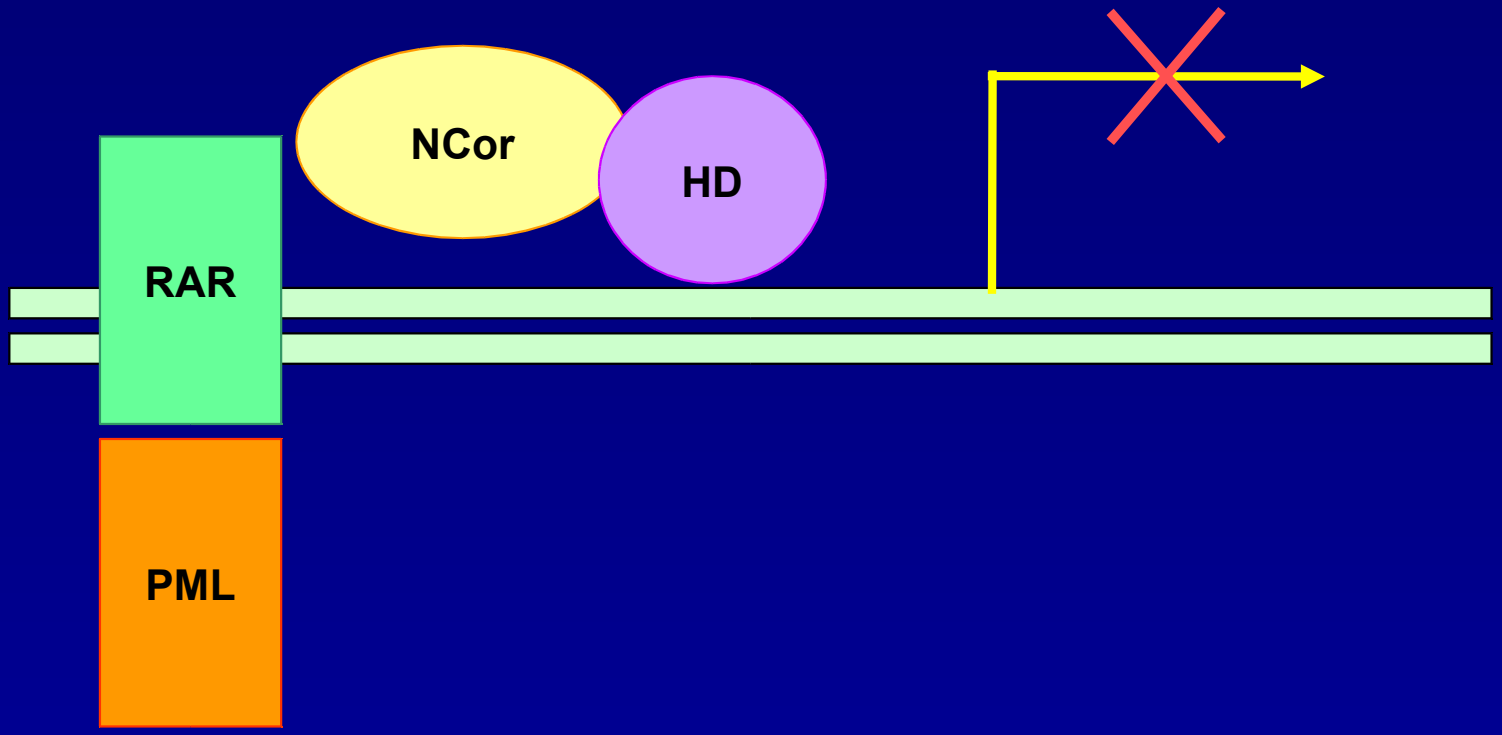


Leucemia acuta a promielociti

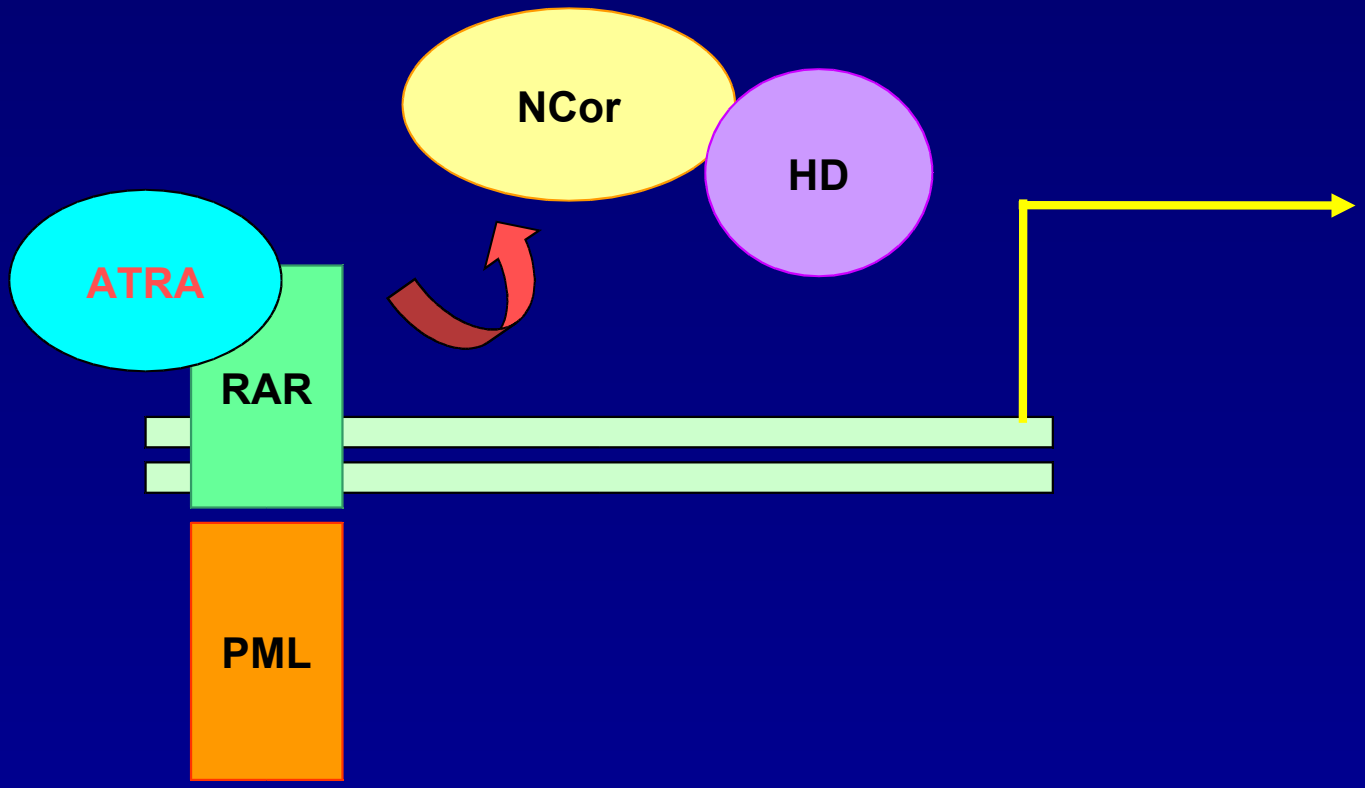


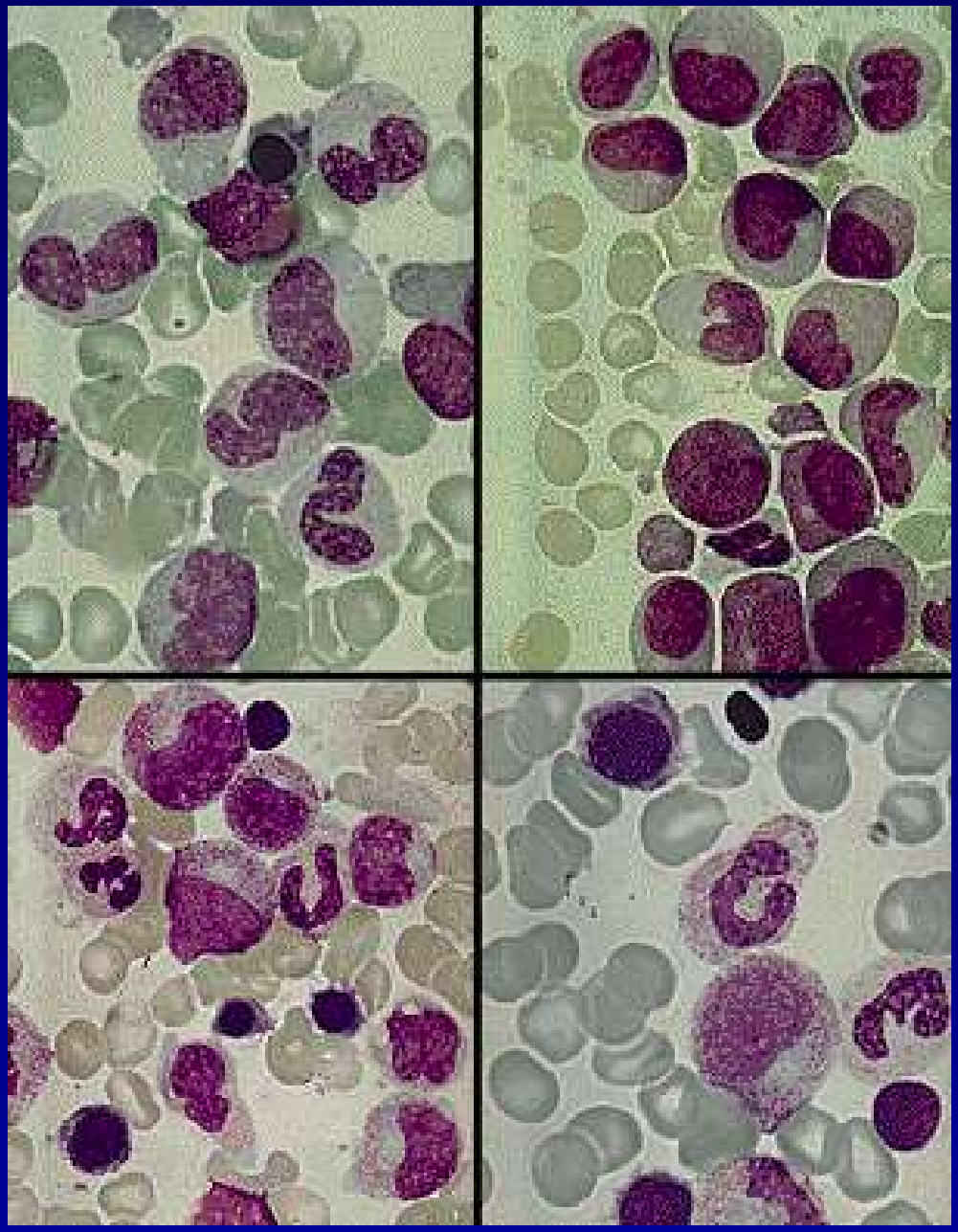

PML gene
Bcr3
Bcr1, Bcr2
Prol.
Cys.
L.Z.
Ser
chrom. 15
chrom. 17
DNA
Ligand
RARα gene
PML /RARα fusion gene
Prol.
Cys.
L.Z.
DNA
Ligand

PML-RARα



PML-RARα





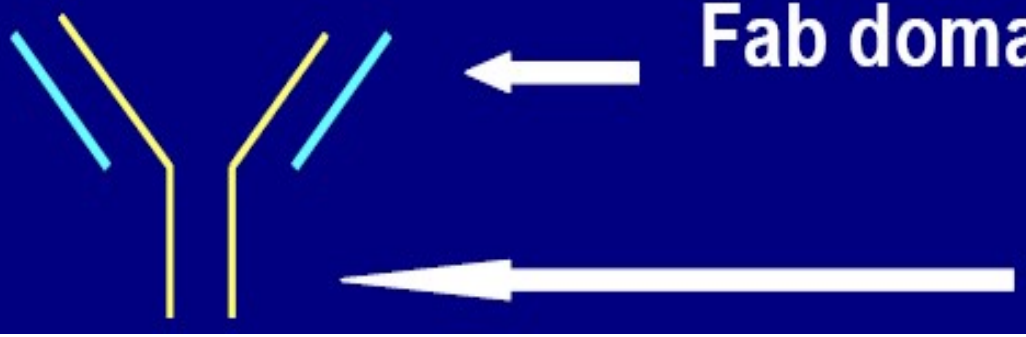
AML- M3
ATRA- differentiation

ANTICORPI

MONOCLONALI

Monoclonal Antibodies: The Basics

- AB are immunoglobulins produced by the B lymphocytes in response to antigenic stimulation
- AB consist of :
  - 2 light chains + 2 heavy chains
  - Fab domain - antigen binding site
  - Fc domain - determines effector function of AB



Elementi da considerare per la costruzione di un Anticorpo Monoclonale

1. Scelta dell'antigene bersaglio
2. Immunogenicità dell'anticorpo
3. Durata della vita media di un anticorpo
4. Interazione tra anticorpo e funzioni effettrici del sistema immunitario del paziente
5. Coniugazione con tossine o radionuclidi

Monoclonal Antibody Therapy: Options

Murine	Chimeric	Humanized
100% foreign glycoprotien	Human: constant Murine: variable	Murine hypervariable regions are spliced into human/primate framework
antigenicity	⇓ antigenicity ⇑ ADCC	⇓ antigenicity

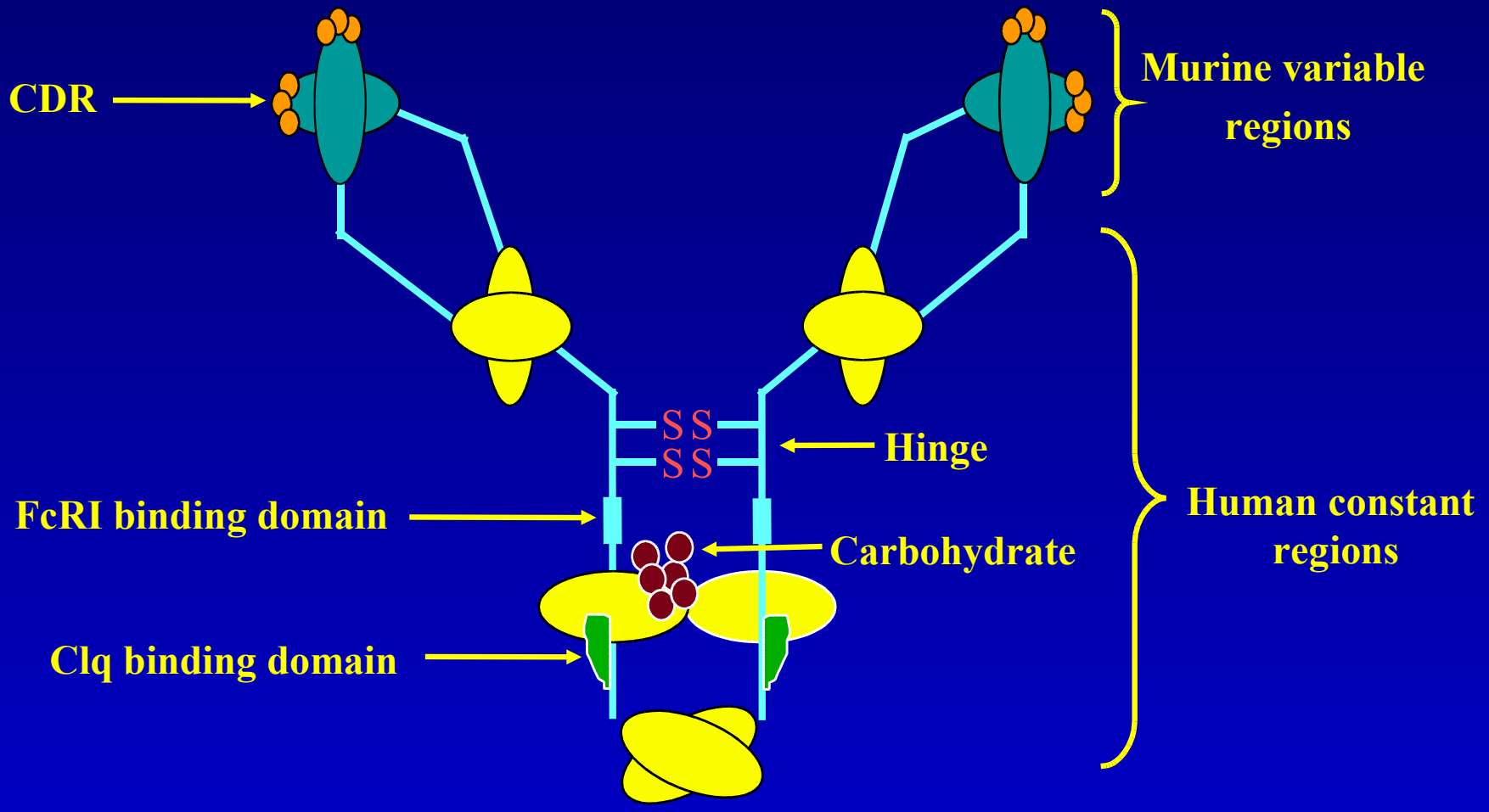
Monoclonal Antibodies: Development and Production

MoAb	Type
Alemtuzumab	humanized IgG_1/κ
Bevacizumab	humanized IgG_1/κ
Cetuximab	chimeric IgG_1/κ
Gemtuzumab ozogamicin	humanized IgG_1/κ-toxin conjugate
Ibritumomab tiuxetan	mouse IgG_1/κ-chelator conjugate
Rituximab	chimeric IgG_1/κ
Tositumomab	mouse IgG_{2a}/λ and ^{131}I-labeled IgG_{2a}/λ
Trastuzumab	humanized IgG_1/κ

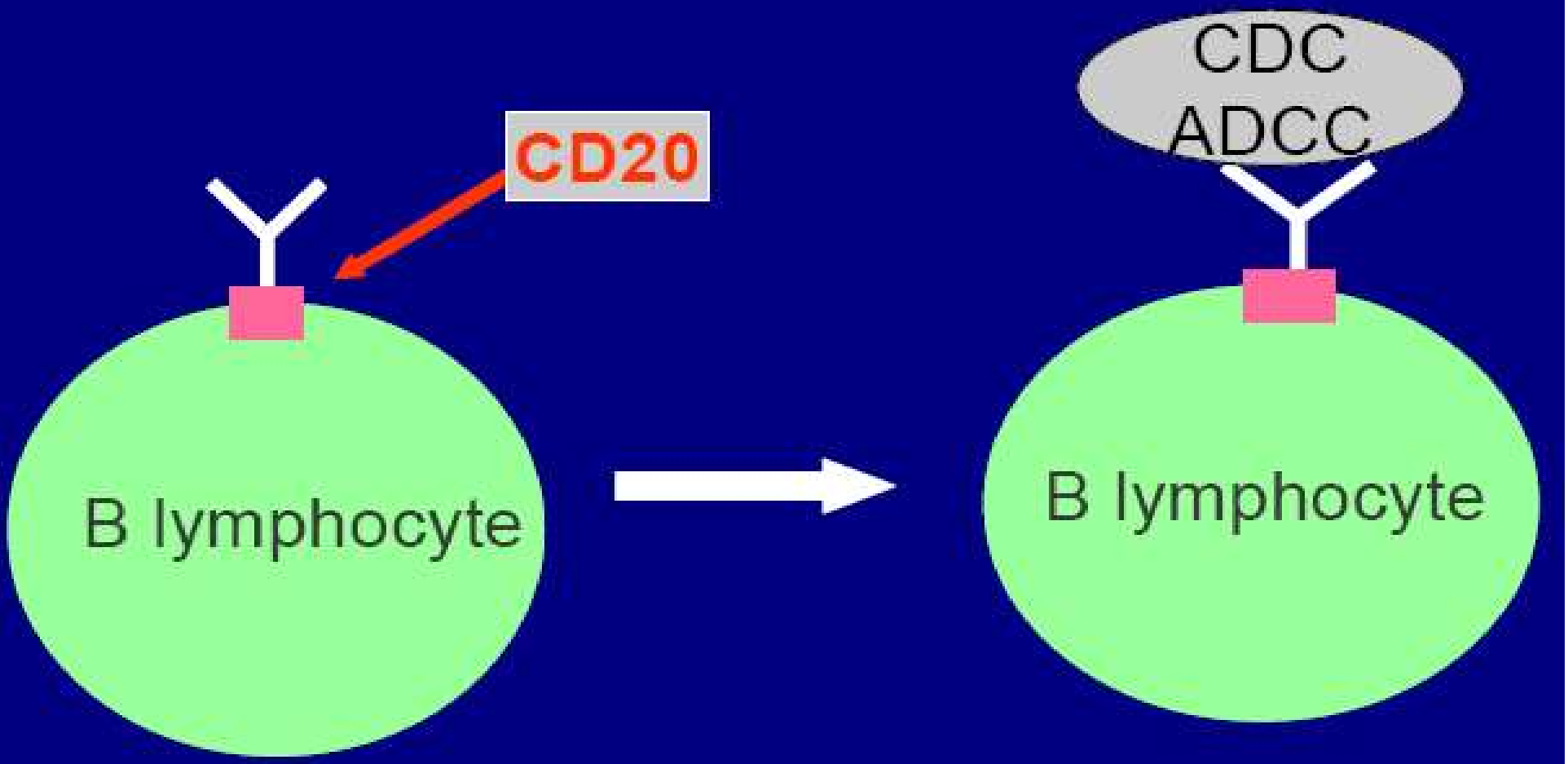
Monoclonal Antibodies: Potential Advantage as Cancer Therapy

- high specificity for tumor antigens
- low-cross sensitivity with normal cells
- design flexibility
- customized effector functions
- specific targeting of toxic therapy

Rituximab


CDR
Murine variable regions
S S
S S
Hinge
FcRI binding domain
Carbohydrate
Human constant regions
Clq binding domain

Rituximab: Mechanism of Action



CD20 Antigen: A Target for Monoclonal Antibody Therapy

CD20 is:

- a membrane-imbedded protein
- restricted to B-cell precursors and mature B cells
- not found on stem cells or committed progenitor cells
- lost when B cells differentiate to form plasma cells
- expressed > 90% of B-cell NHL

Antigen Expression in B-Cell Lineage

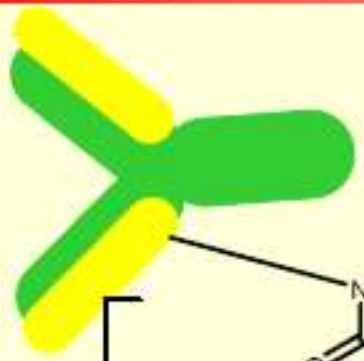
Antibody-Drug Conjugates (ADCs)

ADC are MAbs linked to cell-killing drugs
High affinity and selectivity of Ab to tumor cells

MAbs enter or are internalized within cells
Cell factors or environment cause the drug to be released from the MAb
Released drugs kills the cell

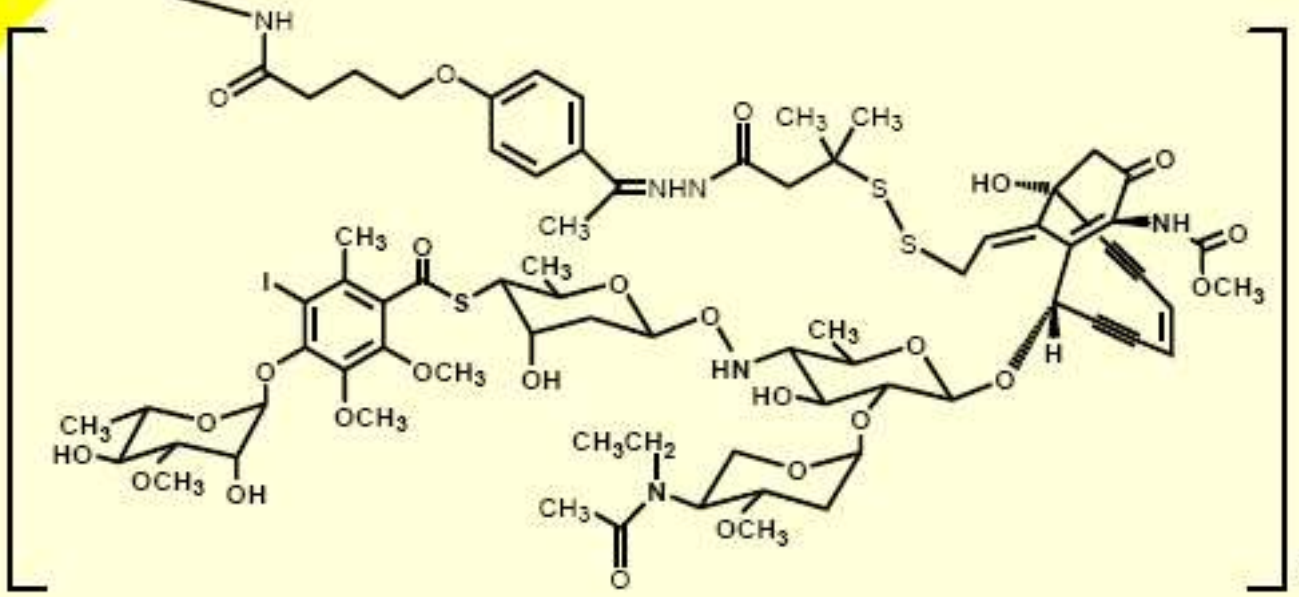
Very specific cell killing. Much different than chemotherapy where all cells are exposed to drug.

Mylotarg™
Gemtuzumab Ozogamicin



Anti-CD33

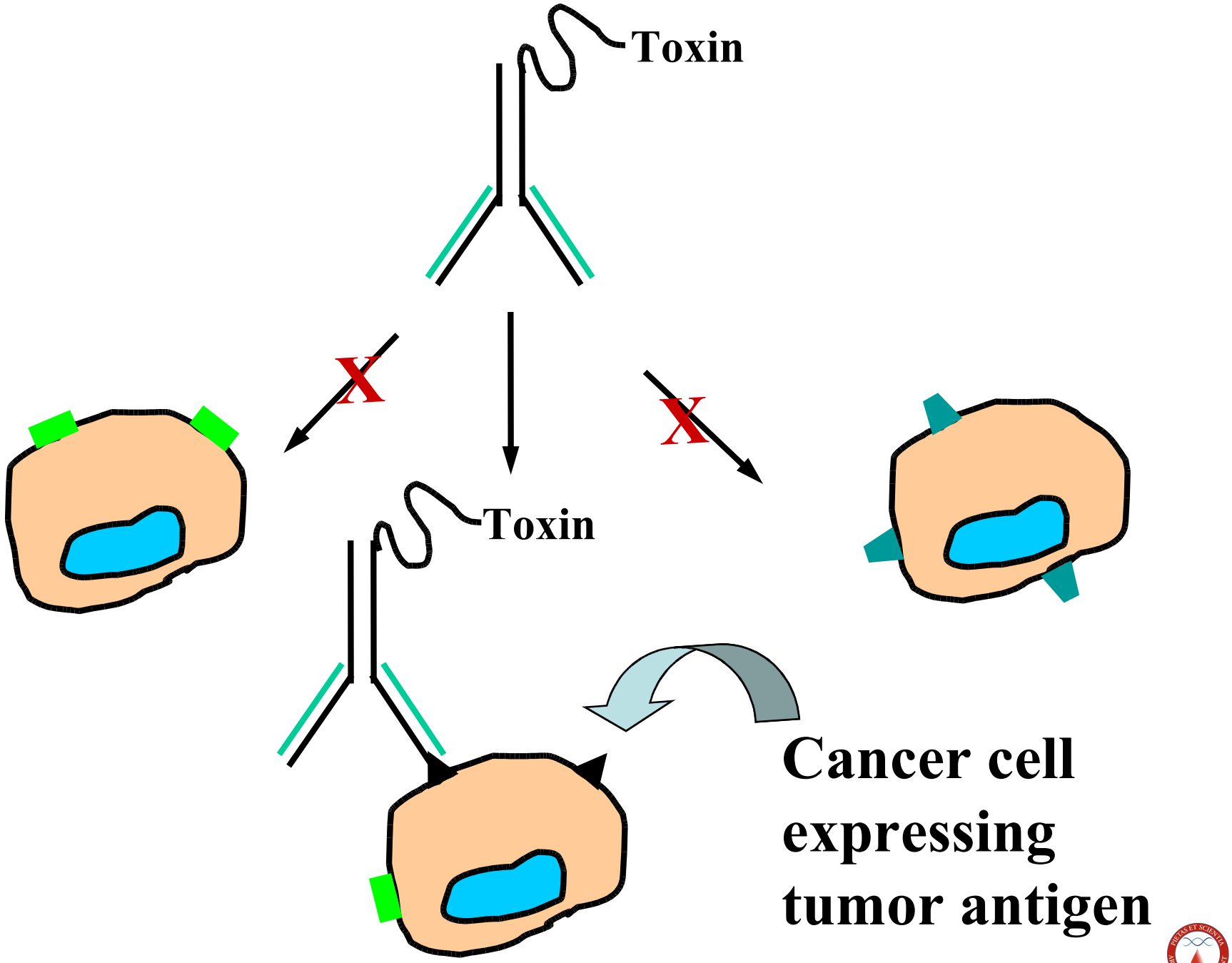
Acute myeloid leukemia



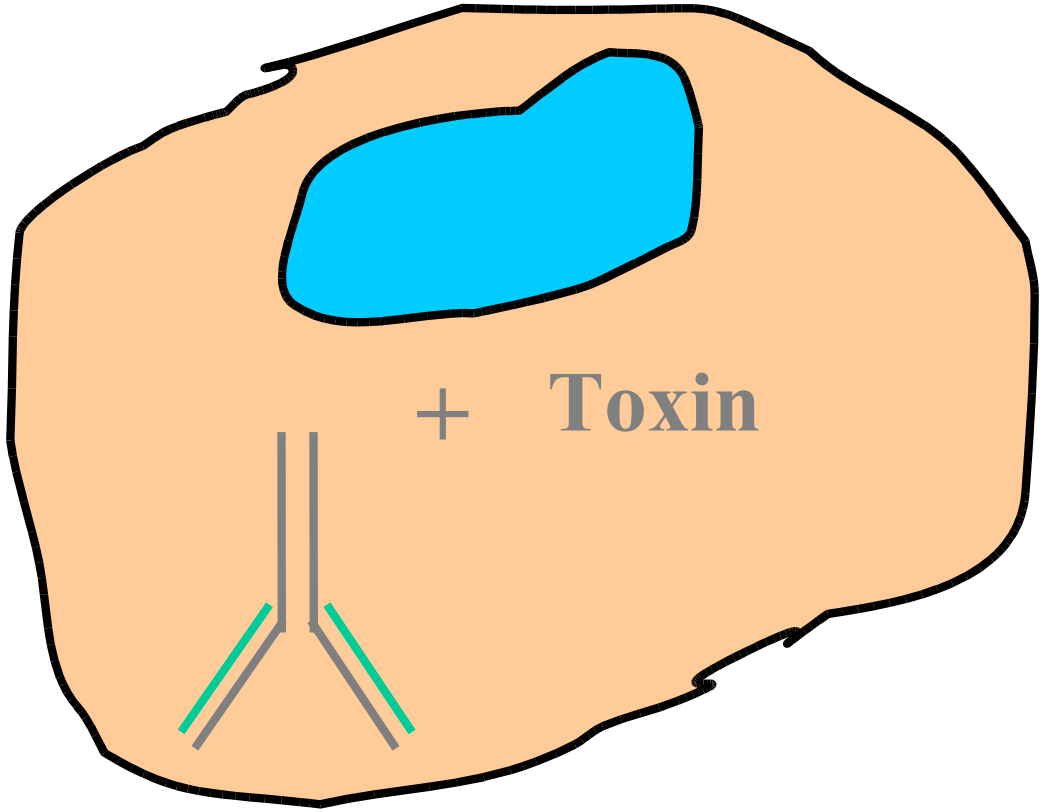
average n = 2 to 3

Introduction

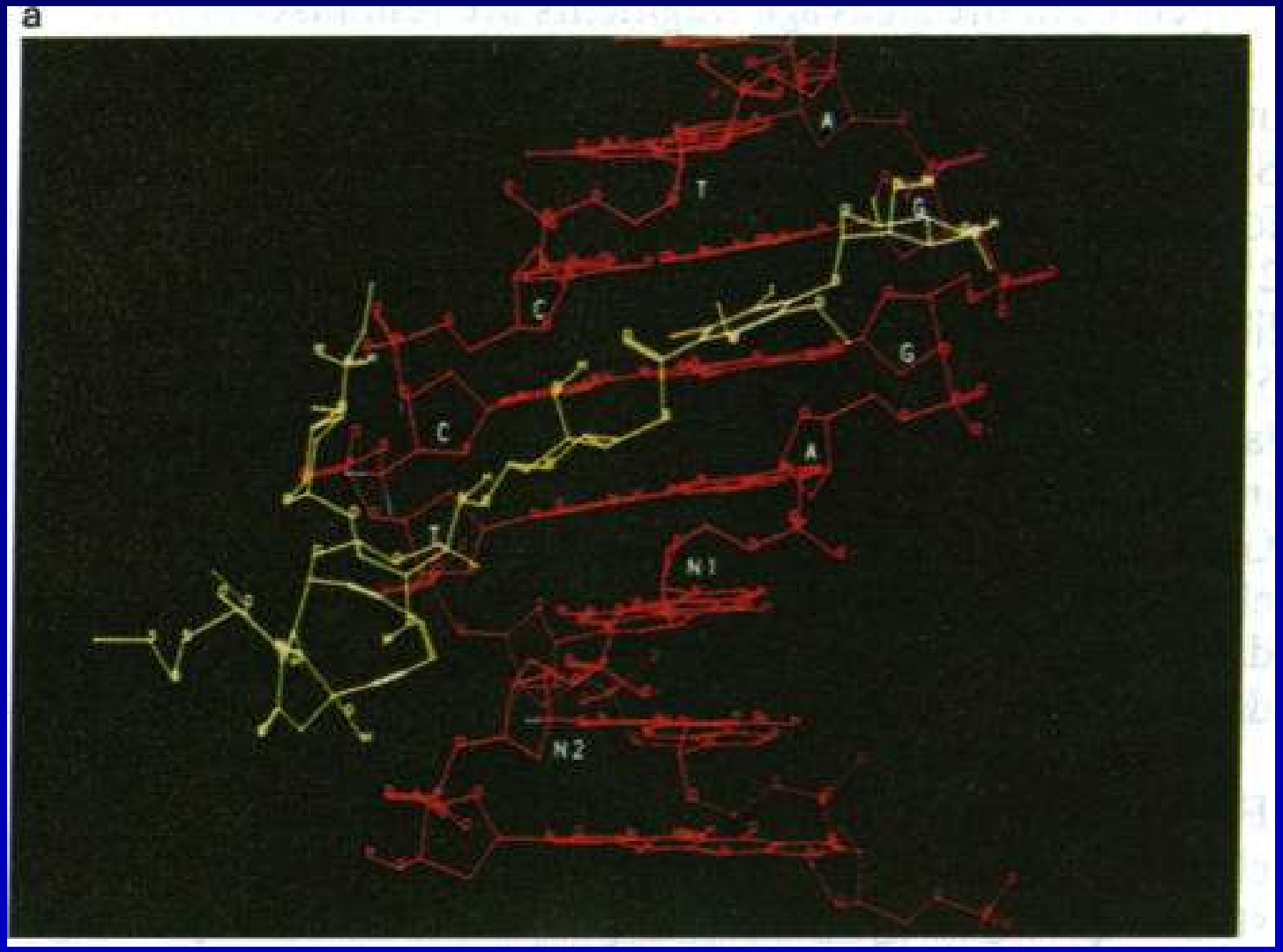
- Indication
  - Mylotarg is indicated for patients with CD33 positive AML in first relapse who are ≥ 60 years of age and not candidates for other cytotoxic chemotherapy
- Mechanism of Action
  - Antibody-targeted chemotherapy
  - Binds CD33 cell surface antigen on myeloid cells
  - Internalization and release of highly potent antitumor enediyne calicheamicin
  - Spares pluripotent stem cell and allows regeneration of normal blood cells following therapy


Toxin
X
X
Toxin
Cancer cell
expressing
tumor antigen

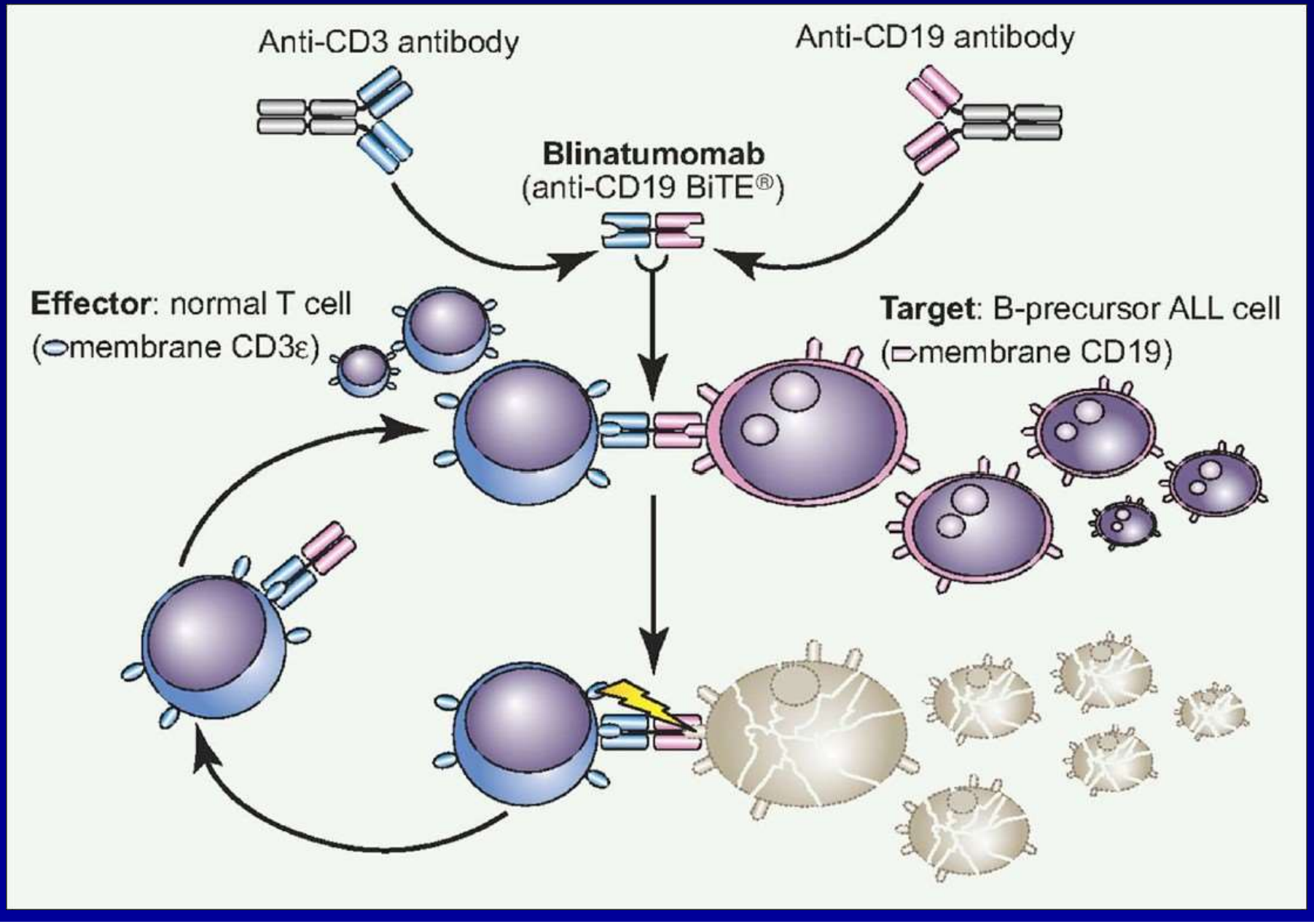
Intracellular breakdown of Antibody linked toxin complex



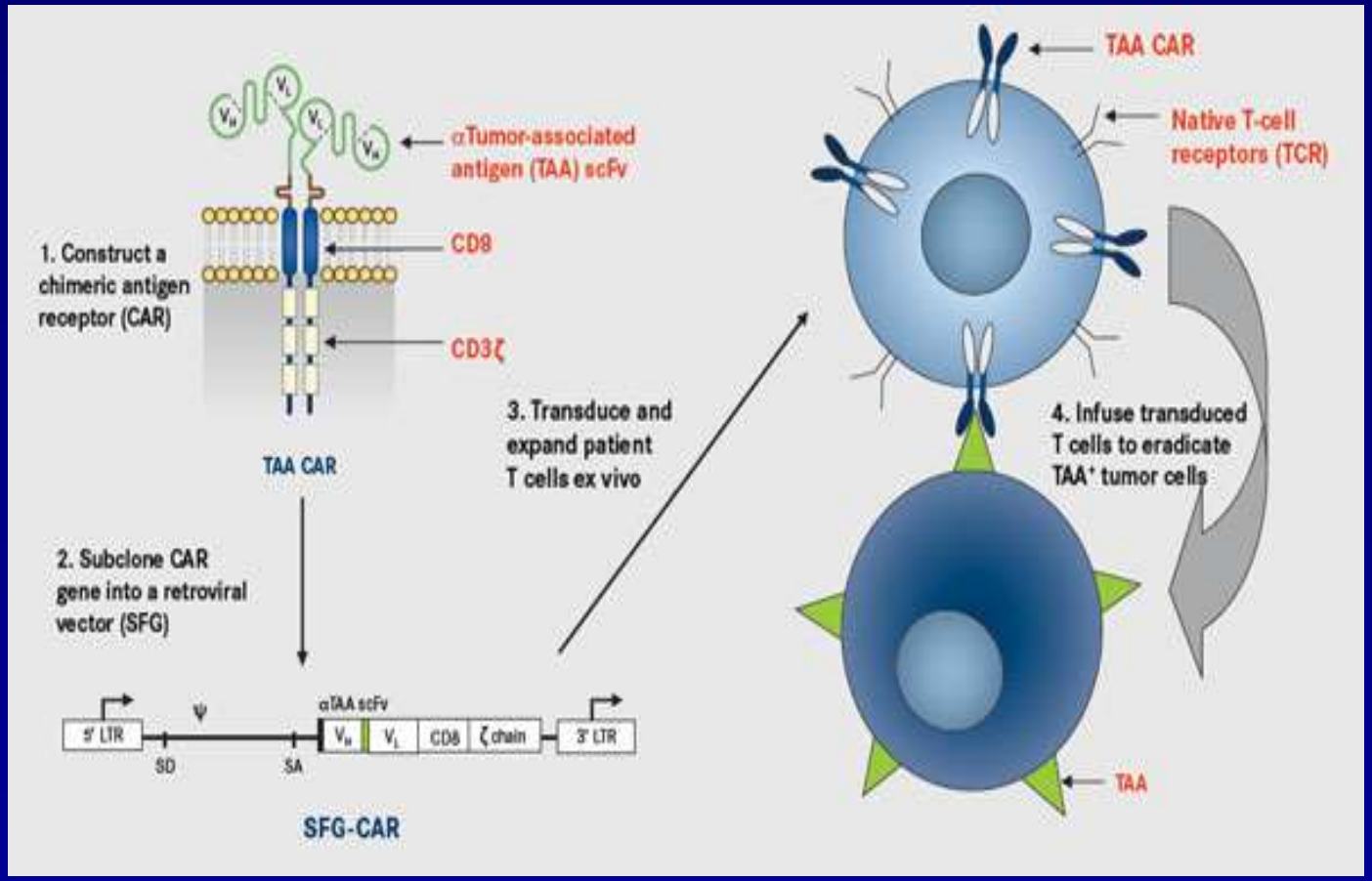
Calcichemicin intercalates into DNA



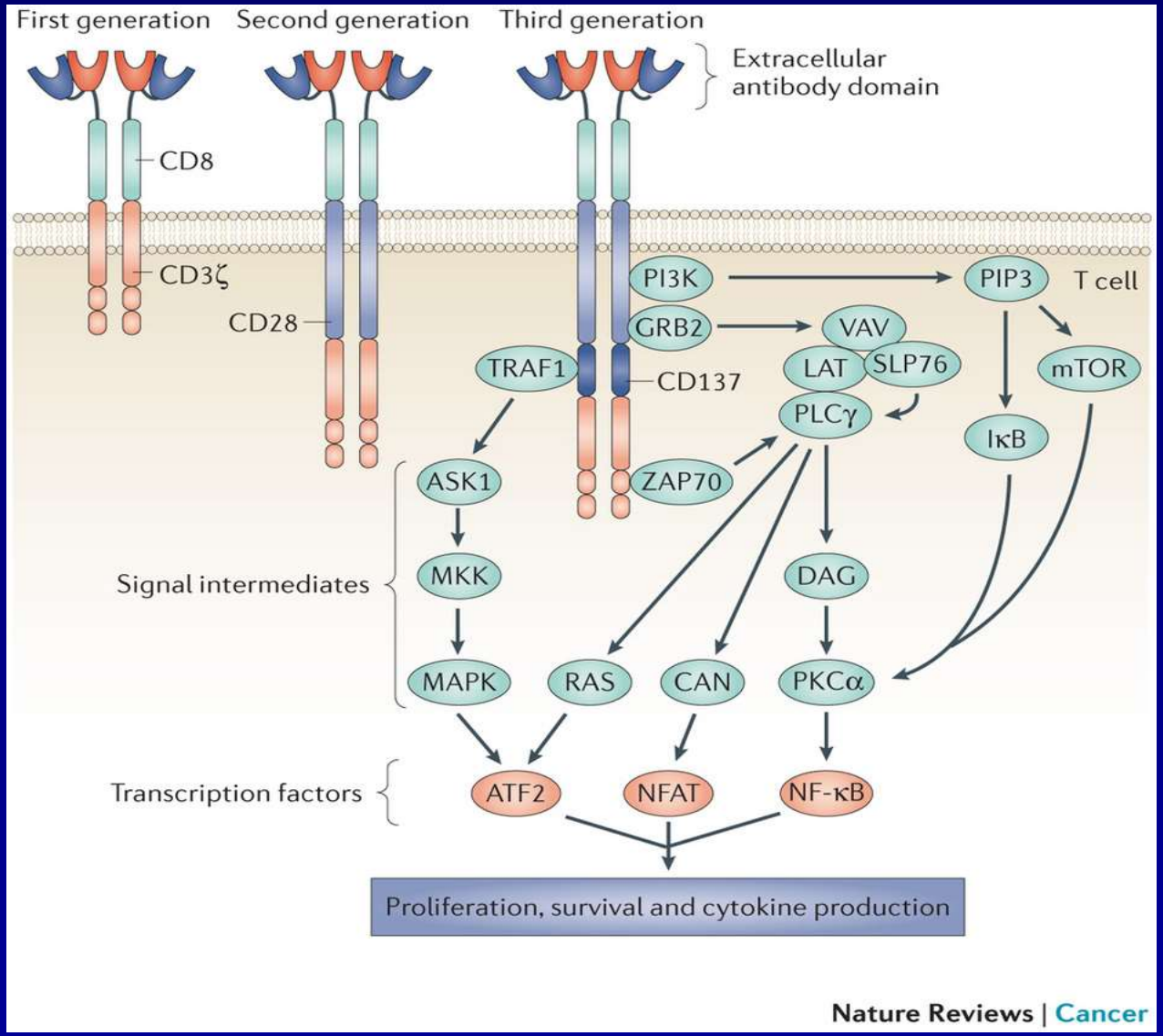
DUAL MONOCLONAL ANTIBODIES



CAR-T CELLS: Chimeric Antigen Receptor



CAR-T CELLS: Chimeric Antigen Receptor



LE SINDROMI MIELODISPLASTICHE

MDS: DEFINIZIONE

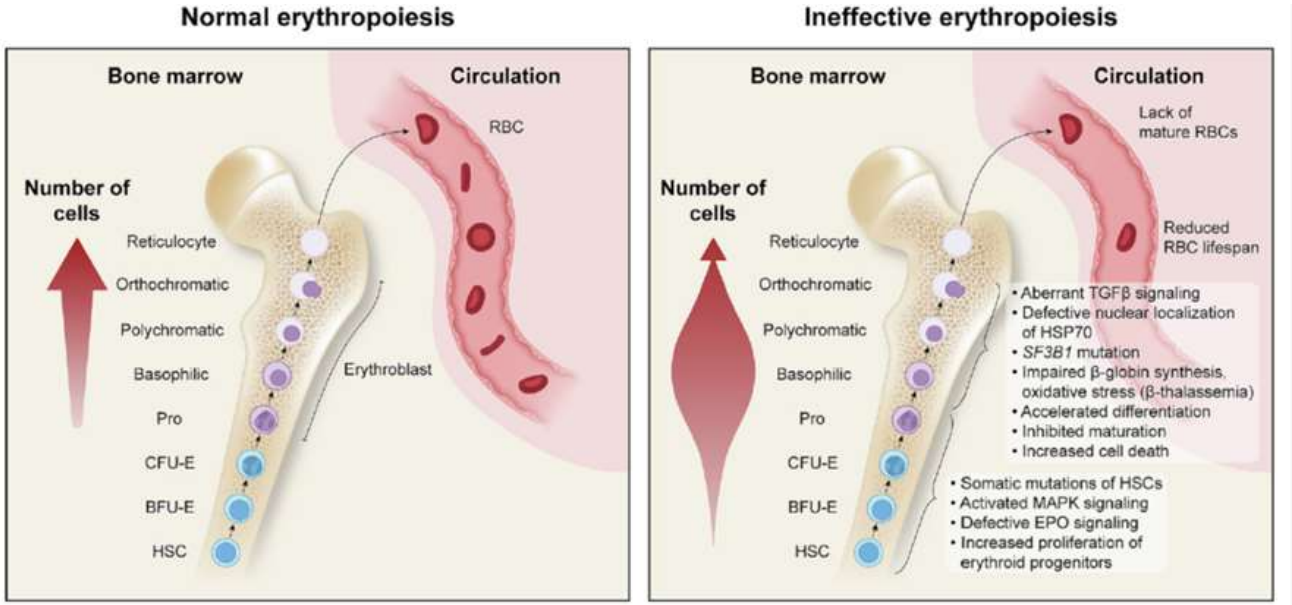
disordini clonali acquisiti della cellula staminale caratterizzati da:

- **emopoiesi inefficace**
- **displasia singola o multilineare**
- **citopenia progressiva**
- **alterazioni citogenetiche e molecolari**
- **evoluzione leucemica**
- **prognosi variabile**

Definition

Myelodysplastic syndrome (MDS), recently ridefined Myelodysplastic Neoplasm, is an eterogeneous myeloid clonal disorder characterized by:

1. Morphologic **dysplasia** (≥ 10%) and bone marrow (BM) cellularity often increased, but potentially also normal od decreased (≤ 25%, age adjusted)
2. Ineffective hematopoiesis and variable **cytopenias** (Hb <13 g/dL in males, < 12 g/dL in females; ANC < 1.8 x10^9/L; PLTs < 150.000 x10^9/L)
3. Peripheral **blasts** (PB) < 20% and/or BM blasts < 20%



Barcellini MD et al. Blood Rev 2023

Epidemiology

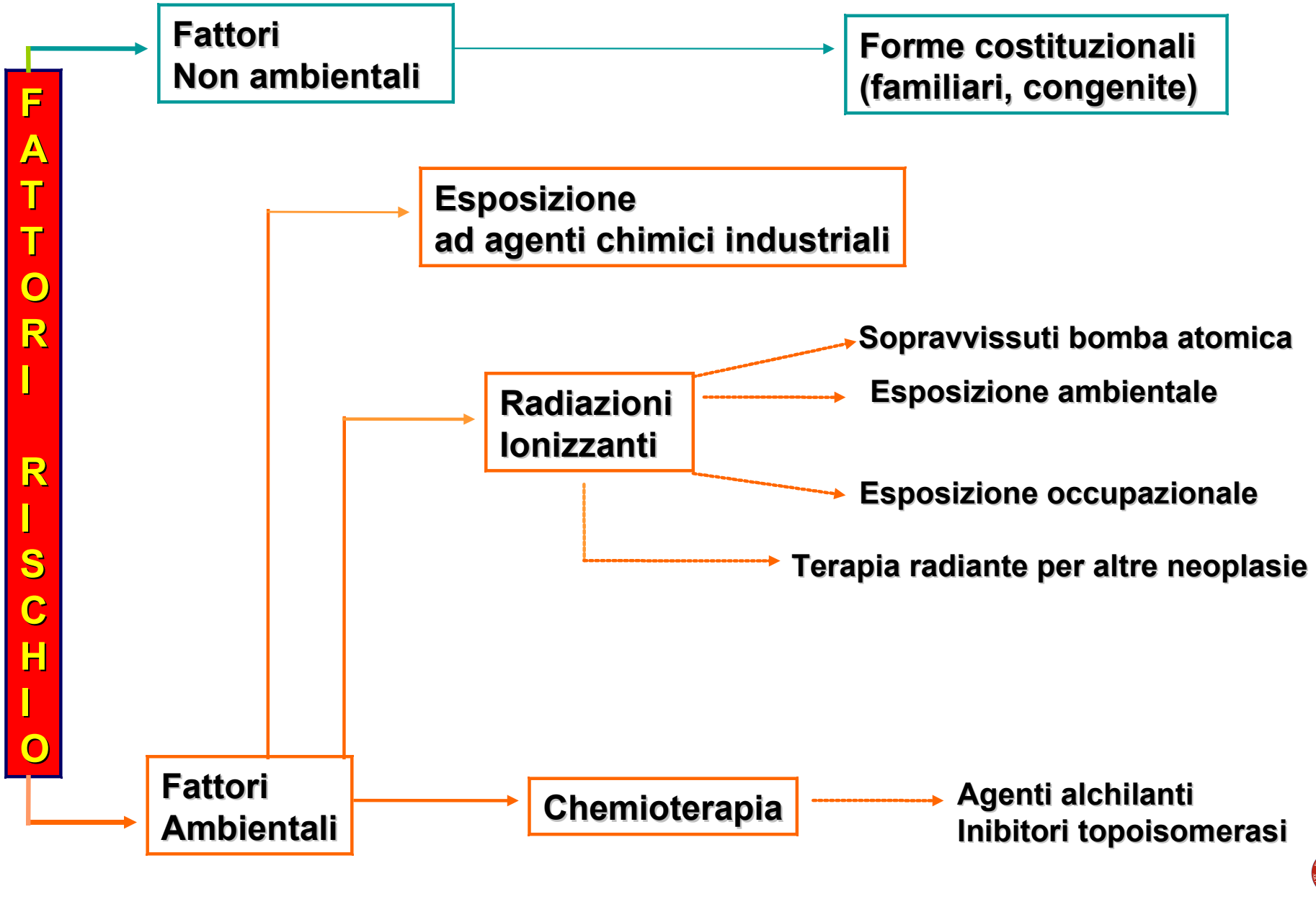
Because of the insidious course of MDS and the refining of their classification exact numbers of the prevalence and incidence of MDS are not available.

In US:

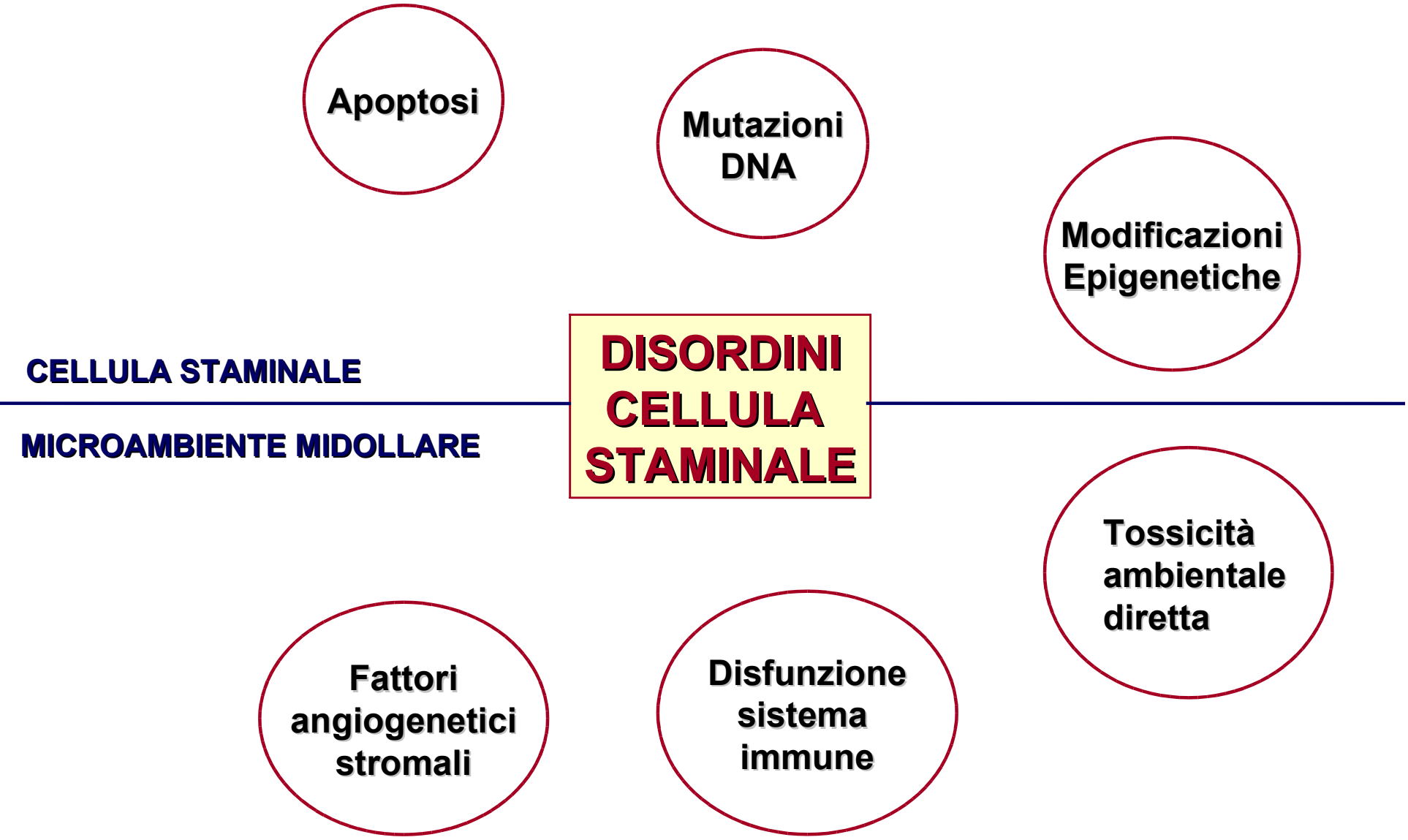
- Overall: **0.1** % US population, **4/100.000/y** (probably understimated)
- median age at diagnosis **76** ys; the incidence increased with age: 1.4-2.6/100.000 for age 50-59 up to 53.3/100.000 > 85 ys
- Disease onset before the age of 40 ys is rare (0.1-0.3/100.000)
- M>F except in MDS with isolated del(5q)

Rotter LK et al. The Cancer J 2023

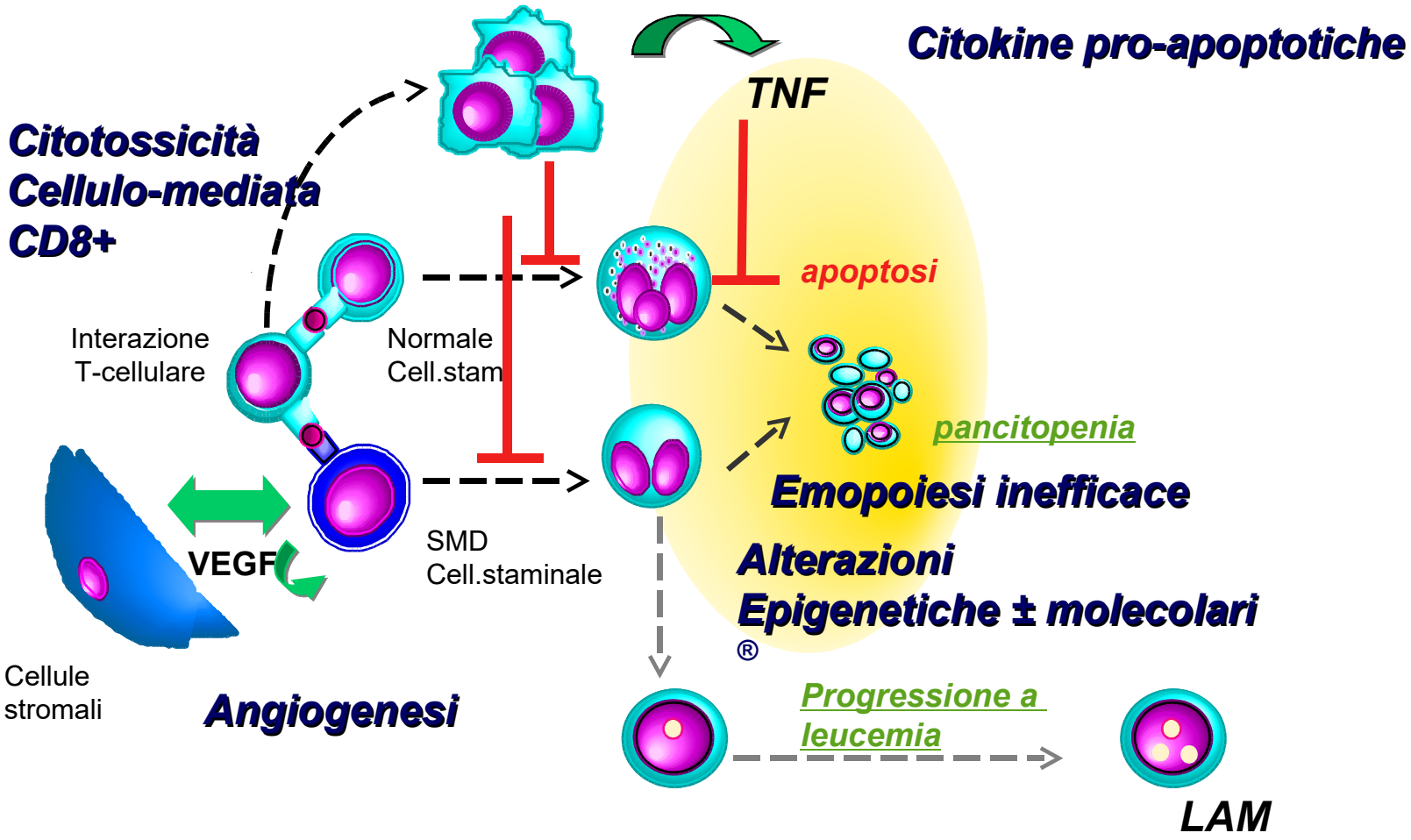
SMD: eziologia



MDS: PATOGENESI



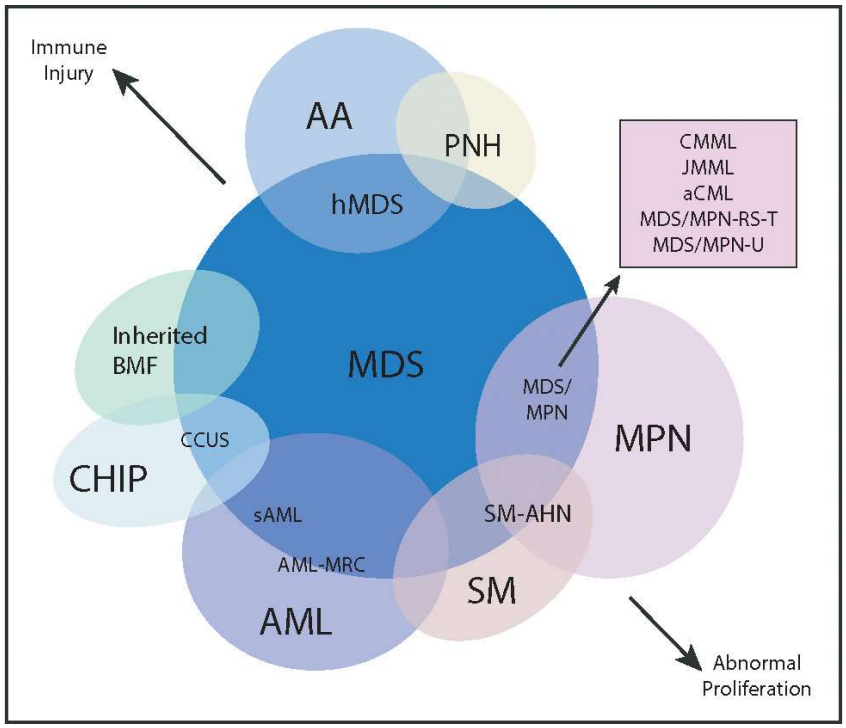
MDS: PATOGENESI



Differential diagnosis

It is probably the most difficult hematologic diagnosis due to:

- complexity of differential diagnosis (non-hematologic and non-clonal cytopenias are much more frequent)
- cytological diagnosis, although quantitatively defined (WHO), is subjective and associated with an inter-observer reproducibility variable
- the boundary between MDS and other clonal hematologic disorders (e.g. AA, MPN, AML) is often thin since the respective diagnostic criteria have historically been established artificially with respect to the pathological continuum

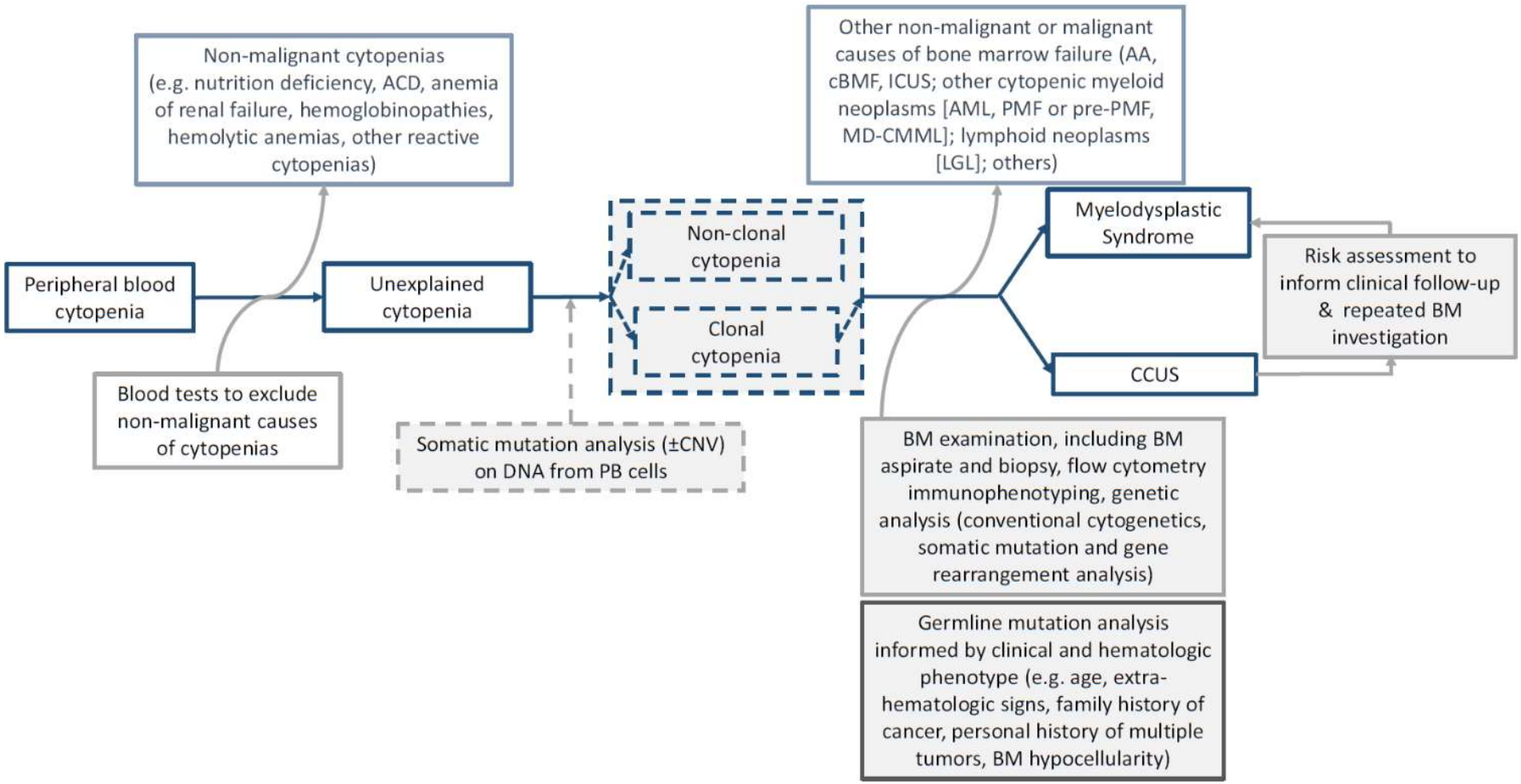


No MDS

Differential diagnosis
Aplastic anemia, pure red cell aplasia
Metastatic carcinoma
Toxic bone marrow injury (alcohol, lead, zinc, copper deficiency, nonsteroidal anti-rheumatic drugs, etc.)
Reactive bone marrow changes (infections e.g. sepsis, HIV, hepatitis, tuberculosis and other chronic infections, autoimmune diseases, thyroid disease, etc.), copper deficiency
Paroxysmal nocturnal hemoglobinuria
Immune thrombocytopenia
Megaloblastic anemia
Hypersplenic syndromes
Acute leukemia (especially erythroleukemia, FAB-M6)
Myeloproliferative diseases (especially CMML, aCML, PMF)
Hairy cell leukemia, large granular lymphocytic leukemia
Congenital dyserythropoietic anemia (rare)
Idiopathic cytopenia of undetermined significance
Clonal cytopenia of undetermined significance

Hellstrom-Lindberg E et al, Haematol 2020

Work-up diagnostico



Hasserjian R, Germing U, Malcovati L. Blood 2023

MDS: classificazione

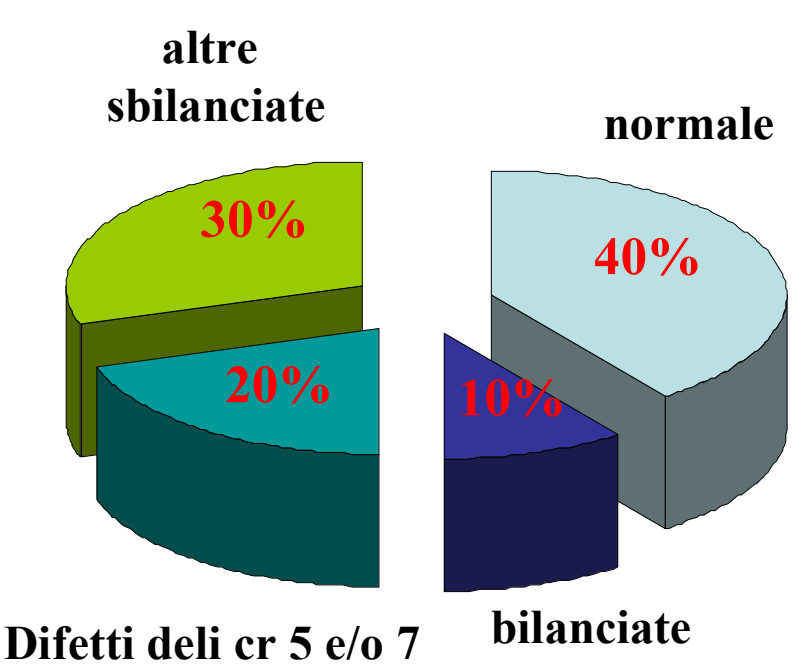
Casistica 2897 pazienti

Primitive	94.4%
Secondarie (terapie correlate)	5.6%

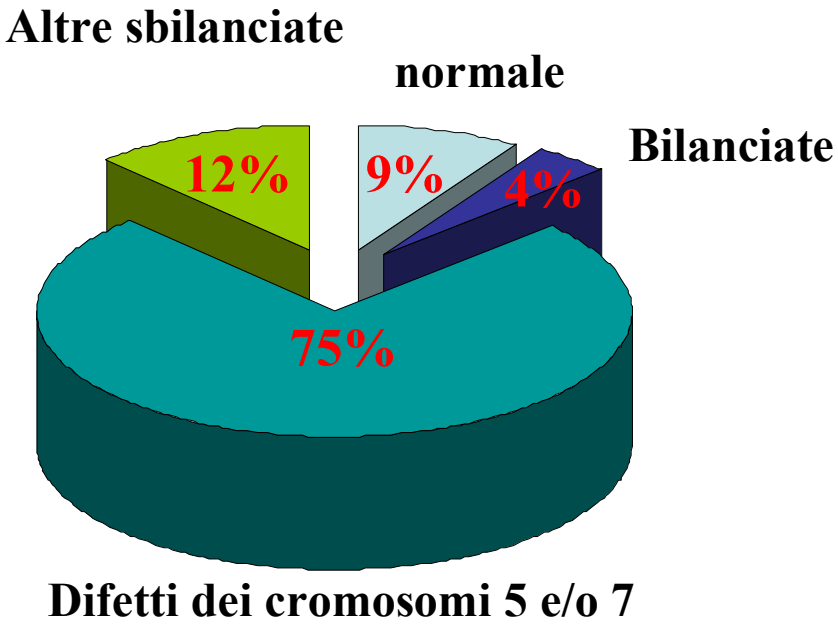
Registro MDS del distretto di Dusseldorf 2006

Anomalie cromosomiche: frequenza

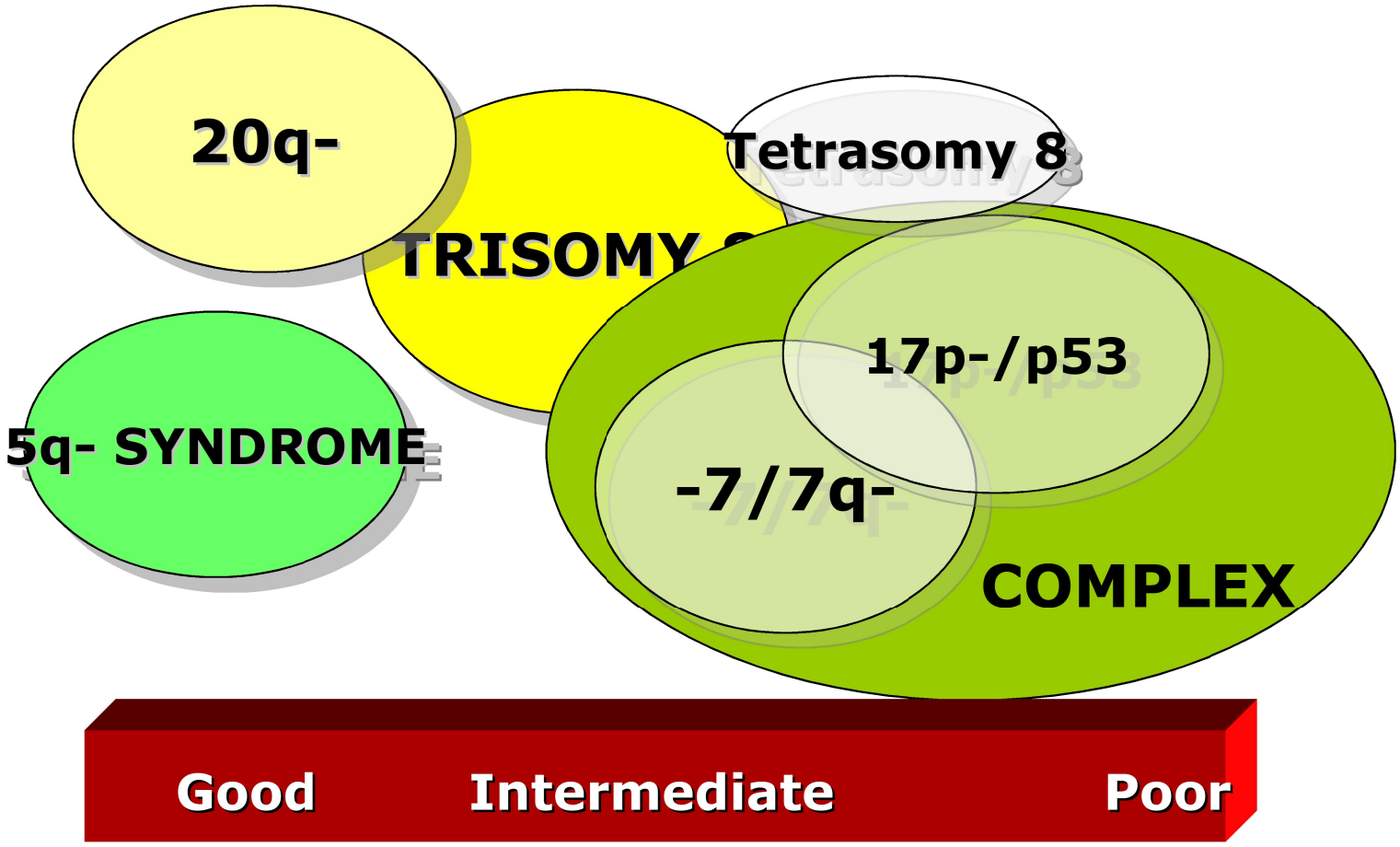
SMD de novo



SMD secondarie

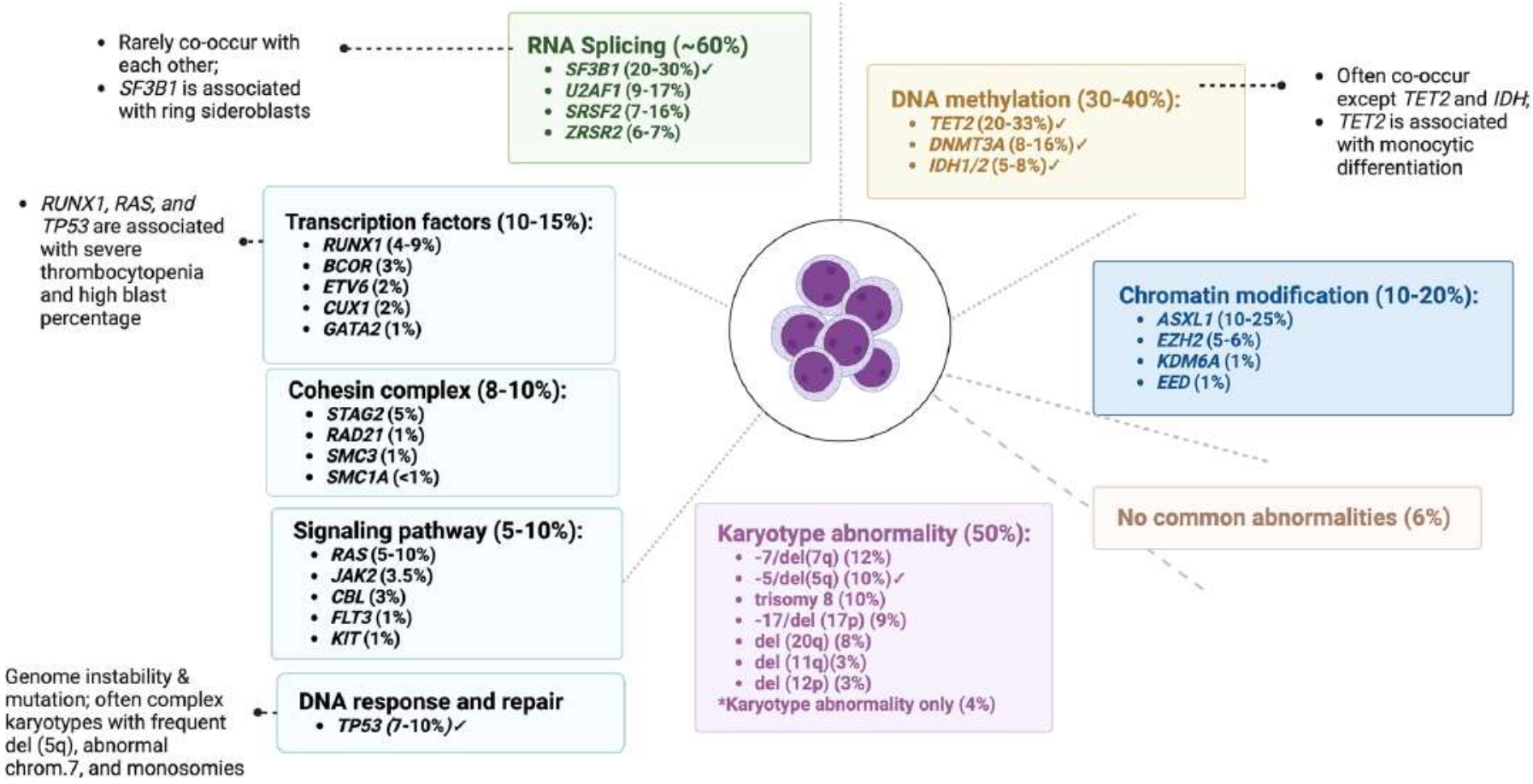


Alterazioni citogenetiche specifiche nelle SMD



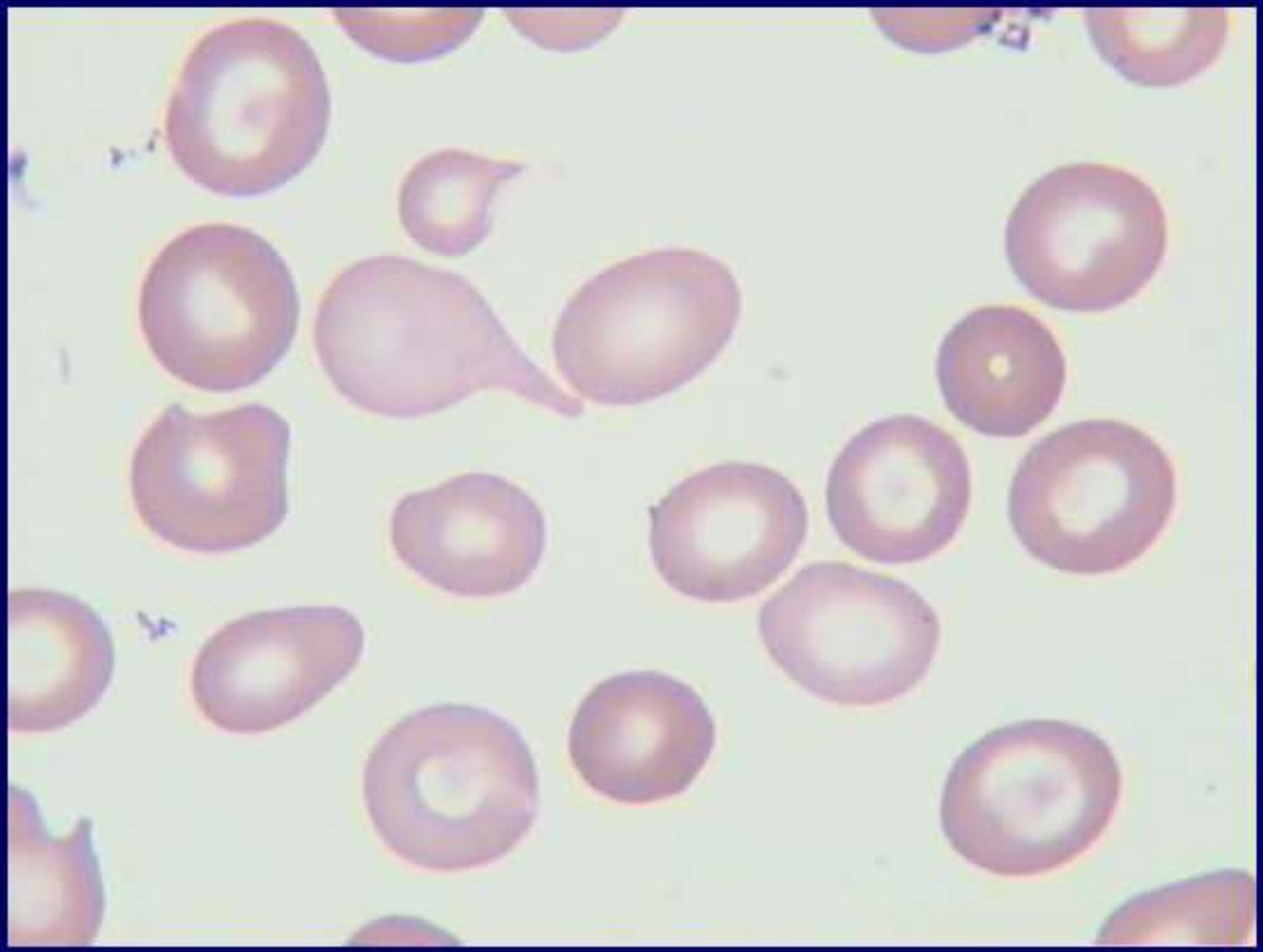
Genetic analysis

- Conventional kayotype (at least 20 metaphases)
- FISH for 17p deletion if TP53 mutation is detected

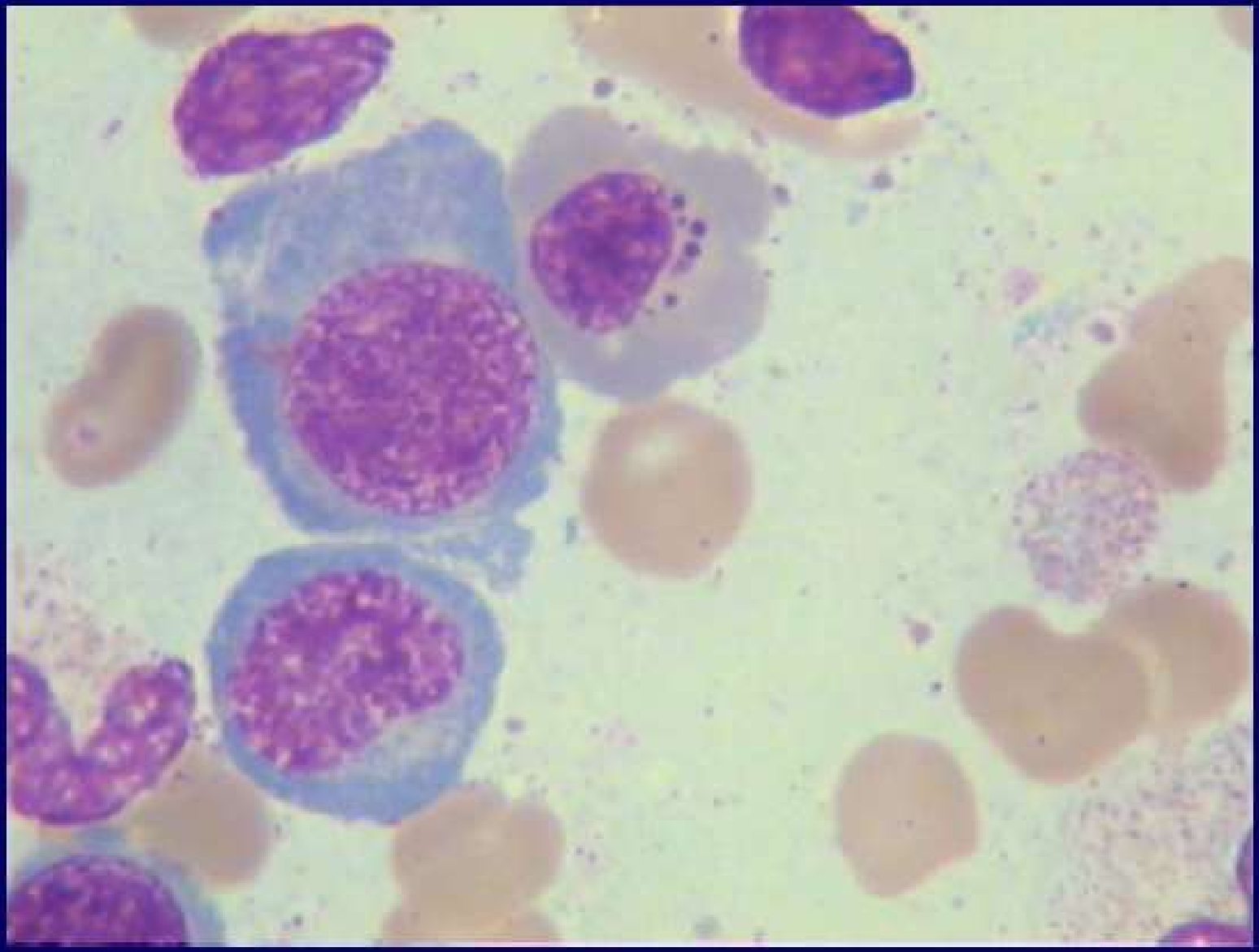


Gurnari C et al. Clin Hematol Intern 2023

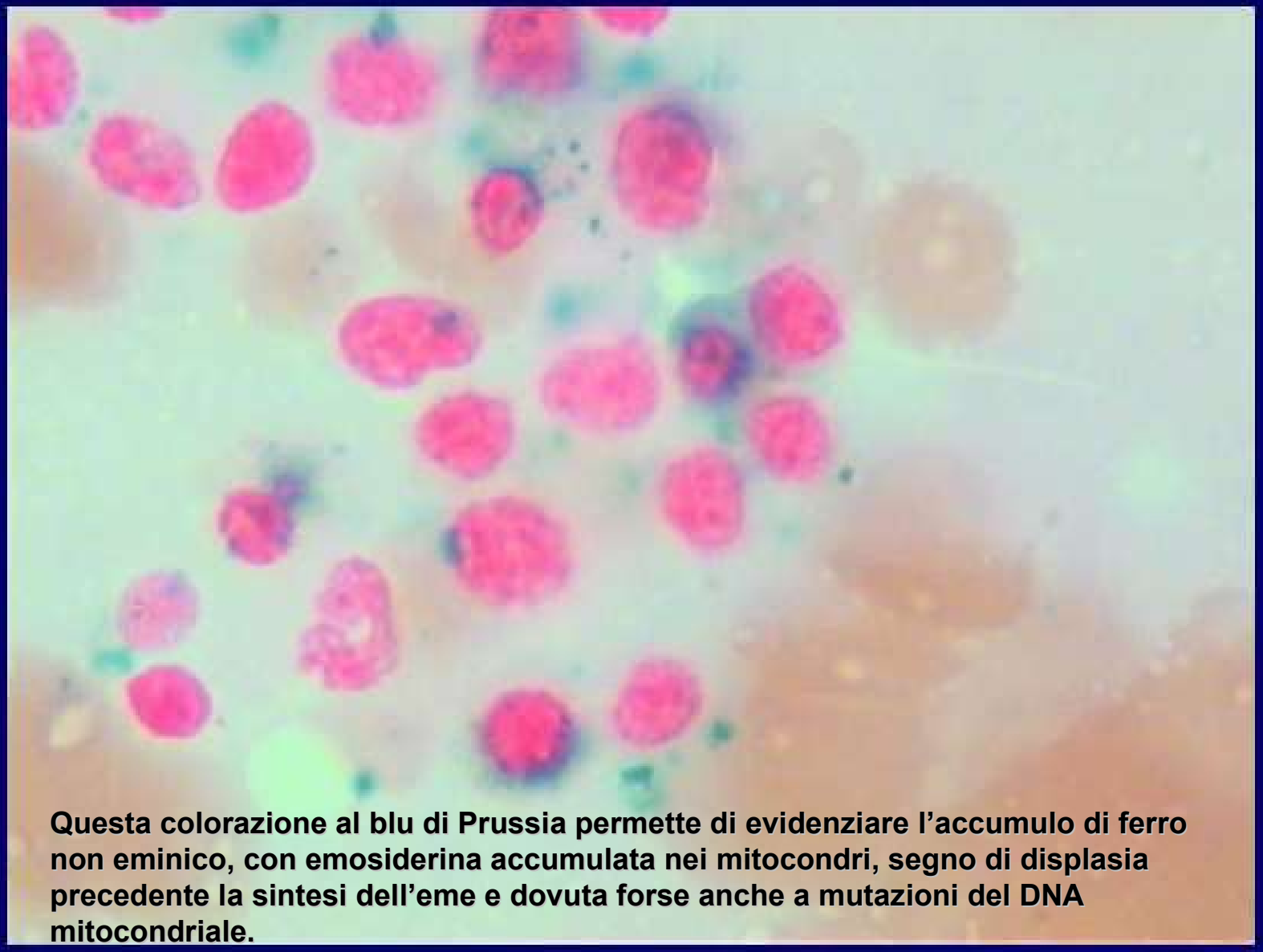
SMD: diseritropoiesi sangue periferico



SMD: diseritropoiesi midollare

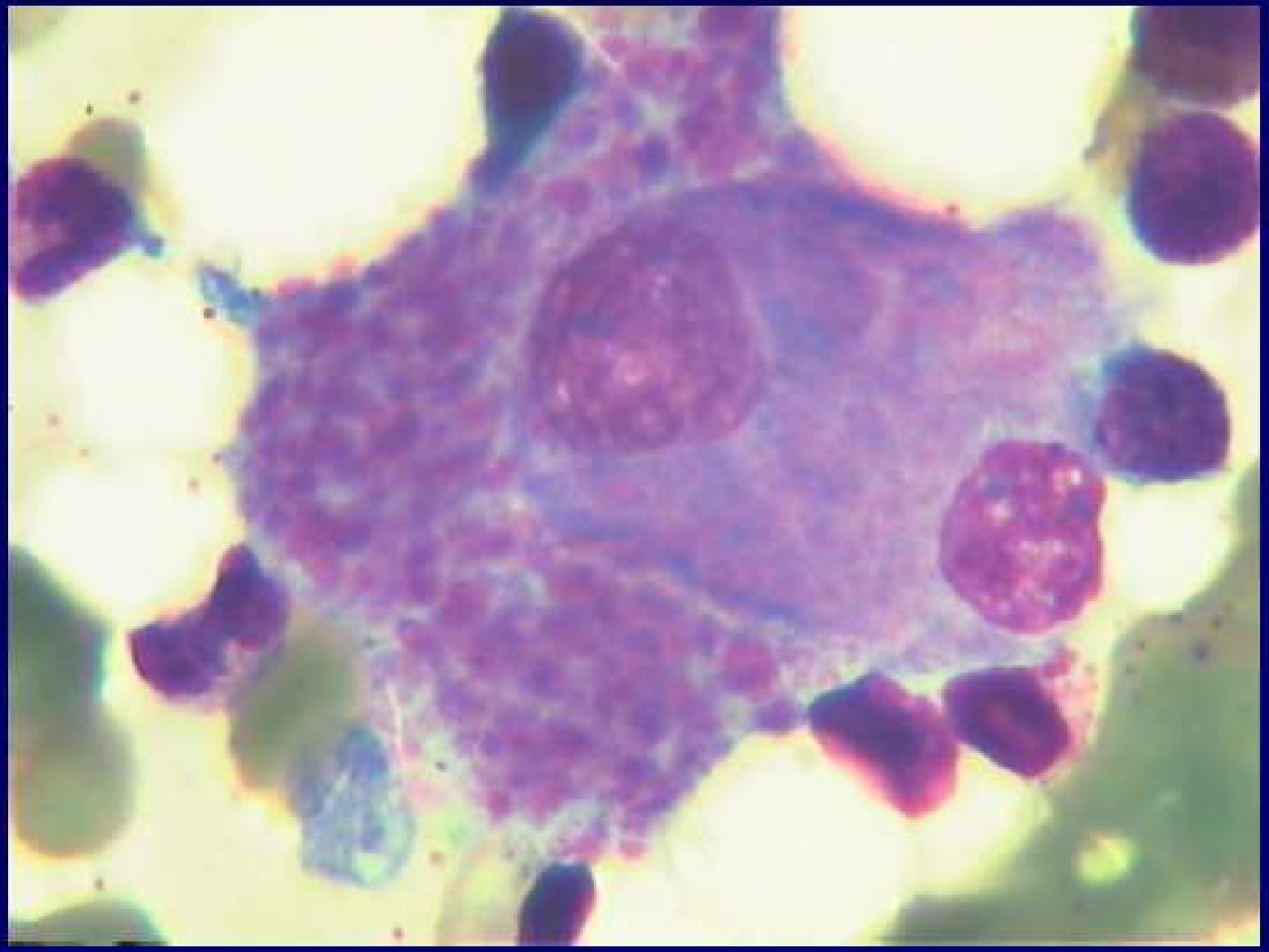


SMD: sideroblasti ad anello con granuli perinucleari di emosiderina (colorazione Pearl)

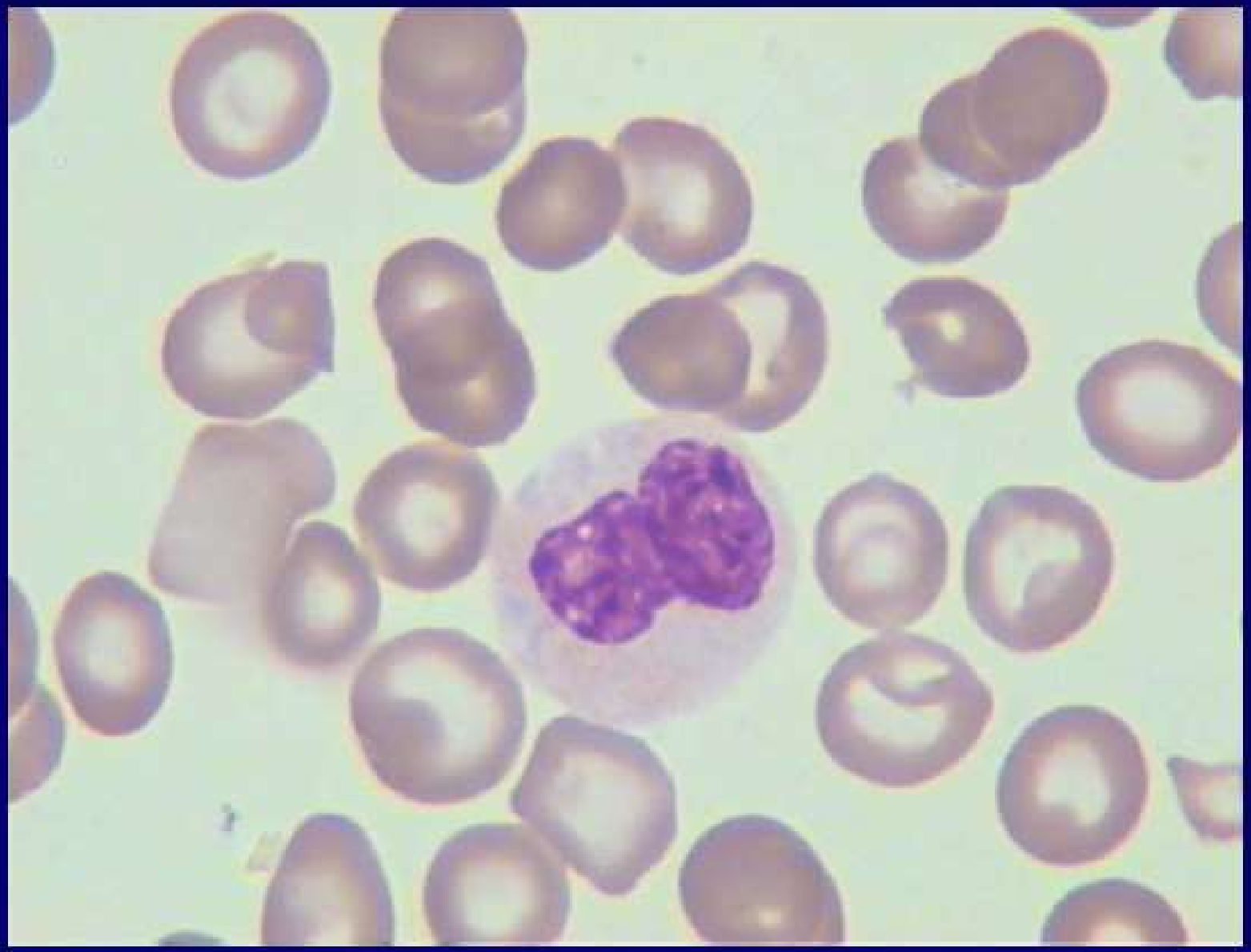


Questa colorazione al blu di Prussia permette di evidenziare l'accumulo di ferro non eminico, con emosiderina accumulata nei mitocondri, segno di displasia precedente la sintesi dell'eme e dovuta forse anche a mutazioni del DNA mitocondriale.

SMD: dismegacariocitopoiesi midollare



SMD: dismielopoiesi



Classification

Once a patient is diagnosed with MDS, the disease is classified into one of several subtypes depending on the morfologic and/or genetic features: MDS classification aim to identiify subgroups with shared molecular patogenesis to inform targeted treatments, which could benefit across different risk categories.

2022

Hasserjian R, Germing U, Malcovati L. Blood 2023

CLASSIFICAZIONE FAB

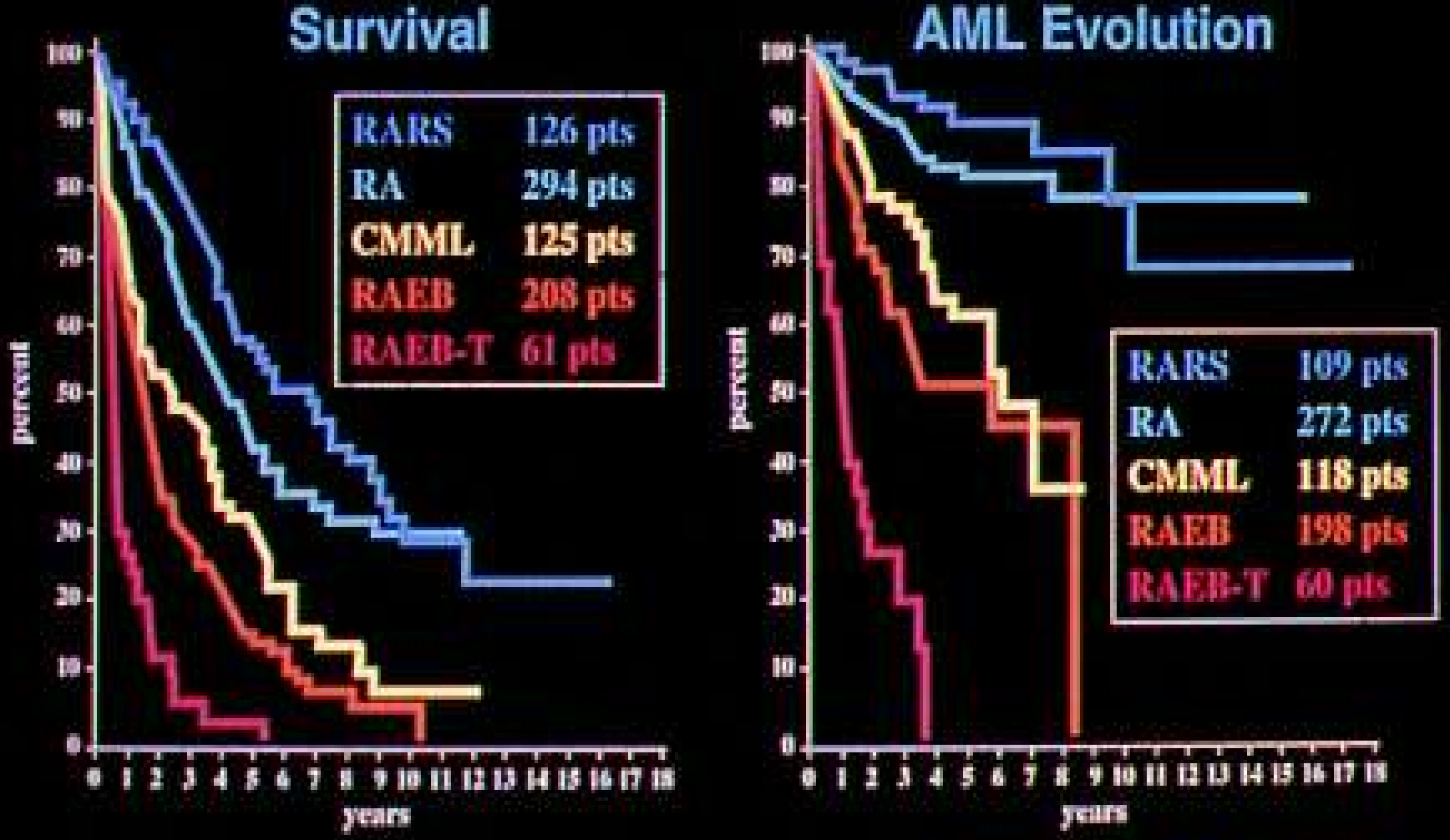
(Bennet et al. 1982, Br.J.Hematol.)

Tipo SMD	%blasti BM	%blasti SP
RA	< 5	< 1
RARS*	< 5	< 1
RAEB	5-20	< 5
RAEB-t	> 20-30	≥ 5
CMML**	< 5-30	< 5

*Sideroblasti ad anello > 15%

**monocitosi (1 x 10^9/L)

FAB Classification



International MDS Workshop
Blood 89: 2079, 1997

CLASSIFICAZIONE WHO 2008

Refractory cytopenia with unilineage dysplasia (RCUD): [Refractory anemia (RA); Refractory neutropenia (RN); Refractory thrombocytopenia (RT)]	Unicytopenia or bicytopenia[1] No or rare blasts (<1%)[2]	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 erythroid precursors are ring sideroblasts Erythroid dysplasia only <5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s) No or rare blasts (<1%)[2] No Auer rods <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[2] No Auer rods <1x10^9/L monocytes	Unilineage or multilineage dysplasia 5-9% blasts[2] No Auer rods
Refractory anemia with excess blasts-2 (RAEB-2)	Cytopenia(s) 5-19% blasts[3] Auer rods ±[3] <1x10^9/L monocytes	Unilineage or multilineage dysplasia 10-19% blasts[3] Auer rods ±[3]
Myelodysplastic syndrome – unclassified (MDS-U)	Cytopenias <1% blasts[2]	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. (See Table 6) <5% blasts
MDS associated with isolated del(5q)	Anemia Usually normal or increased platelet count No or rare blasts (<1%)	Normal to increased megakaryocytes with hypolobated nuclei <5% blasts Isolated del(5q) cytogenetic abnormality No Auer rods

Sindrome del 5q-

Identificata da van den Berghe nel 1985

Riconosciuta come entità a se stante dalla classificazione WHO

Correlata ad una diagnosi di RA/RARS

Colpisce maggiormente il sesso femminile: ratio F:M = 2:1

Età mediana alla presentazione 60 anni

Anemia macrocitica con valori piastrinici normali o aumentati

Sindrome del 5q-

Nel midollo megacariociti ipolobulati

Elevato fabbisogno trasfusionale

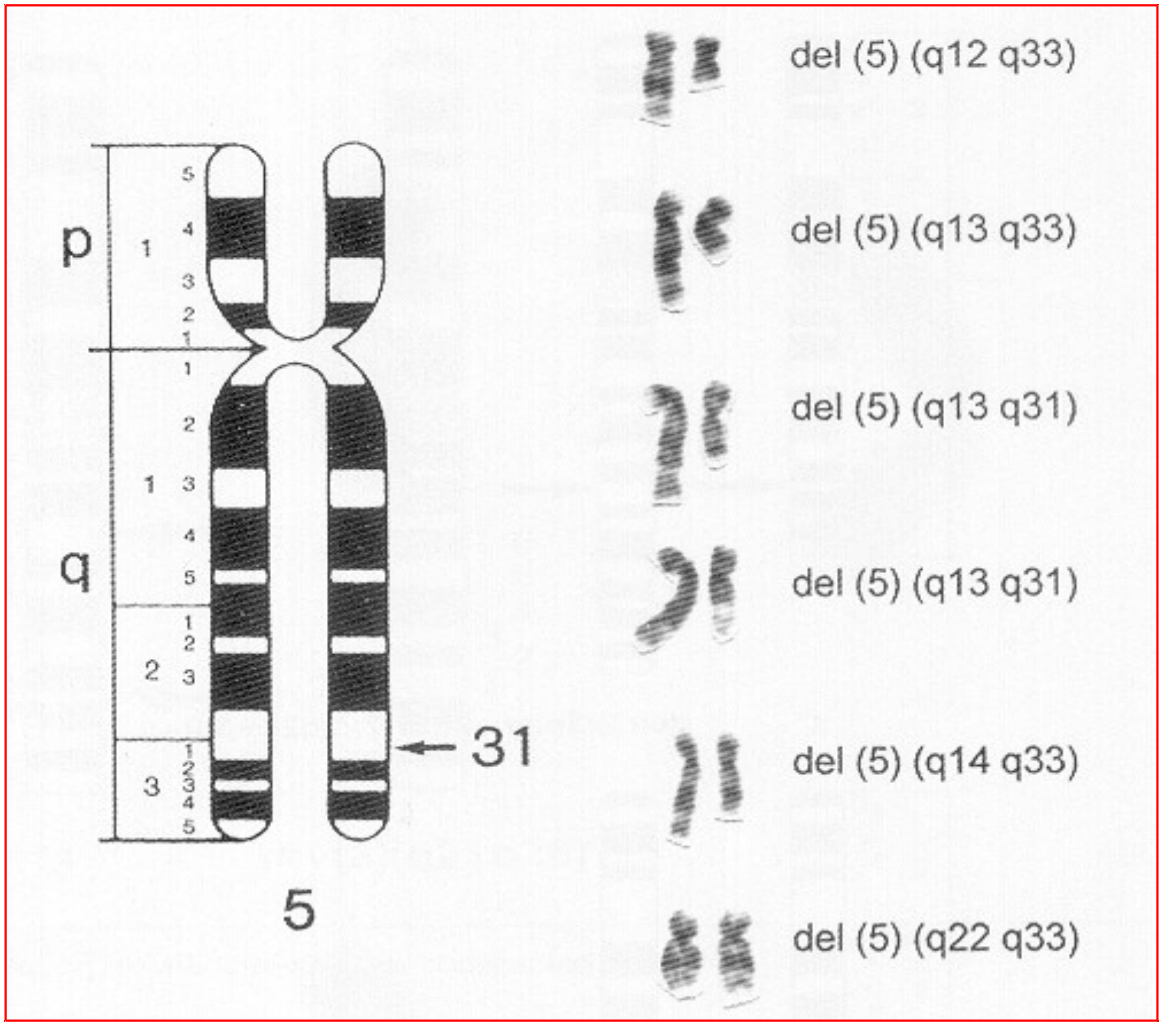
Numero di leucociti normale o leggermente diminuito

Sopravvivenza media prolungata

Blasti nel midollo < 5%

Prognosi favorevole: evoluzione clinica e citogenetica rara (2% dei pazienti)

Delezione 5 q come unica anomalia


p
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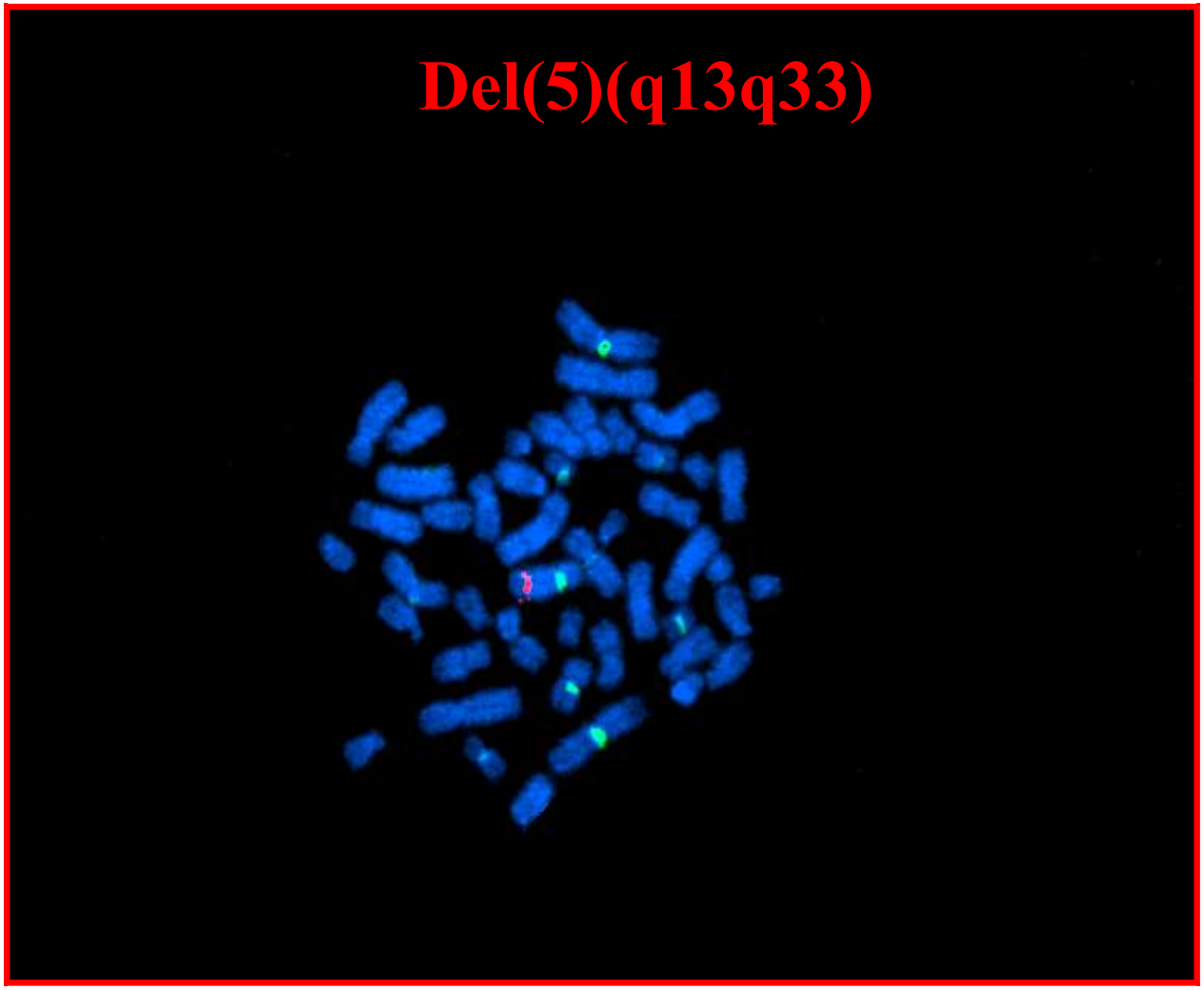
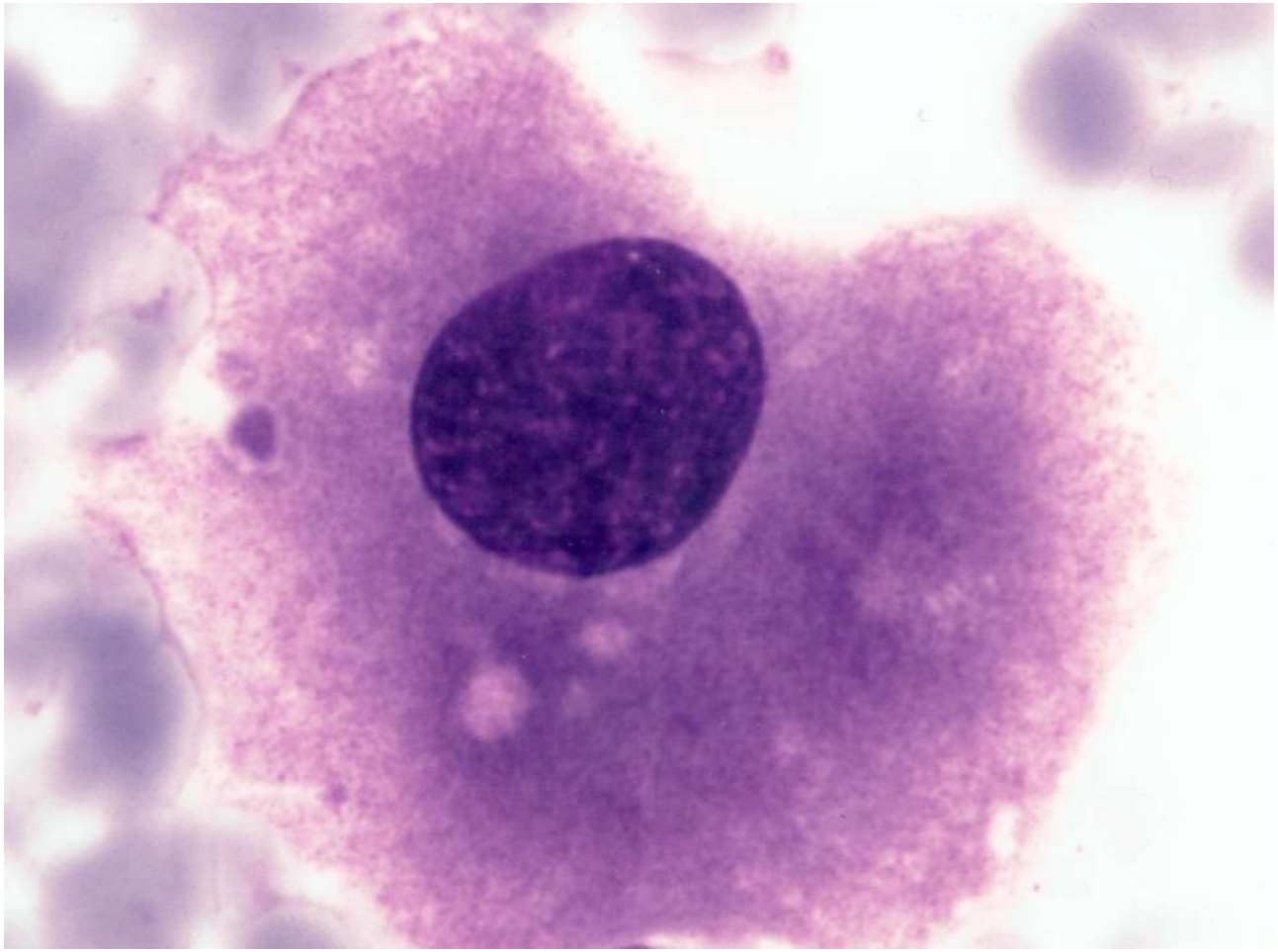
5
del (5) (q12 q33)
del (5) (q13 q33)
del (5) (q13 q31)
del (5) (q13 q31)
del (5) (q14 q33)
del (5) (q22 q33)

Del(5)(q13q33)



Sindrome del 5q-: megacariocito ipolobulato



International Prognostic Scoring System (IPSS)

- The most practical and validated MDS classification system currently available to clinicians is the IPSS which predicts both survival and risk of transformation to AML based on:
  - Marrow blast %
  - Cytogenetics
  - And number of cytopenias.

(A) International Prognostic Scoring System: derivation of patient score. **(Greenberg et al.1997,Blood)**

	Score value				
	0	0·5	1	1·5	2
BM blasts percentage	< 5	5–10		11–20	21–30
Karyotype	Good	Intermediate	Poor		
Cytopenias	0/1	2/3			

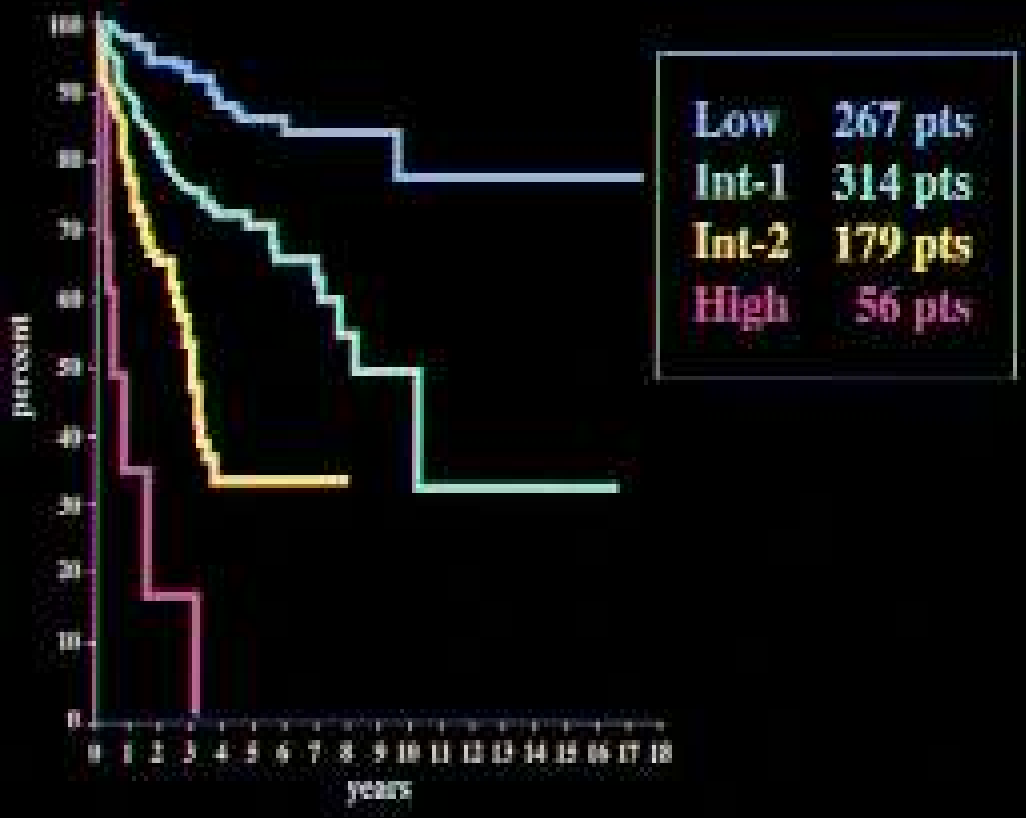
Score for risk groups are as follows: Low 0; INT-1 0·5–1·0; INT-2 1·5–2·0; High ≥ 2·5. Karyotype: Good, normal, –Y, del(5q), del(20q); Poor, complex (≥ 3 abnormalities) or chromosome 7 anomalies; Intermediate, other abnormalities. Cytopenias defined as haemoglobin concentration < 10 g/dl, neutrophils $< 1{\cdot}8 \times 10^9/l$ and platelets $< 100 \times 10^9/l$.

(B) Median survival: IPSS score.

	Median survival (years)			
	≤ 60 years ($n = 205$)	> 60 years ($n = 611$)	≤ 70 years ($n = 445$)	> 70 years ($n = 371$)
Low ($n = 267$)	11·8	4·8	9	3·9
INT-1 ($n = 314$)	5·2	2·7	4·4	2·4
INT-2 ($n = 176$)	1·8	1·1	1·3	1·2
High ($n = 59$)	0·3	0·5	0·4	0·4

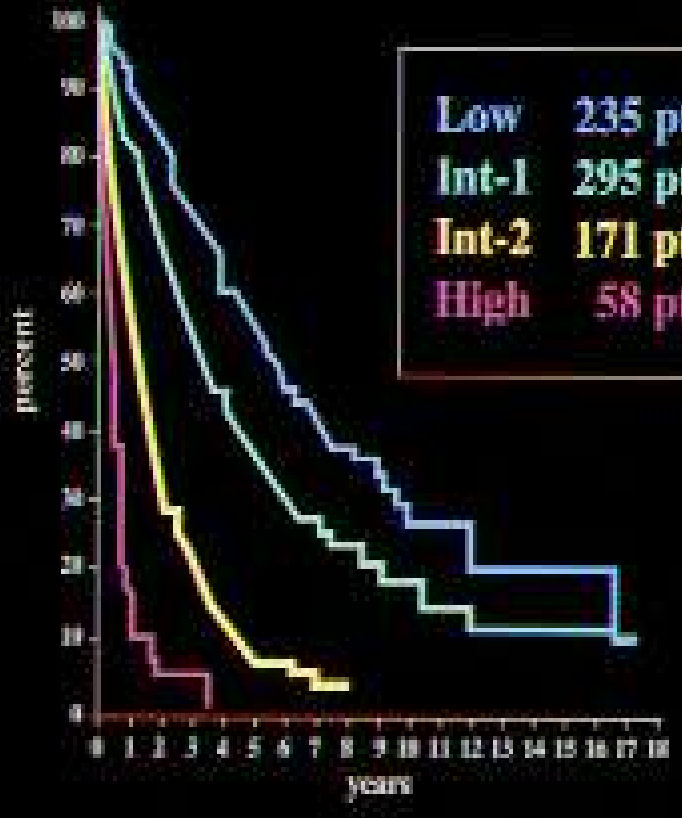
International MDS Risk Classification

Survival



Low	267 pts
Int-1	314 pts
Int-2	179 pts
High	56 pts

AML Evolution



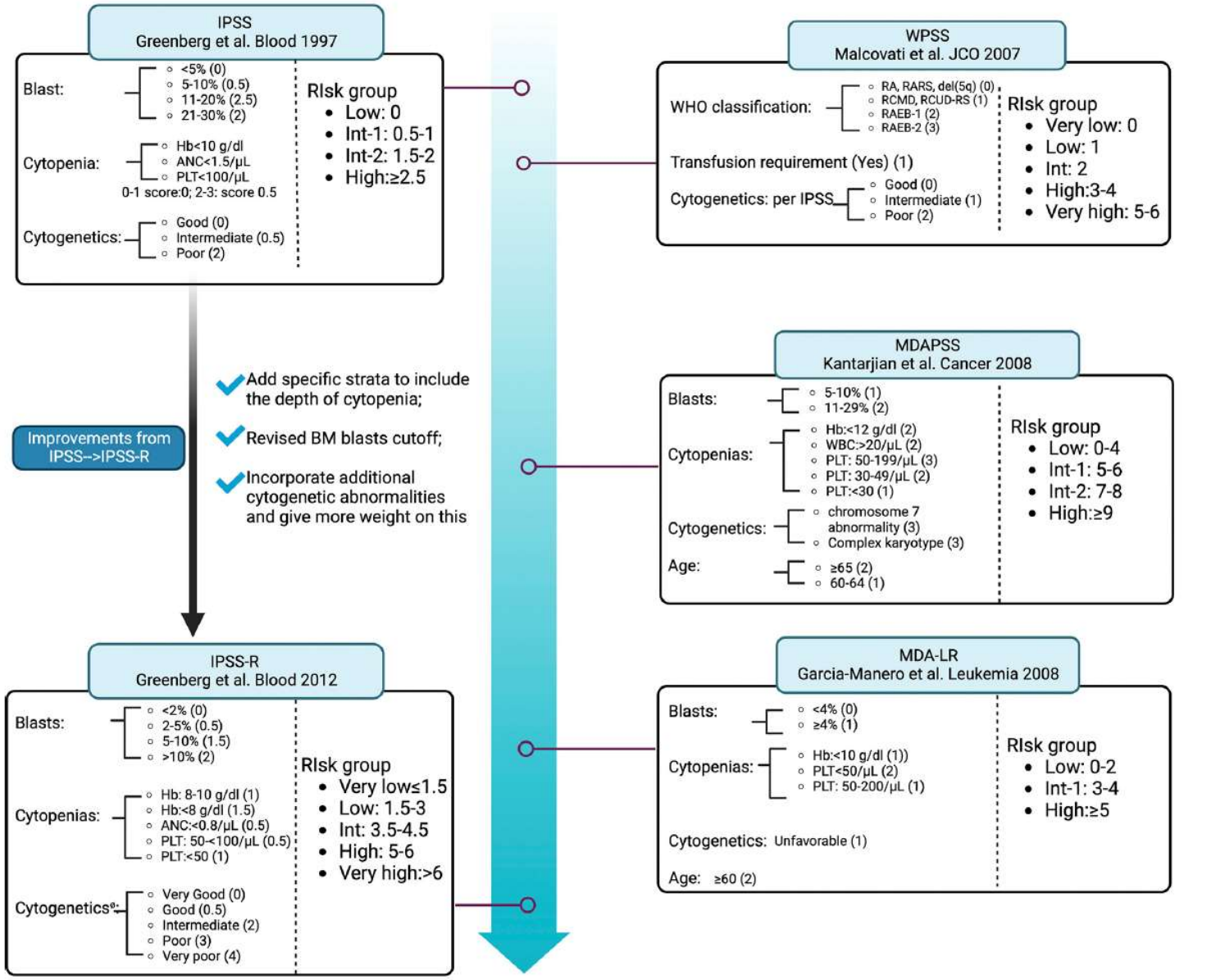
Low	235 pts
Int-1	295 pts
Int-2	171 pts
High	58 pts

International MDS Workshop
Blood 89: 2079, 1997

International MDS Risk Classification

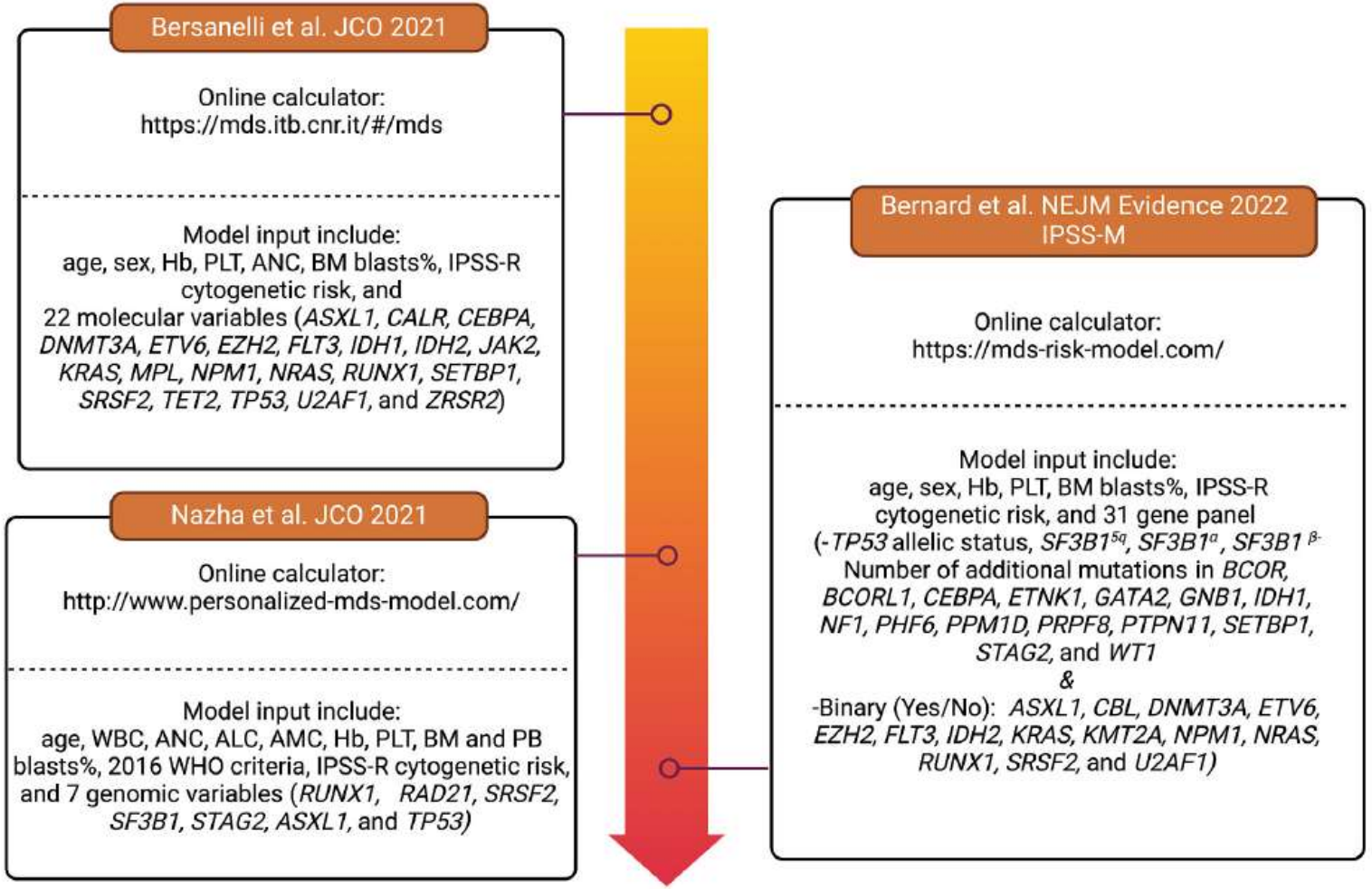
International MDS Workshop
Blood 89: 2079, 1997

Prognosis



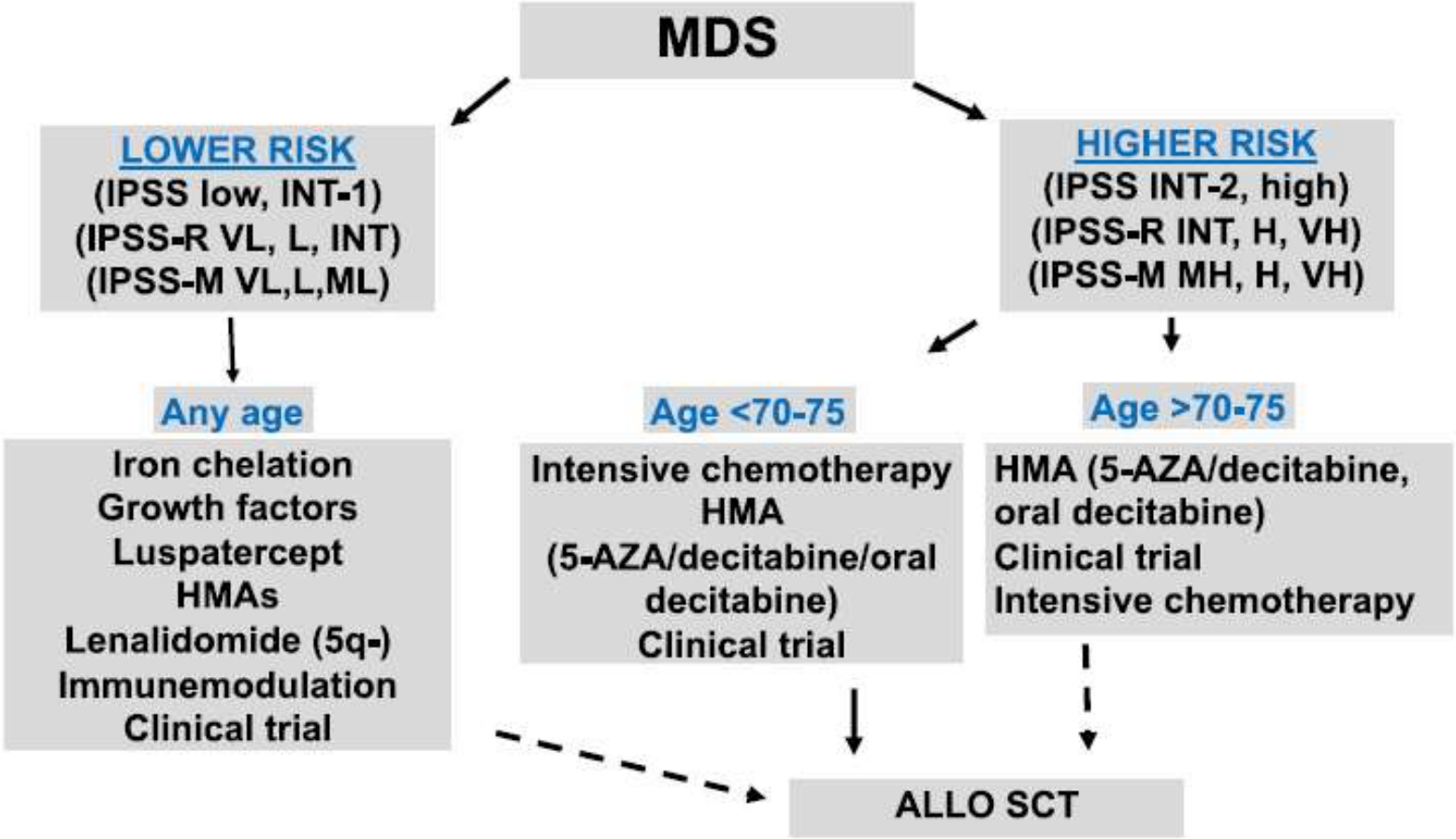
Stahl M et al. Blood Rev 2023

Prognosis



Stahl M et al. Blood Rev 2023

Proposed treatment algorithm for patients with MDS 2023



Garcia-Manero G. AJH 2023

"Targets" terapeutici nelle SMD: diversità biologica

	LOW / INT-1	INT-2 / HIGH
Patogenesi	MALATTIA-SPECIFICA: • segnale citokinico • segnali proapoptotici • citogenetica (5q-)	MALATTIA SPECIFICA: • alterazioni molecolari es.mutazioni "gain of function" (RAS) • silenziamento epigenetico • VEGF
Apoptosi	**aumentata**	**diminuita**
Traguardo terapeutico	**emopoiesi**	**sopravvivenza**
Obiettivi clinici	**Miglioramento ematologico Qualità di vita**	**Remissione completa Risposta citogenetica**

Terapia

Terapia di Supporto

- Trasfusioni, eritropoietina, G-CSF e antibiotici
- Chelazione del ferro in eccesso

Chemioterapia e trapianto allogenico (giovani)

Terapie "epigenetiche"

- 5-azacitidina
- Decitabina
- Lenalidomide 5q-
- Histone de-acetilase inibitori (HDAC)
- Modificatori biologici e terapia sperimentale

Terapia

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Modificazioni epigenetiche:

Modificazioni ereditabili che non alterano la sequenza nucleotidica dei geni ma ne alterano l'attivita' modulandone l'espressione:

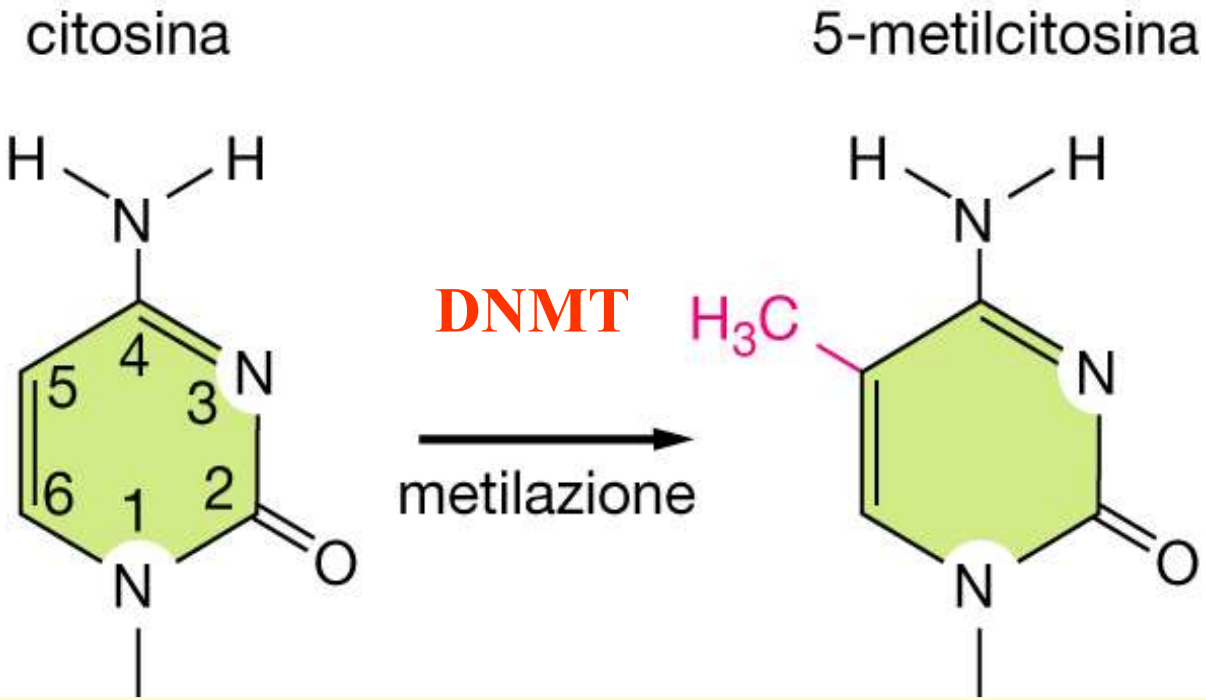
1. METILAZIONE del DNA

2. MODIFICAZIONE DEGLI ISTONI (acetilazione, metilazione, fosforilazione, ubiquitinazione…..).

Responsabili cambiamenti conformazionali cromatina.

1. METILAZIONE DNA

Nei vertebrati la metilazione i[illegible]na sul dinucleotide CpG ad opera di enzimi **DNA-metiltr**[illegible]ngono un gruppo metile al C5 della citosina: il risultato è la 5-metilcitosina.

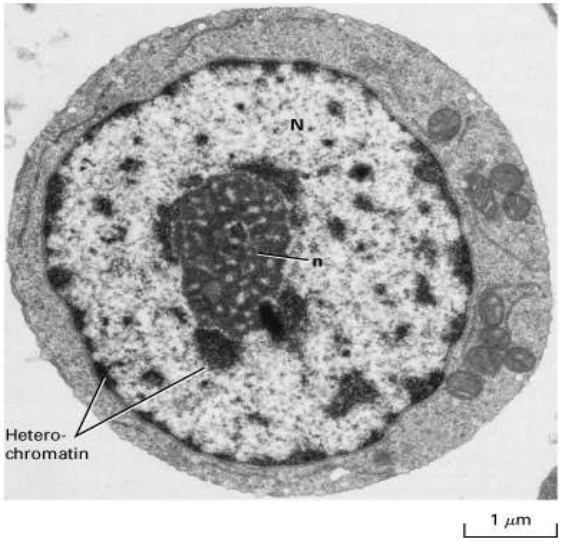


Le sequenze CpG sono sotto-rappresentate nel genoma (probabilmente per la tendenza della 5-metilcitosina a venire deaminata e mutata in T), ma abbondanti nelle regioni promotrici dei geni (**isole CpG**)

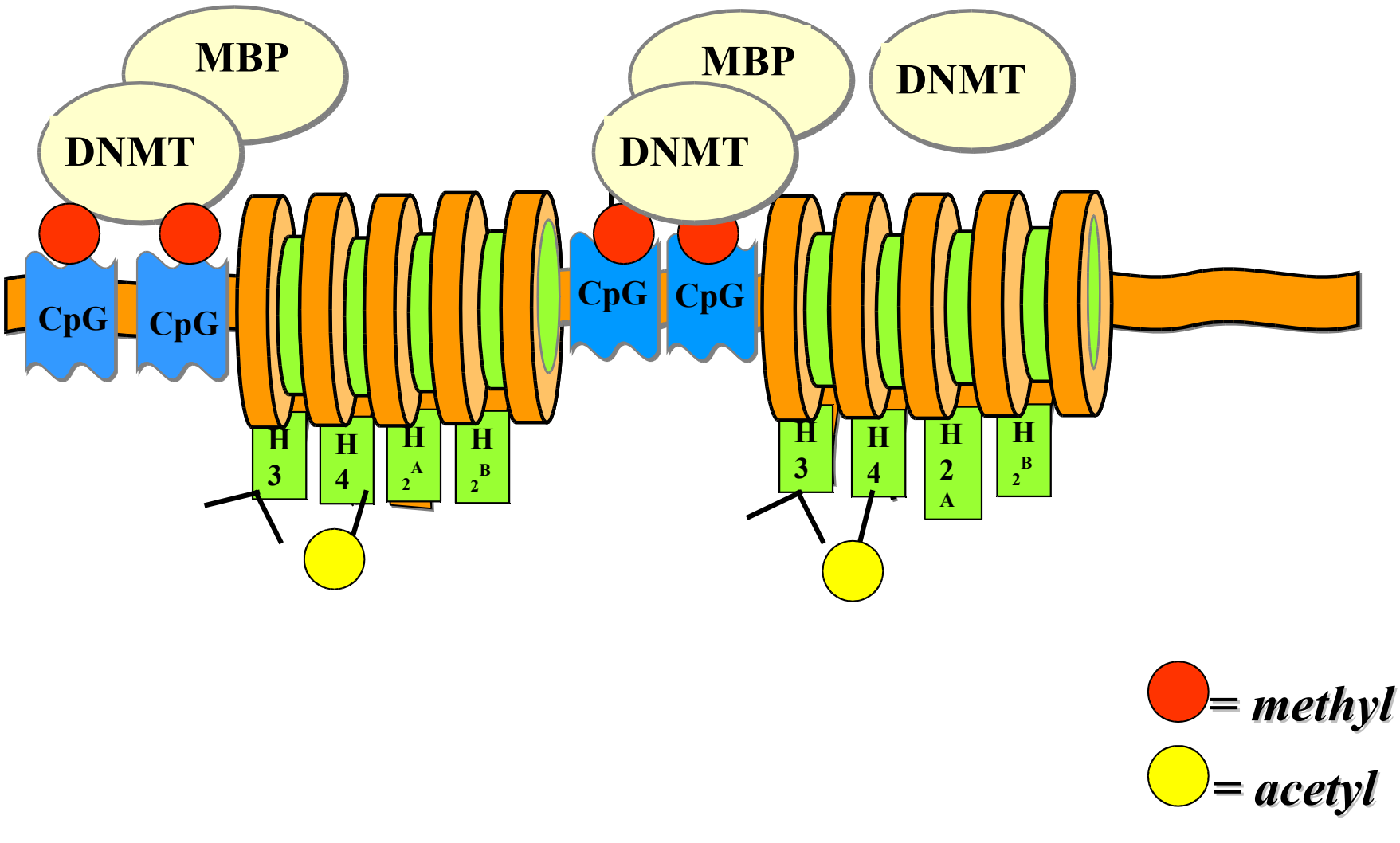
Le isole CpG nella regione promoter sono usualmente demetilate e la loro **metilazione determina una inibizione dell'espressione genica**.

CARATTERISTICHE DELLA CROMATINA

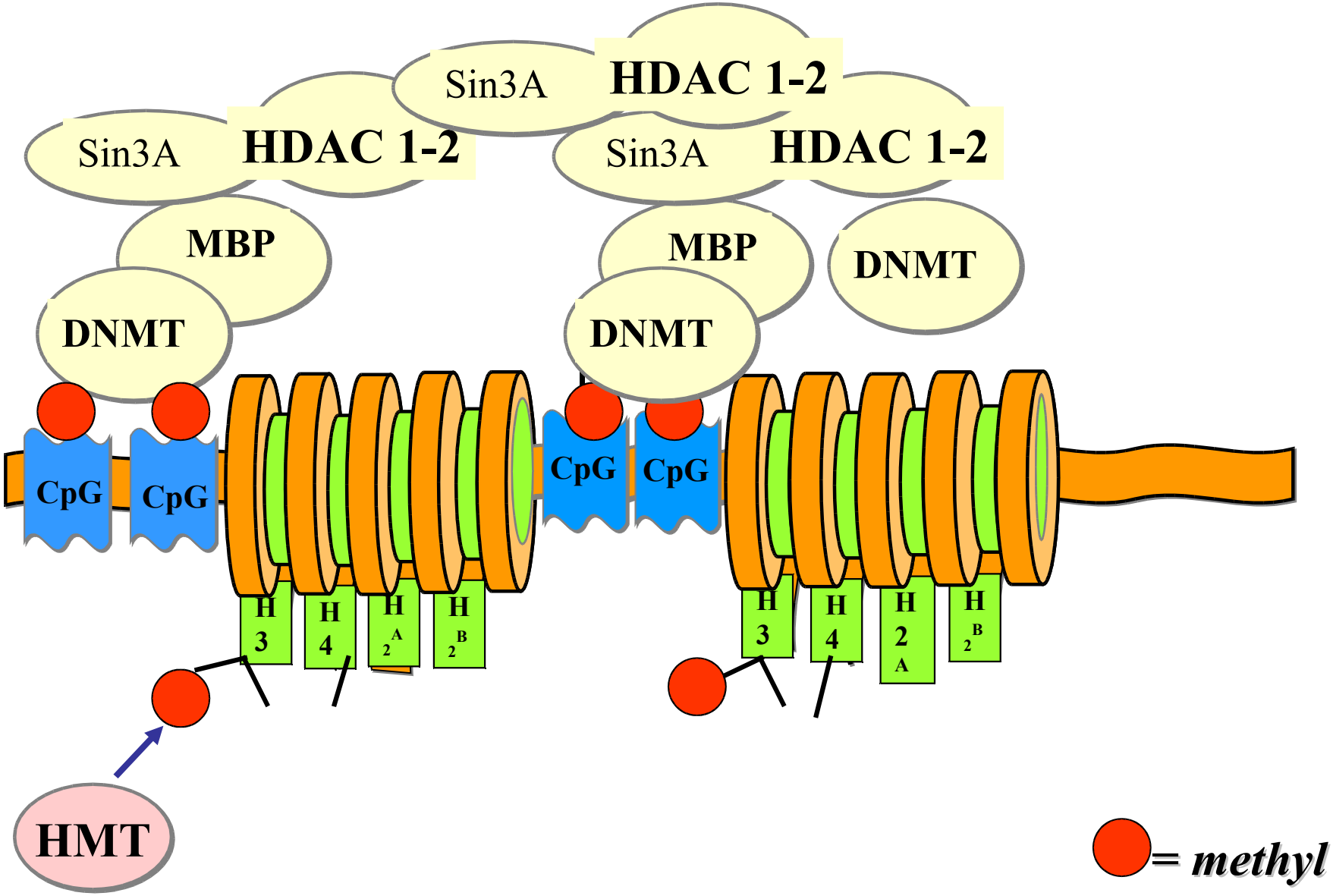
Caratteristica	Cromatina attiva (GENI HOUSEKEEPING)	Cromatina inattiva (GENI TESSUTO-SPECIFICI)
Conformazione della cromatina	Estesa, aperta	Condensata
Metilazione del DNA	Poco metilata specialmente nelle regioni del promotore	Metilata



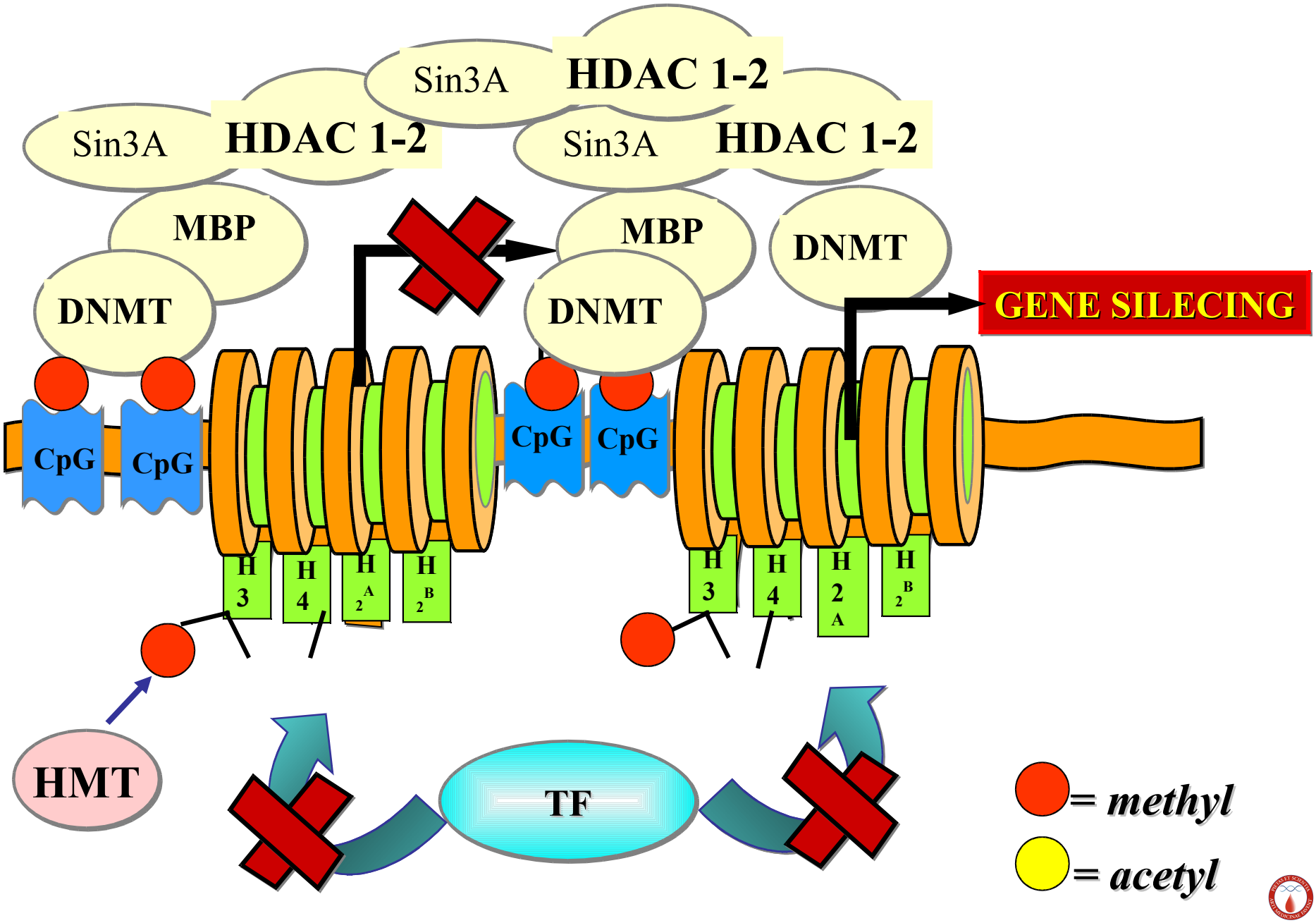
DNA hypermethylation in neoplastic cells



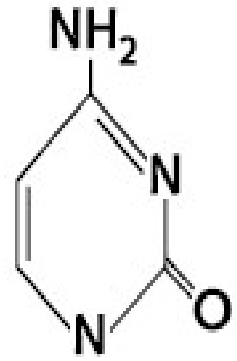
DNA hypermethylation in neoplastic cells



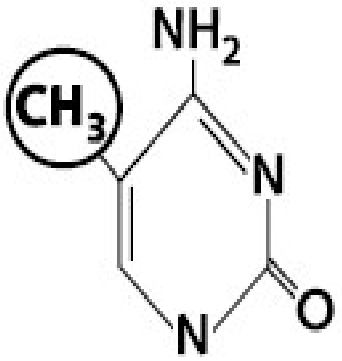
DNA hypermethylation in neoplastic cells



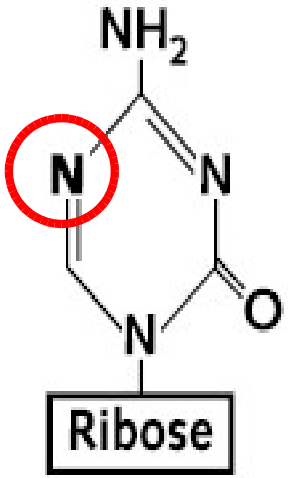
Epigenetic DRUGS



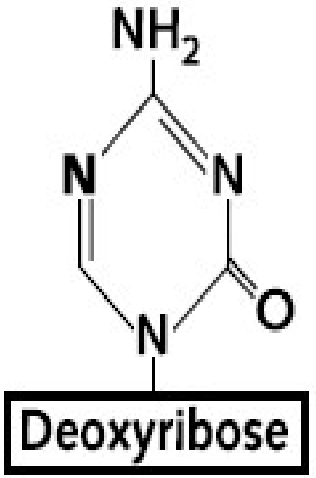
Cytosine



5-methyl-cytosine

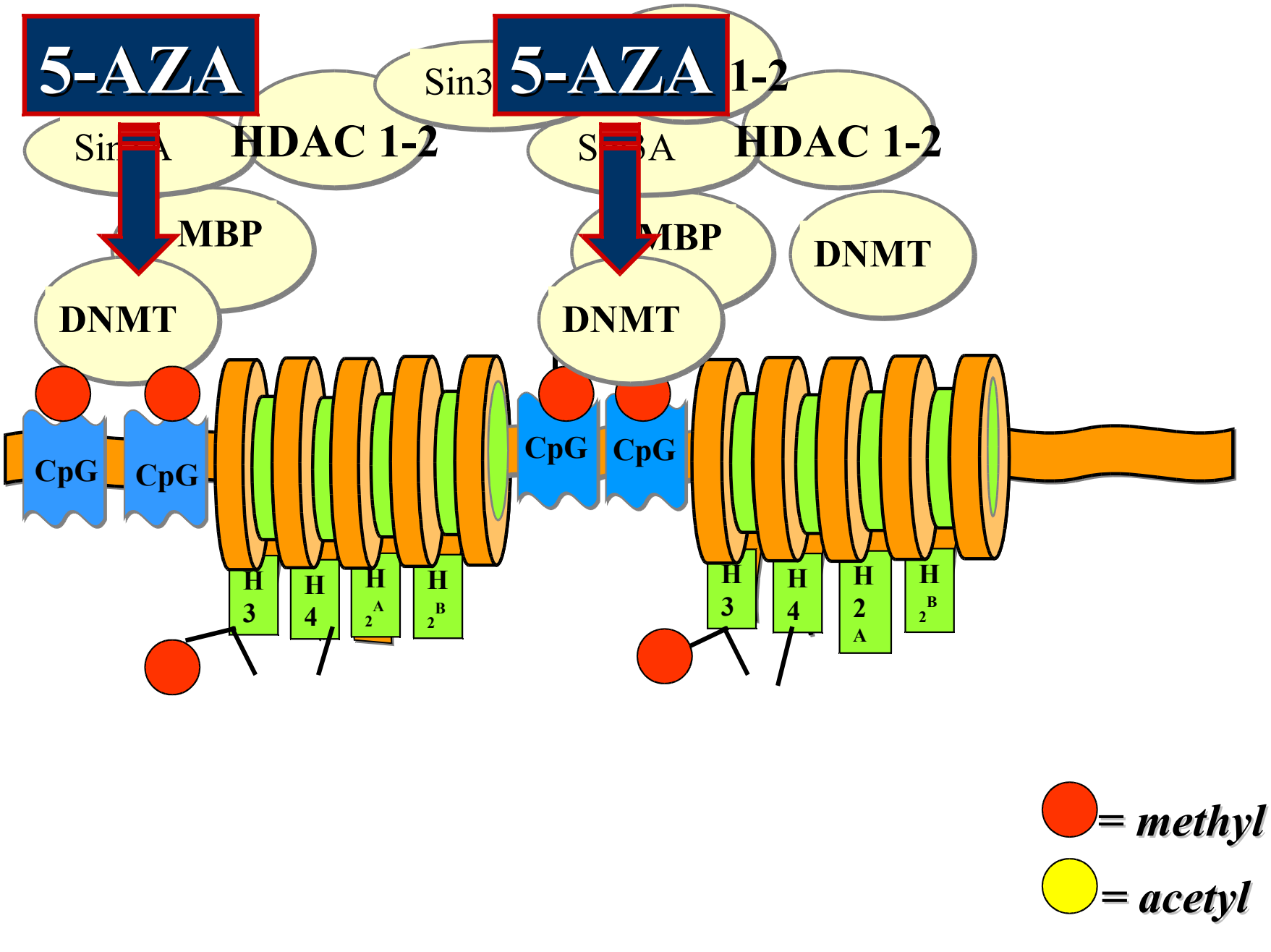


5-aza-cytidine

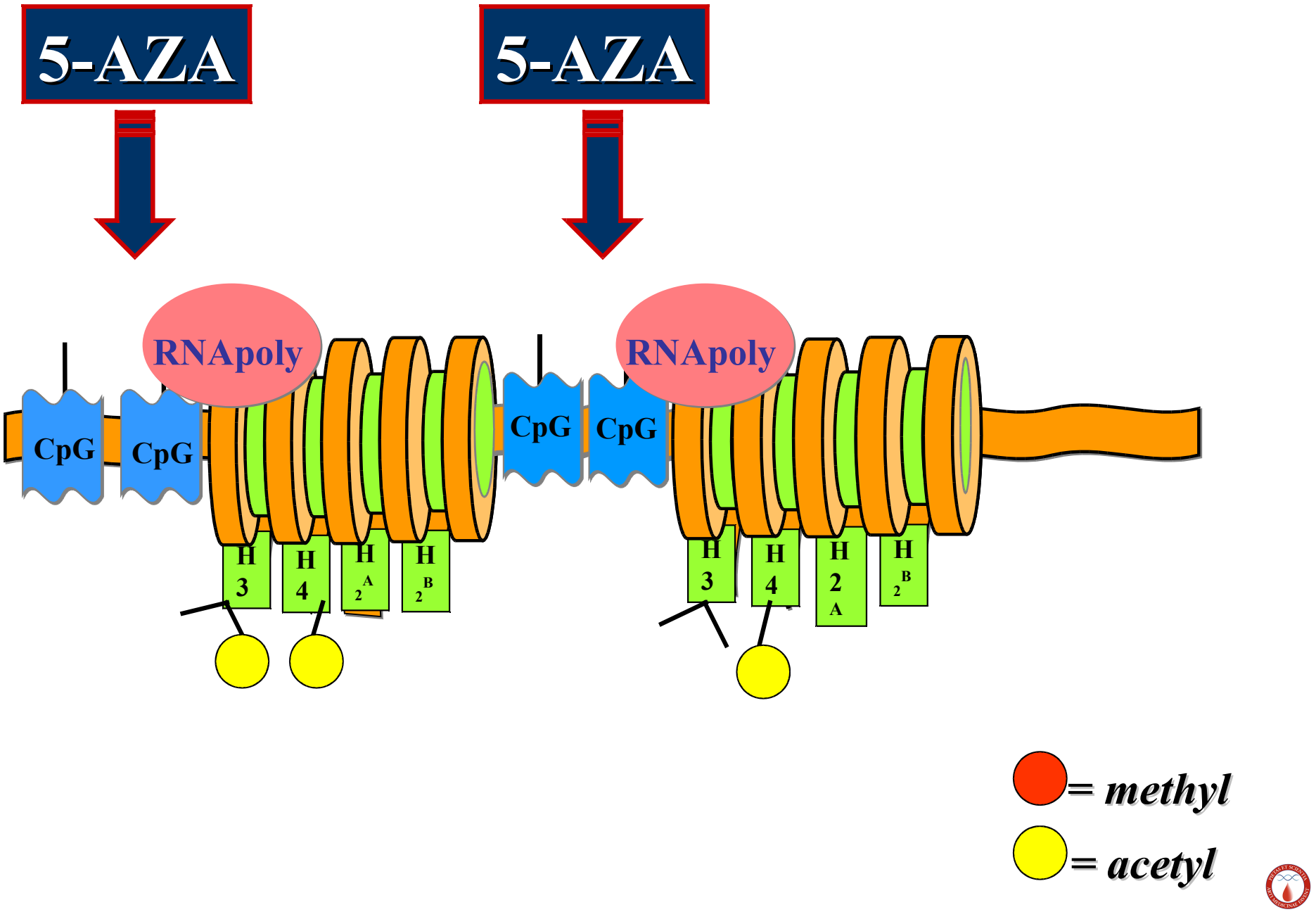


5-aza-2'-deoxy-cytidine

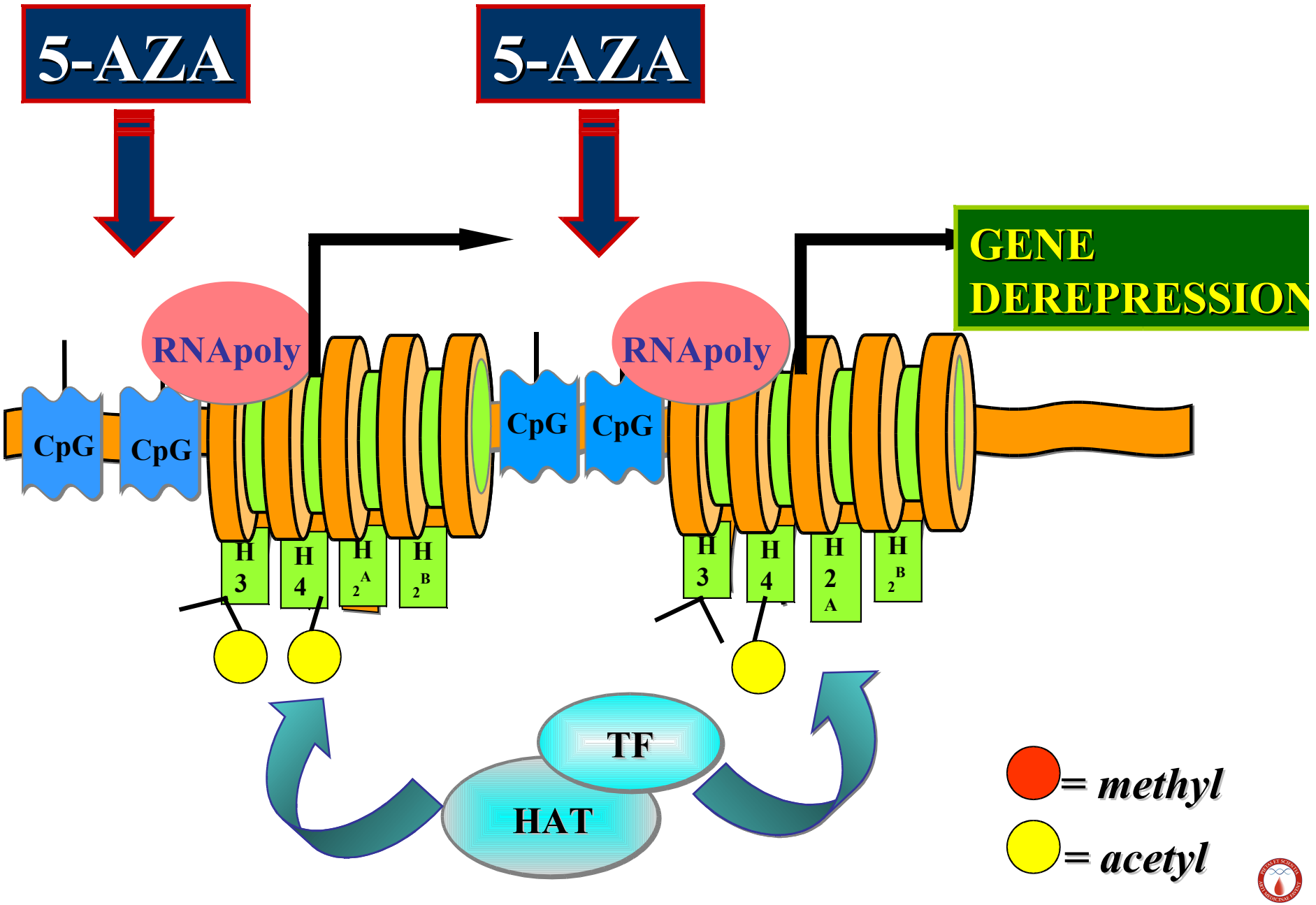
Epigenetic therapy



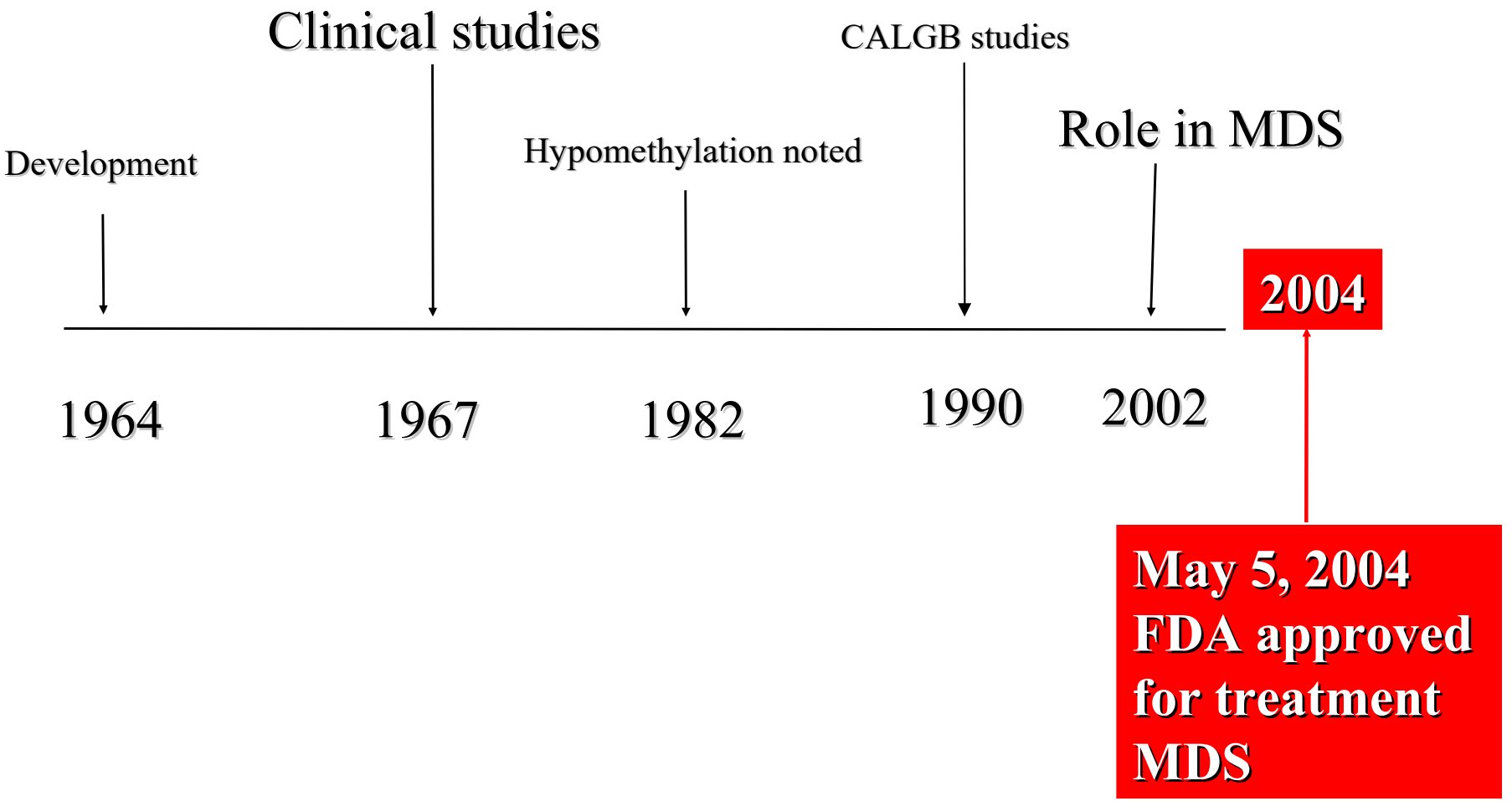
Epigenetic therapy



Epigenetic therapy

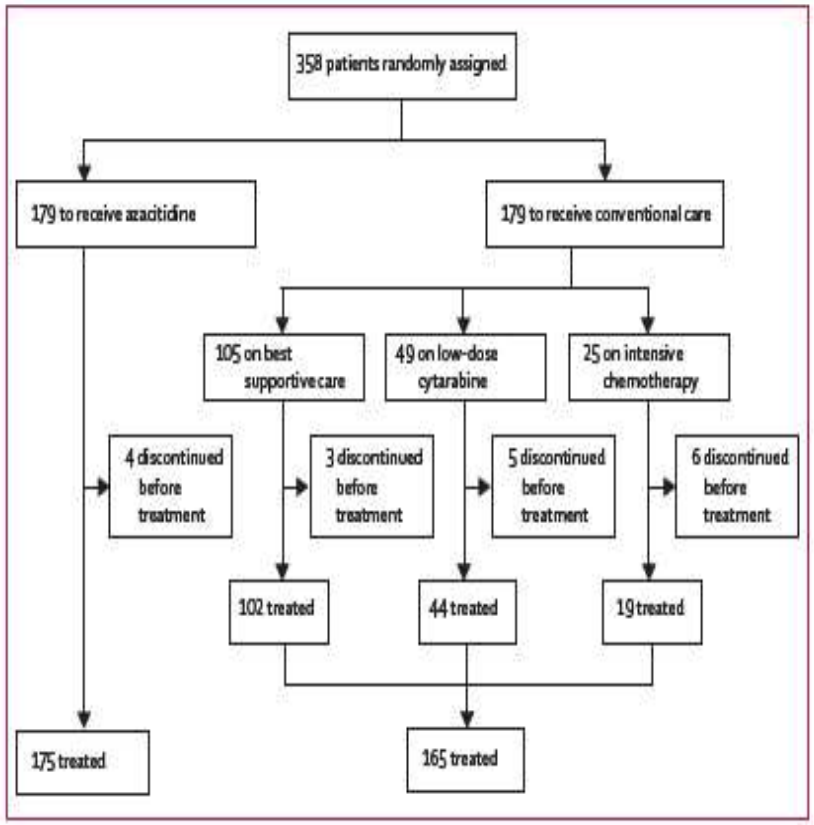


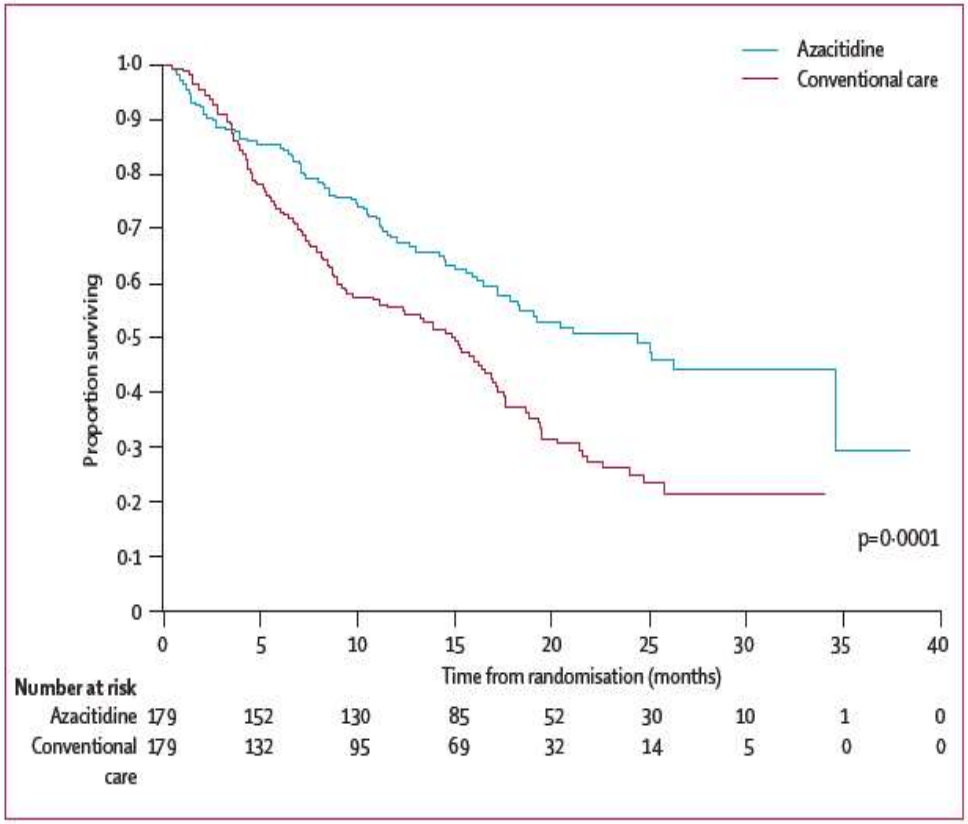
Azacitidine Time Line



Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study

Pierre Fenaux, Ghulam J Mufti, Eva Hellstrom-Lindberg, Valeria Santini, Carlo Finelli, Aristoteles Giagounidis, Robert Schoch, Norbert Gattermann, Guillermo Sanz, Alan List, Steven D Gore, John F Seymour, John M Bennett, John Byrd, Jay Backstrom, Linda Zimmerman, David McKenzie, CL Beach, Lewis R Silverman, for the International Vidaza High-Risk MDS Survival Study Group





Lancet Oncol 2009; 10: 223–32

Gene	%	Location	Function
SF3B1	28	2q33	Splicing factor
TET2	21	4q24	Control of cytosine hydroxymethylation
ASXL1	14	20q11	Epigenetic regulator
SRSF2	12	17q25	Splicing factor
RUNX1	9	21q22	Transcription factor
TP53	8	17p13	Transcription factor
U2AF1	7	21q22	Splicing factor
EZH2	6	7q36	Polycomb group protein
NRAS	4	1p13	Signal transduction
JAK2	3	9p24	Tyrosine kinase
ETV6	3	12p13	Transcription factor
CBL	2	11q23	Signal transduction
IDH2	2	15q26	Cell metabolism, epigenetic regulation
NPM1	2	5q35	Phosphoprotein
IDH1	1	2q33	As IDH1
KRAS	<1	12p12	Signal transduction
GNAS	<1	20q13	G protein
PTPN11	<1	12q24	Protein phosphatase
BRAF	<1	7q34	Raf kinase
PTEN	<1	10q23	Phosphatase
CDKN2A	<1	9q121	Cell cycle control

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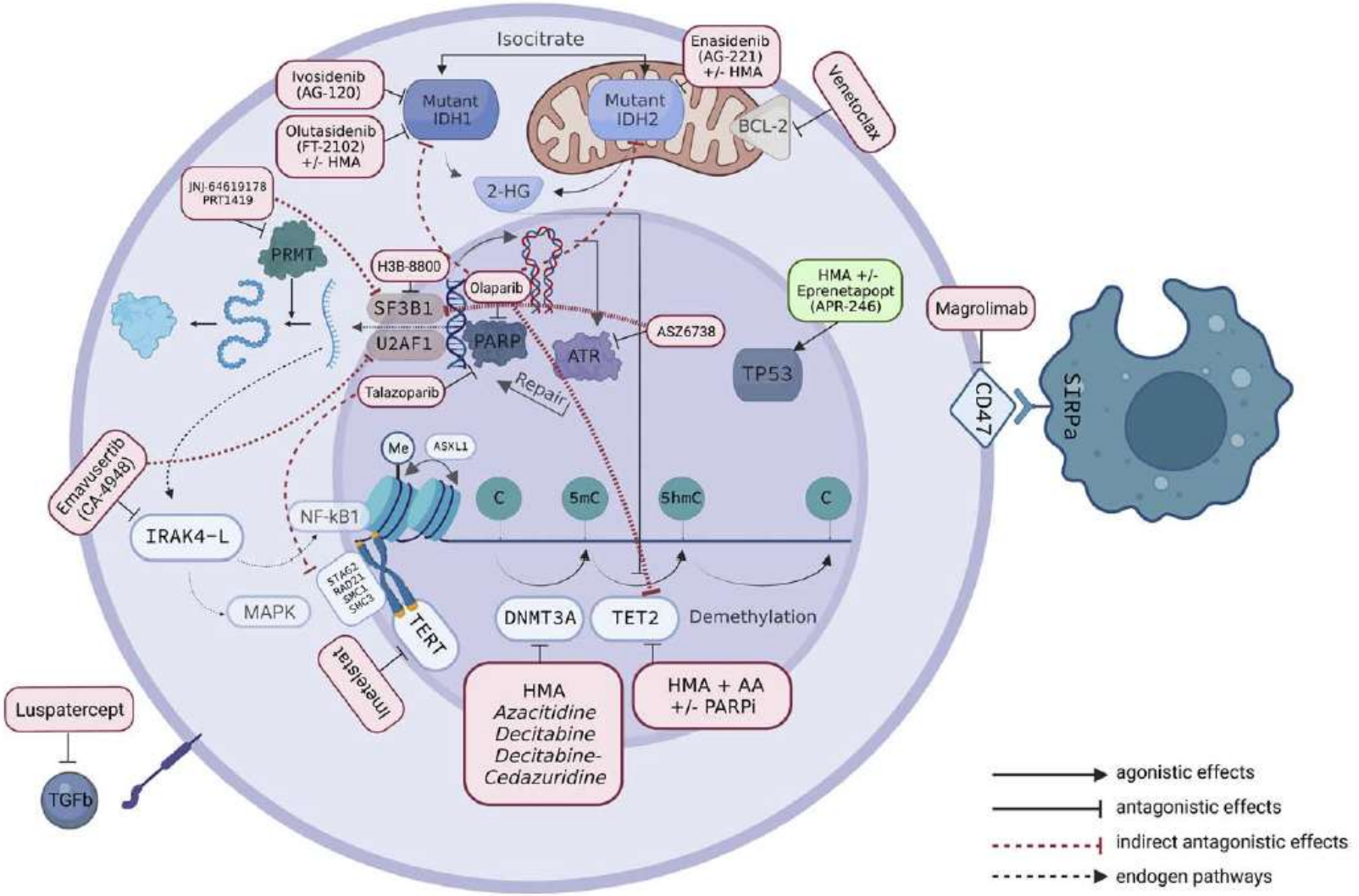
Treatment



Rotter LK et al. The Cancer J 2023