

LEUCEMIE ACUTE

Prof. MICHELE MALAGOLA



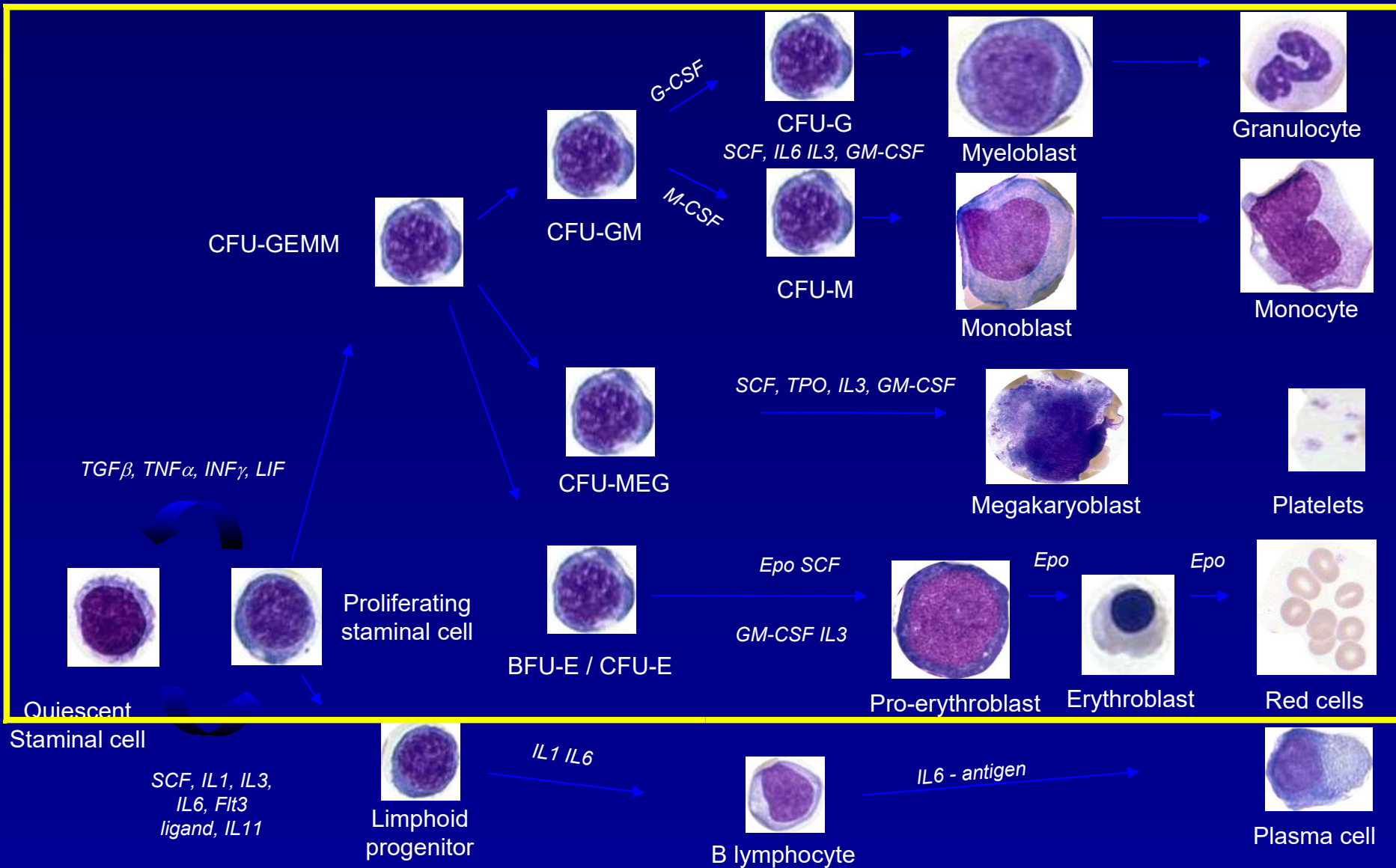
Cattedra di Ematologia
USD-Trapianti di Midollo Osseo per Adulti

SELF RENEWAL

COMMITMENT

PRECURSOR EXPANSION

TERMINAL DIFFERENTIATION



Emopoiesi normale

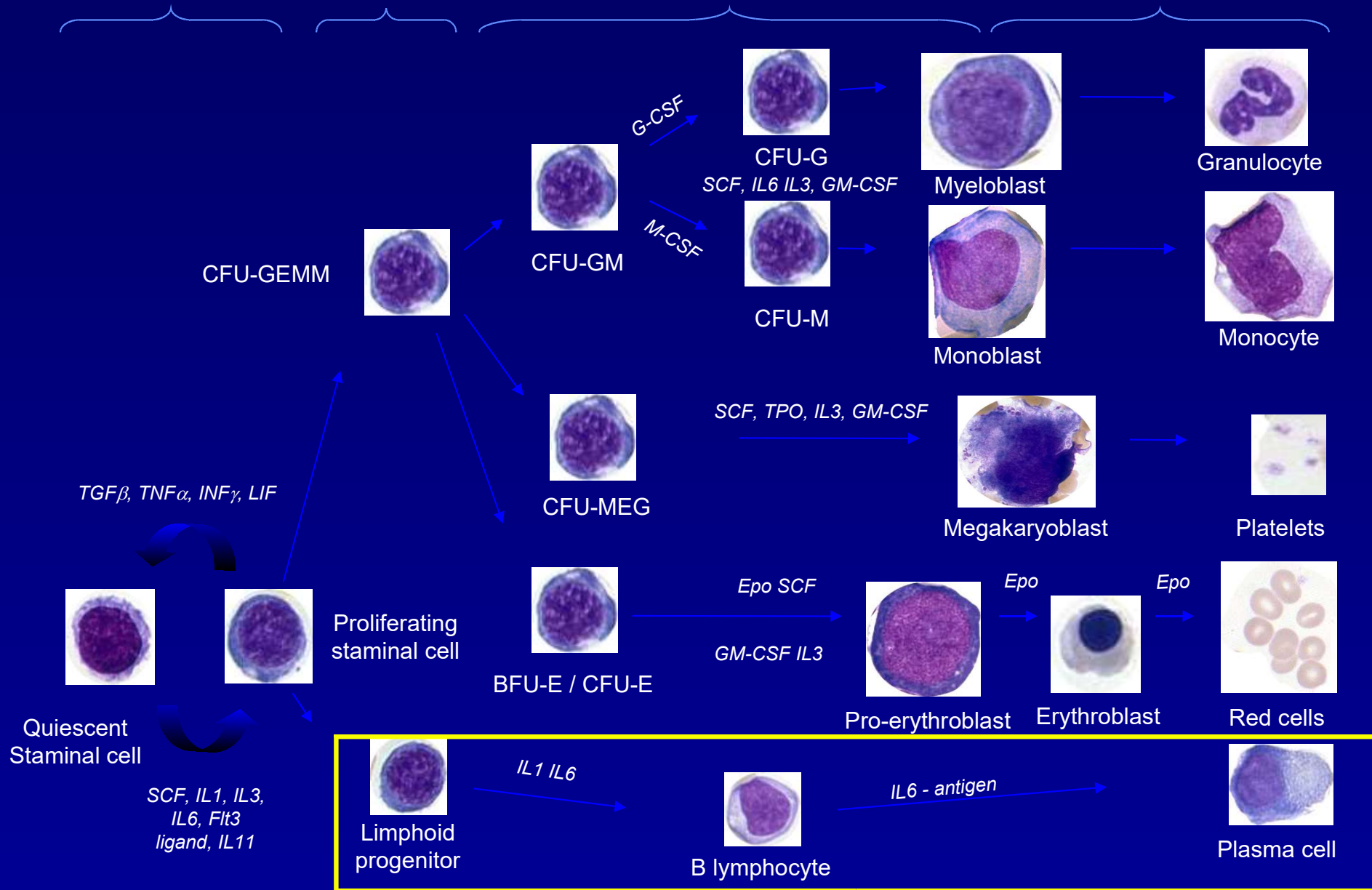


SELF RENEWAL

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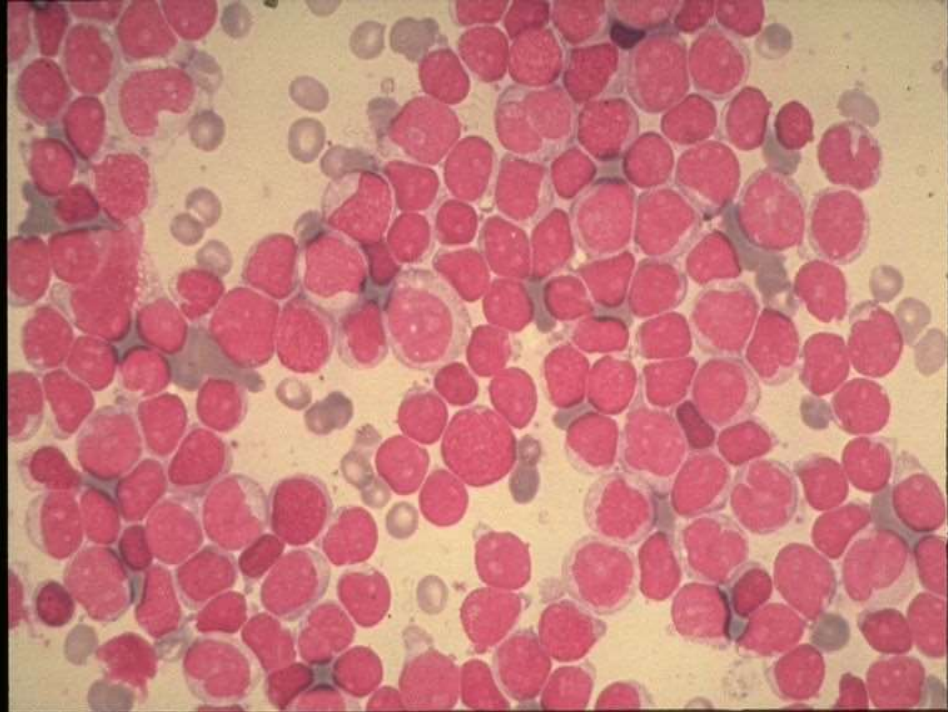
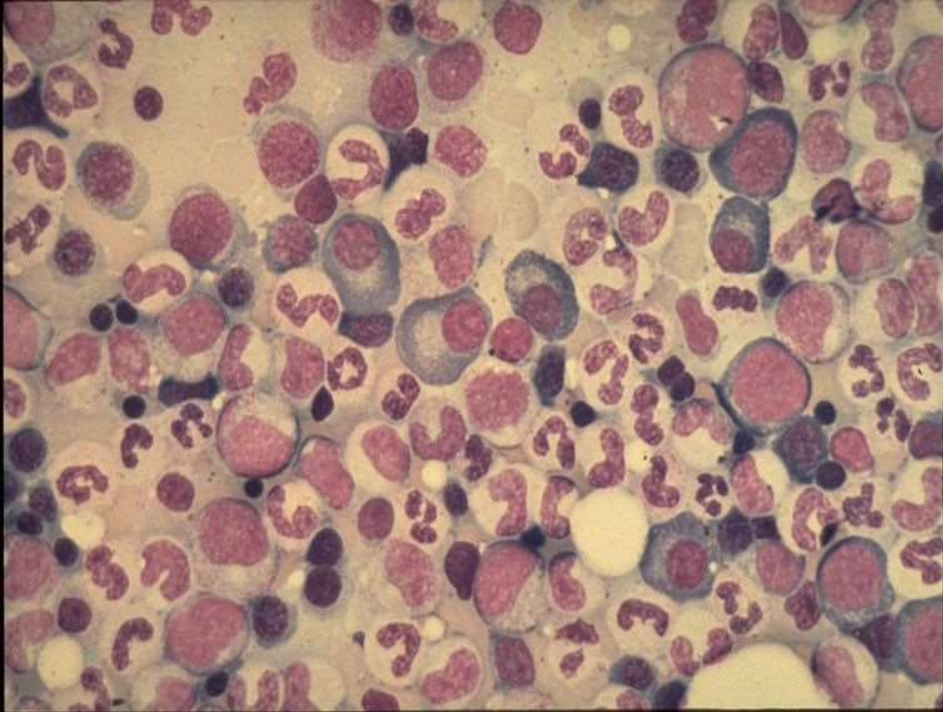
PRECURSOR EXPANSION

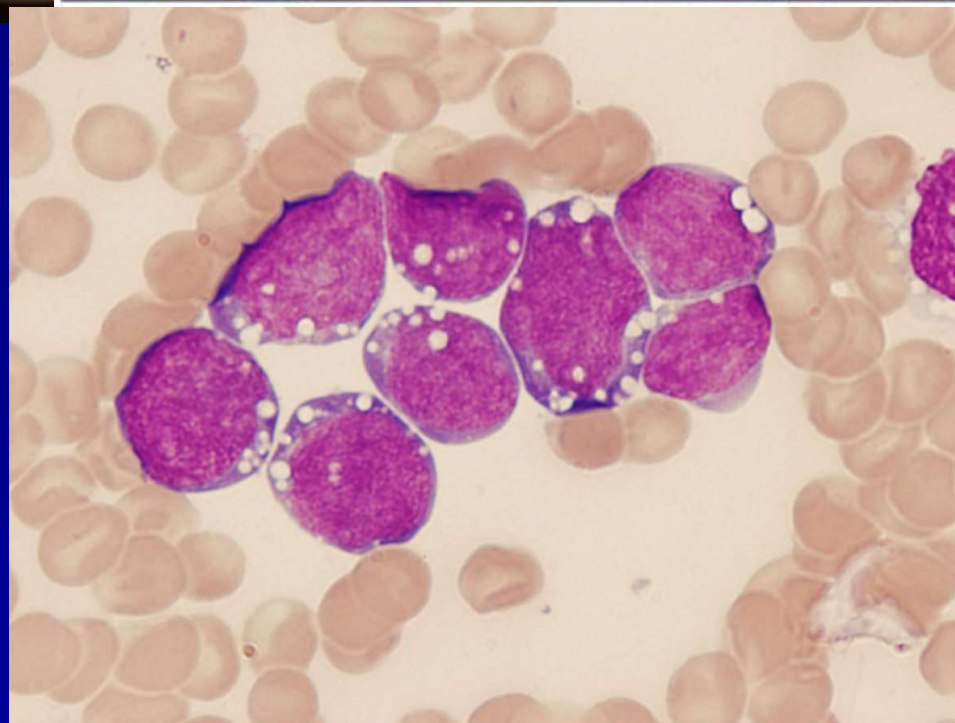
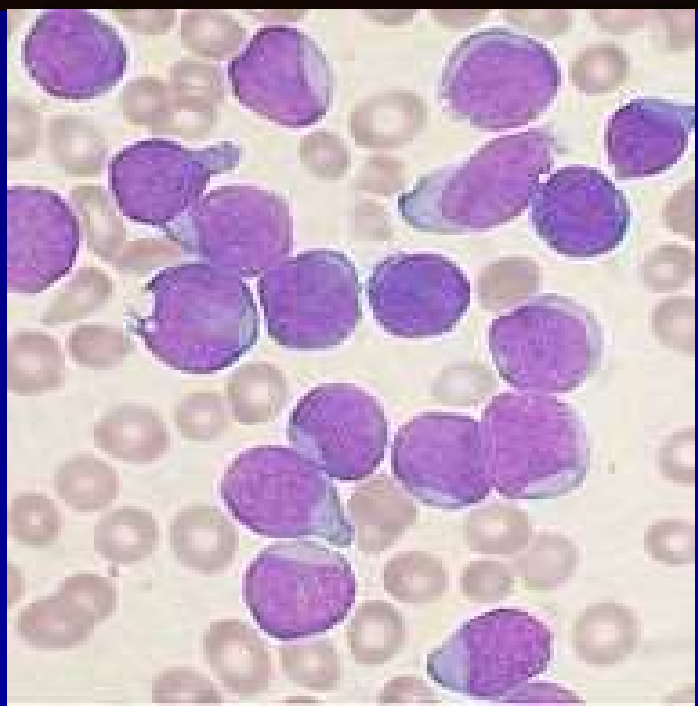
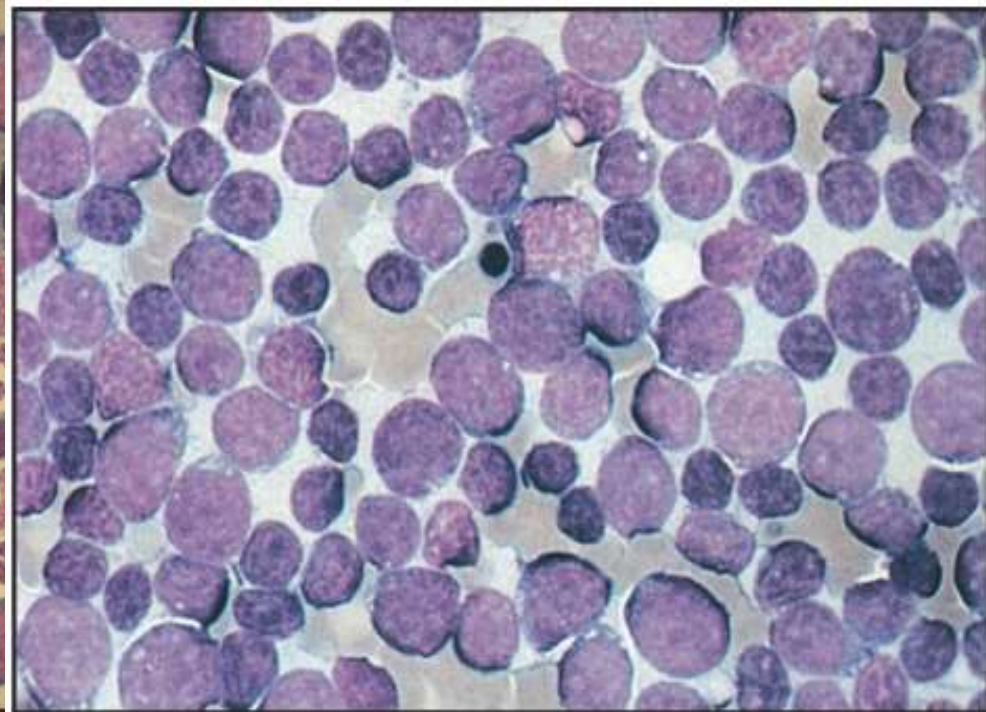
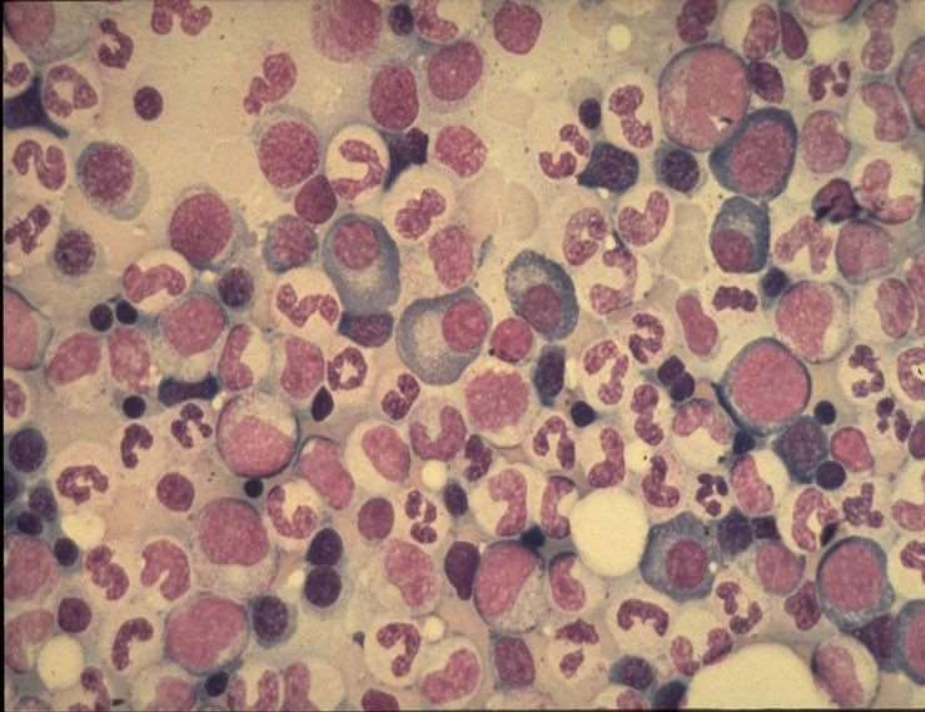
TERMINAL DIFFERENTIATION



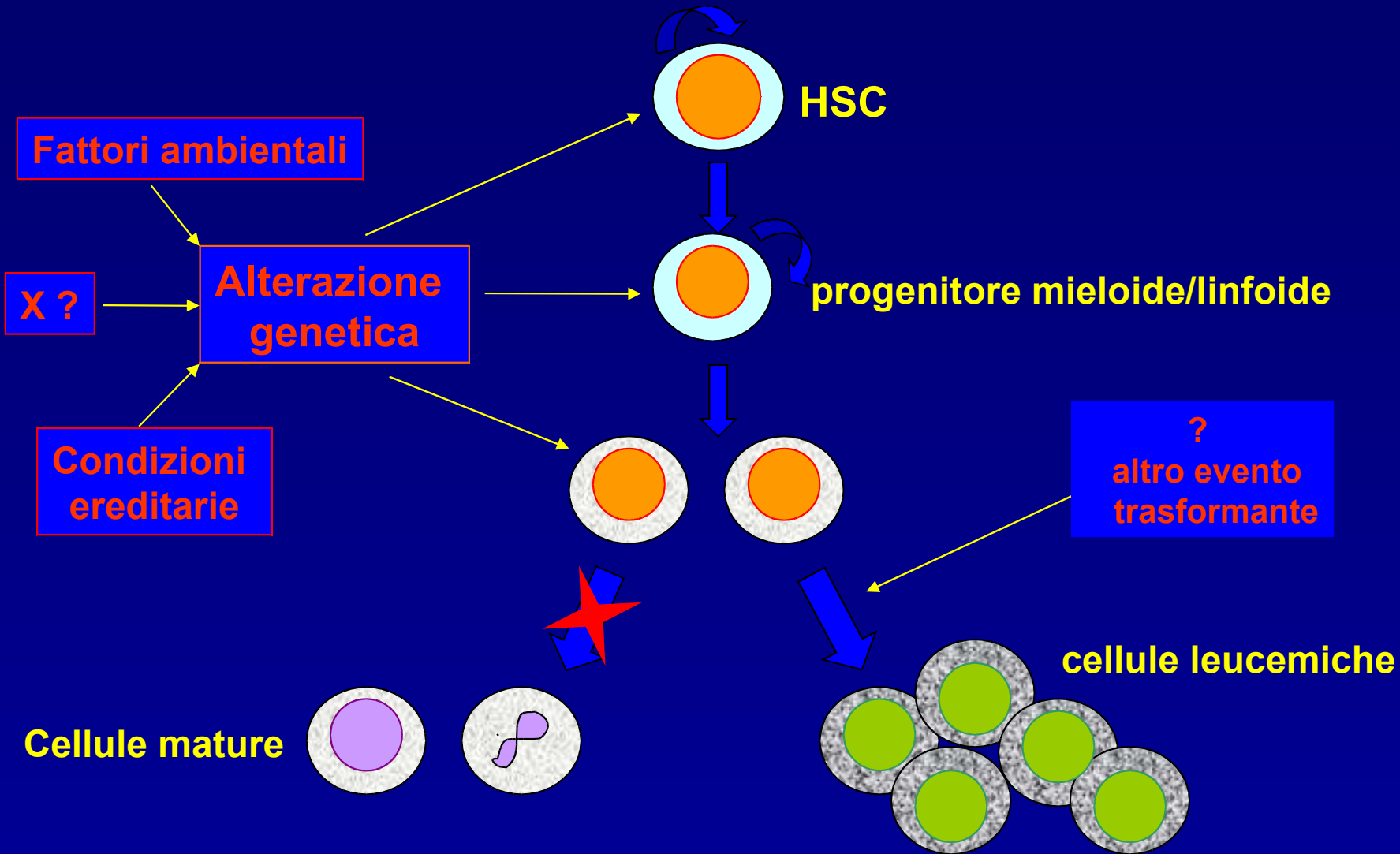
Emopoiesi normale



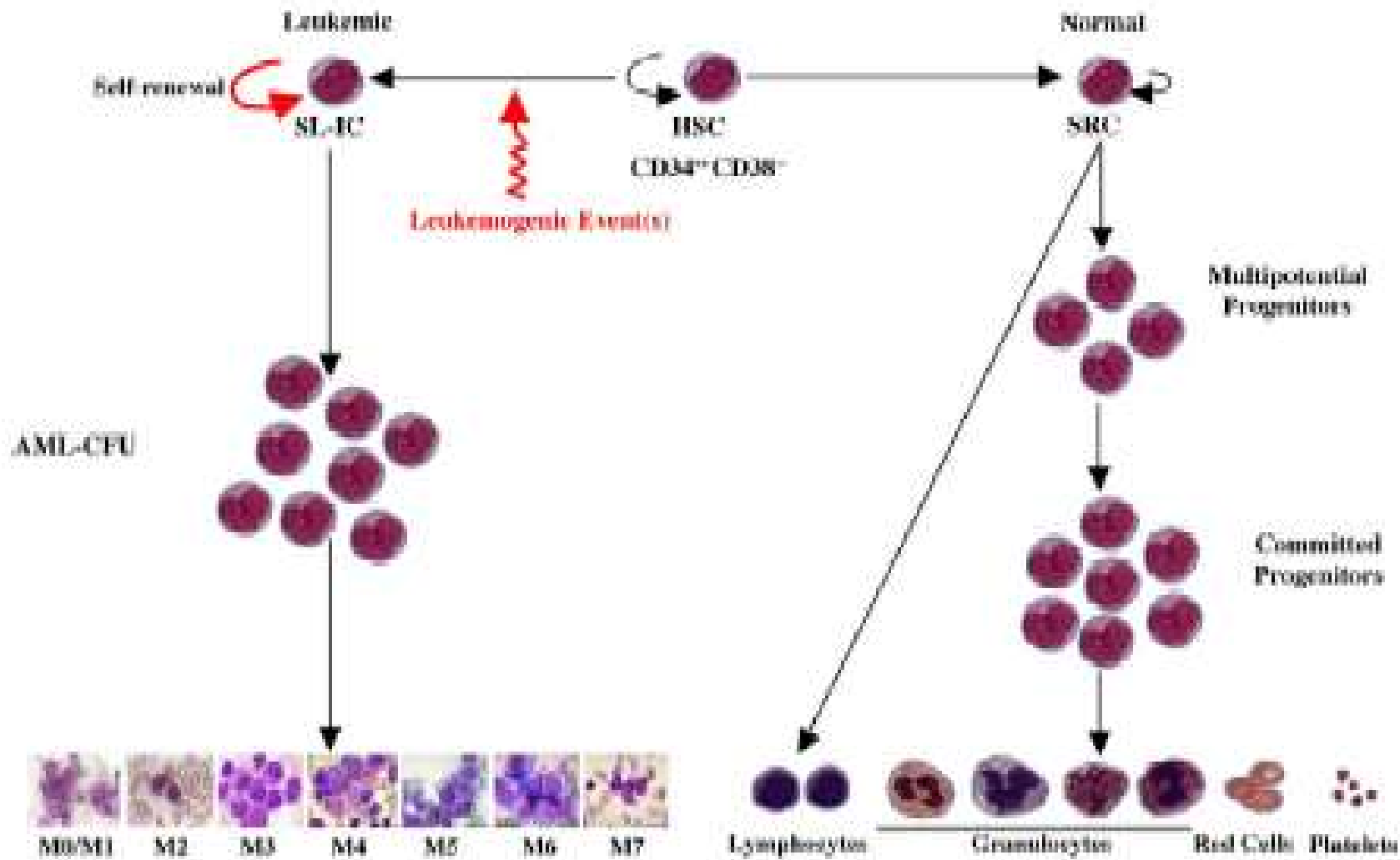




EZIOPATOGENESI DELLE LEUCEMIE ACUTE



LEUCEMOGENESI



1. A model of AML heterogeneity that postulates leukemogenic events occurring in primitive stem cells resulting in increased self-renewal, a brogation of self 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**)



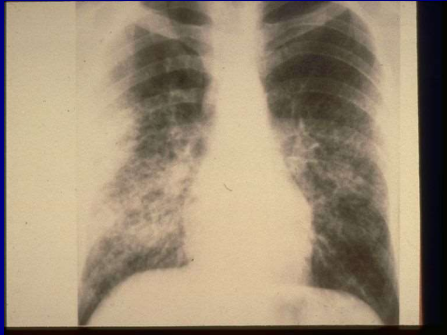
Leucemia Acuta

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 10⁹/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

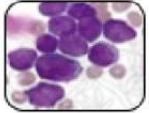


Analisi citofluorimetrica nelle leucemie acute: immunofenotipo

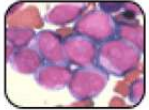
- **Diagnosi** delle leucemie acute
- **Classificazione** delle leucemie acute
- **Monitoraggio** malattia minima residua (sensibilità 10^{-3} – 10^{-4})



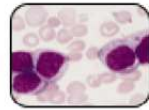
FAB CLASSIFICATION



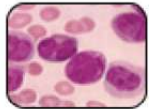
M0: Undifferentiated acute myeloblastic leukemia (5%)



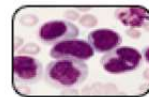
M1: Greater number of myeloblasts with <10% granulocytic differentiation.



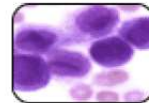
M2: Myeloblasts in great number with granulocytic differentiation >10%, NSE <20%.



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.



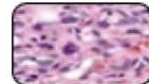
M4: >20% but <80% NSE-butyrate positivity in Monocytic cells



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

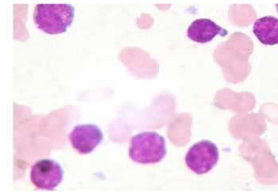


M6: >30% myeloblasts with more than 50% erythroblasts eliminating the erythroid cells.



M7: Acute megakaryoblastic leukemia <5%

FAB classification of lymphoblastic leukaemia



L1 Lymphoblastic leukaemia with homogeneous structure

Frequency:

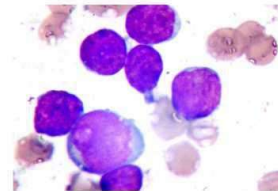
Between 25% and 30% of cases in adults, and 85% of cases in children.

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:

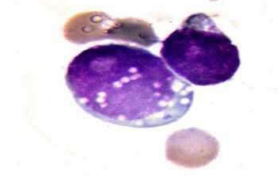
Accounts for 70% of cases in adults, and 14% in children.

Morphology:

Nucleus is irregular, heterogeneous chromatin structure, large nucleoli.

Immunophenotype

B:	T:
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L3 Burkitt's leukaemia

Frequency:

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

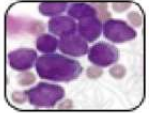
Morphology:

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

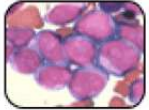
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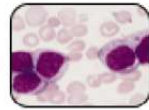
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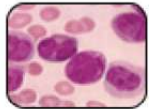
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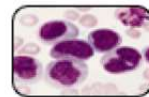
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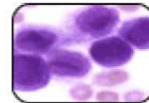
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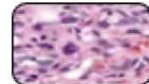
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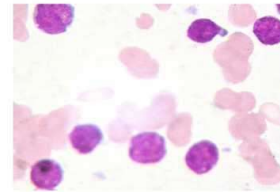
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Molecular classification

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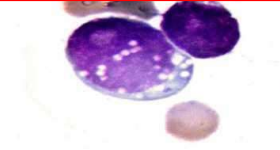
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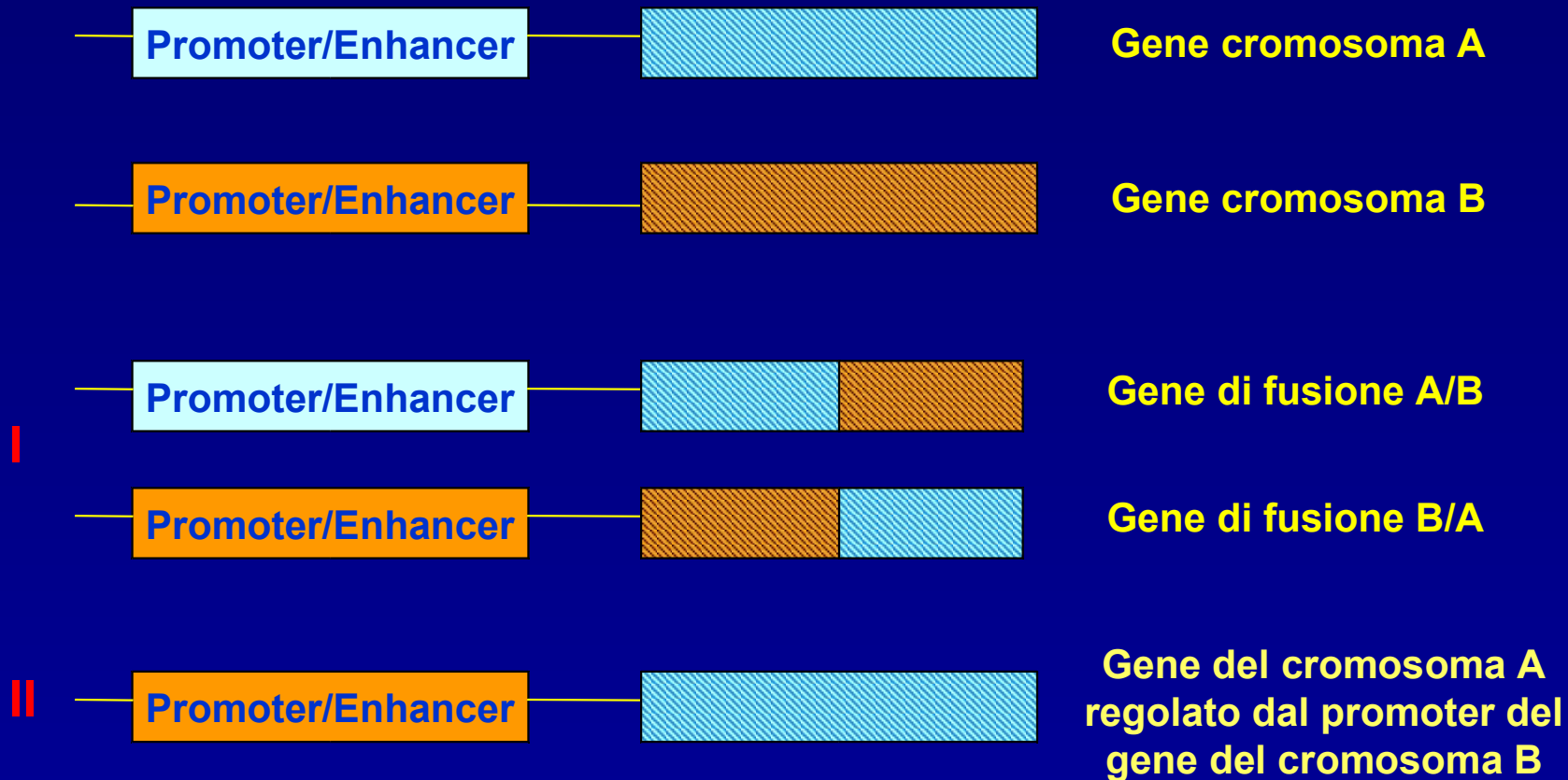
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*CD20	*CD4
*Cytoplasmic or superficial immunoglobulin	

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

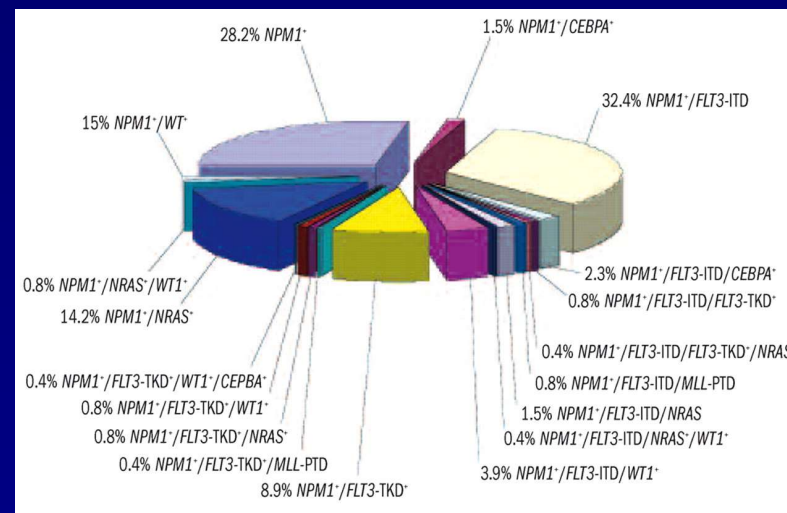
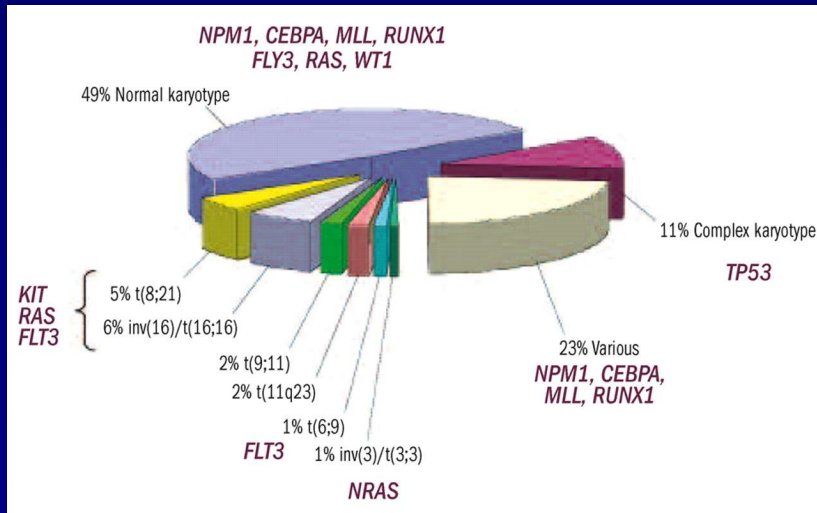
- B-lymphoblastic leukaemia/lymphoma, not otherwise specified
- B-lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities
 - B-lymphoblastic leukaemia/lymphoma with t(9;22)(q34.1;q11.2); BCR-ABL1
 - B-lymphoblastic leukaemia/lymphoma with t(v;11q23.3); KMT2A-rearranged
 - B-lymphoblastic 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

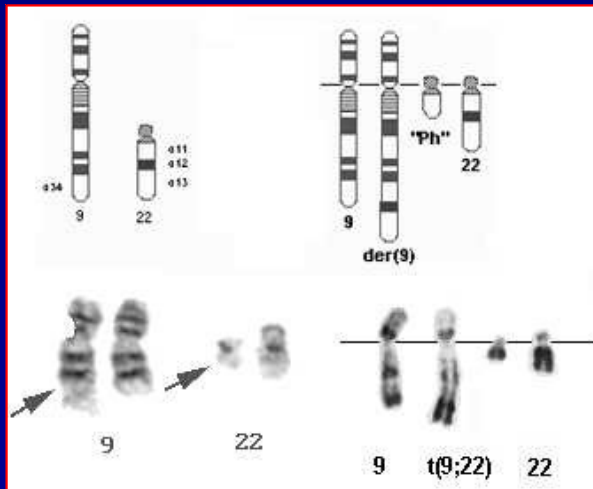
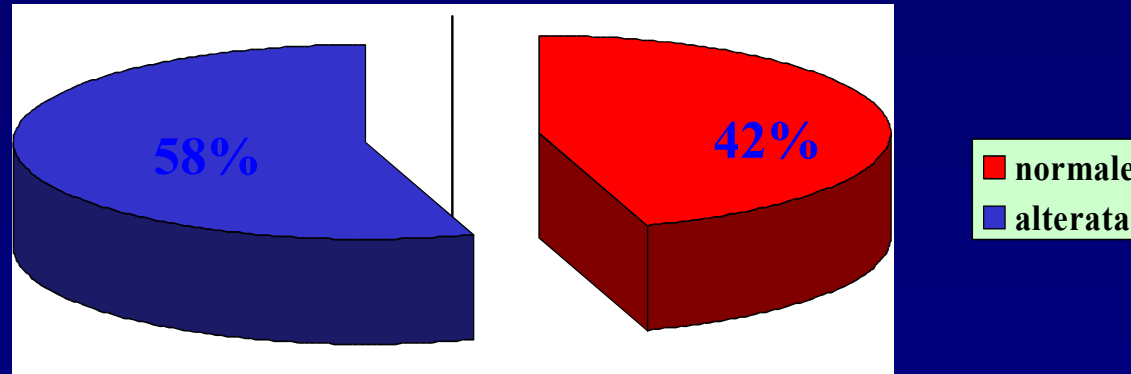


LAM - Citogenetica

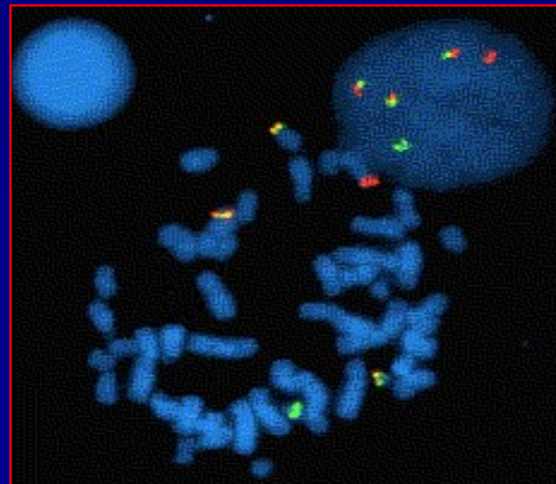


1612 casi LAM diagnosi

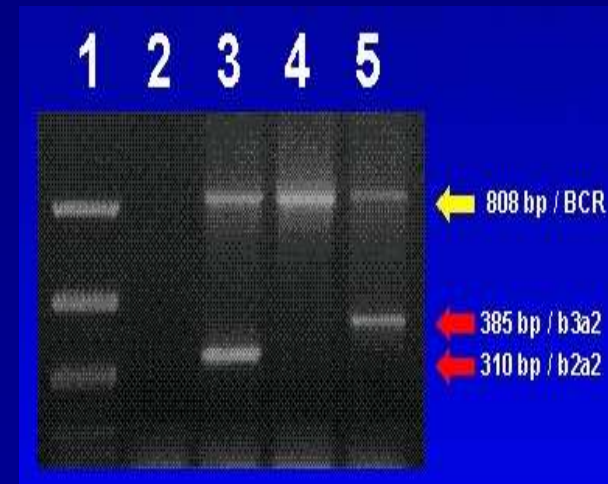
Grimwade, Blood 1998



Citogenetica Convenzionale



Citogenetica Molecolare (FISH)

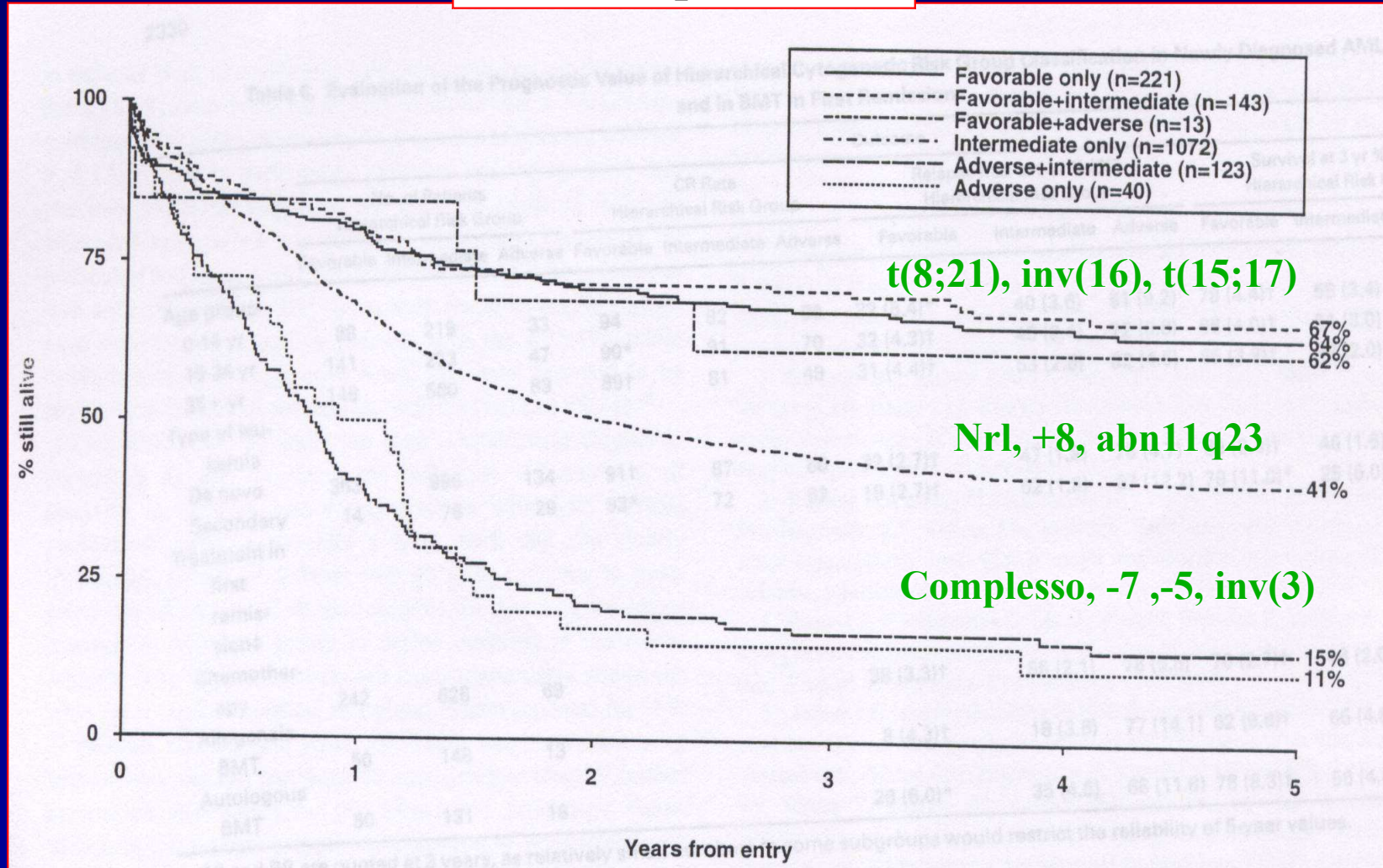


Biologia Molecolare

I parametri biologici e la prognosi delle LA

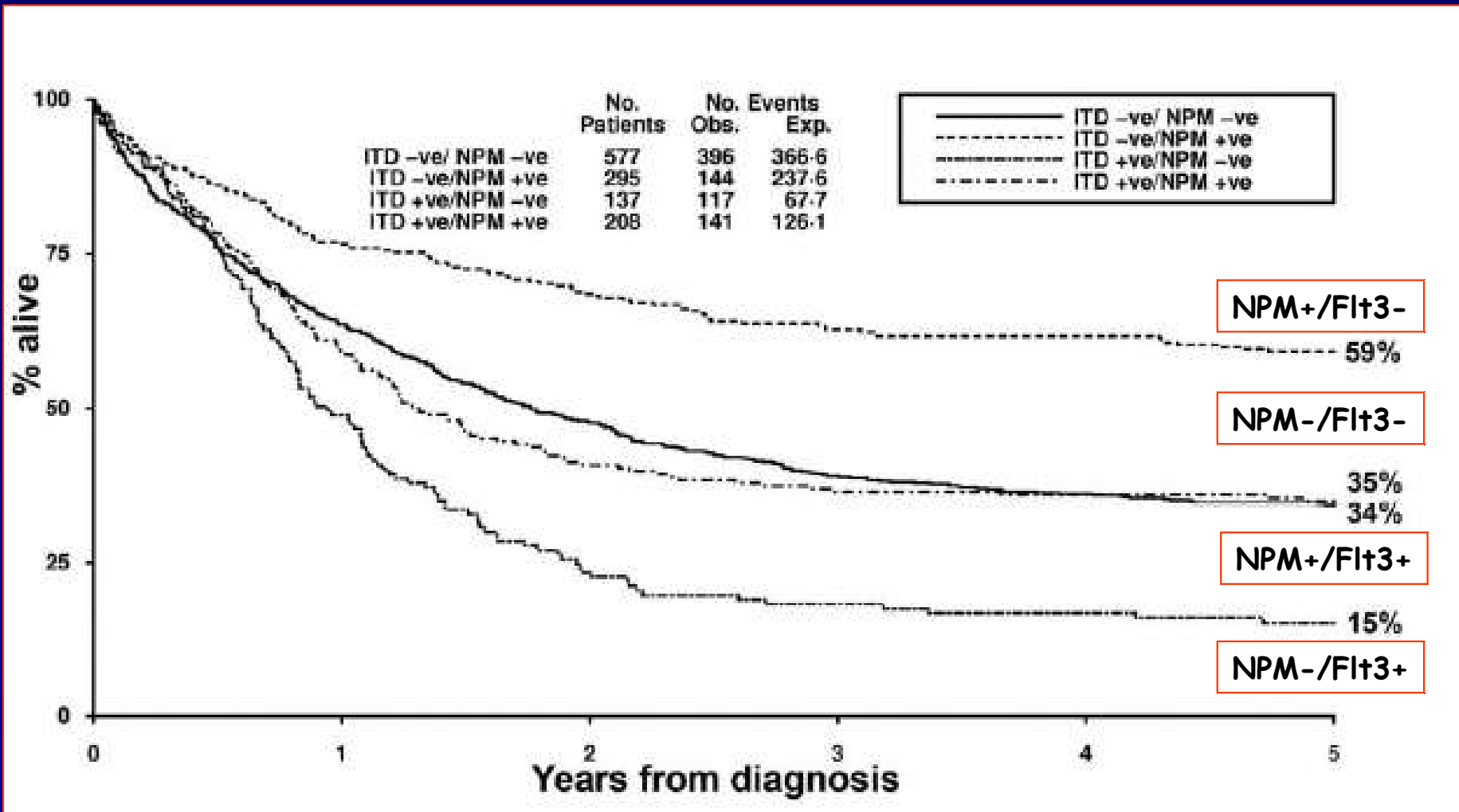
OS 1612 pz < 55 aa

Grimwade et al. Blood 1998



Cy-NPM e Flt3-ITD (3)

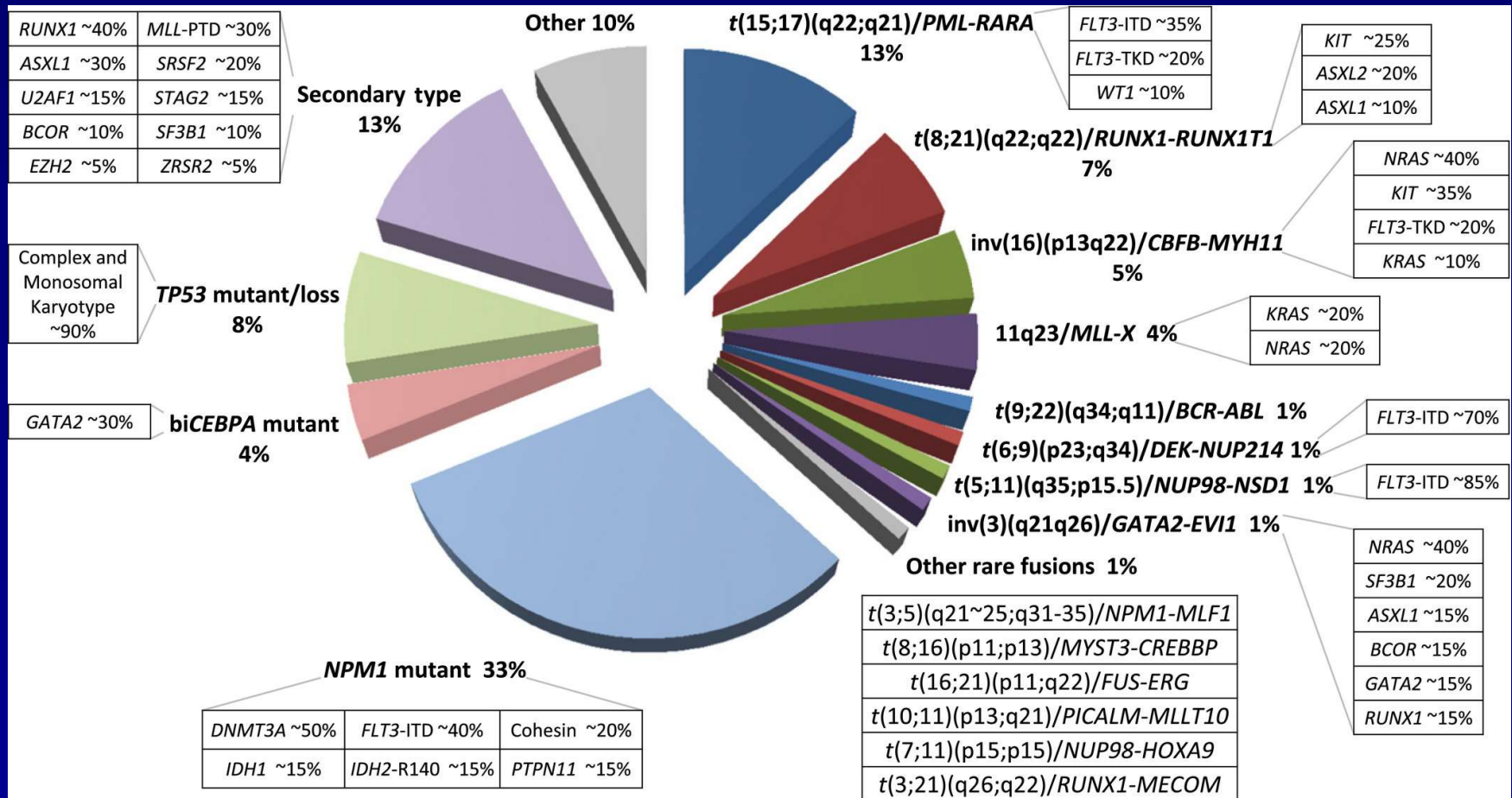
OS



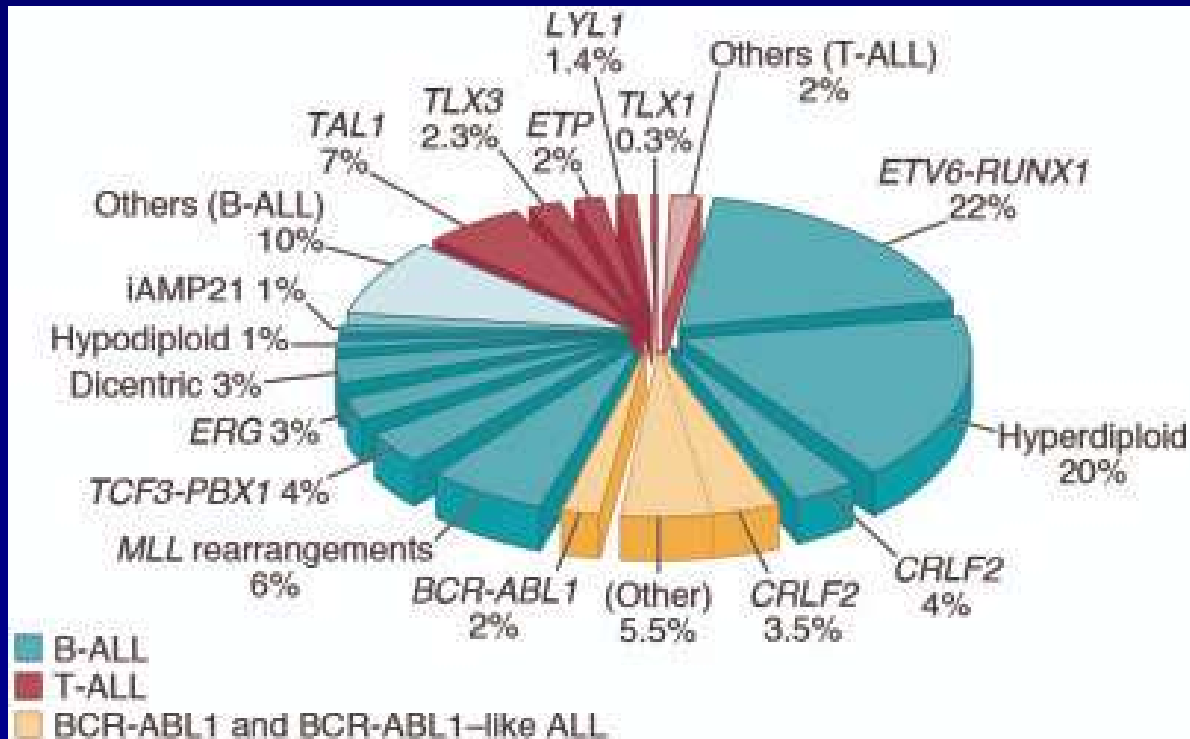
Gale R, Blood 2008 (MRC)

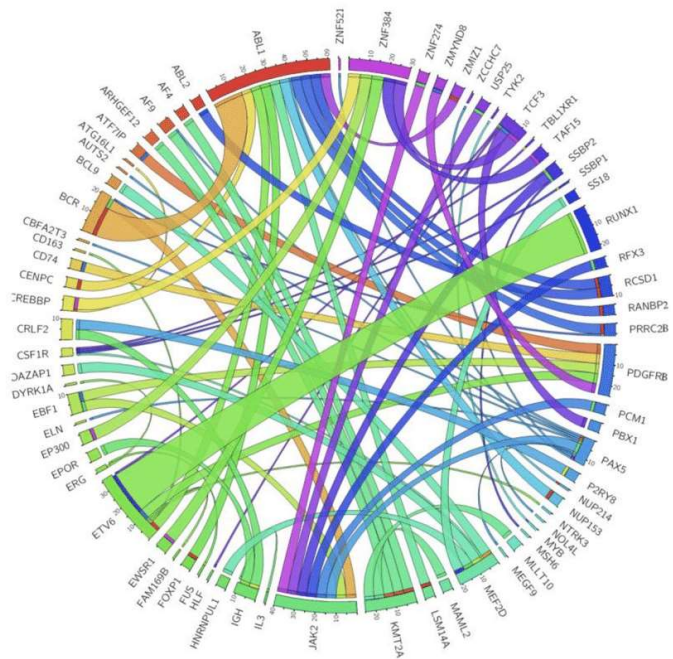


Genomic alterations by NGS - AML

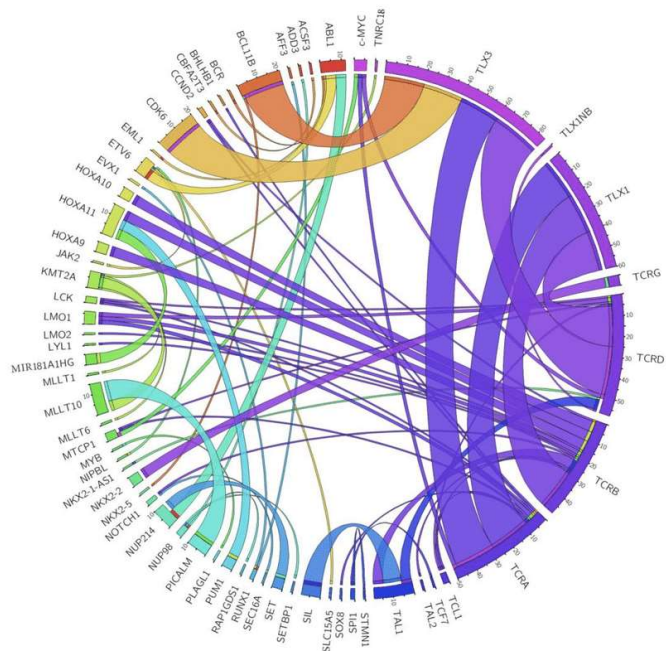


Genomic alterations - ALL





(A)



(B)

Genomic alterations by NGS - ALL

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

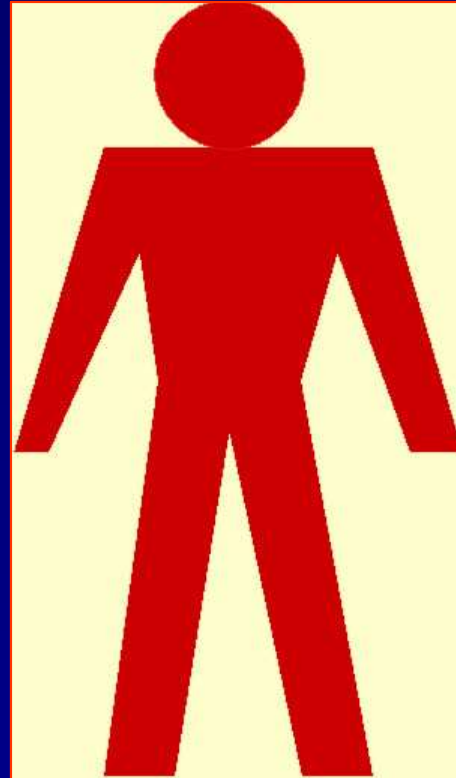
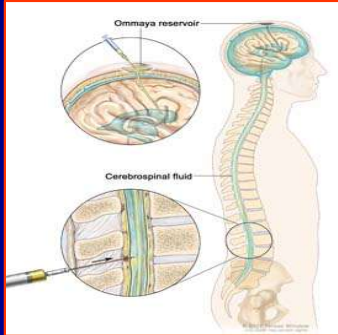


I "vecchi" (Storici) fattori prognostici clinici

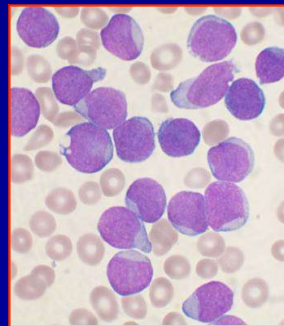
Localizzazioni cutanee



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:

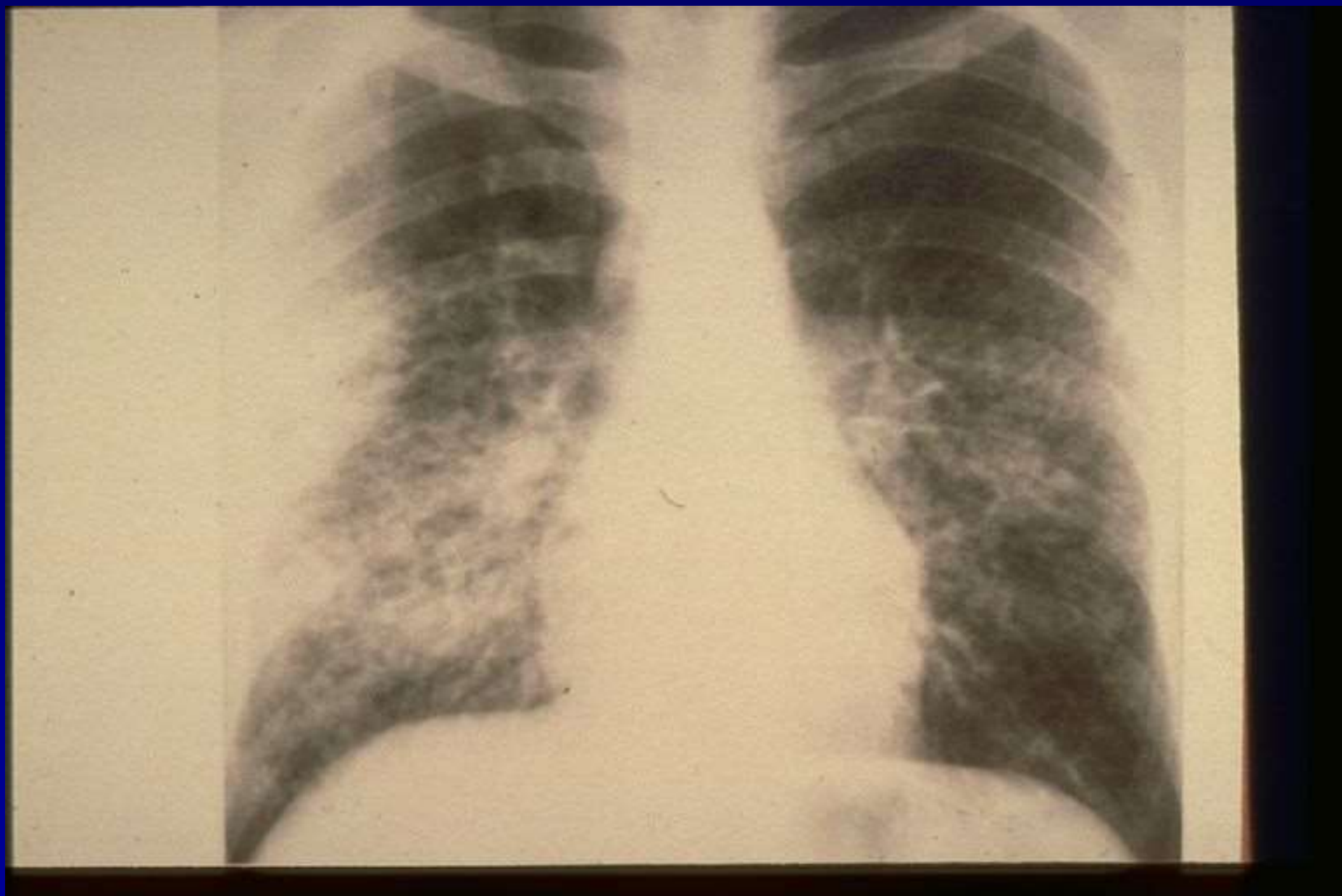
TRASFUSIONE DI UNITA' DI GLOBULI ROSSI CONCENTRATI.

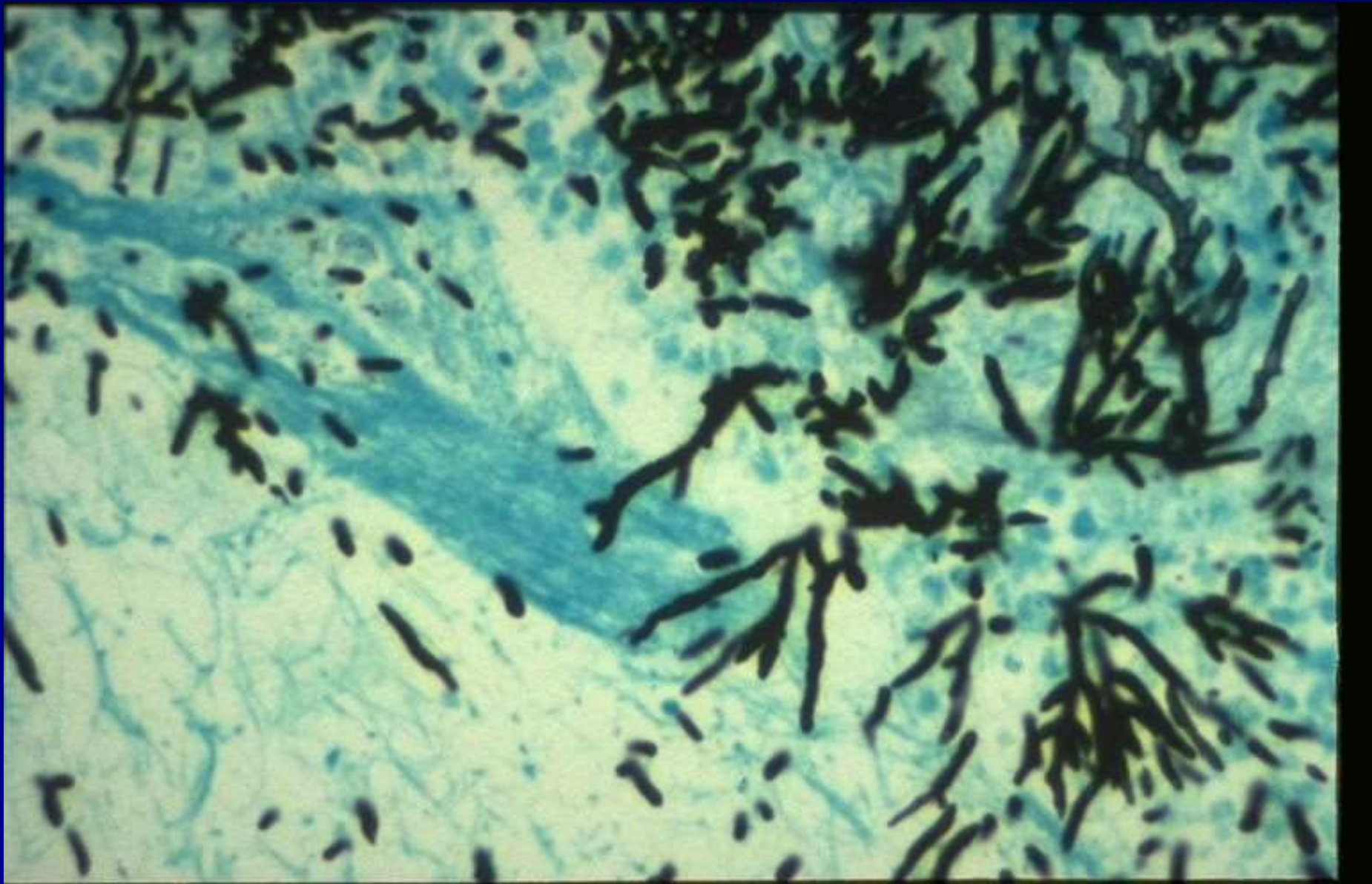
Hb >9g/dl

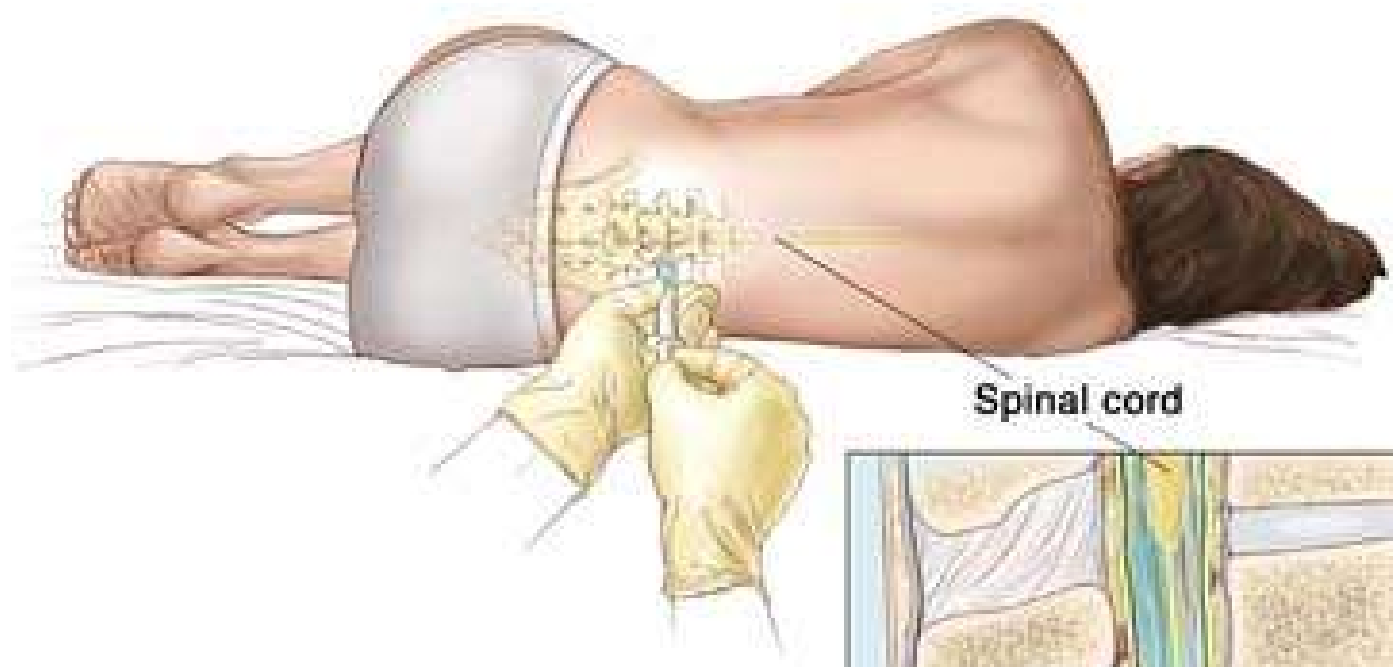
PIASTRINOPENIA:

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





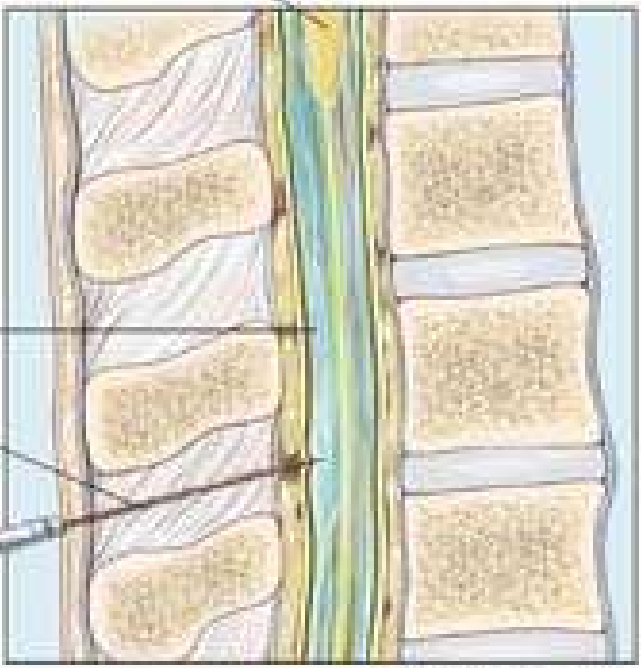




Spinal cord

Cerebrospinal fluid

Spinal needle



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LEUCEMIE ACUTE : I RISULTATI

LAM

LAM-M3

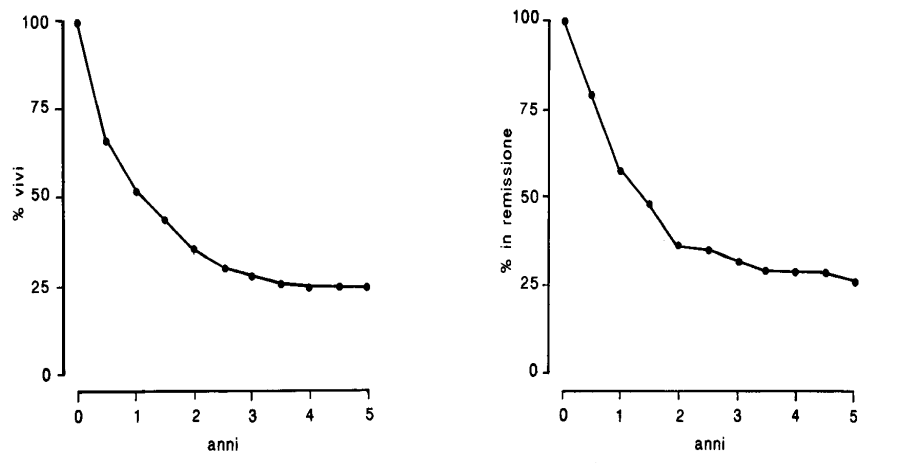
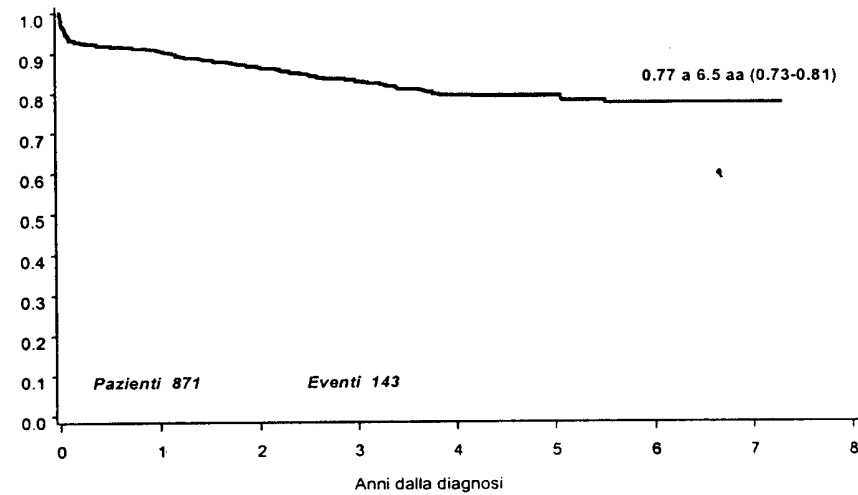
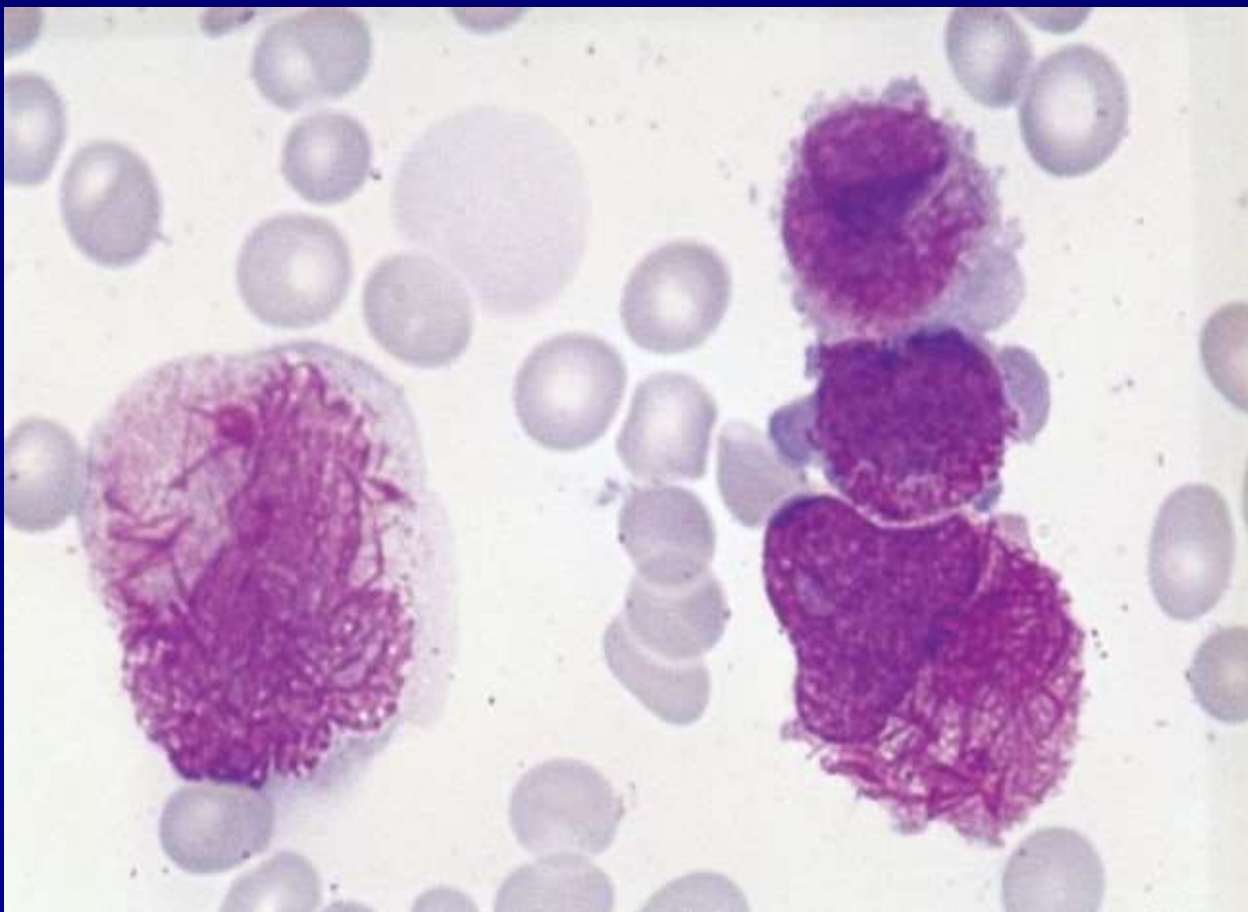


Figura 3.9 - Curve attuariali di durata della vita (a sinistra) e di durata della prima remissione (a destra) di 466 casi di LAM. Le due curve sono simili e poi diventano praticamente identiche perché i pazienti che restano in remissione possono diventare lunghi sopravvissuti. Dopo 5 anni le ricadute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

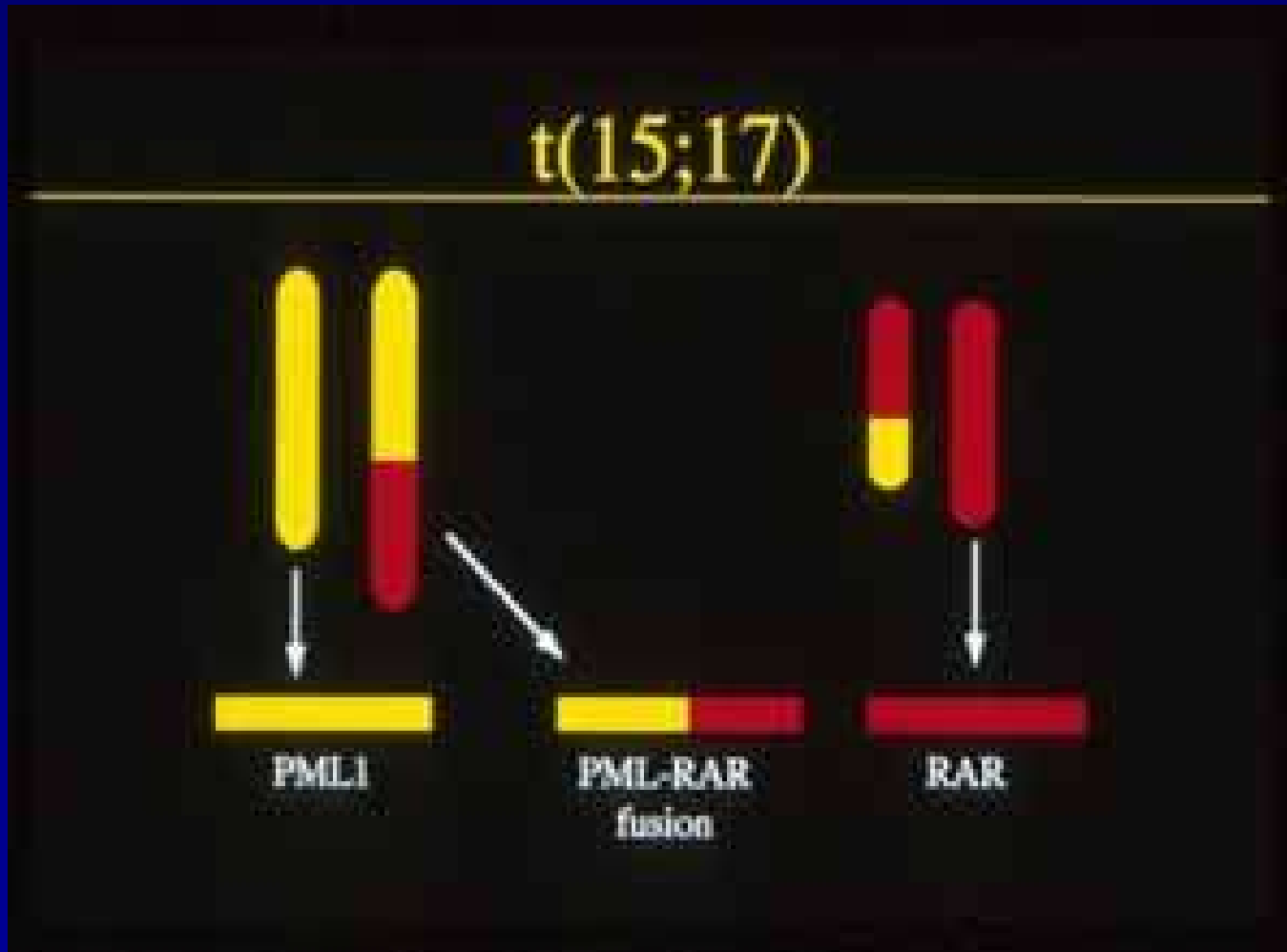
LAP0493: Sopravvivenza



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

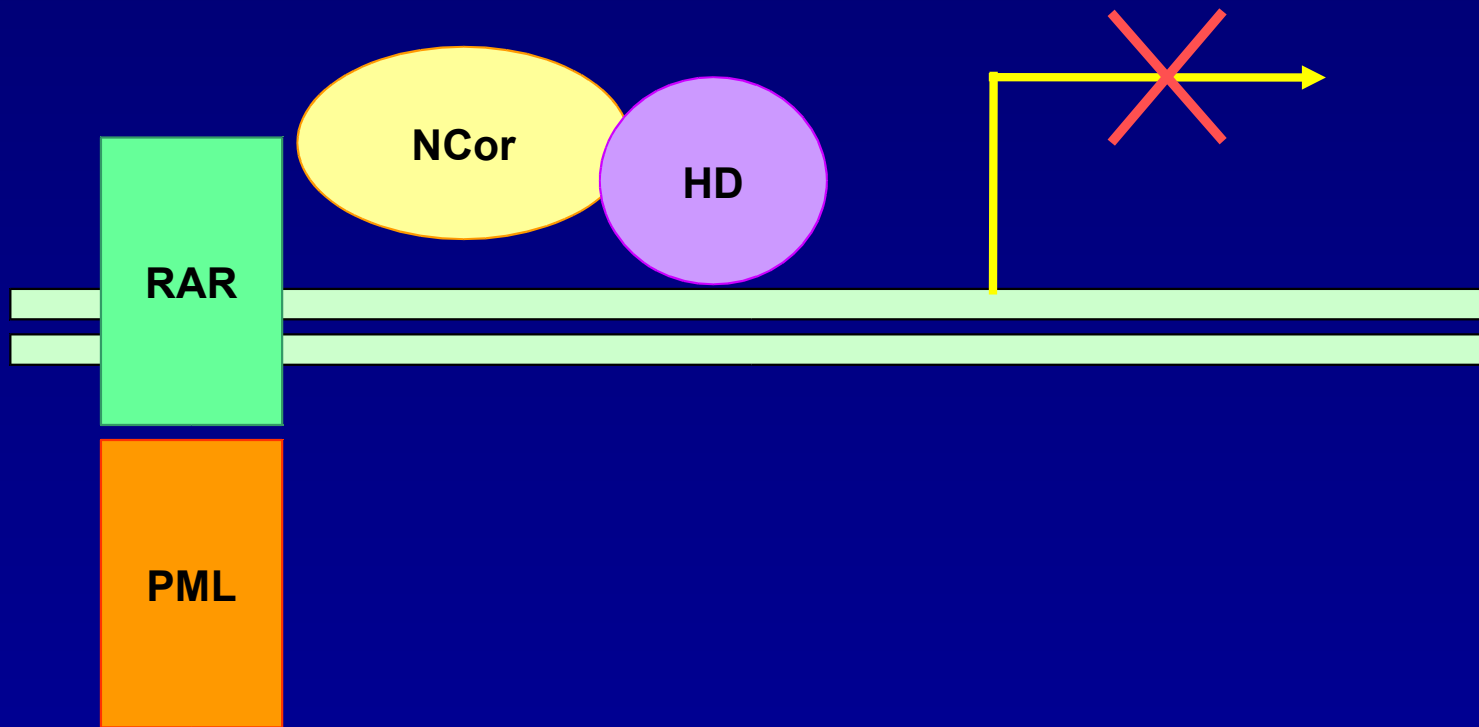
Prol. Cys. L.Z.

DNA

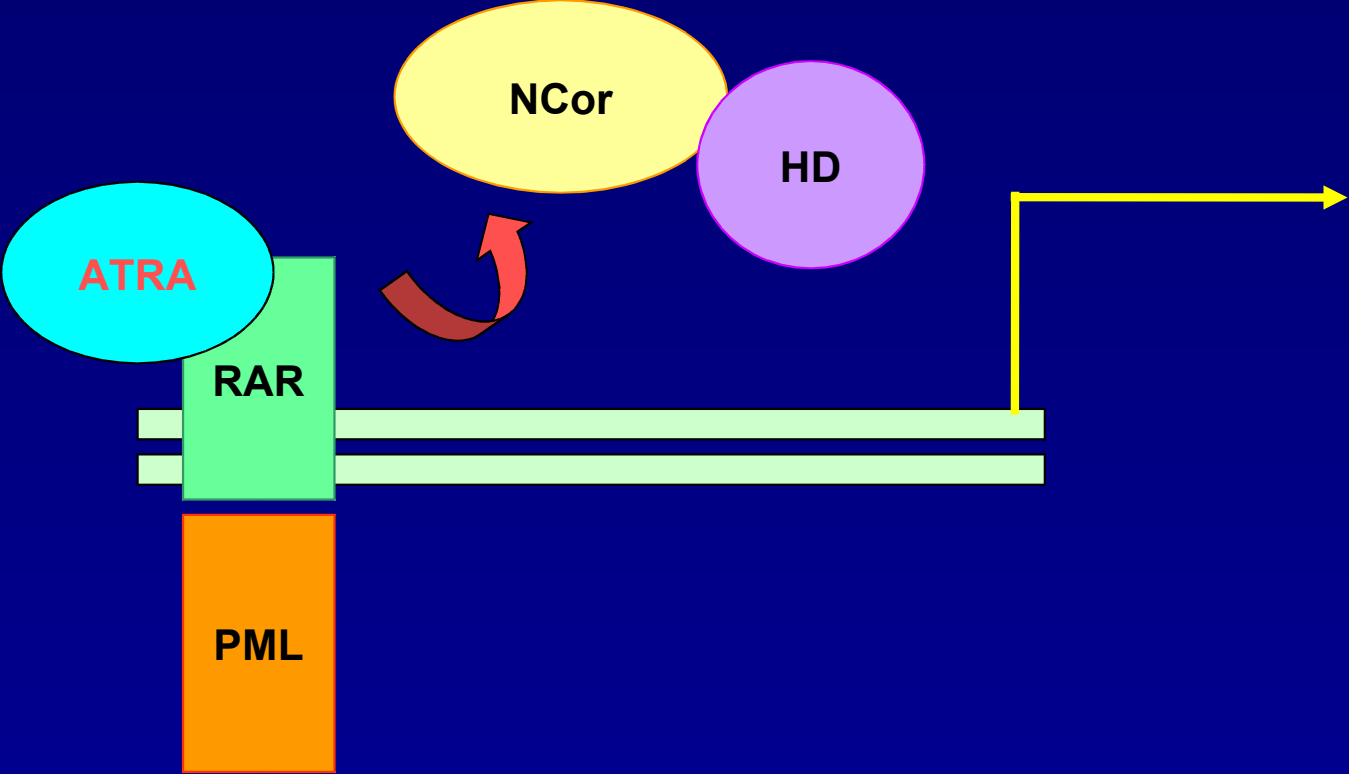
Ligand

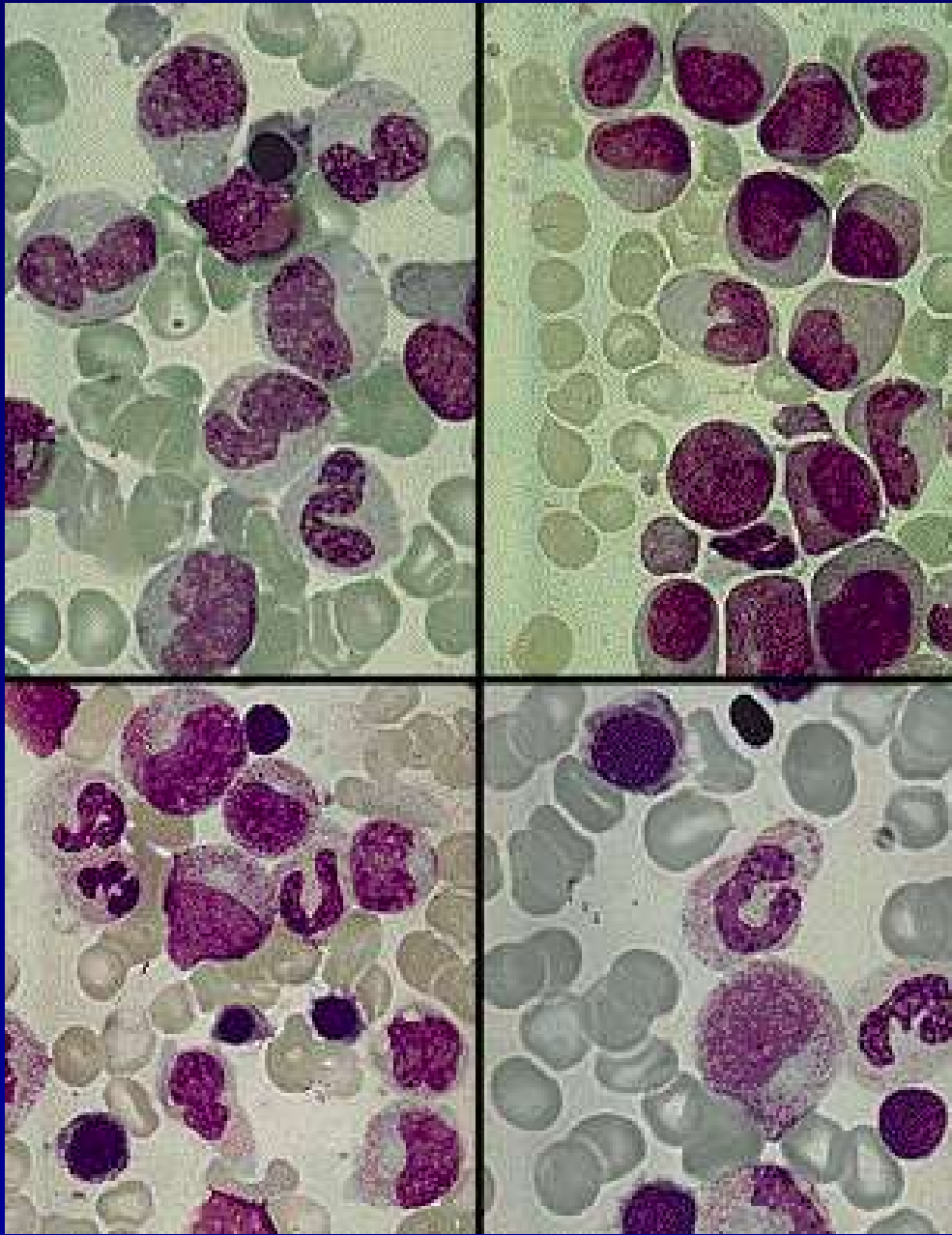


PML-RAR α



PML-RAR α





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

100% foreign glycoprotein

antigenicity

Chimeric

Human: constant
Murine: variable

↓ antigenicity
↑ ADCC

Humanized

Murine hypervariable regions are spliced into human/primate framework

↓ antigenicity



Monoclonal Antibodies: Development and Production

MoAb

Alemtuzumab

Bevacizumab

Cetuximab

Gemtuzumab ozogamicin

Ibritumomab tiuxetan

Rituximab

Tositumomab

Trastuzumab

Type

humanized IgG₁/κ

humanized IgG₁/κ

chimeric IgG₁/κ

humanized IgG₁/κ-toxin conjugate

mouse IgG₁/κ-chelator conjugate

chimeric IgG₁/κ

mouse IgG_{2a}/λ and ¹³¹I-labeled IgG_{2a}/λ

humanized IgG₁/κ

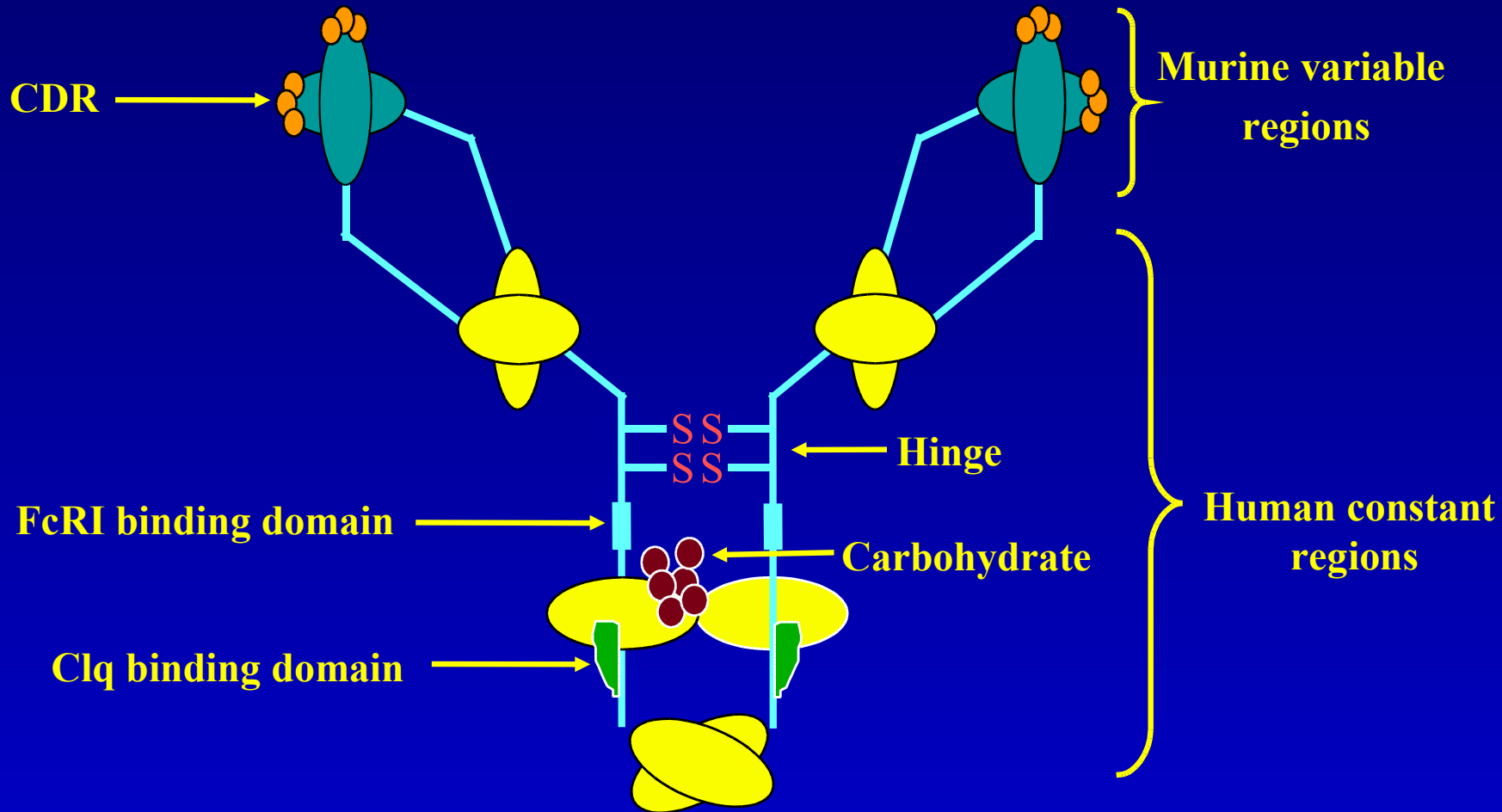


Monoclonal Antibodies: Potential Advantage as Cancer Therapy

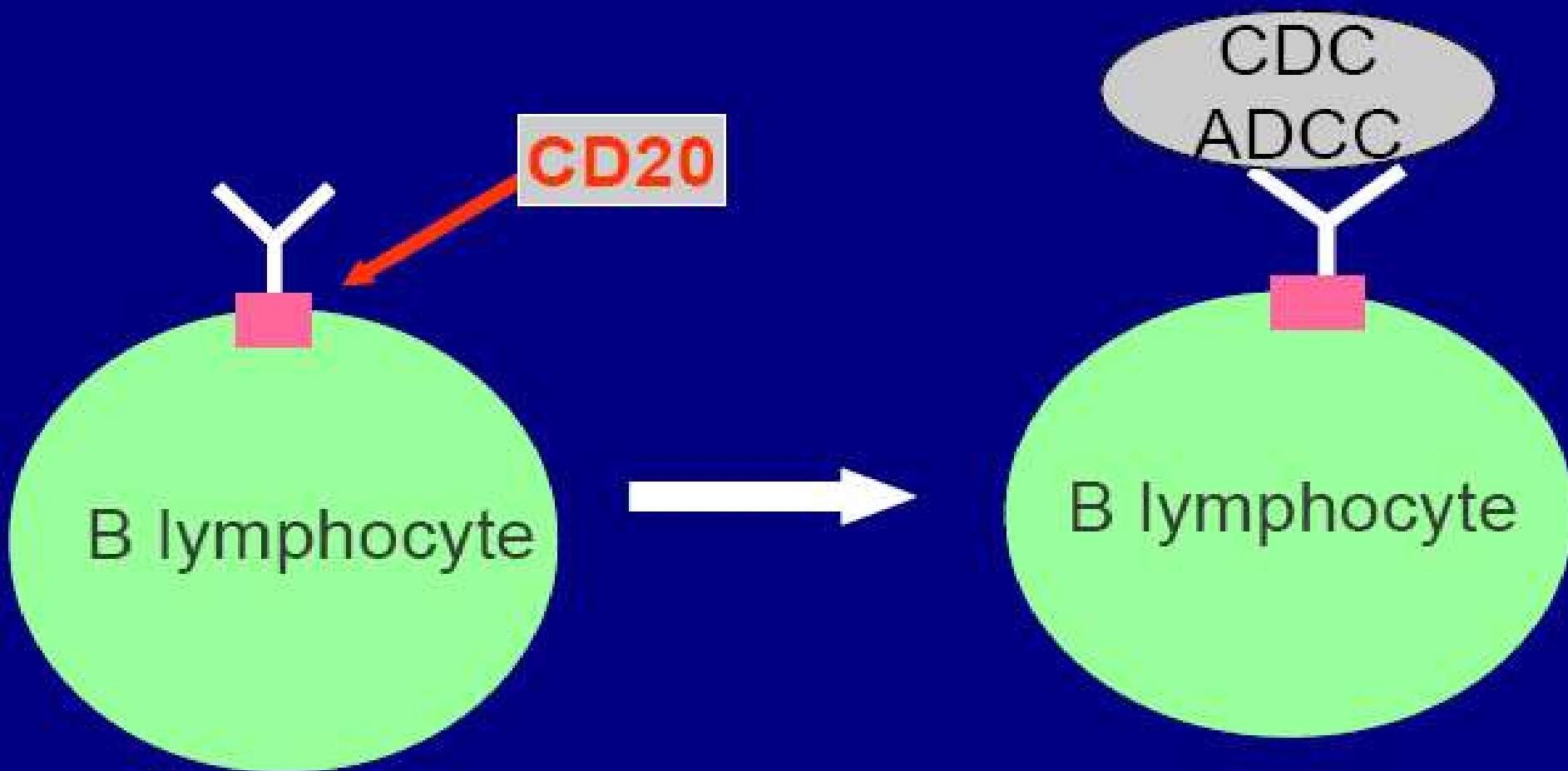
- **high specificity for tumor antigens**
- **low-cross sensitivity with normal cells**
- **design flexibility**
- **customized effector functions**
- **specific targeting of toxic therapy**



Rituximab



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

ALL, CLL, PLL

Burkitts, FL, DLCL, HCL

WM

MM



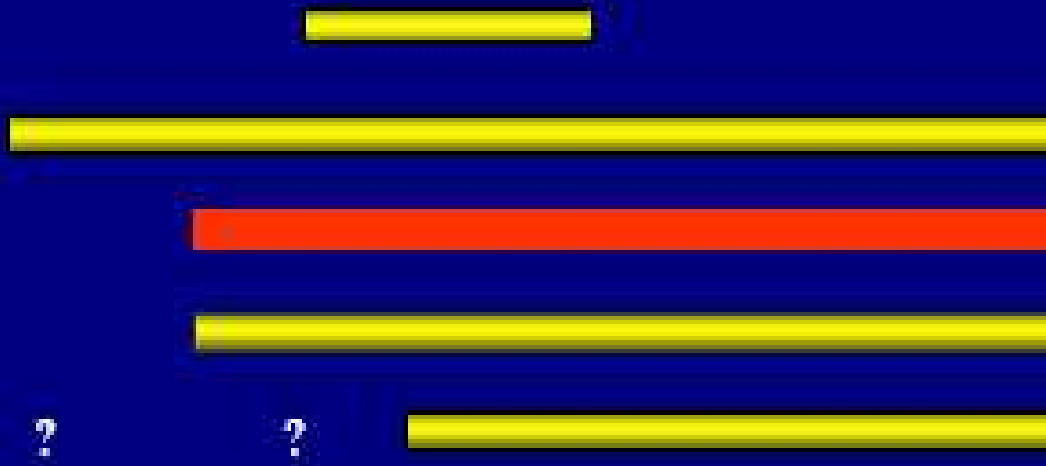
±CD5

CD19

CD20

CD22

CD52



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**

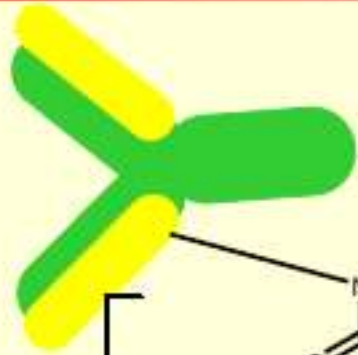
Released drugs kills the cell

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



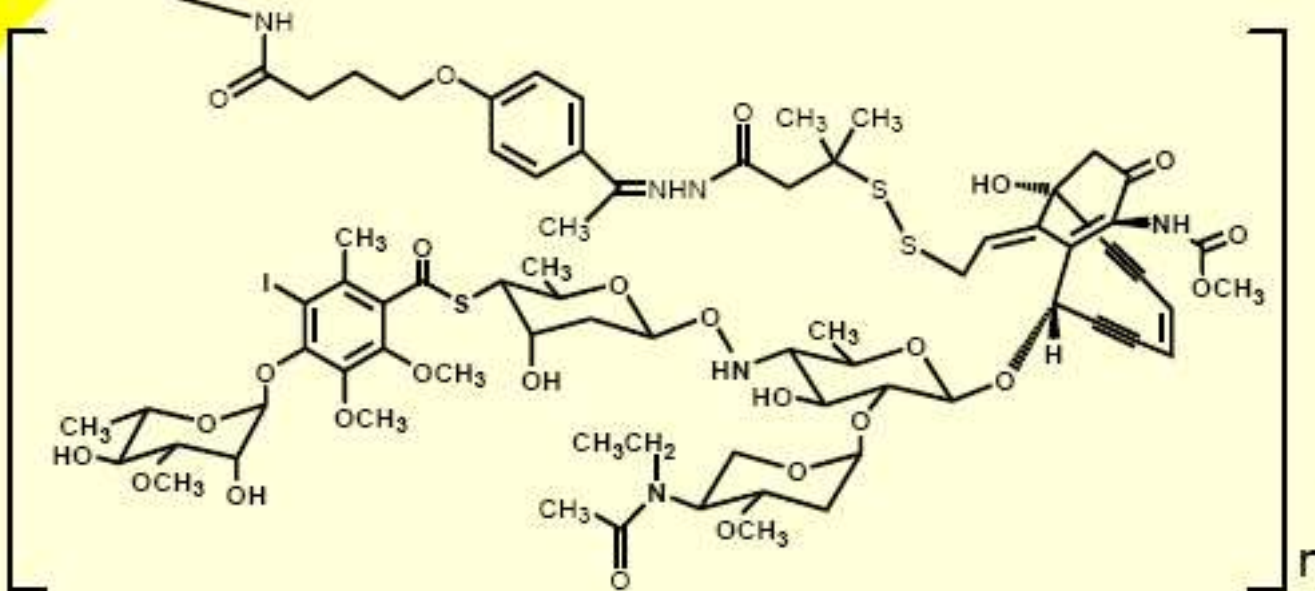
Mylotarg™

Gemtuzumab Ozogamicin



Anti-CD33

Acute myeloid
leukemia



average n
= 2 to 3



Introduction

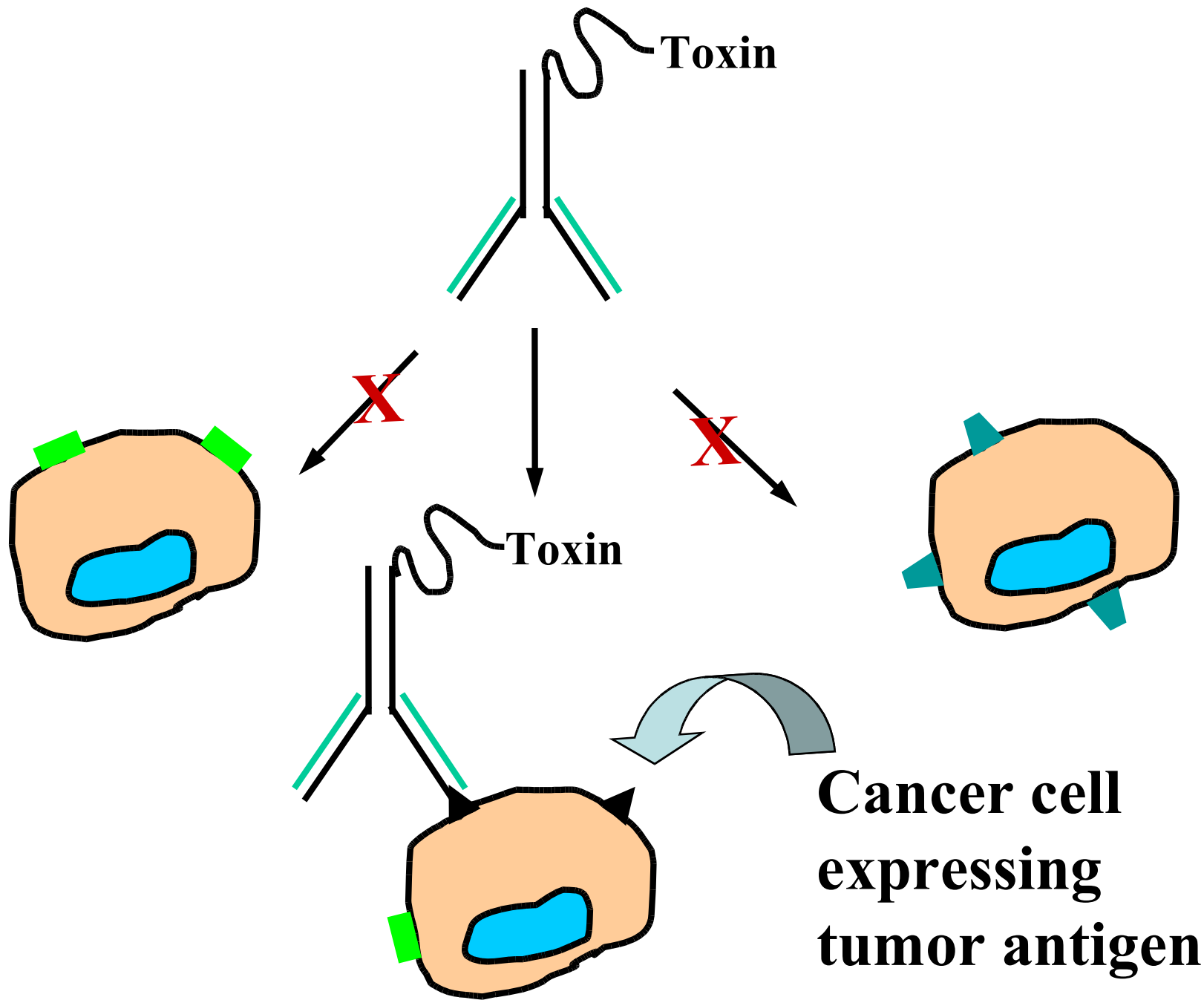
- **Indication**

- ▶ 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

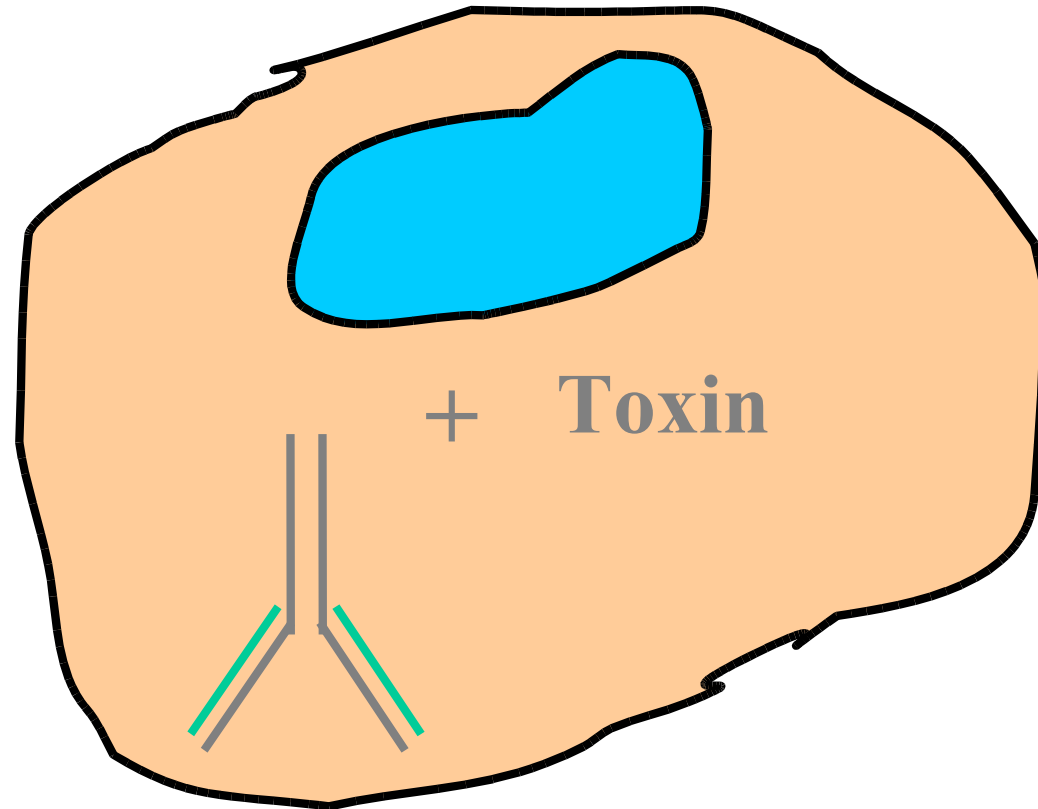
- **Mechanism of Action**

- ▶ Antibody-targeted chemotherapy
- ▶ **Binds CD33 cell surface antigen on myeloid cells**
- ▶ Internalization and release of highly potent antitumor enediyne calicheamicin
- ▶ **Spare pluripotent stem cell and allows regeneration of normal blood cells following therapy**

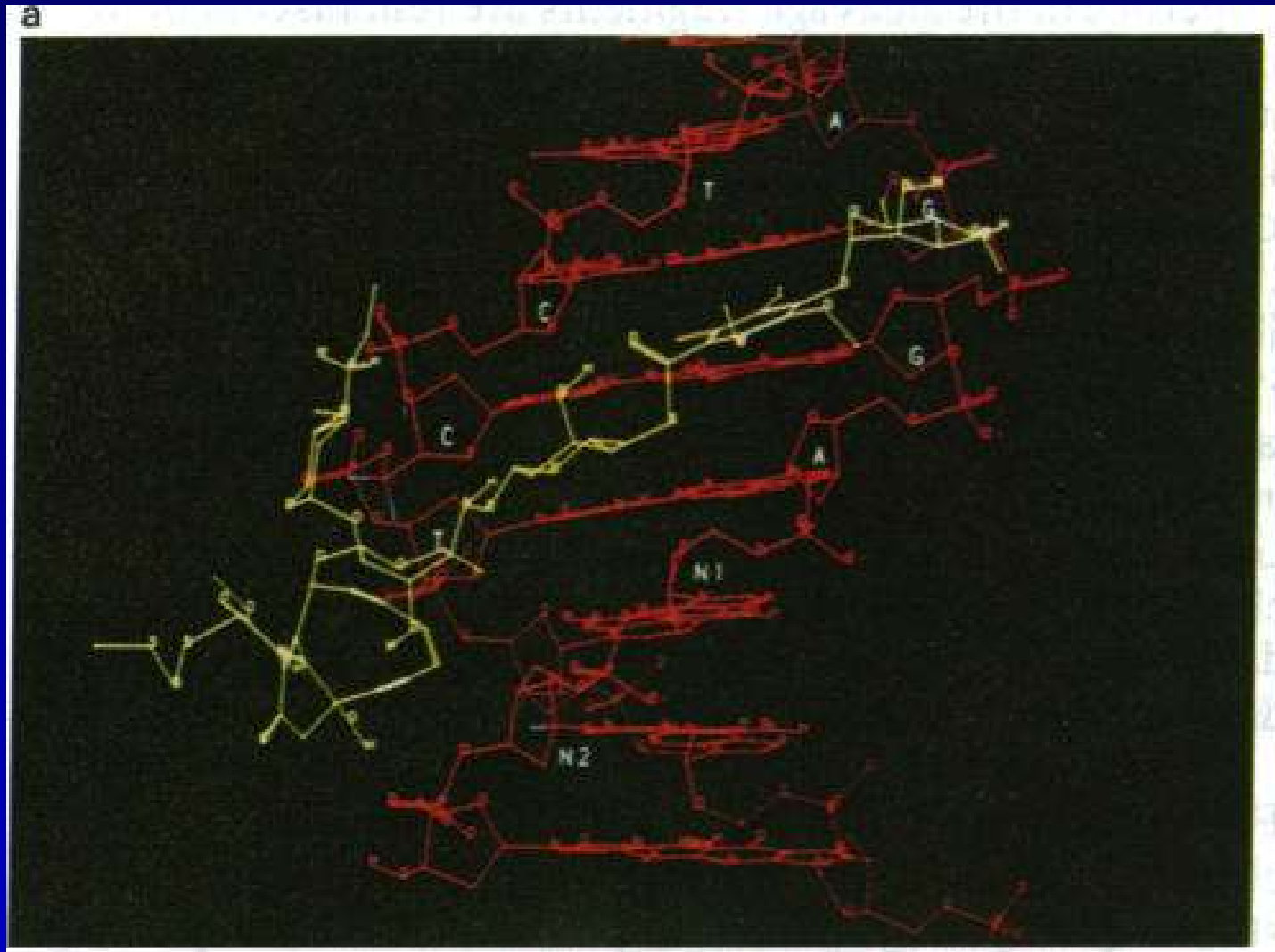




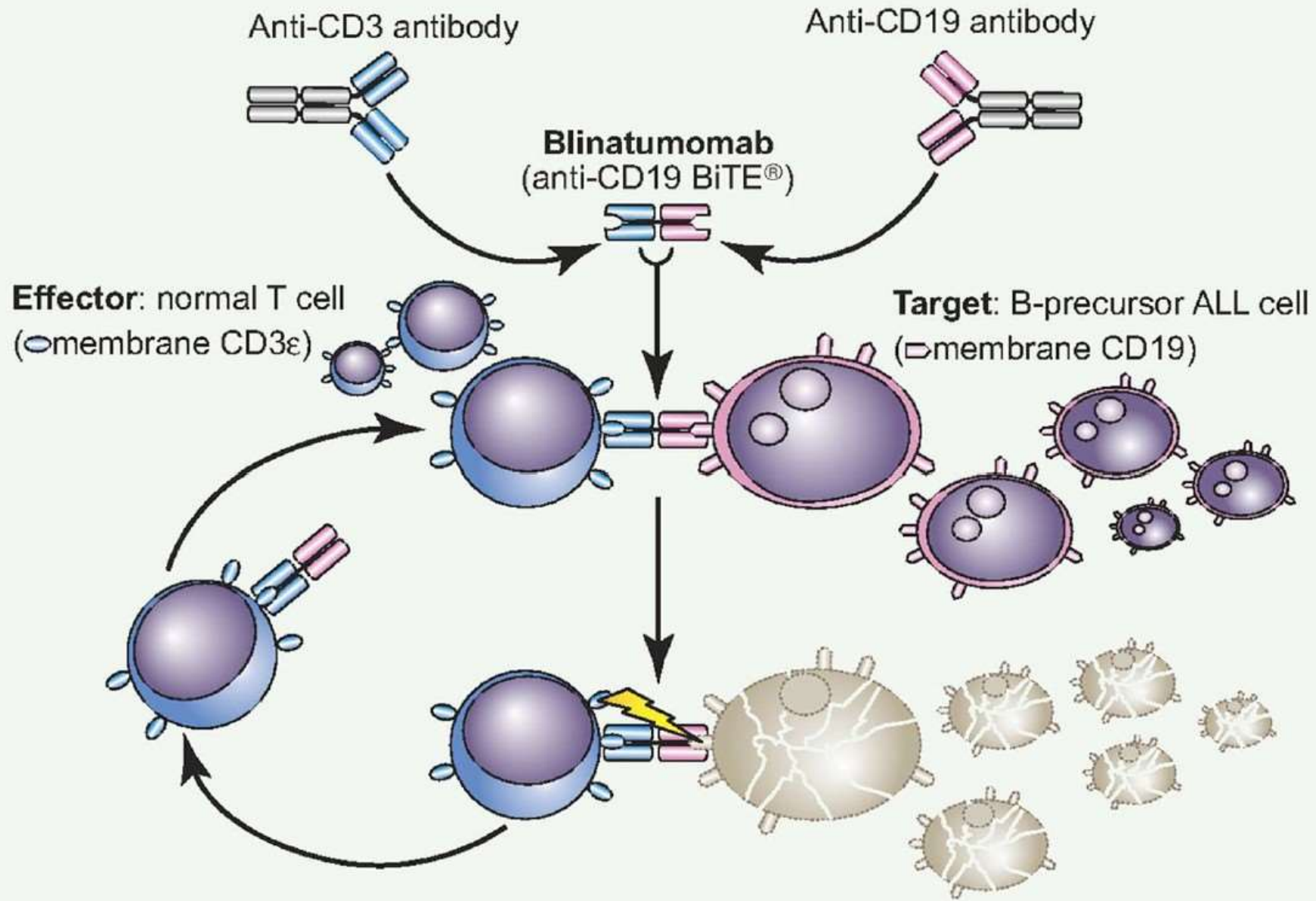
Intracellular breakdown of Antibody linked toxin complex



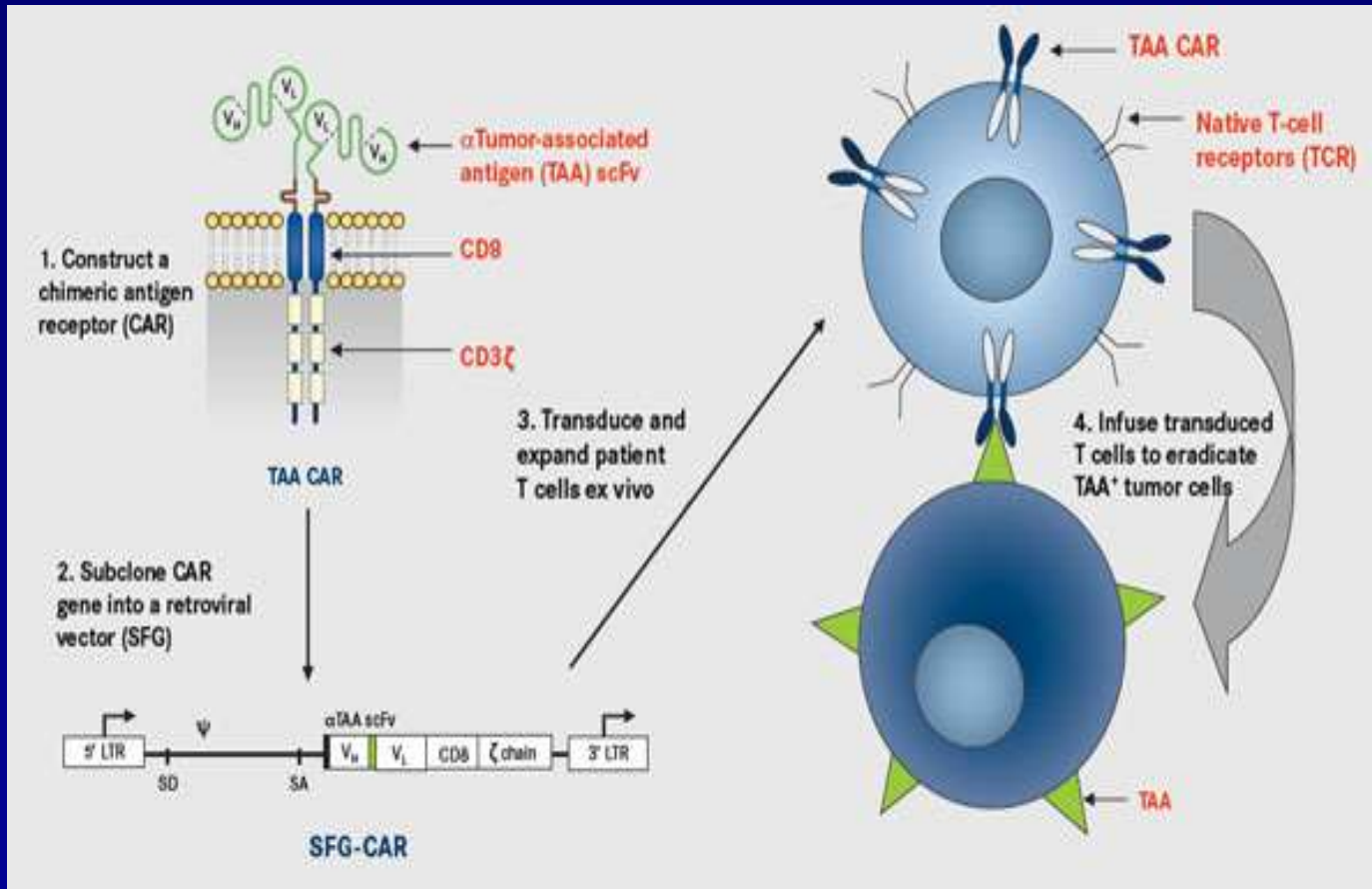
Calcichemicin intercalates into DNA



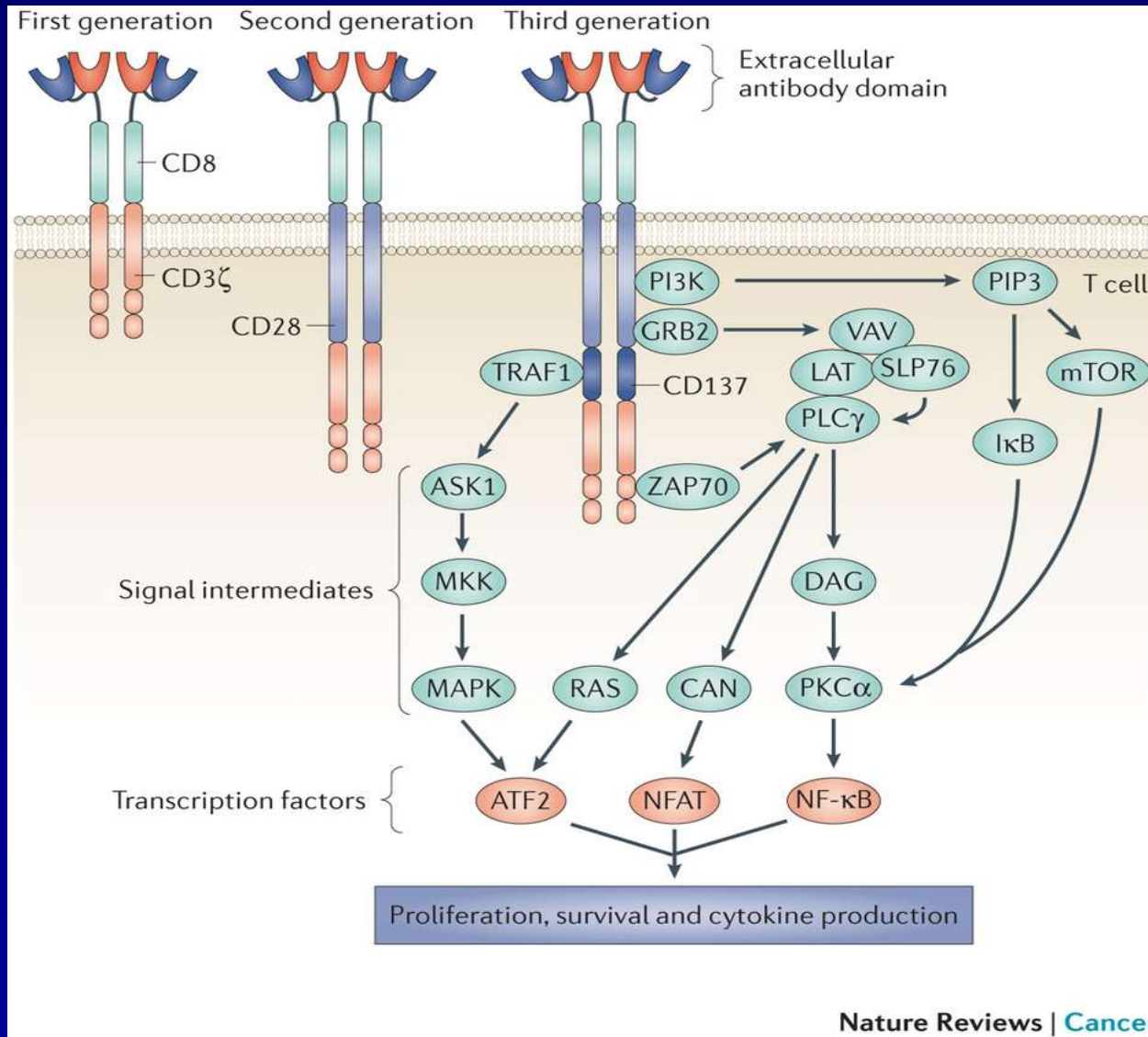
DUAL MONOCLONAL ANTIBODIES



CAR-T CELLS: Chimeric Antigen Receptor



CAR-T CELLS: Chimeric Antigen Receptor



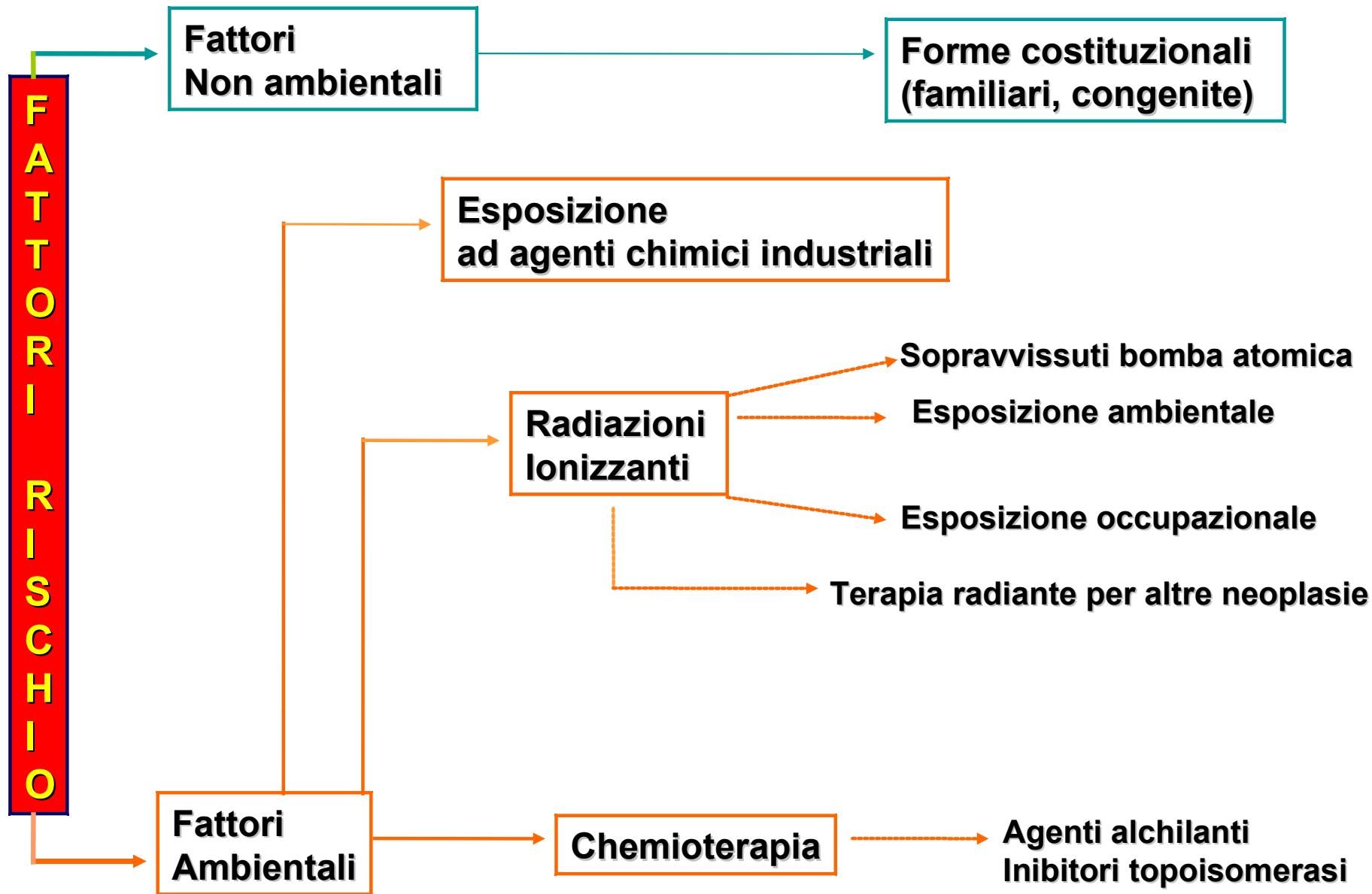
LE SINDROMI MIELODISPLASTICHE

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**





MDS: PATOGENESI

Apoptosi

Mutazioni
DNA

Modificazioni
Epigenetiche

CELLULA STAMINALE

**DISORDINI
CELLULA
STAMINALE**

MICROAMBIENTE MIDOLLARE

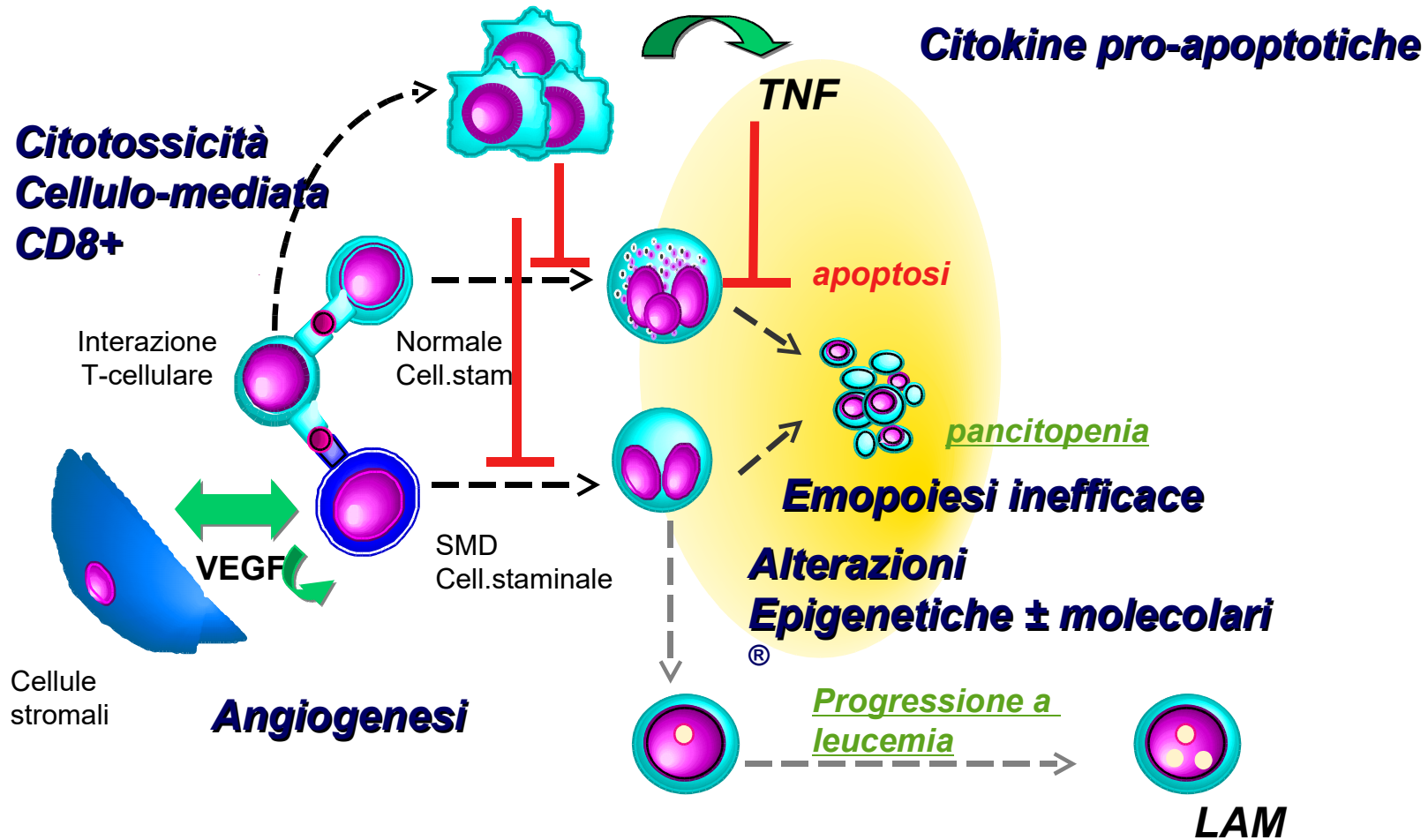
Tossicità
ambientale
diretta

Fattori
angiogenetici
stromali

Disfunzione
sistema
immune



MDS: PATOGENESI



MDS: classificazione

Casistica 2897 pazienti

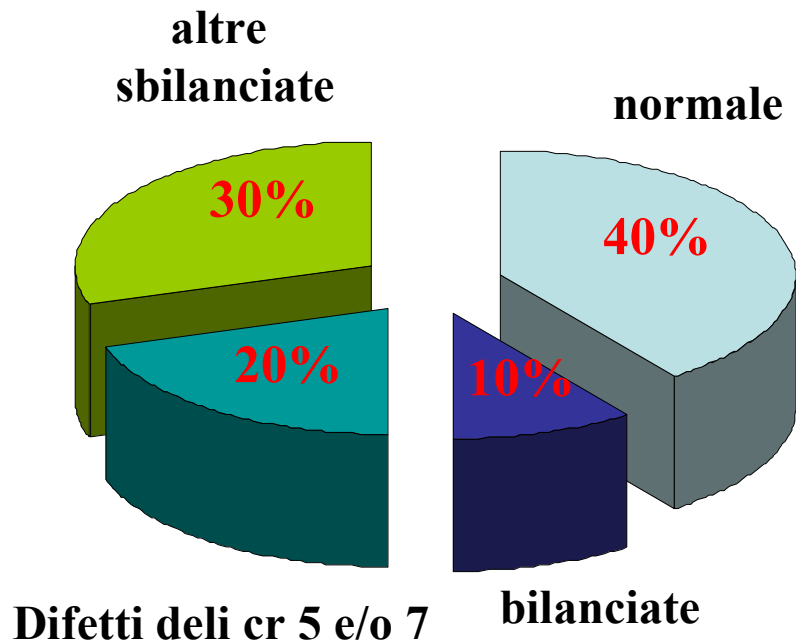
Primitive	94.4%
Secondarie (terapie correlate)	5.6%

Registro MDS del distretto di Dusseldorf 2006

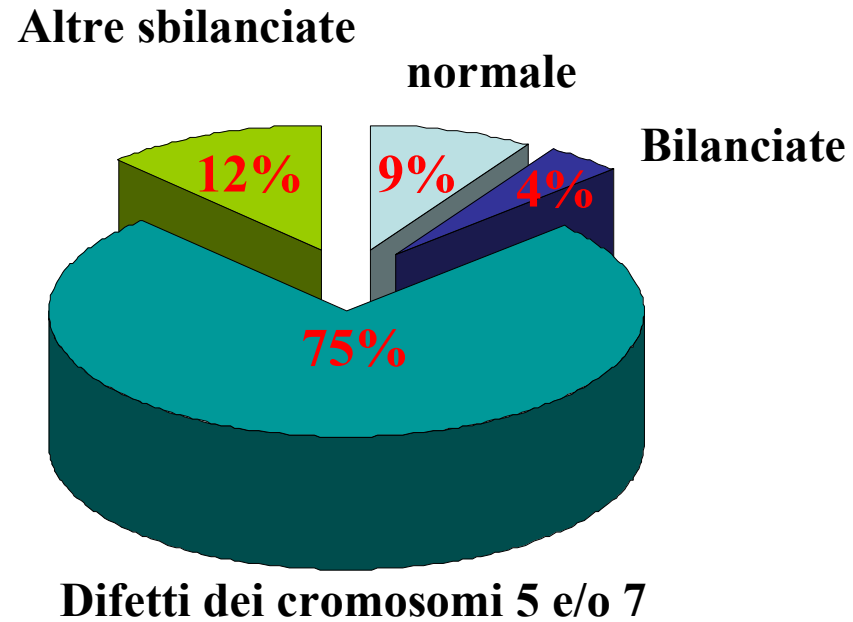


Anomalie cromosomiche: frequenza

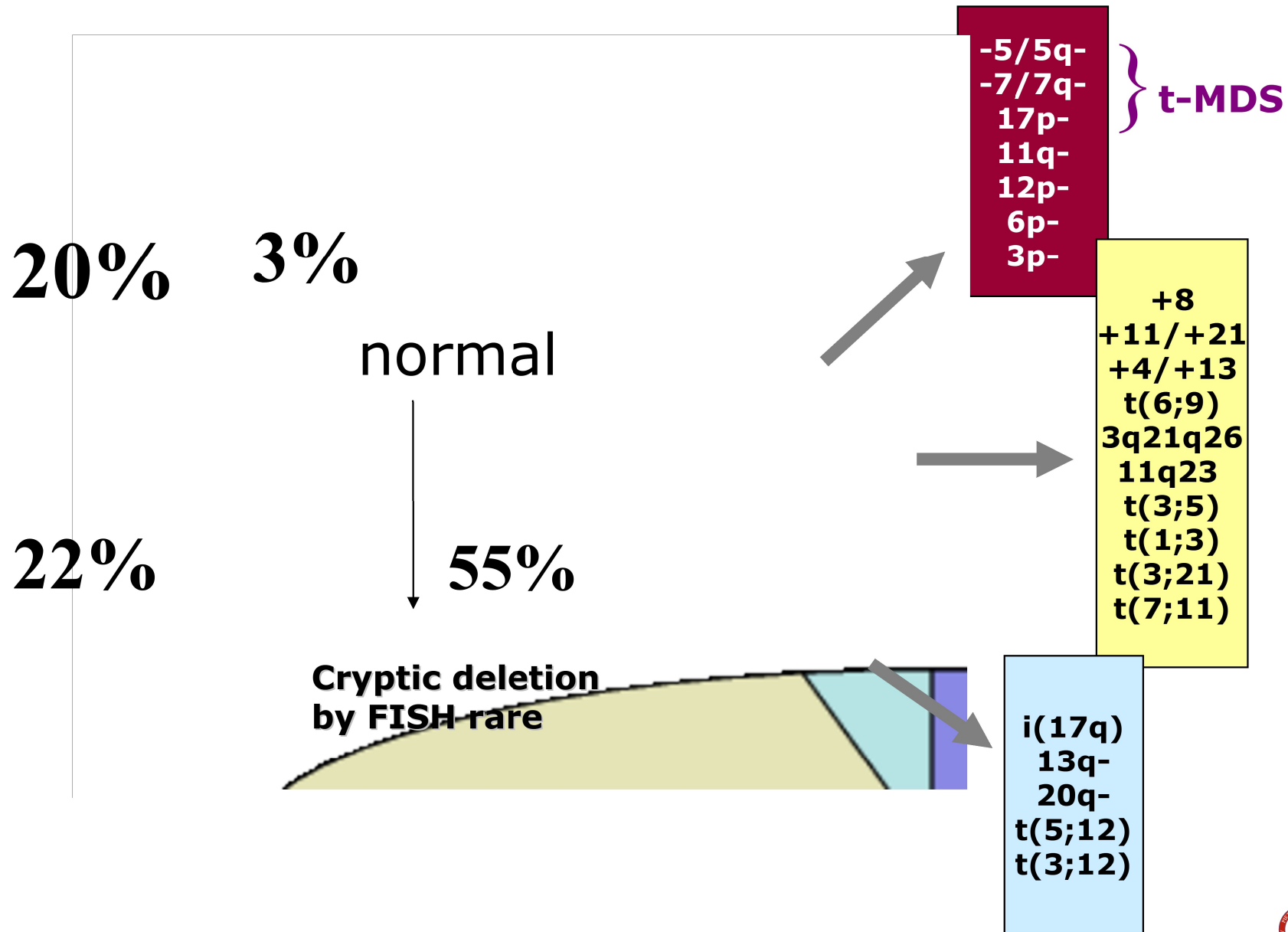
SMD de novo



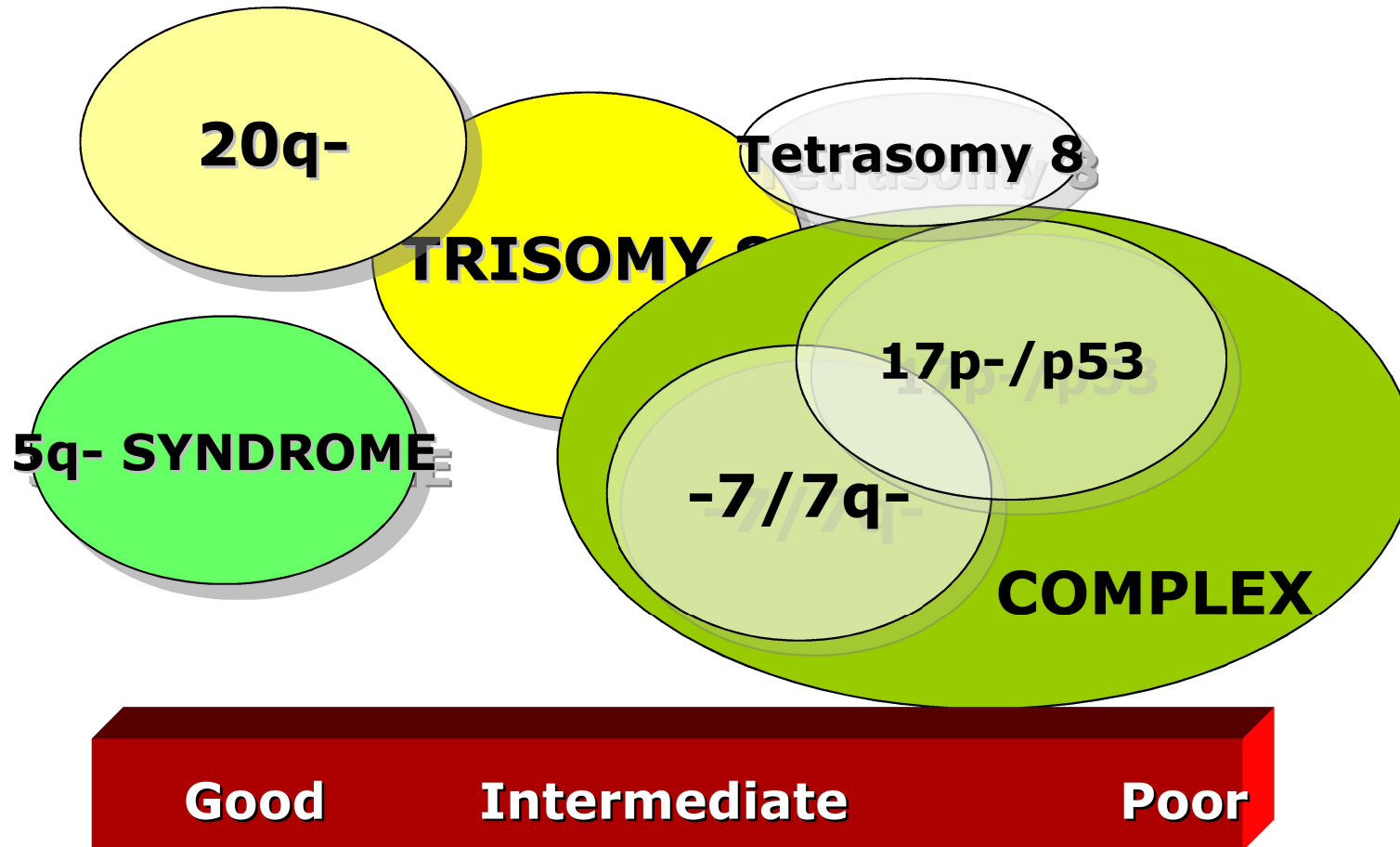
SMD secondarie



Importanza dell'analisi del cariotipo nelle MDS



Alterazioni citogenetiche specifiche nelle SMD



Difetto cromosomico e morfologia

Difetto

Morfologia

Inv(3)/t(3;3)

Micromegacariociti

Del(5)(q13q33)

Megacariociti ipolobulati

+6

SMD ipoplastica

-7/del(7q)

Displasia trilineare

Del(11)(q14q23)

Sideroblasti ad anello

t(12;?)(p13;?)

Eosinofili aumentati

17p-

Neutrofili vacuolati con anomalia di Pelger

Del(20q)

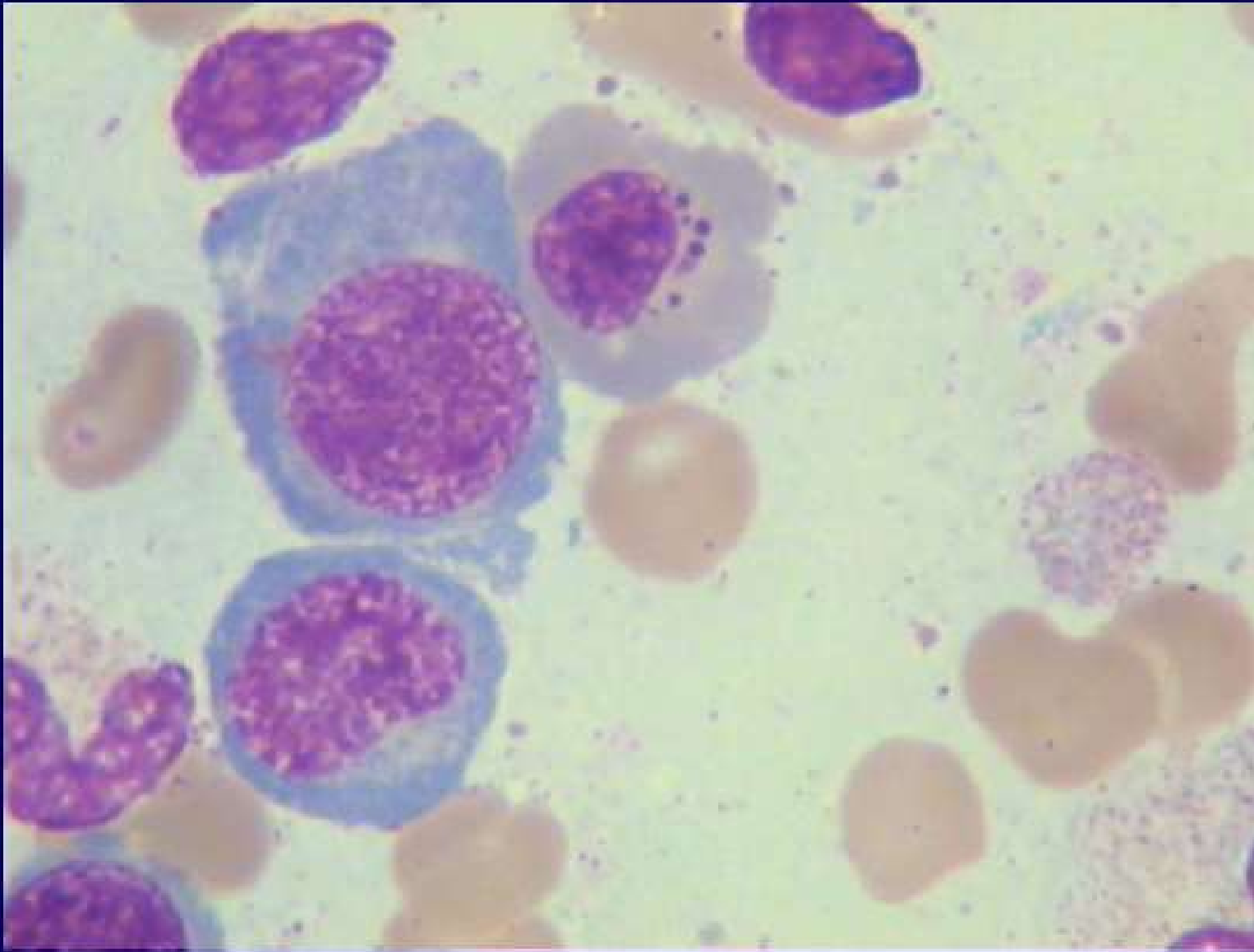
Diseritro- e dismegacariocitopoiesi



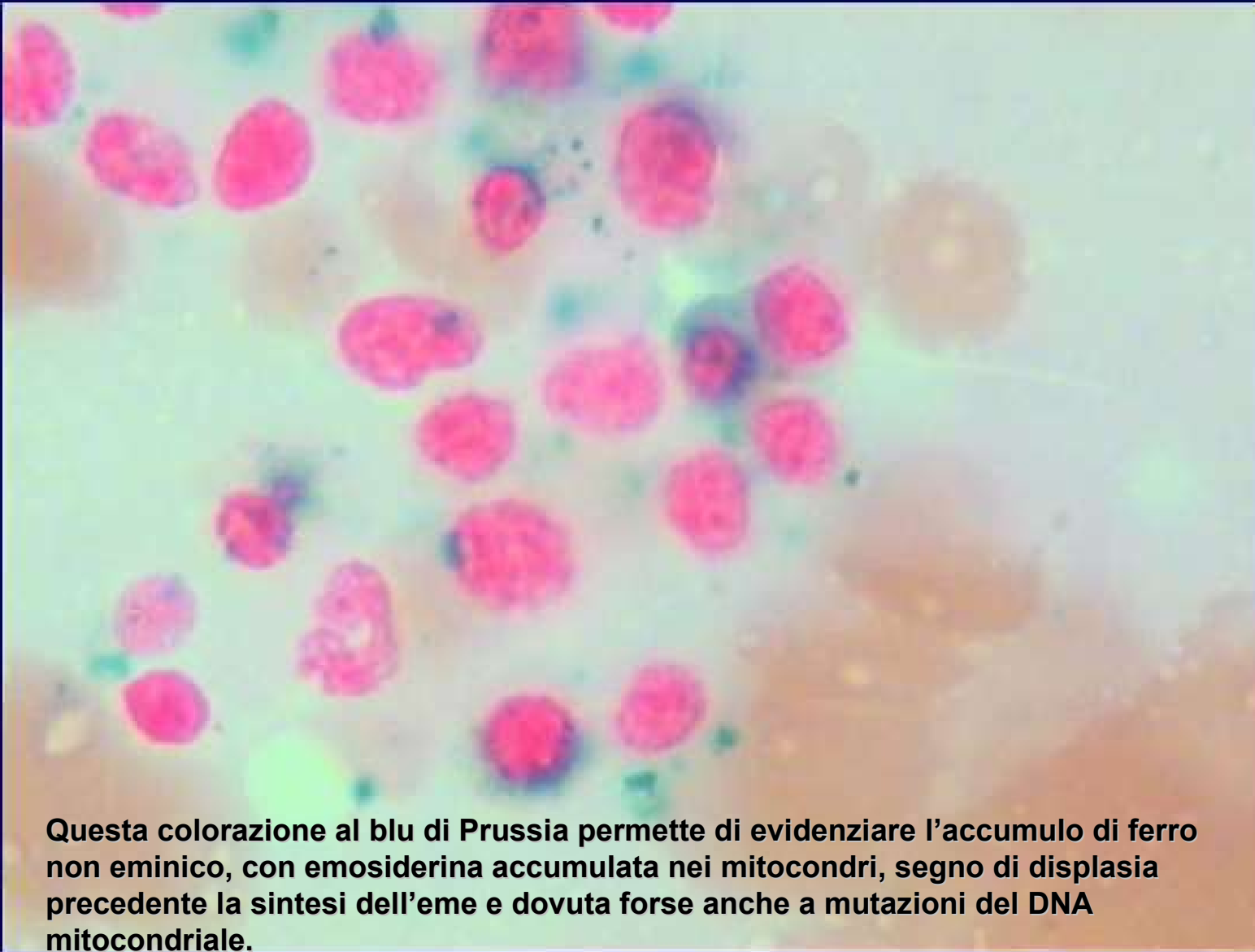
SMD: diseritropoiesi sangue periferico



SMD: diseritropoiesi midollare

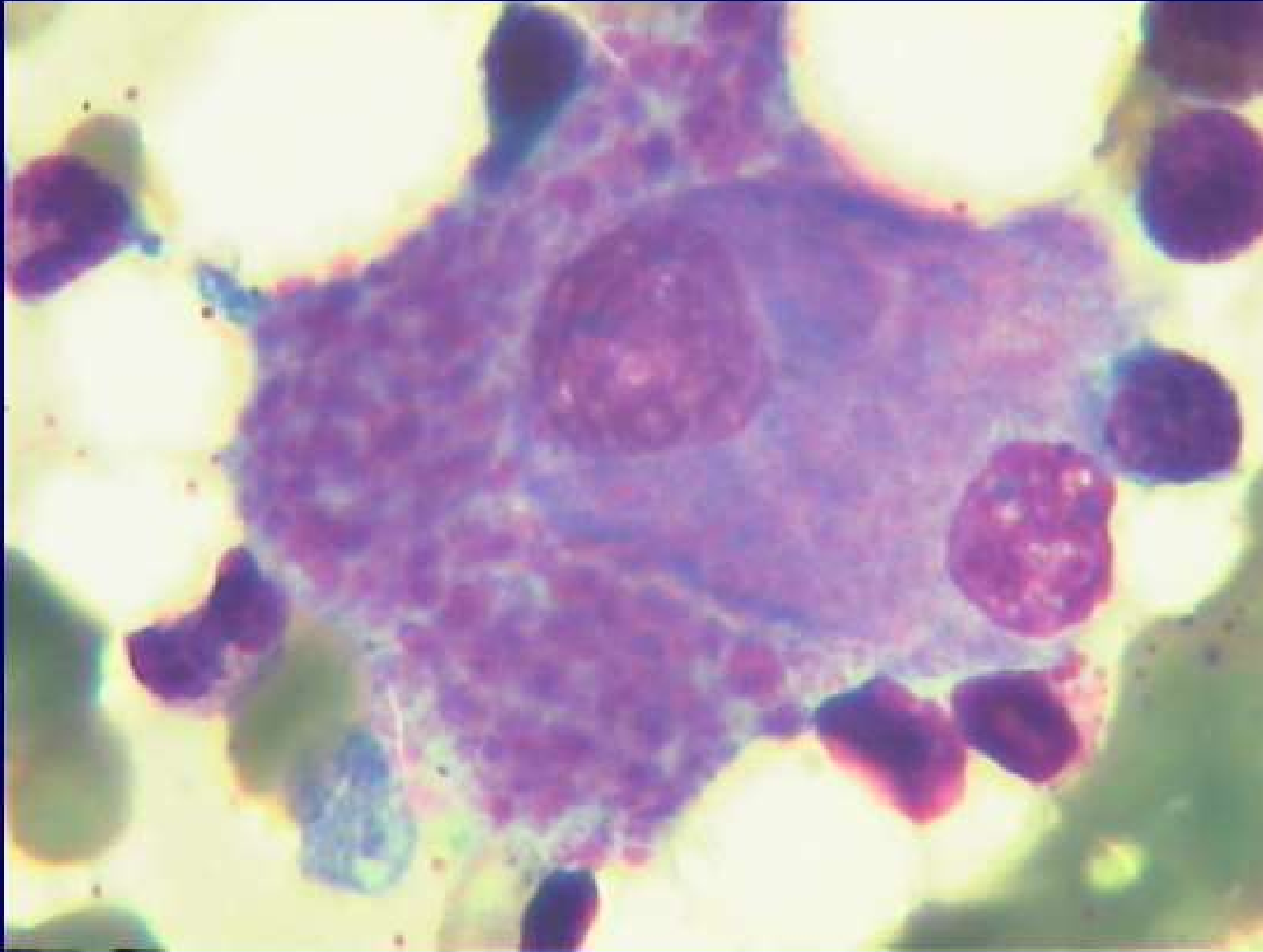


SMD: sideroblasti ad anello con granuli perinucleari di emosiderina (colorazione Pearl)

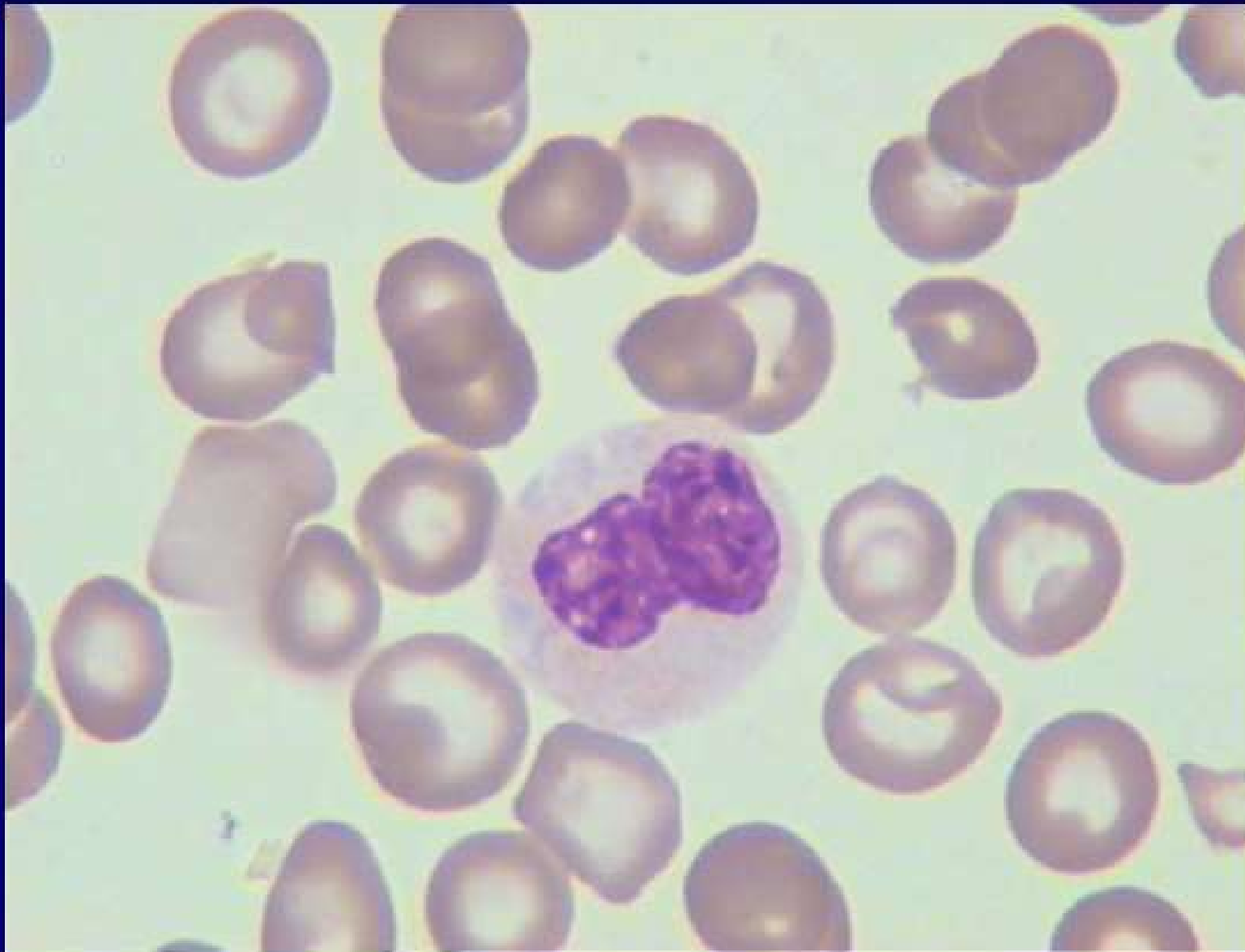


Questa colorazione al blu di Prussia permette di evidenziare l'accumulo di ferro non emnico, con emosiderina accumulata nei mitocondri, segno di displasia precedente la sintesi dell'eme e dovuta forse anche a mutazioni del DNA mitocondriale.

SMD: dismegacariocitopoesi midollare



SMD: dismielopoiesi



SMD: DIAGNOSI

- **MORFOLOGIA:** 1. midollo osseo ipercellulare
2. displasia emopoiesi mono o multi-lineare;
3. alla BOM possibile valutazione ALIP ("abnormal localization of immature precursors")
(aspirato midollare + biopsia)
-

- **CITOPENIA:** anemia, leuco-neutropenia,
trombocitopenia
-

- **CARIOTIPO:** 40-70% pazienti presenta anomalie citogenetiche al momento diagnosi
(citogenetica convenzionale; FISH?)
-

- **(INDAGINI MOLECOLARI):** mutazioni puntiformi
alterazioni epigenetiche
?

SMD: ANALISI CITOFUORIMETRICA

VANTAGGI:

- metodo accurato per la valutazione qualitativa e quantitativa delle cellule emopoietiche;
- evidenzia le anomalie fenotipiche presenti nelle SMD;

SVANTAGGI:

- mancanza di un singolo parametro immunofenotipico specifico per le SMD; panel di Ab per l'analisi delle linee troppo esteso;
- difficoltà di valutare la displasia eritroide.



CITOFUORIMETRIA



metodica NON di screening per la diagnosi delle SMD

... ma ...

da eseguire quando morfologia e analisi citogenetica risultano non informative

(es. diagnosi differenziale anemia aplastica e MDS ipoplasica)

CLASSIFICAZIONE FAB

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

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

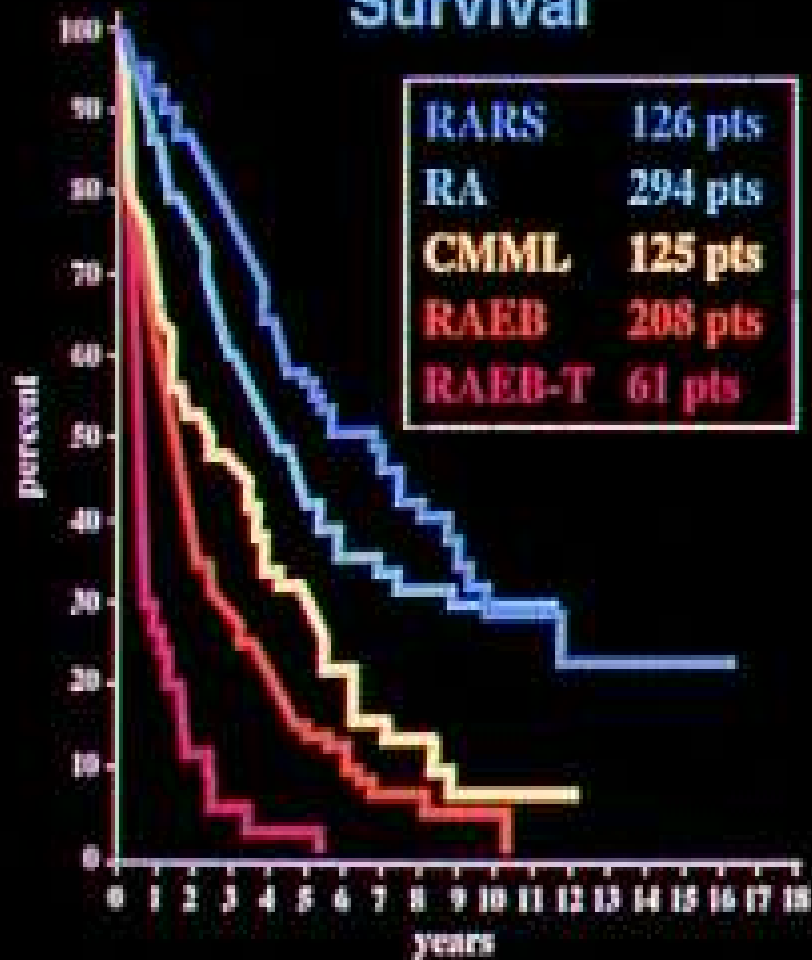
*Sideroblasti ad anello > 15%

**monocitosi ($1 \times 10^9/L$)

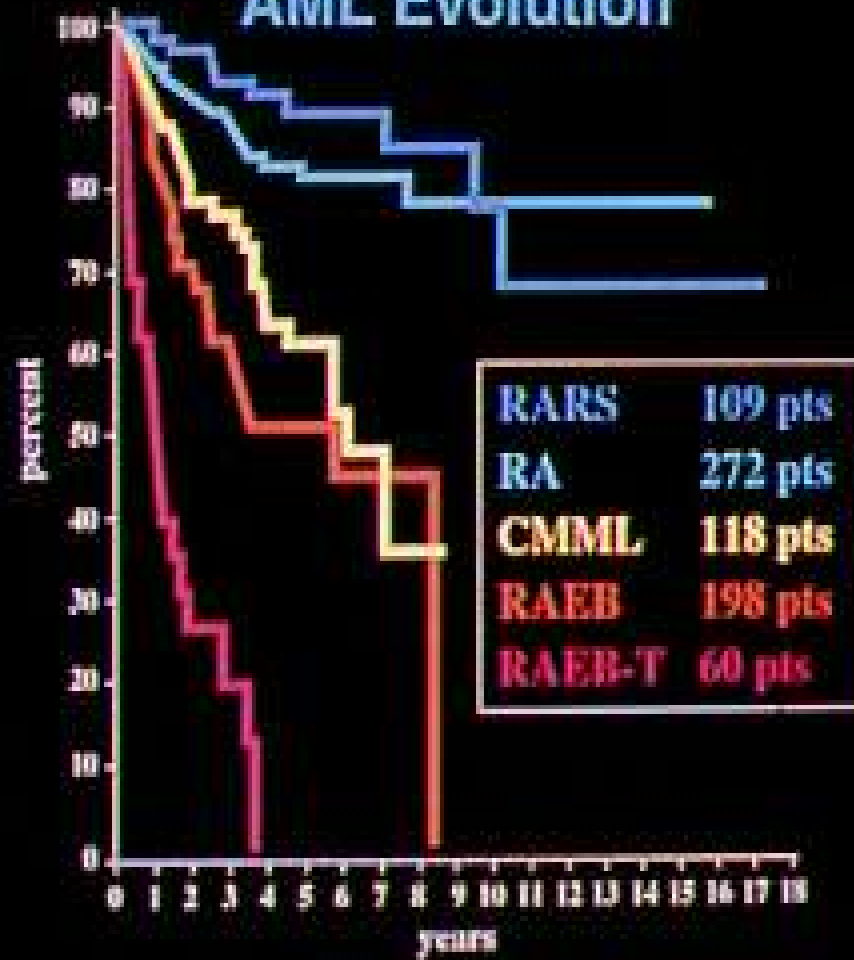


FAB Classification

Survival



AML Evolution



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

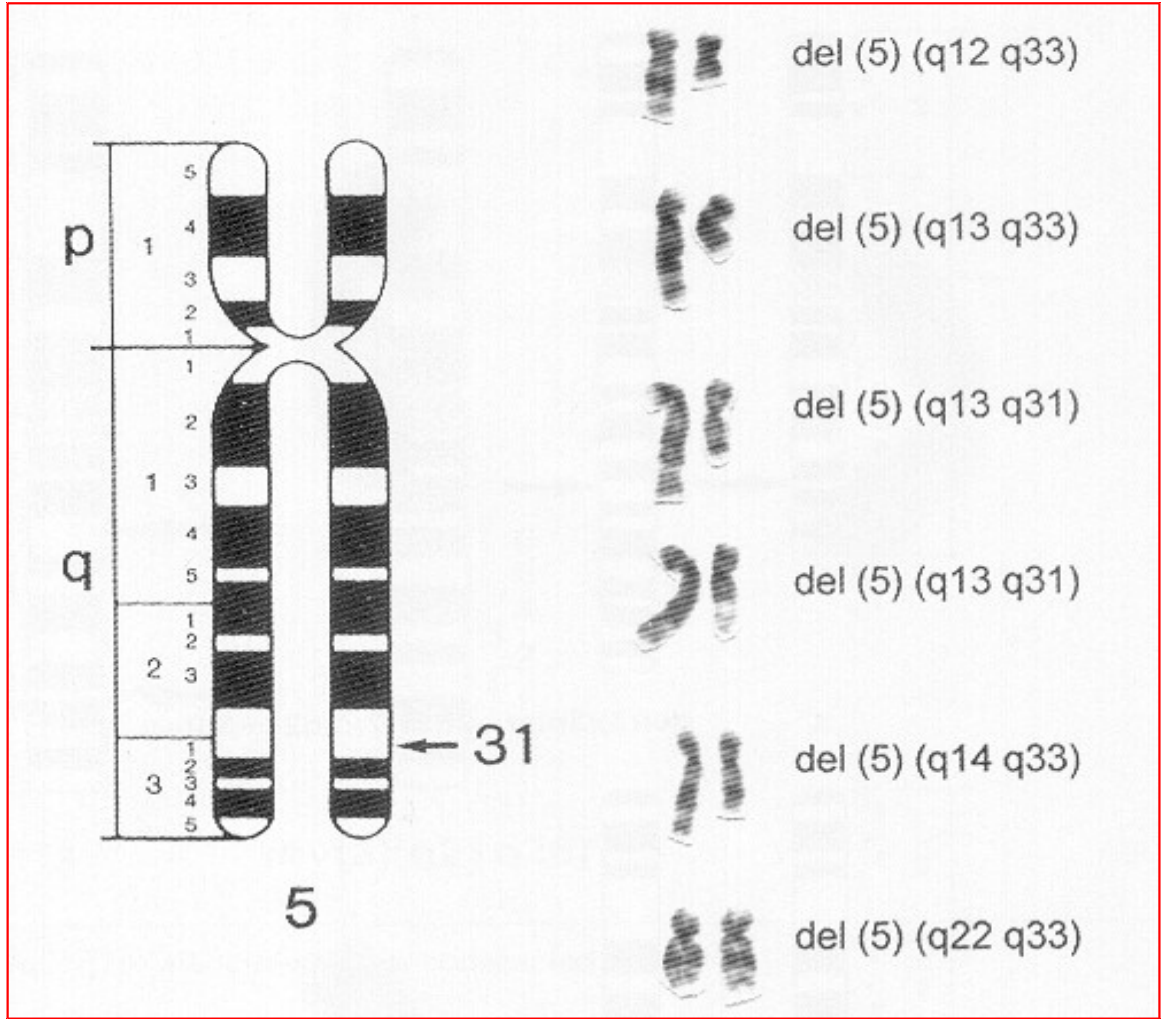
Sopravvivenza media prolungata

Blasti nel midollo < 5%

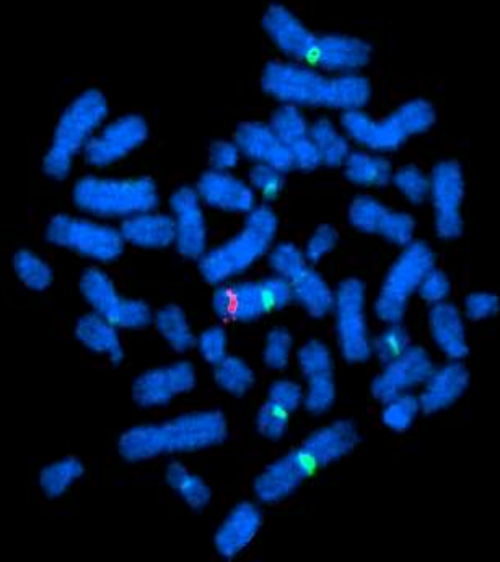
Prognosi favorevole: evoluzione clinica e citogenetica rara (2% dei pazienti)

Delezione 5 q come unica anomalia

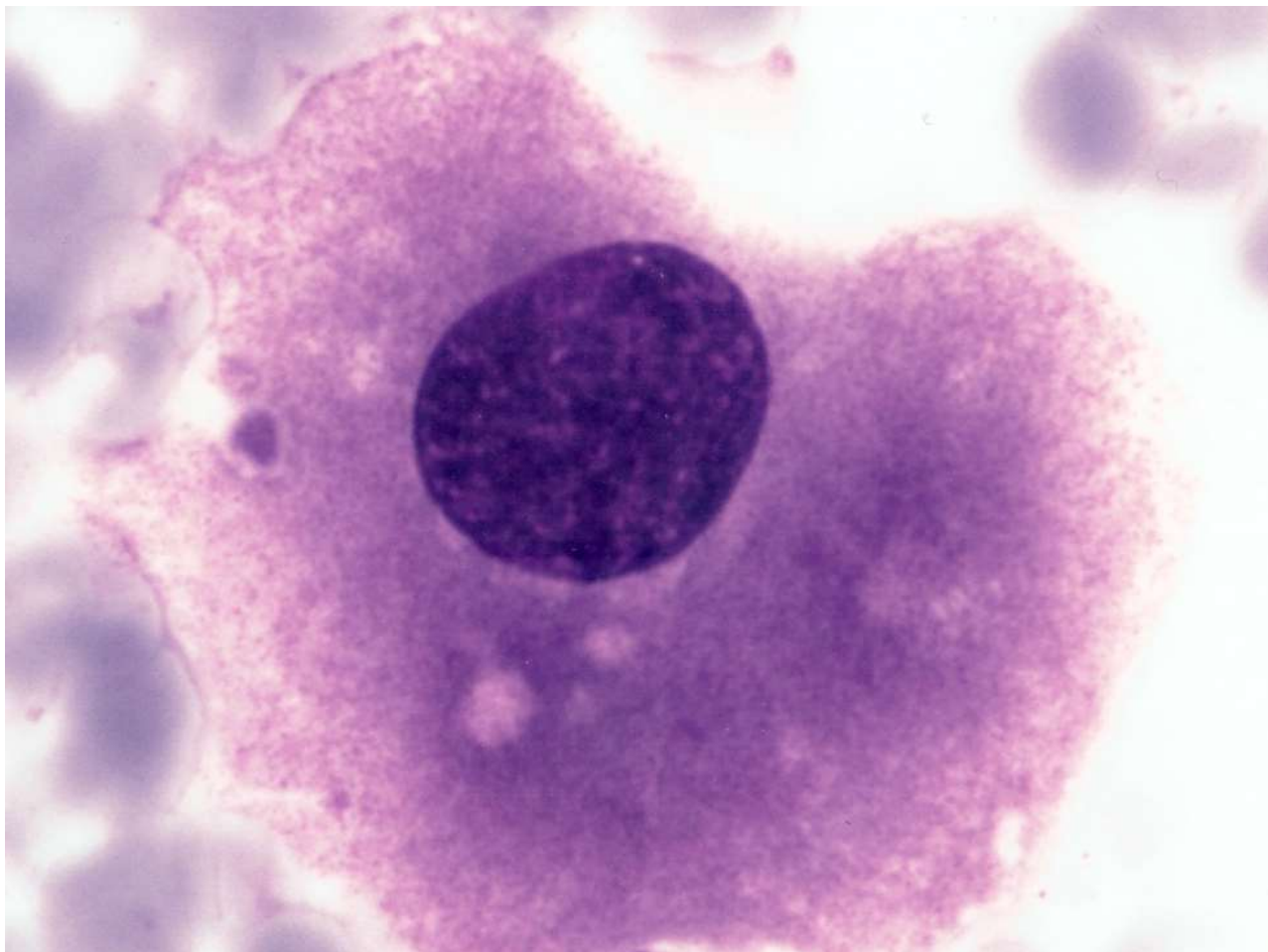




Del(5)(q13q33)



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, $-Y$, $\text{del}(5q)$, $\text{del}(20q)$; Poor, complex (≥ 3 abnormalities) or chromosome 7 anomalies; Intermediate, other abnormalities. Cytopenias defined as haemoglobin concentration < 10 g/dl, neutrophils $< 1.8 \times 10^9/\text{l}$ and platelets $< 100 \times 10^9/\text{l}$.

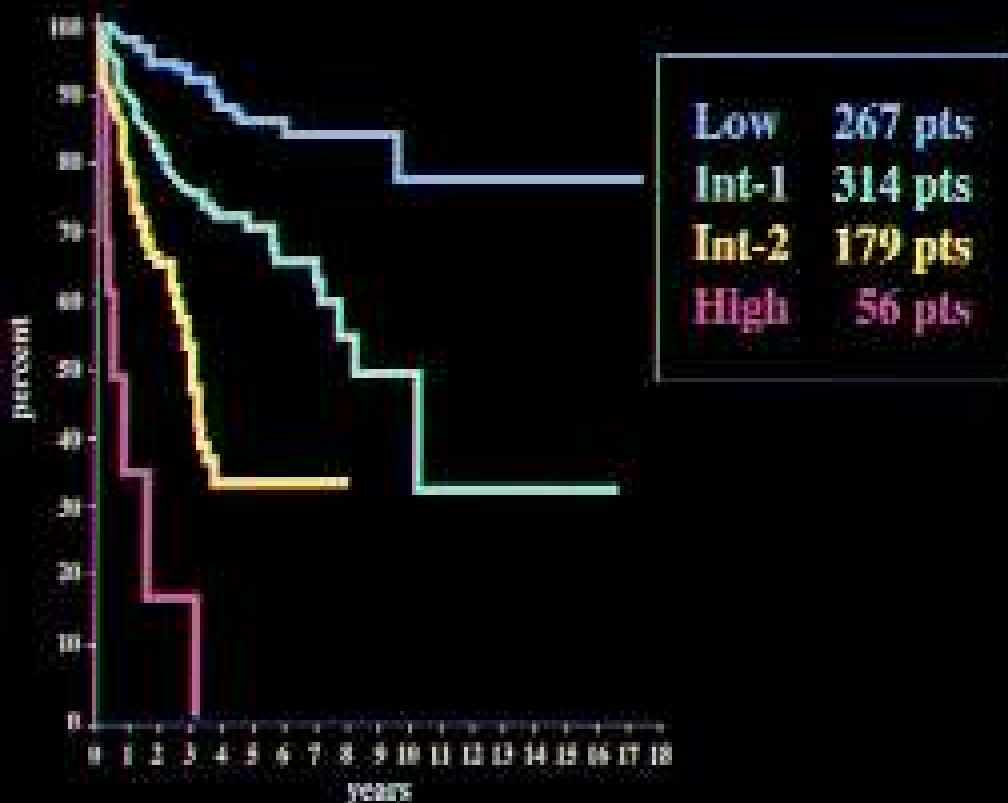
(B) Median survival: IPSS score.

	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

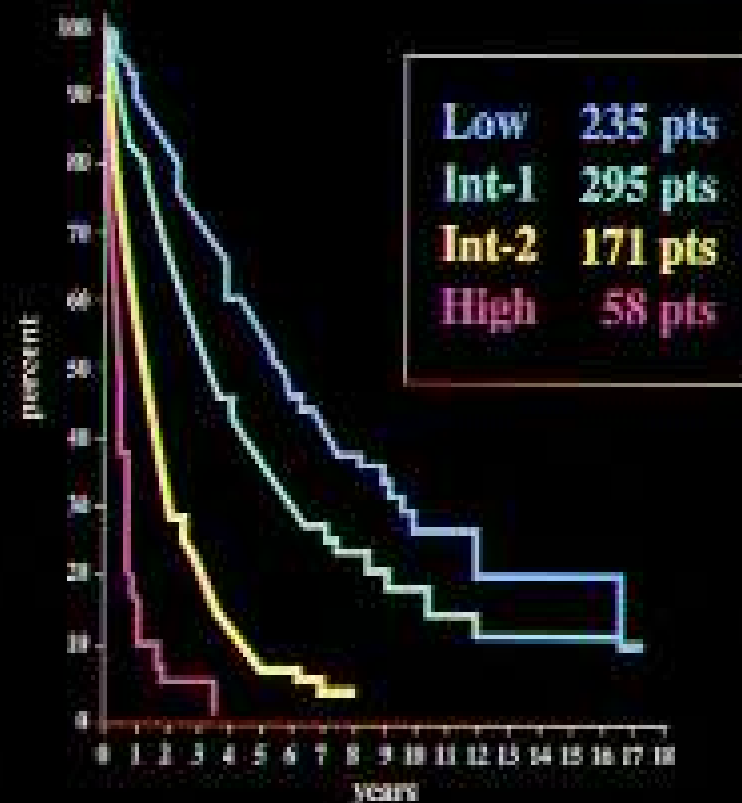


International MDS Risk Classification

Survival



AML Evolution



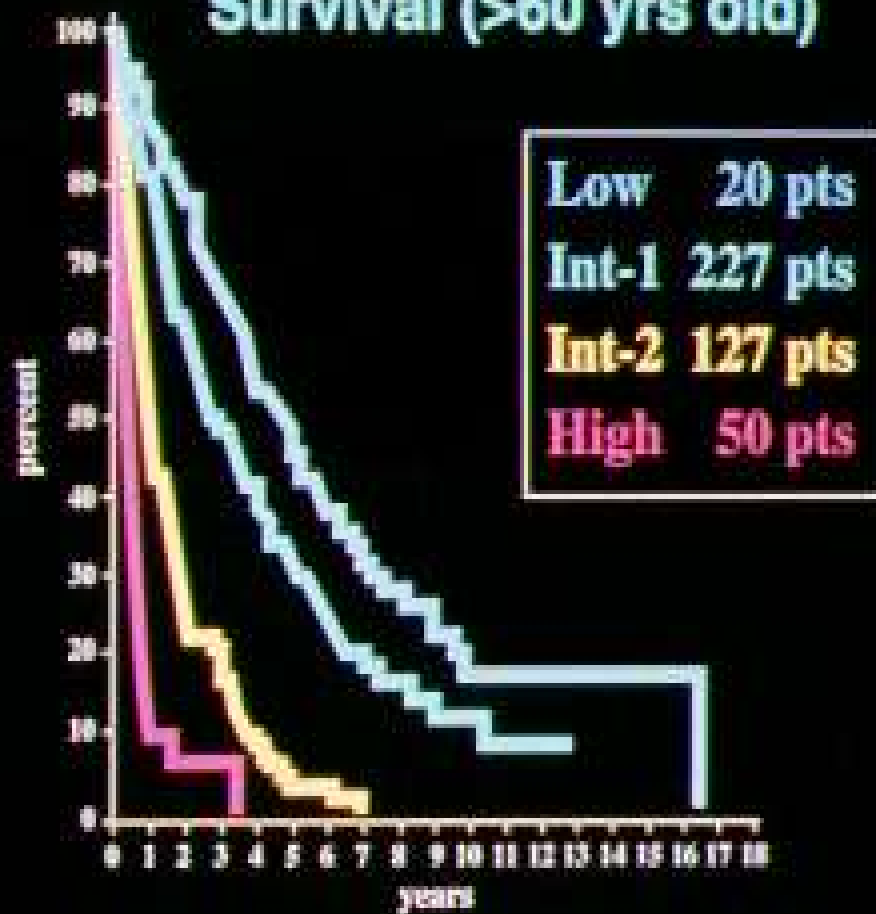
International MDS Workshop
Blood 89: 2079, 1997

International MDS Risk Classification

Survival (≤ 60 yrs old)



Survival (>60 yrs old)



International MDS Workshop
Blood 89: 2079, 1997

“Targets” terapeutici nelle SMD: diversità biologica

	LOW / INT-1	INT-2 / HIGH
Patogenesi	<p>MALATTIA-SPECIFICA:</p> <ul style="list-style-type: none"> • segnale citokinico • segnali proapoptotici • citogenetica (5q-) 	<p>MALATTIA SPECIFICA:</p> <ul style="list-style-type: none"> • 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
- Decitabine
- Lenalidomide 5q-
- Histone de-acetilase inibitori (HDAC)
- Modificatori biologici e terapia sperimentale



Terapia

Terapia di Supporto

- Trasfusioni, eritropoietina, G-CSF e antibiotici
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- Modificatori biologici e terapia sperimentale



Modificazioni epigenetiche:

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

1. METILAZIONE del DNA

2. MODIFICAZIONE DEGLI

ISTONI (acetilazione, metilazione, fosforilazione, ubiquitinazione.....).

Responsabili cambiamenti conformazionali cromatina.

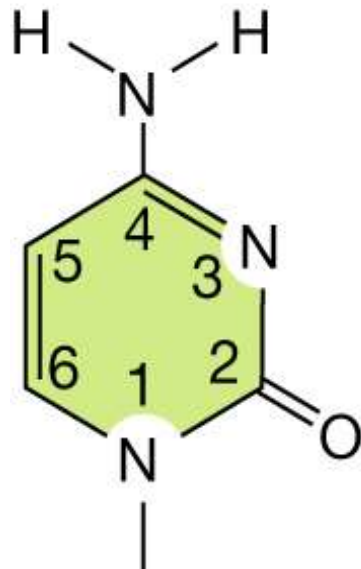


1. METILAZIONE DNA

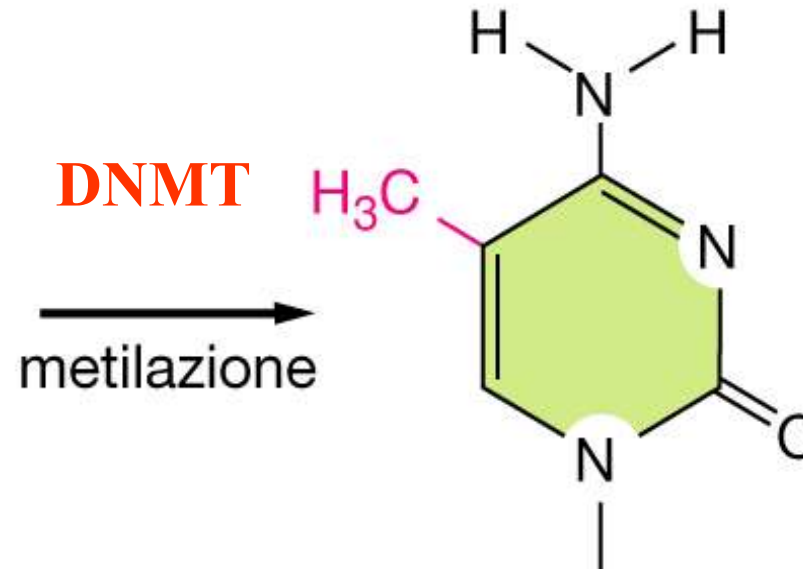
Nei vertebrati la metilazione opera di enzimi **DNA-metiltransferasi** sulla citosina: il risultato è la 5-metilcitosina.

na sul dinucleotide CpG aggiungono un gruppo metile al C5

citosina



5-metilcitosina

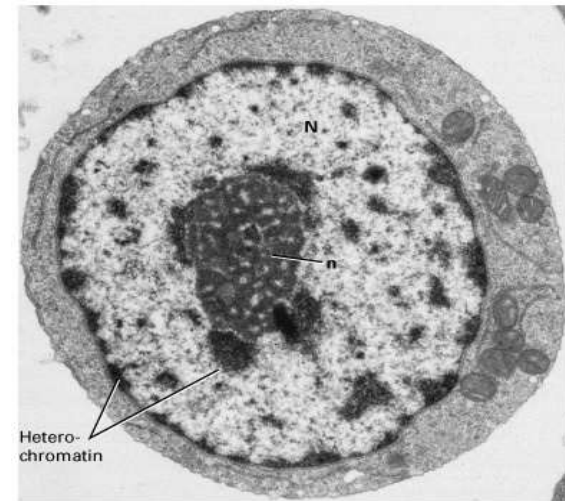
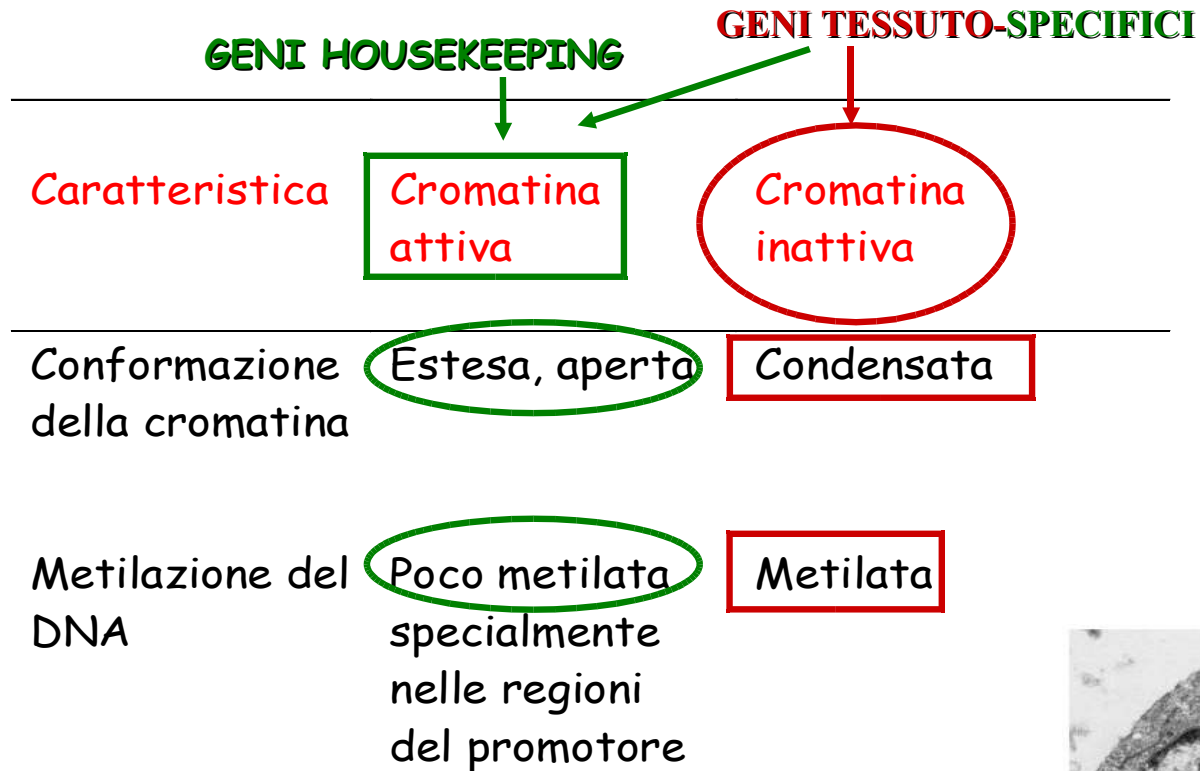


Le sequenze CpG sono sotto-rappresentate nel genoma (probabilmente per la tendenza della 5-metilcitosina a venire deaminata e mutata in T), ma abbondanti nelle regioni promotrici dei geni (**isole CpG**)

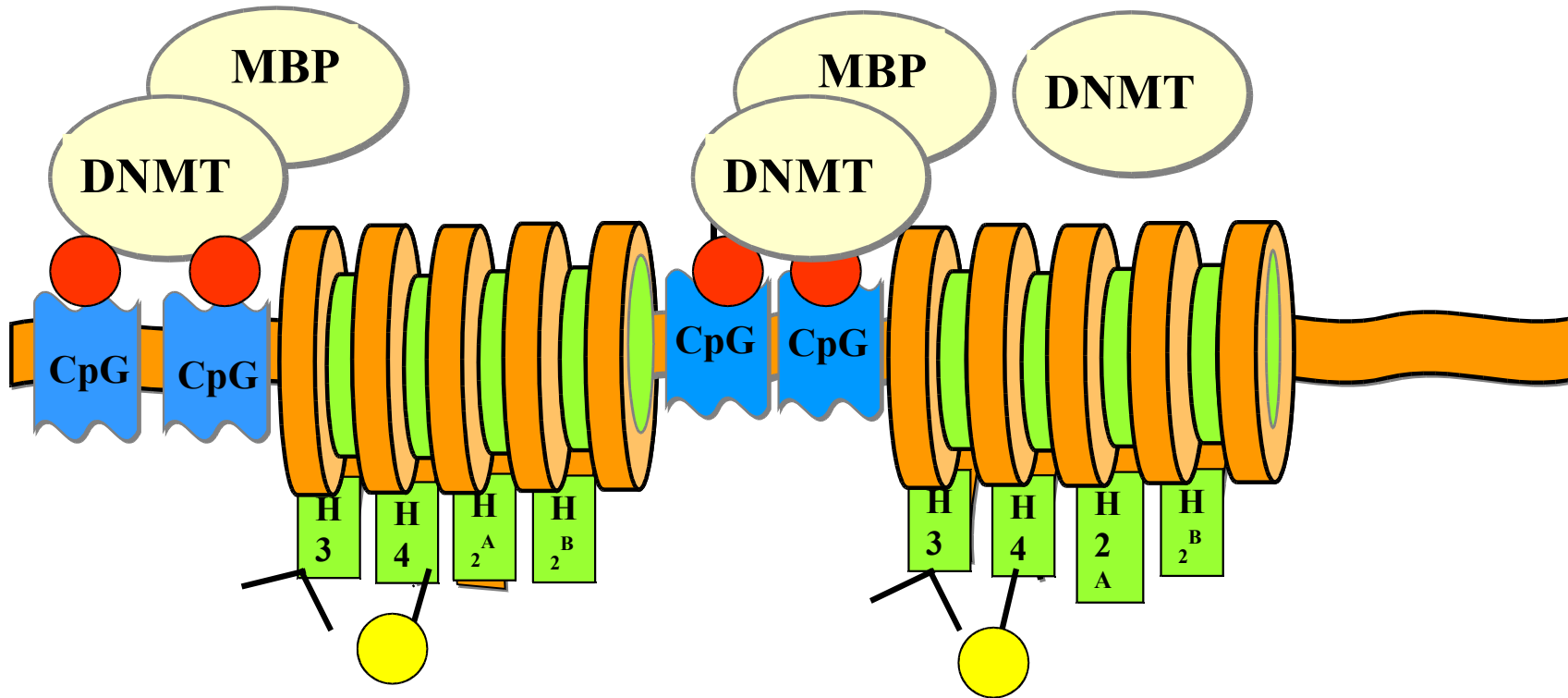
Le isole CpG nella regione promoter sono usualmente demetilate e la loro **metilazione determina una inibizione dell'espressione genica.**



CARATTERISTICHE DELLA CROMATINA



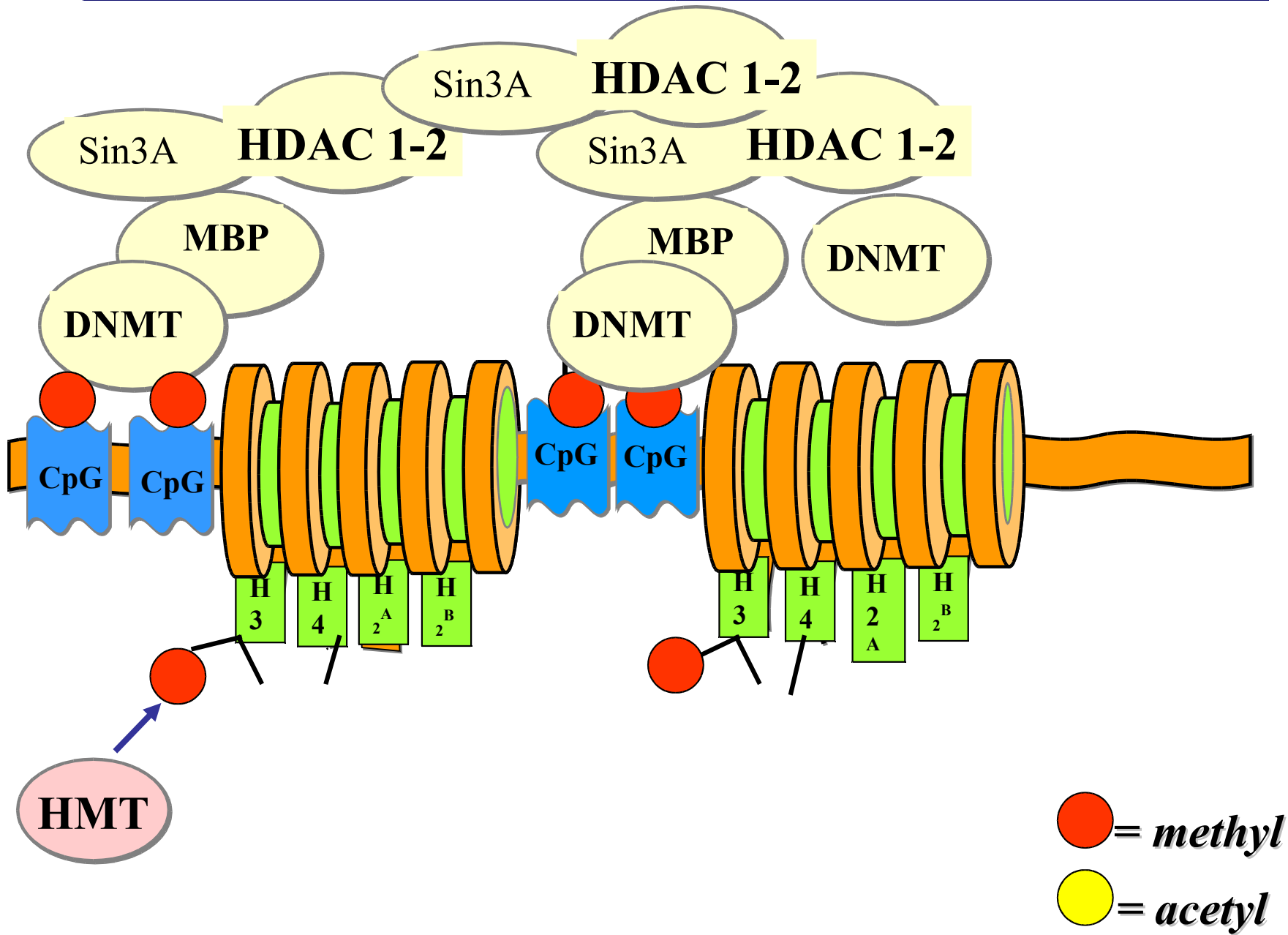
DNA hypermethylation in neoplastic cells



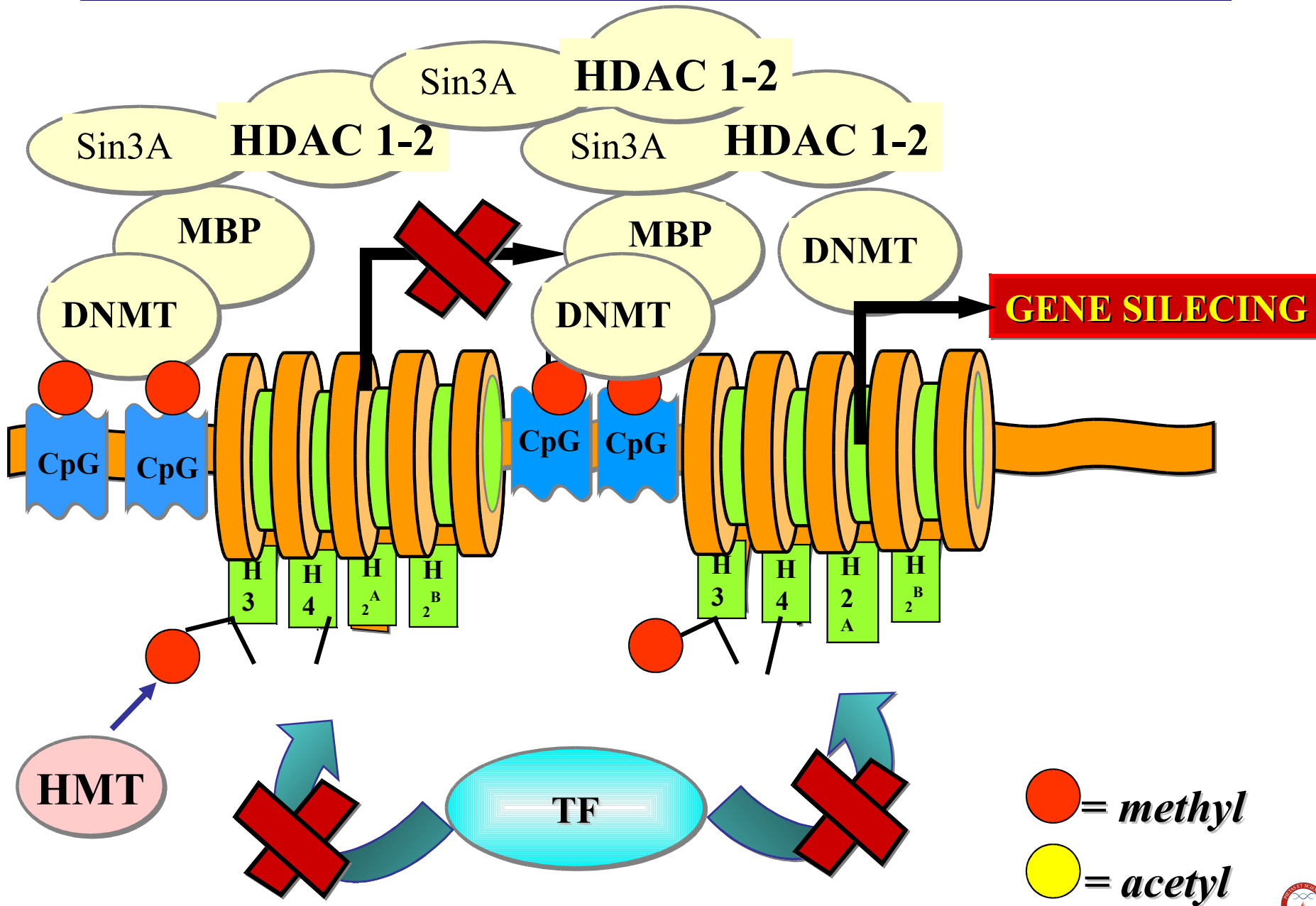
● = *methyl*
● = *acetyl*



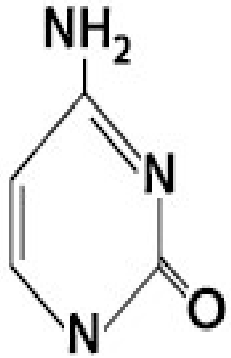
DNA hypermethylation in neoplastic cells



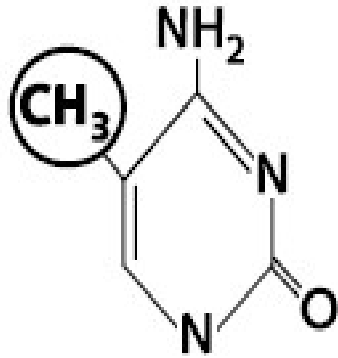
DNA hypermethylation in neoplastic cells



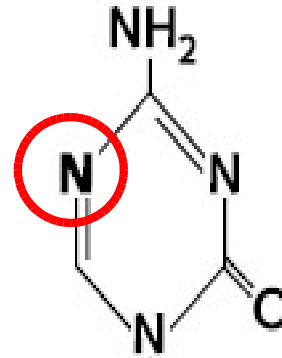
Epigenetic DRUGS



Cytosine

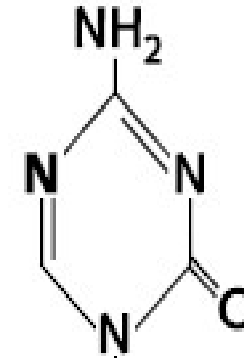


5-methyl-
cytosine



Ribose

5-aza-
cytidine

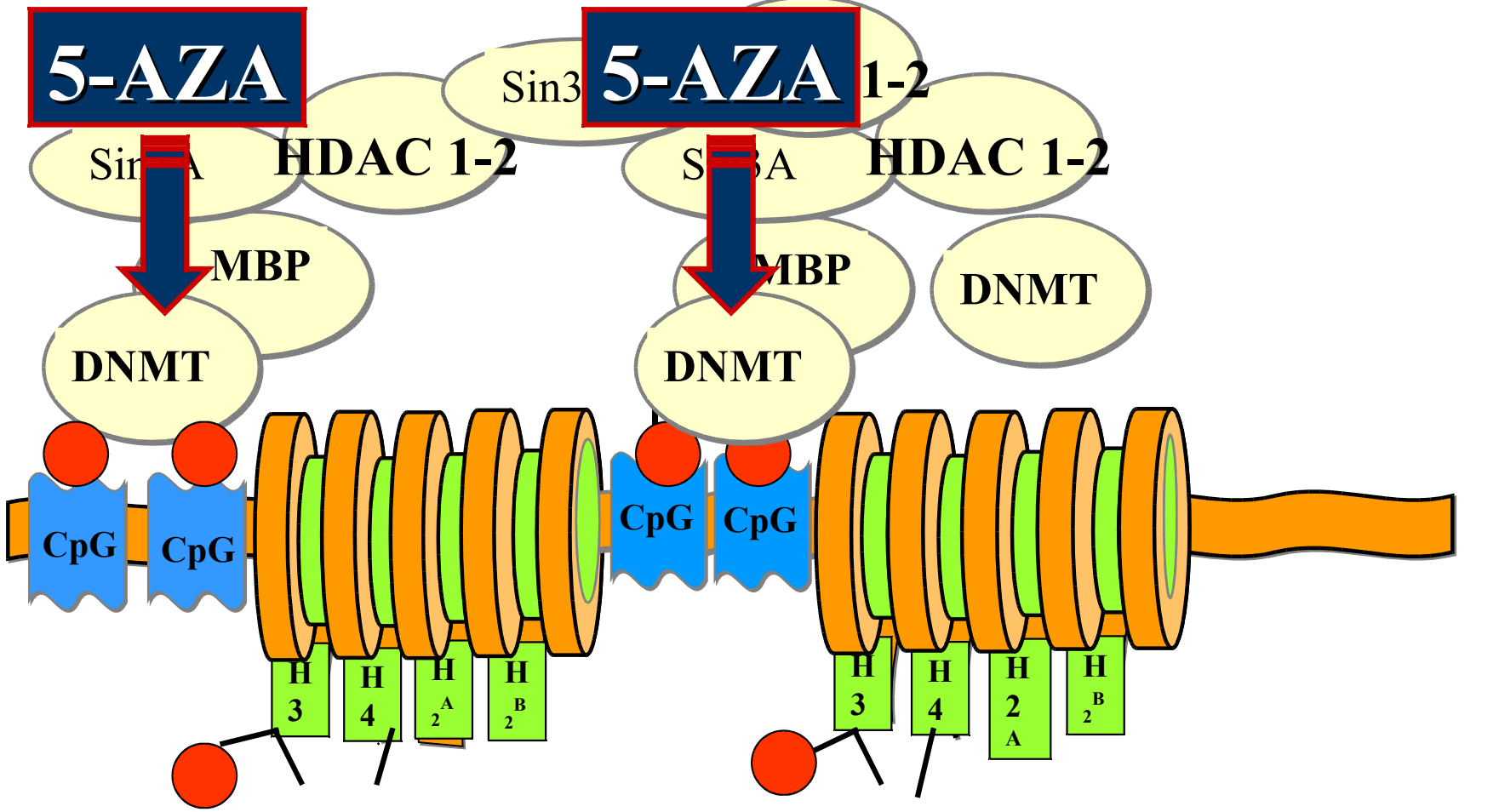


Deoxyribose

5-aza-2'-deoxy-
cytidine



Epigenetic therapy



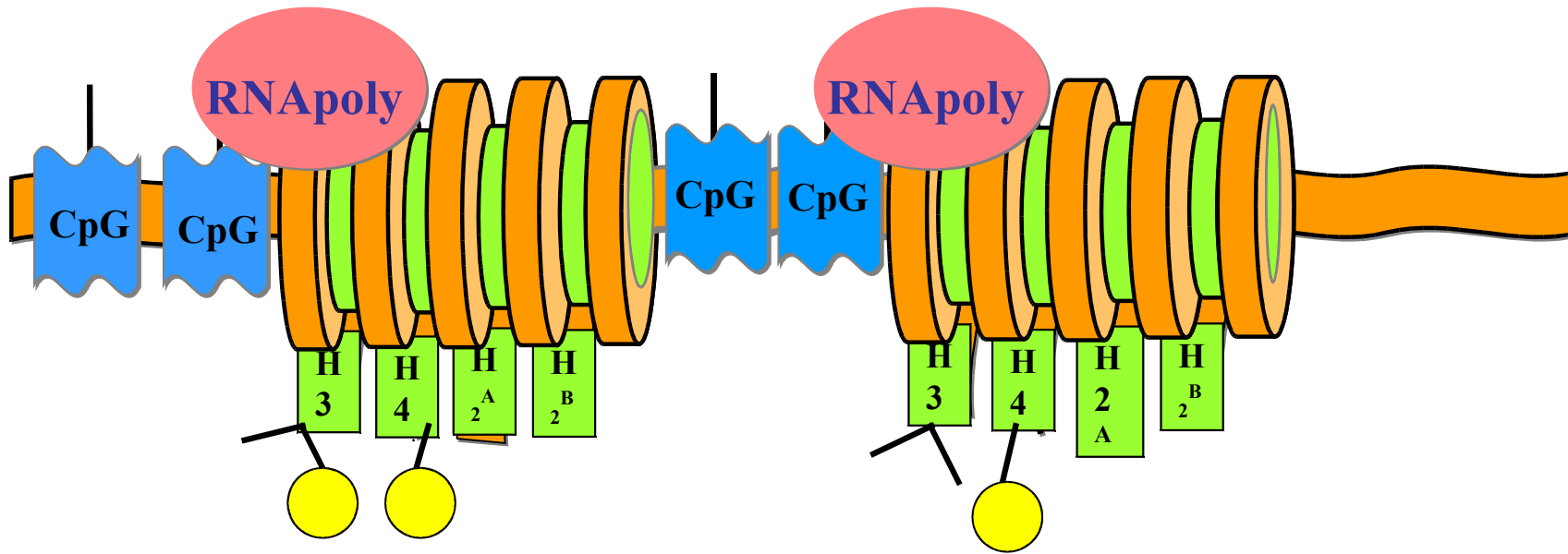
● = methyl
● = acetyl



Epigenetic therapy

5-AZA

5-AZA



● = *methyl*

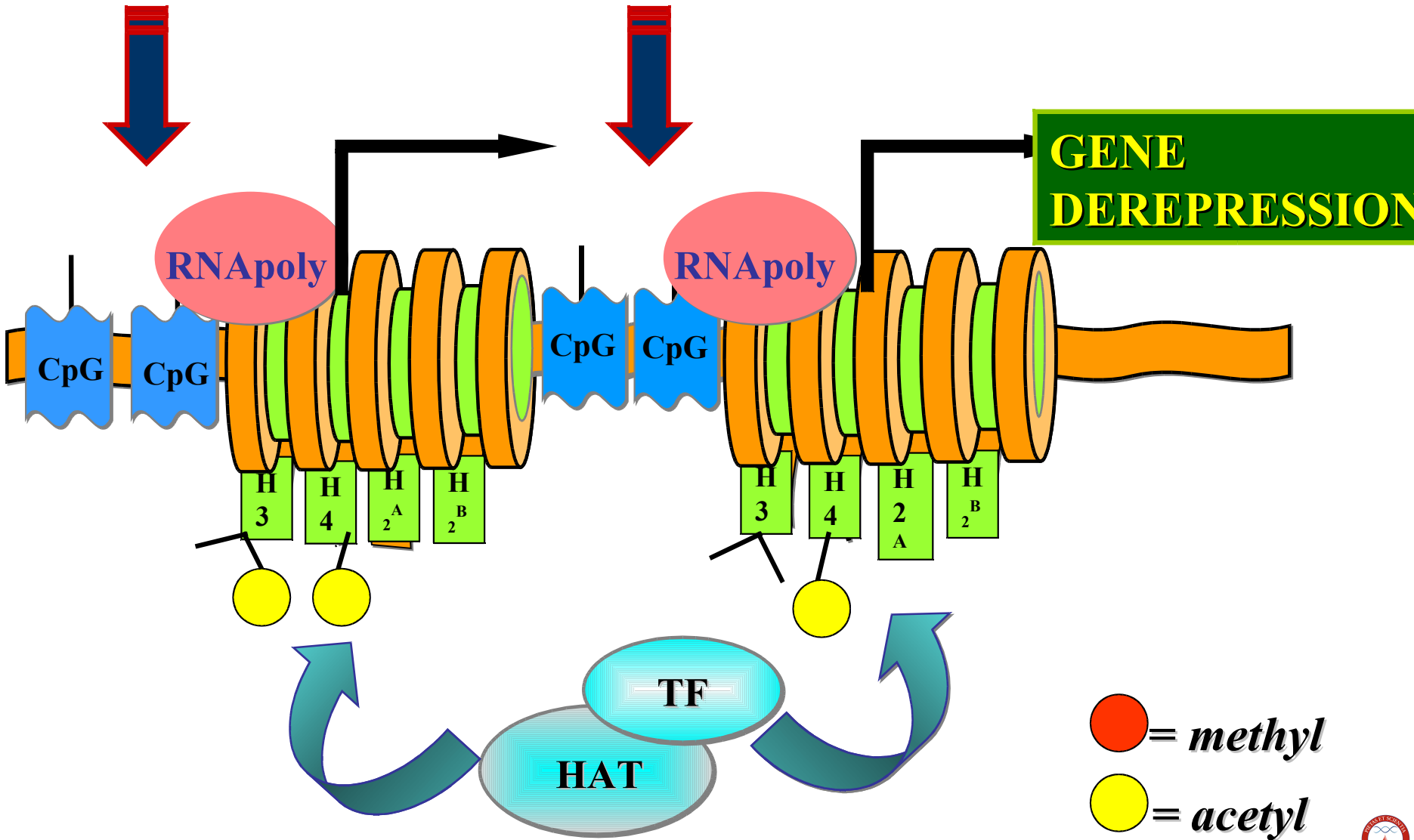
● = *acetyl*



Epigenetic therapy

5-AZA

5-AZA

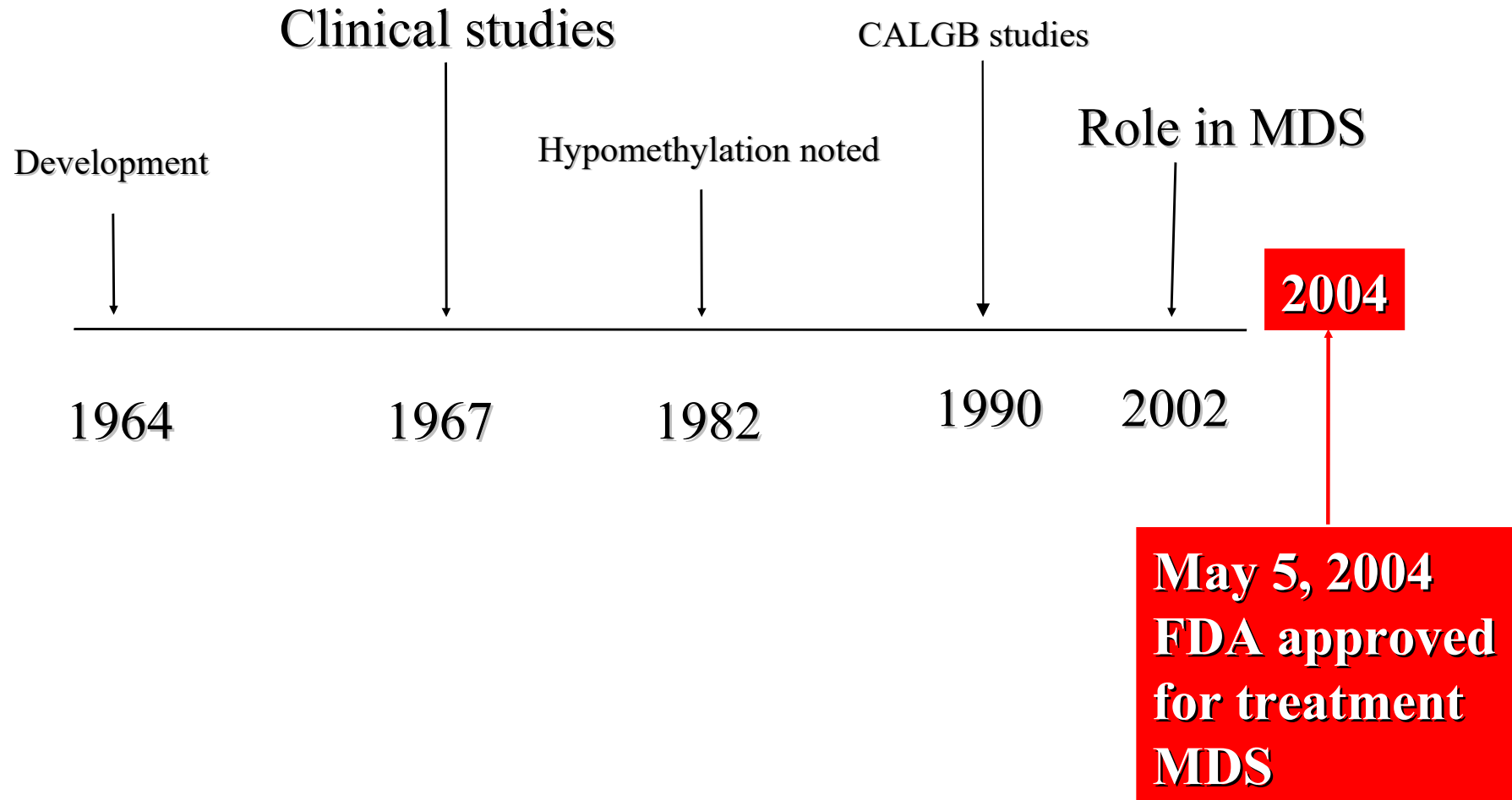


GENE
DEREPRESSION

● = methyl
● = acetyl

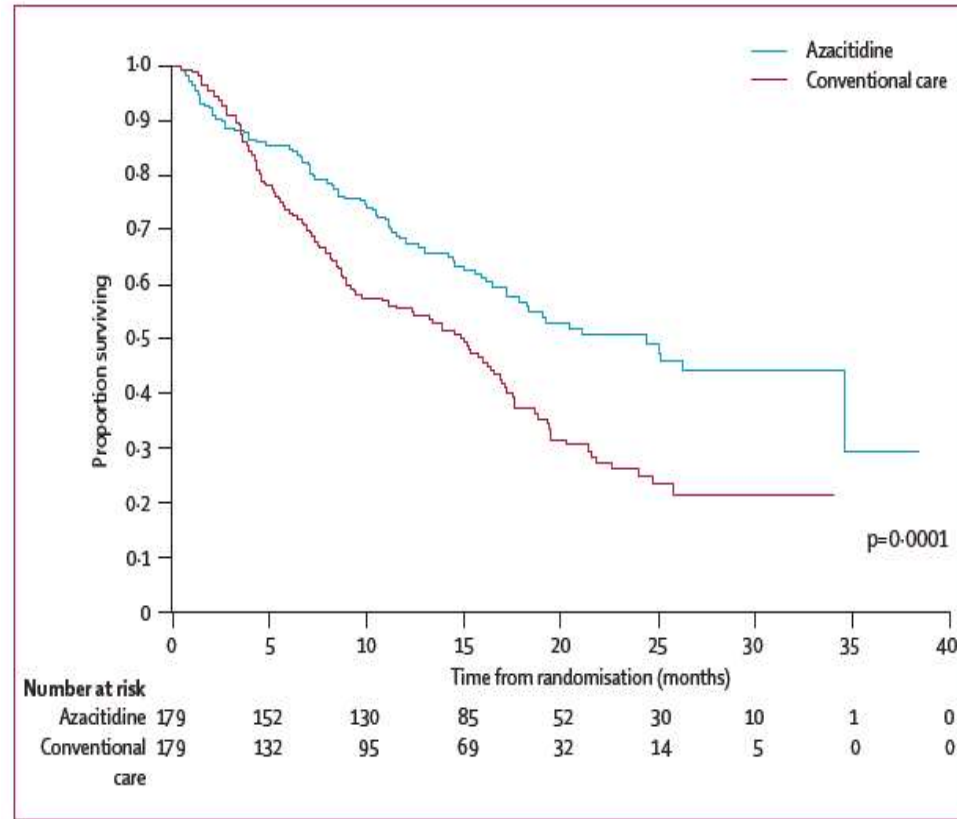
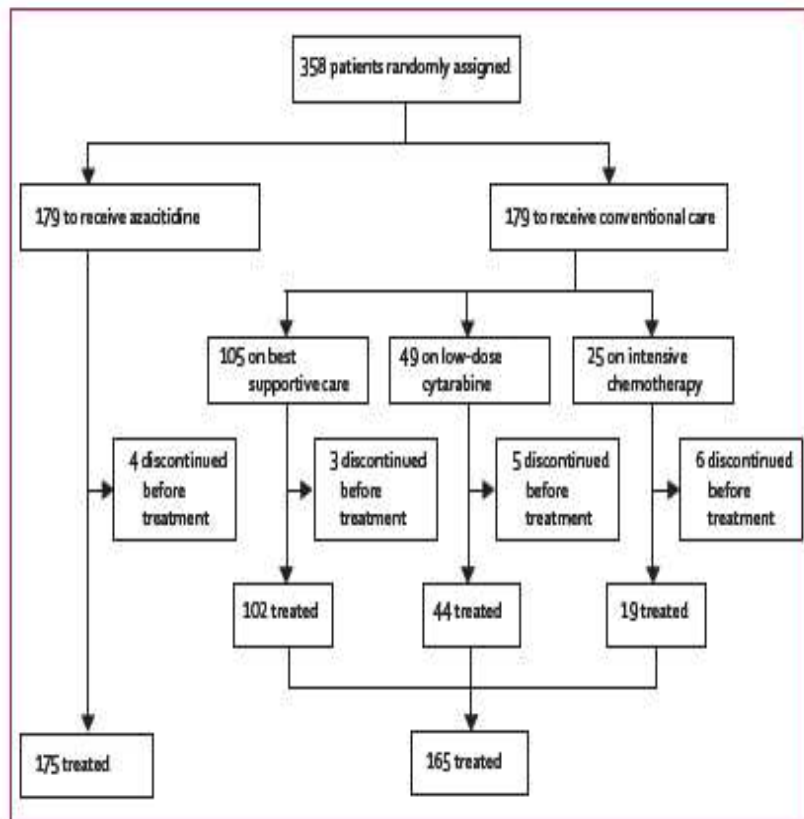


Azacitidine Time Line



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

