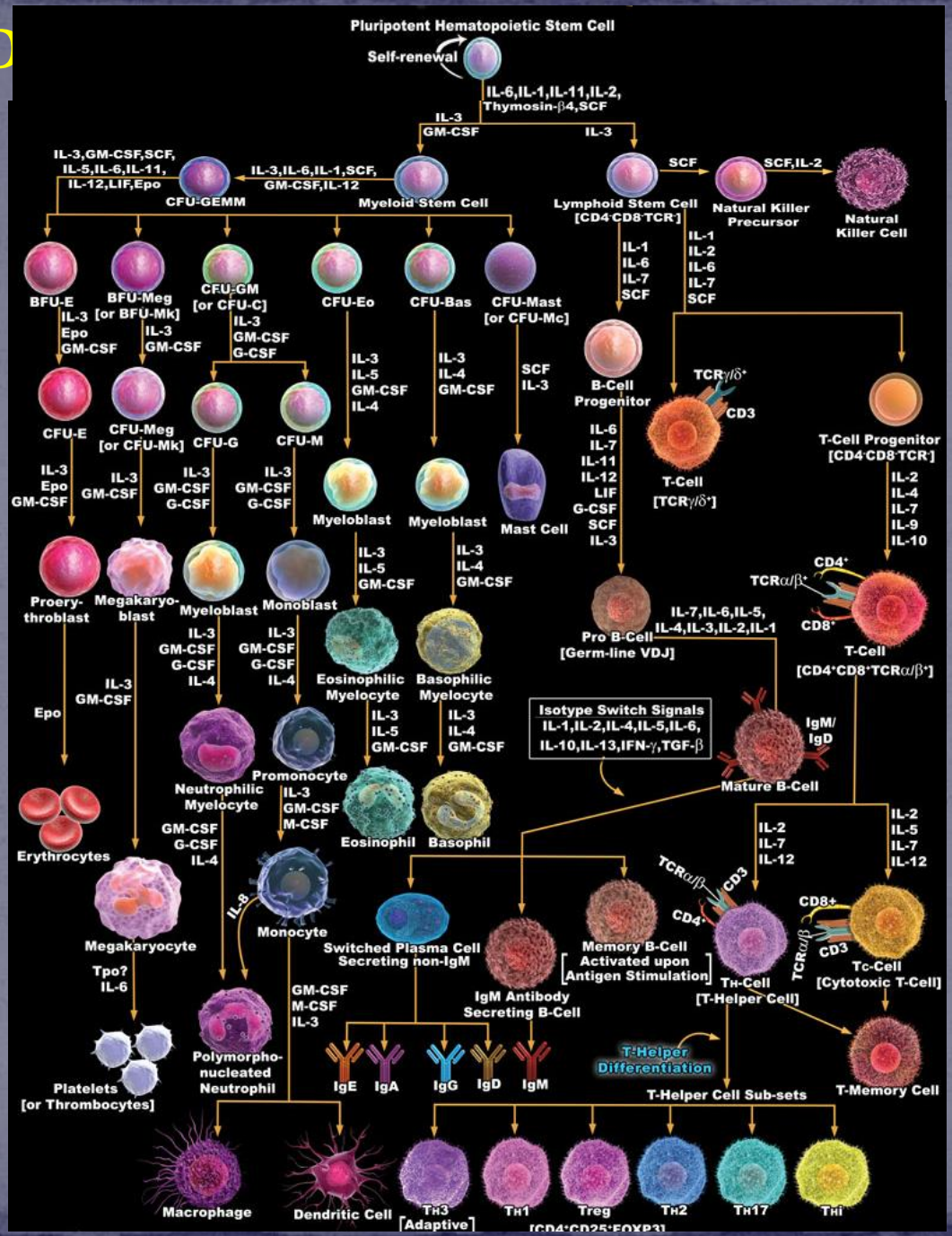
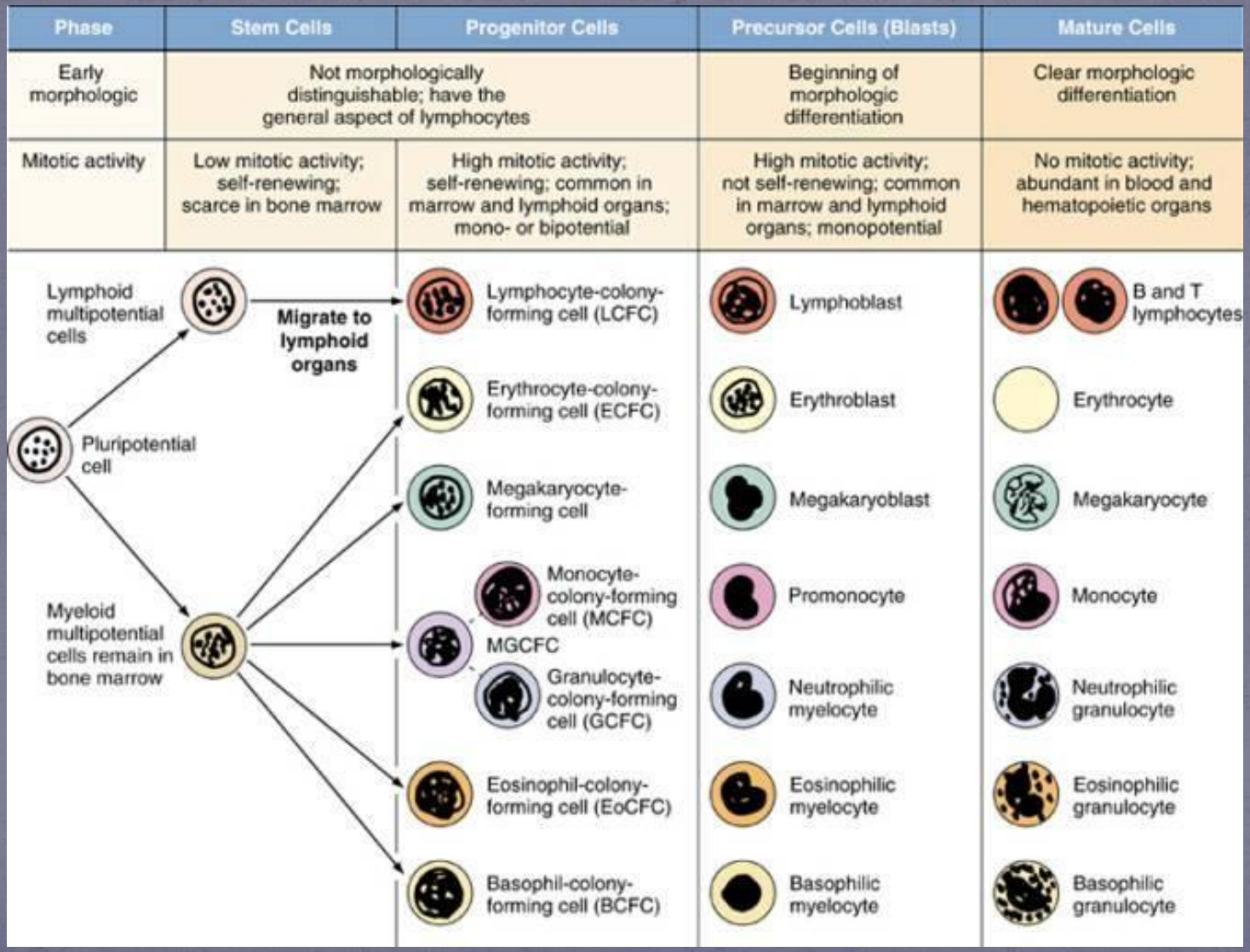
Disordini ematopoietici neoplastici

Maria Elena Gelain

PROD CELLULE

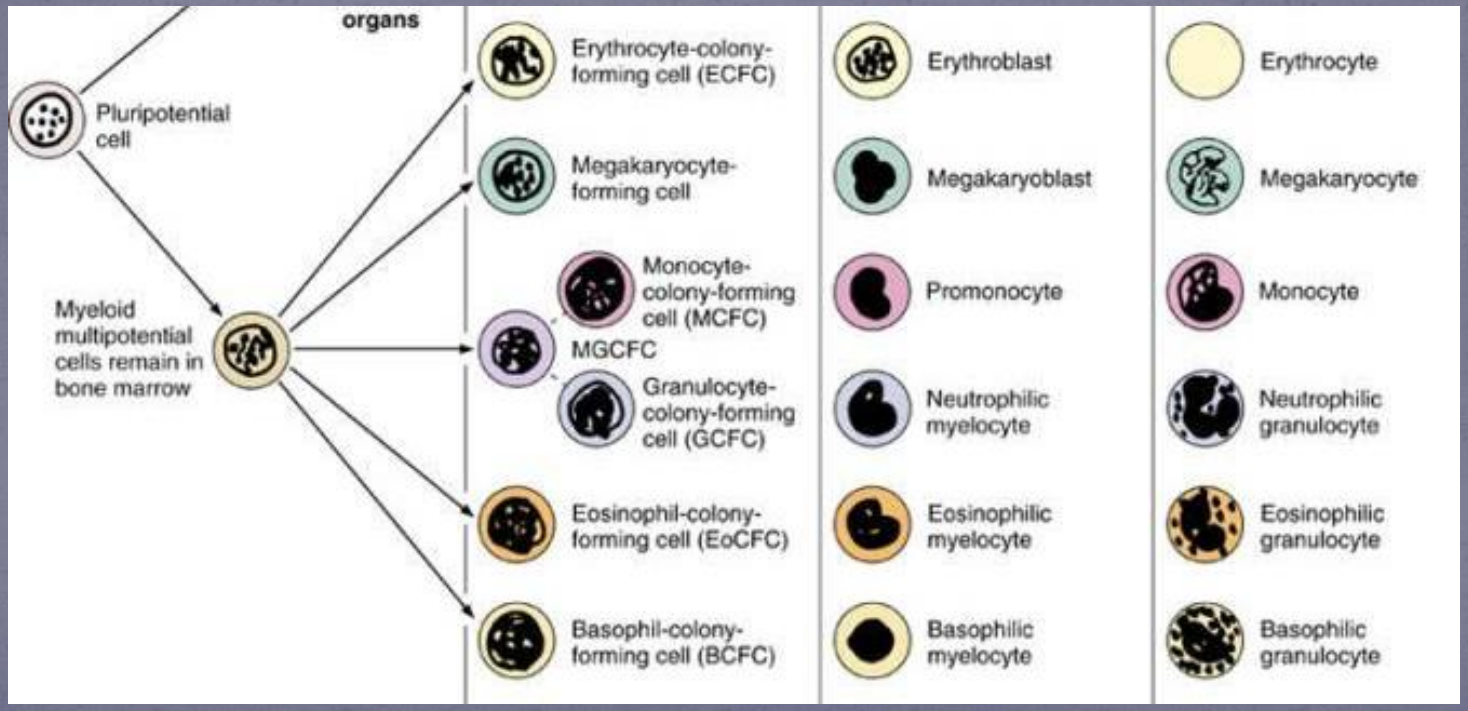


PRODUZIONE E MATURAZIONE DELLE CELLULE EMATOPOIETICHE



PRODUZIONE E MATURAZIONE DELLE CELLULE EMATOPOIETICHE

Phase	Stem Cells	Progenitor Cells	Precursor Cells (Blasts)	Mature Cells
Early morphologic	Not morphologically distinguishable; have the general aspect of lymphocytes		Beginning of morphologic differentiation	Clear morphologic differentiation
Mitotic activity	Low mitotic activity; self-renewing; scarce in bone marrow	High mitotic activity; self-renewing; common in marrow and lymphoid organs; mono- or bipotential	High mitotic activity; not self-renewing; common in marrow and lymphoid organs; monopotential	No mitotic activity; abundant in blood and hematopoietic organs

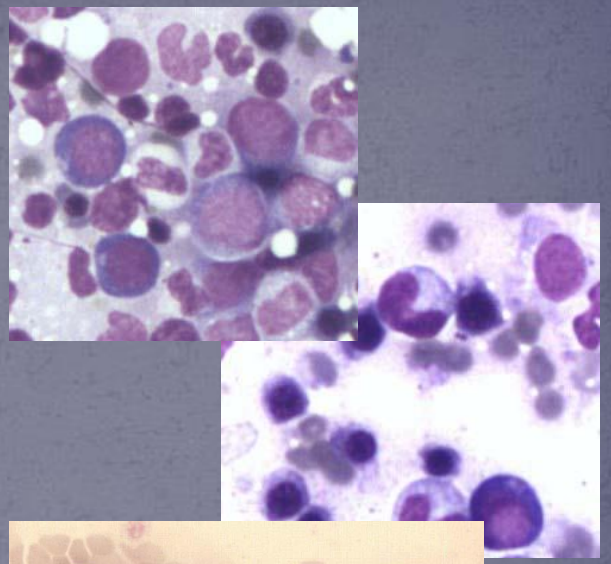


Cellule mieloidi

Midollo

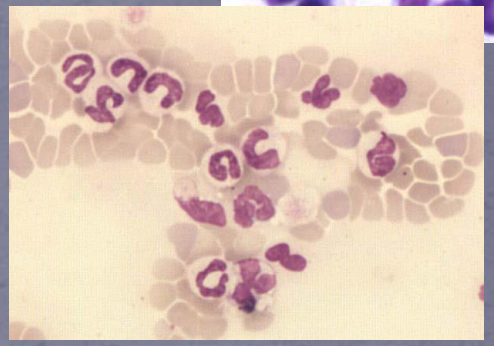
Pool proliferativo

Pool maturativo



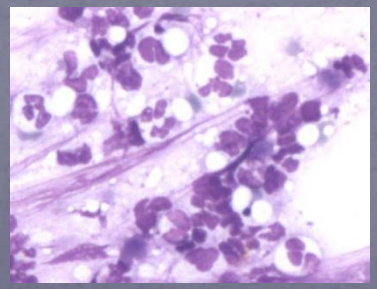
Sangue periferico

Pool circolante



tessuti

Distribuzione nei tessuti



MATURAZIONE CELLULE MIELODI

VARIAZIONI MORFOLOGICHE

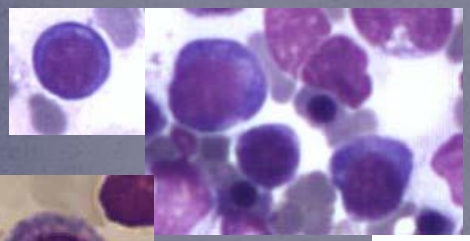
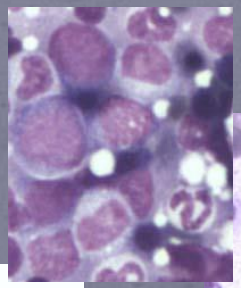
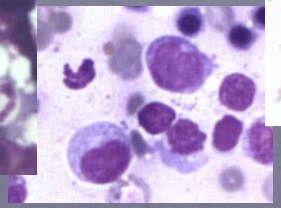
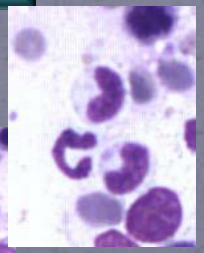
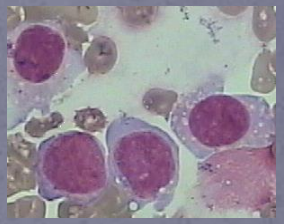
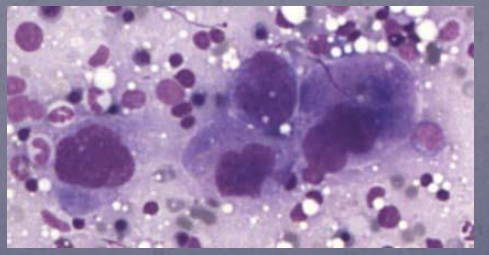
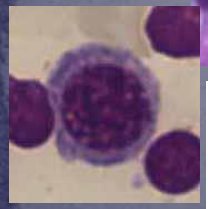
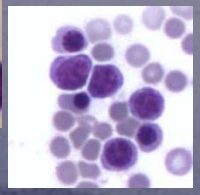
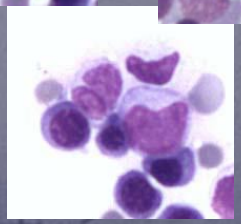
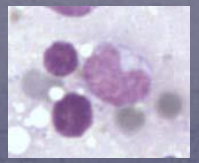
- rubriblasto
- prorubrocita
- rubrocita
- metarubrocita
- reticolocita
- eritrocita

- mieloblasto
- promielocita
- mielocita
- Metamielocita
- Granulocita a banda
- Granulocita segmentato

VARIAZIONI IMMUNOFENOTIPICHE

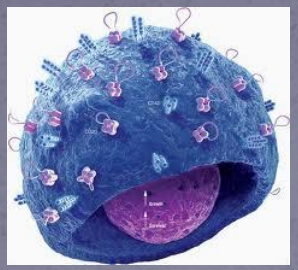
- monoblasto
- promonocita
- monocita

- megacarioblasto
- megacariocita
- piastrine

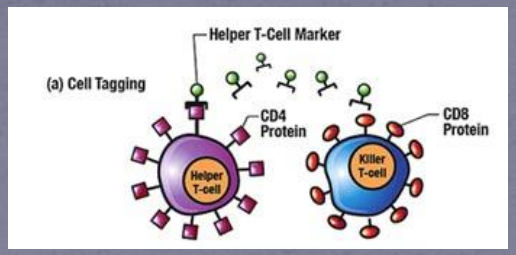











MATURAZIONE CELLULE MIELODI

VARIAZIONI MORFOLOGICHE



VARIAZIONI IMMUNOFENOTIPICHE



Antigeni dei precursori
CD34-CD117

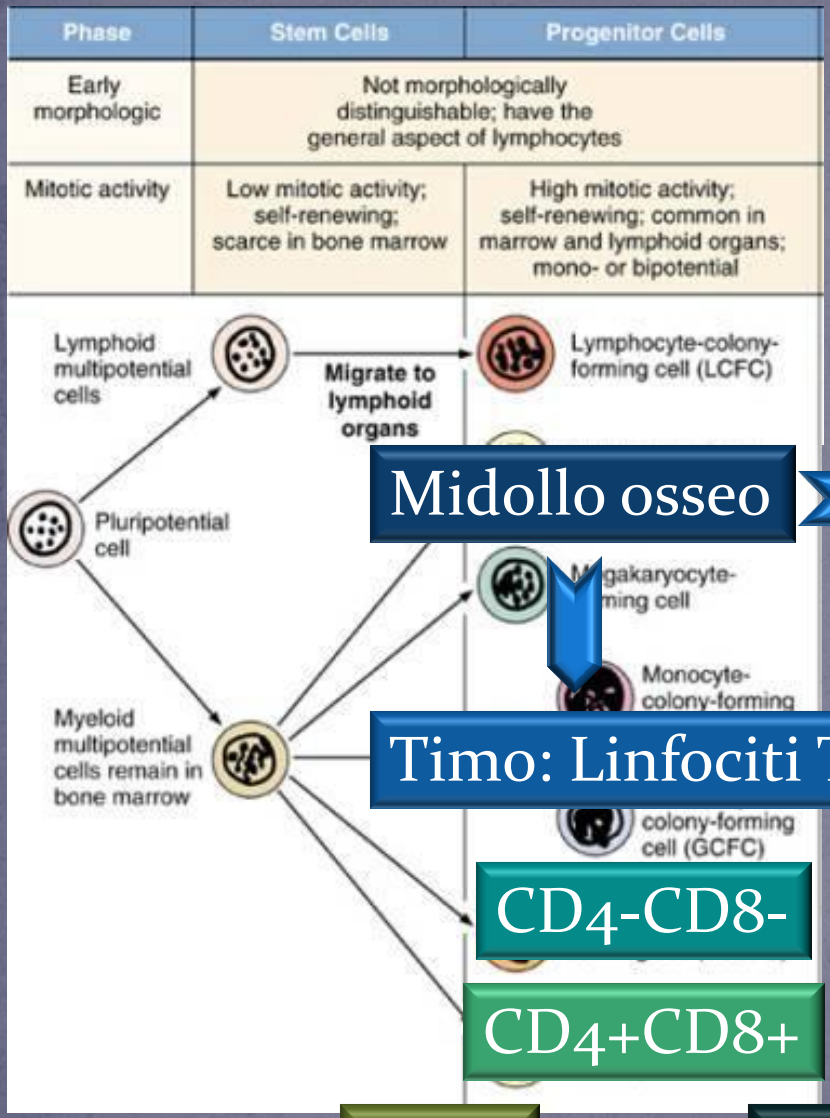
Antigeni panleucocitari
CD45-CD18

Antigeni specifici

- MIELOIDI:
  - CD11c: cellule mielodi
  - CD11B: granulociti neutrofili
  - CD4: granulociti neutrofili (cane)
  - CD14: monociti
  - CD41-61: megacariocita/piastrine

ISTIOCITARI
- CD1, CD11c, CD11d

Maturazione dei linfociti



Midollo osseo → Linfociti B → plasmacellule

Timo: Linfociti T

CD4-CD8-

CD4+CD8+

CD4+ CD8+

Maturazione dei linfociti

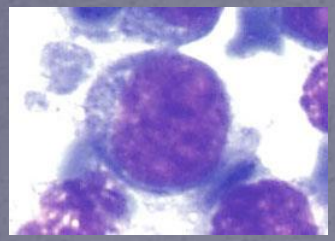
VARIAZIONI MORFOLOGICHE

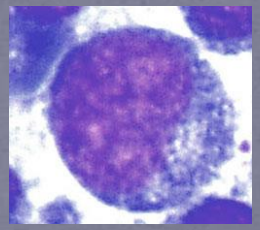
VARIAZIONI IMMUNOFENOTIPICHE

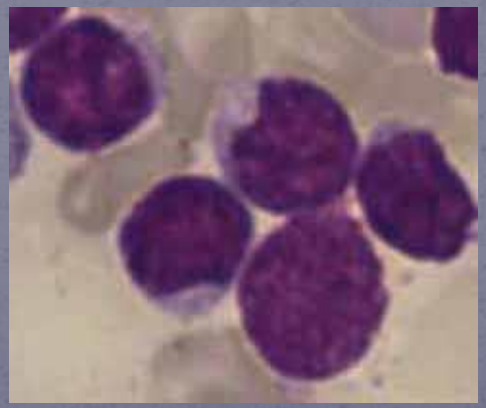
Linfoblasto

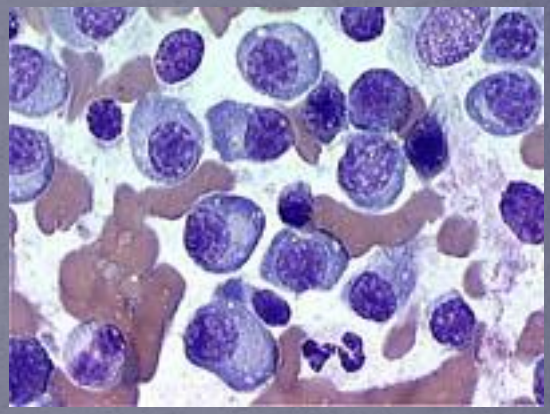
linfocita

plasmacellula









Maturazione dei linfociti

VARIAZIONI MORFOLOGICHE

VARIAZIONI IMMUNOFENOTIPICHE

Antigeni dei precursori
CD34-CD117

Antigeni panleucocitari
CD45-CD18

- T-CELL:
  - CD3: Linfociti T
  - CD5: Linfociti T/subset linfociti B
  - CD4: Linfociti T helper
  - CD8: linfociti T citotossici

- B-CELL:
  - CD21: linfociti B maturi
  - CD79a: linfociti B
  - IgM
  - IgG

A COSA SERVE TUTTO QUESTO?

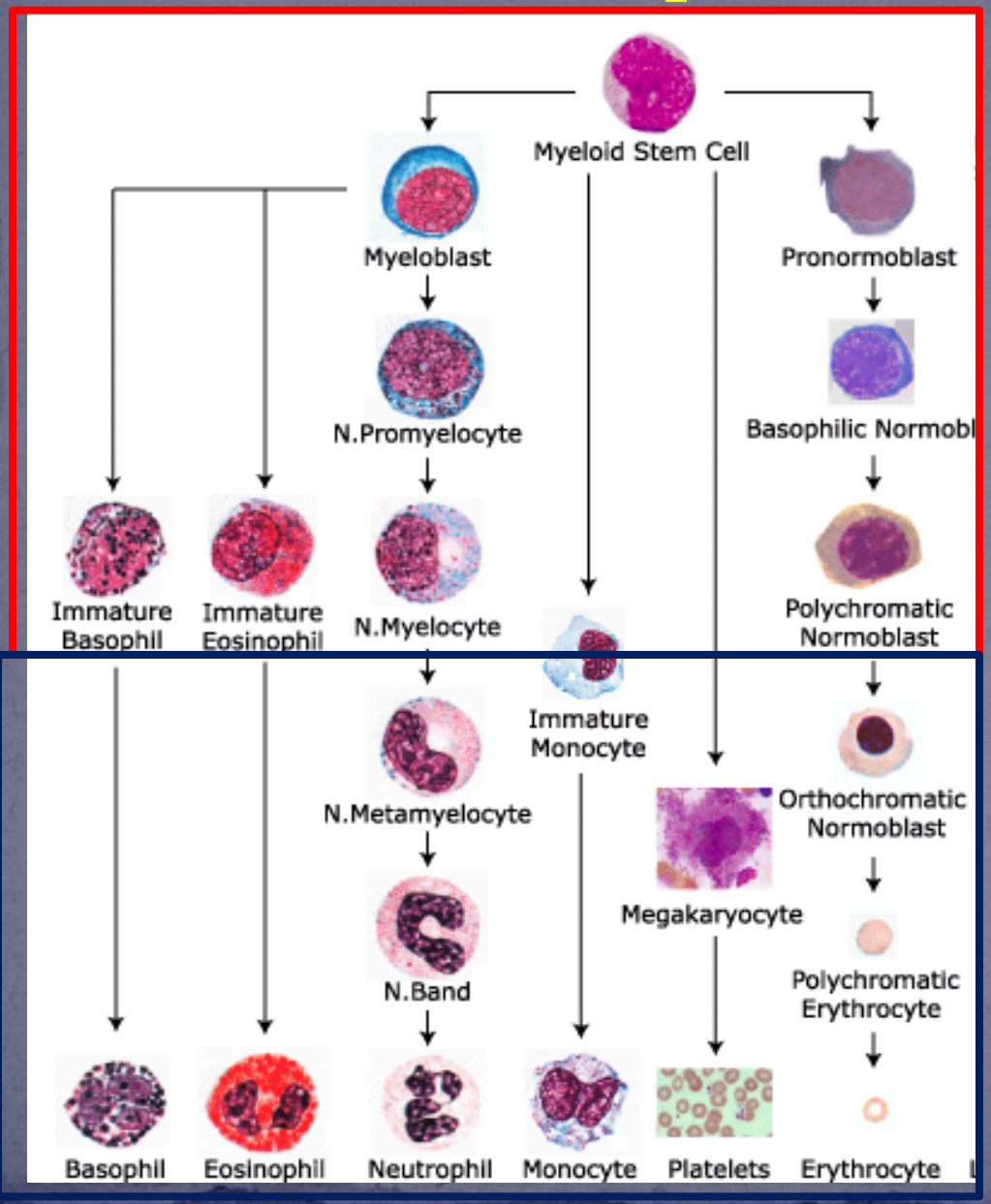
Trasformazione neoplastica in ogni step

A seconda del momento

Neoplasie diverse per

- **Morfologia cellulare**
- **Fenotipo cellulare**
- **Comportamento biologico**
- **Presentazione clinica**
- **Prognosi**
- **terapia**

Neoplasie mielodi



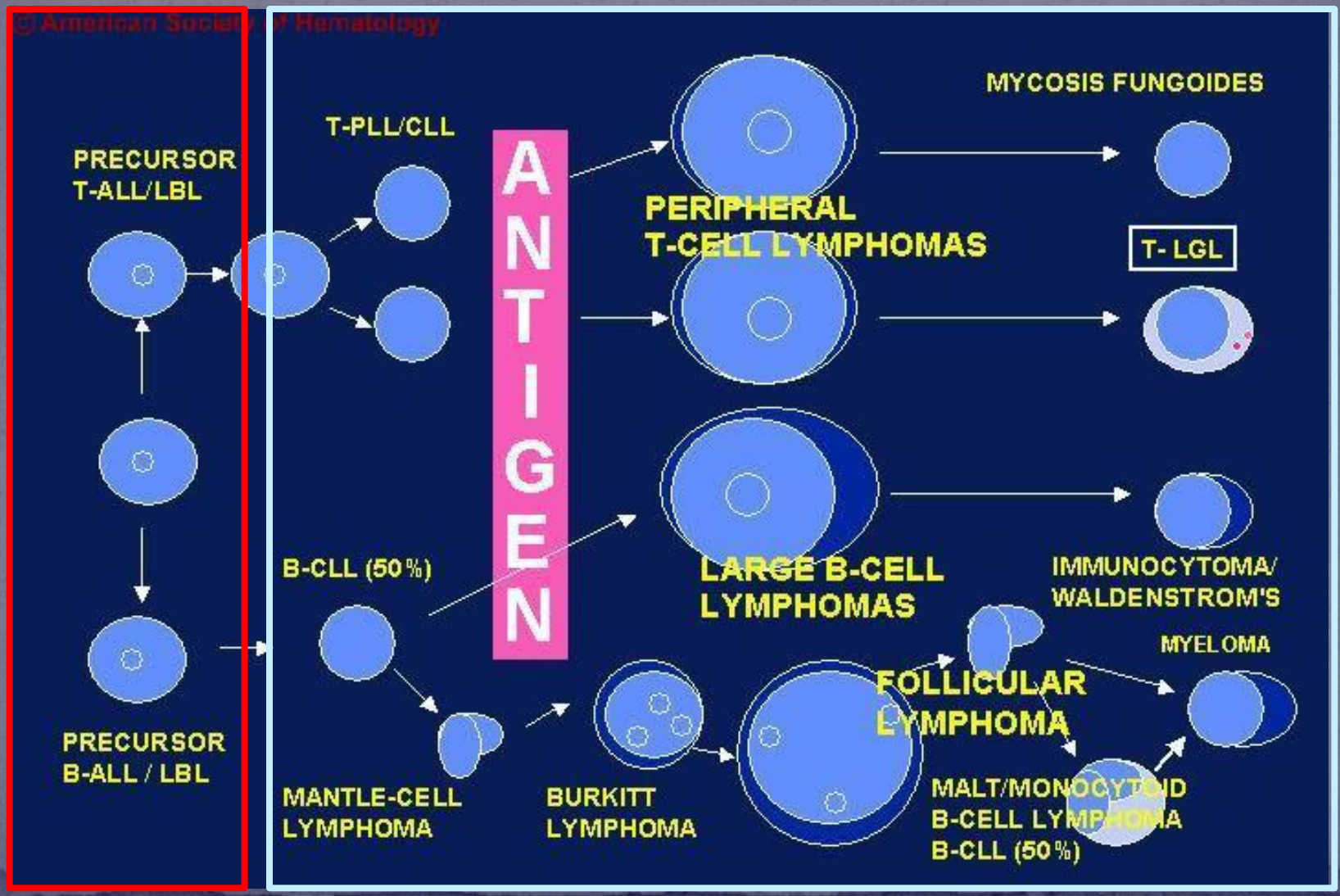
Leucemie mieloidi acute

Leucemie mieloidi croniche (neoplasie mieloproliferative)

Neoplasie linfoidi

Leucemie linfoidi acute
Linfomi linfoblastici

Leucemie linfocitiche croniche
Linfomi



PERCHE' DIFFERENZIARE?

Informazioni prognostiche ⟷ **Scelte terapeutiche**

- **Origine**
  - **Neoplastiche**
  - **Reattive**

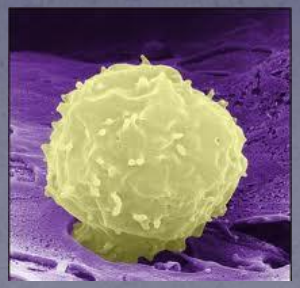
Lo stadio maturativo
- **Precursori: acute**
- **Cellule mature: croniche**

linea cellulare d'appartenenza
- **Linfoide**
- **mieloide**

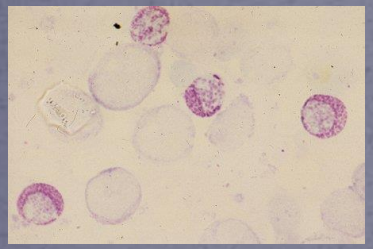
Staging della neoplasia
- **Ricerca delle stesse cellule in altri distretti**

COME DIFFERENZIARE?

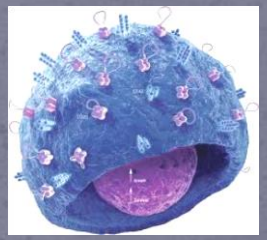
- **MORFOLOGIA**



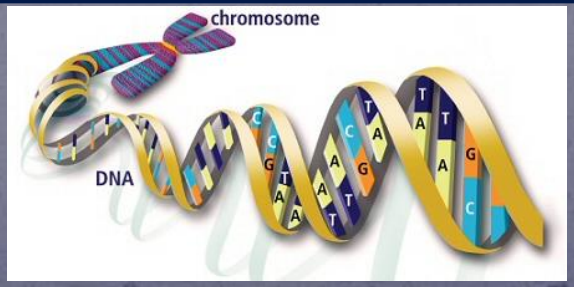
CITOCHIMICA



FENOTIPO



GENETICA

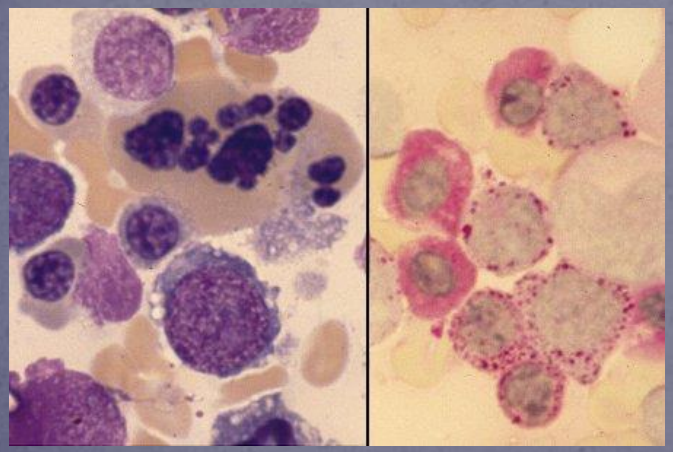


2-CITOCHIMICA
Linea d'appartenenza

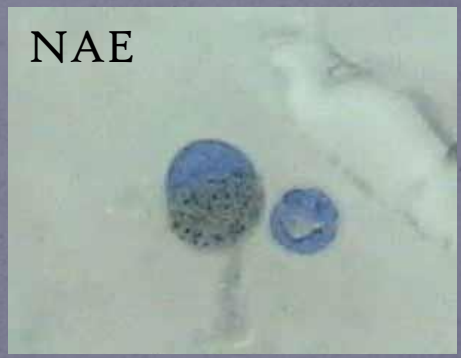
Speciali colorazioni per: enzimi, lipidi, carboidrati

Strisci, aspirati, citogentrifugati
preparati freschi, fissati , congelati

mieloide



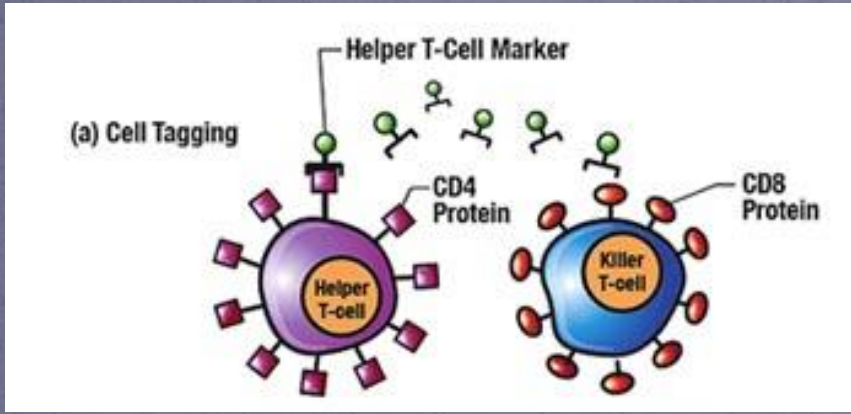
linfoide



3-IMMUNOFENOTIPIZZAZIONE
LINEA D' APPARTENENZA

Cellule neoplastiche:
stesse caratteristiche antigeniche della loro controparte neoplastica

Sulla superficie e nel citoplasma : stesse proteine



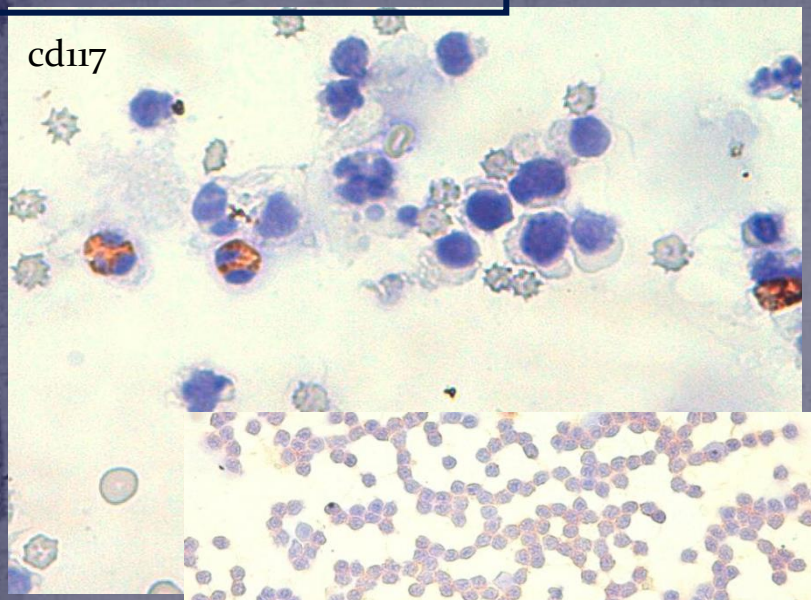
Identificate con anticorpi monoclonali

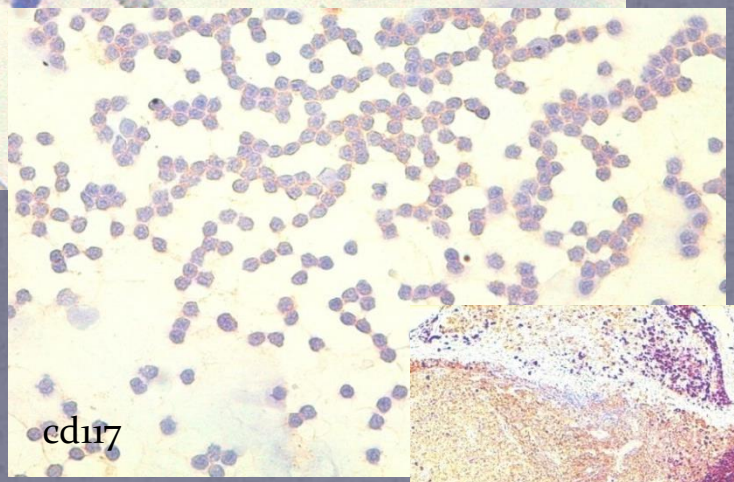
Immunocitochimica
immunoistochimica

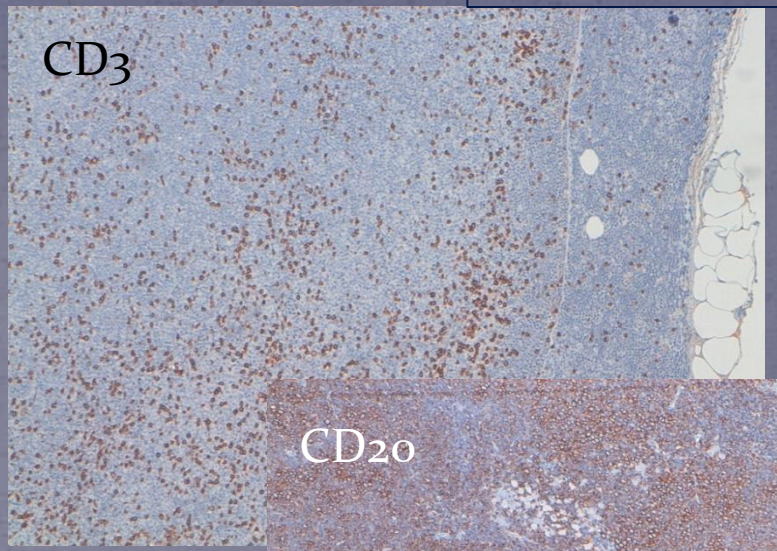
Citometria a flusso

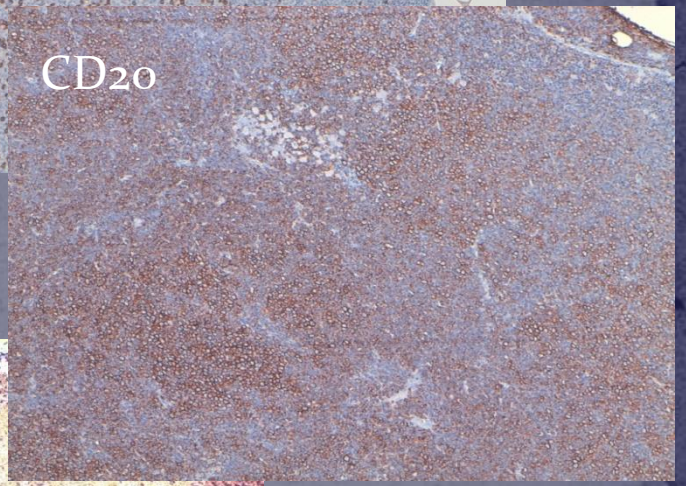
Immunocitochimica

immunoistochimica









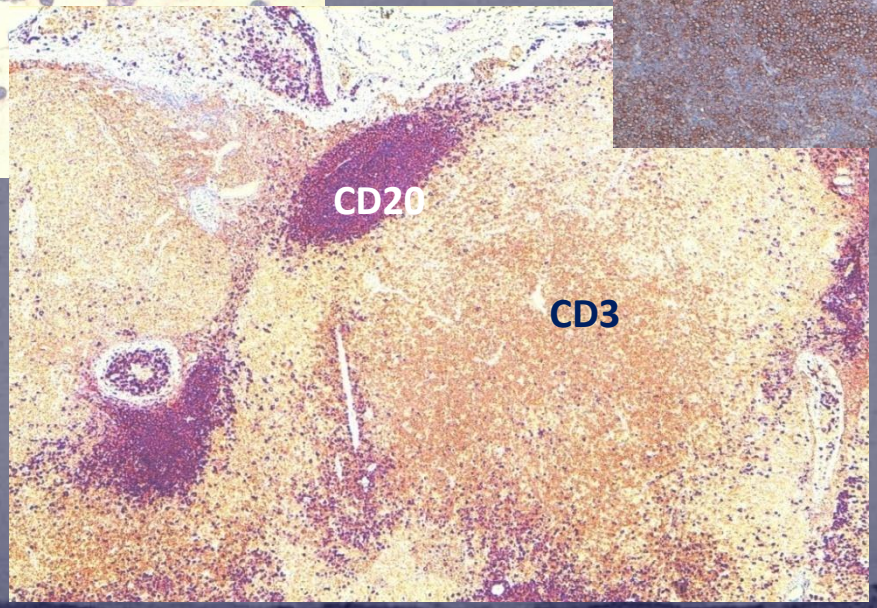
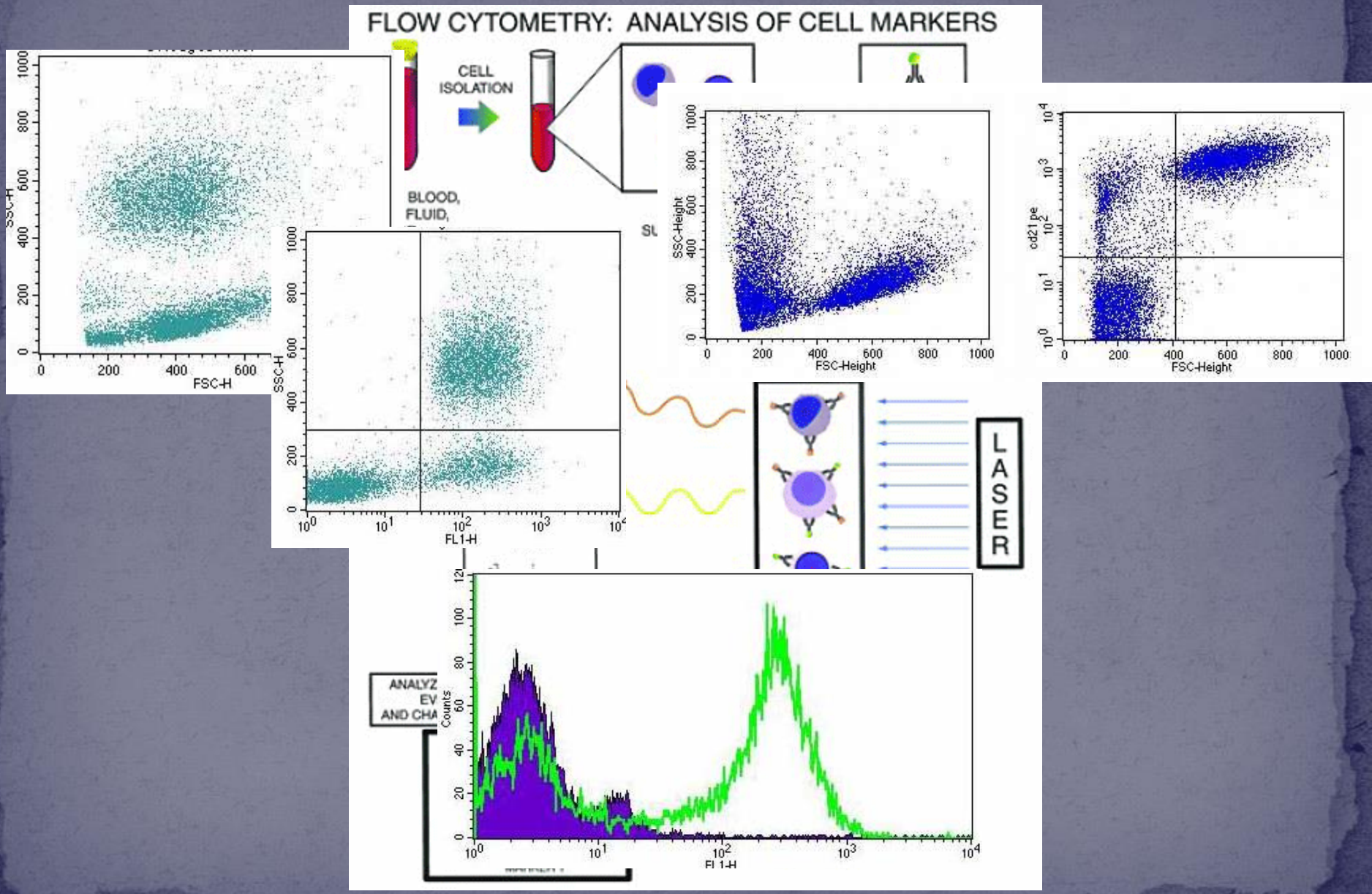


Foto Dr.ssa A. Aricò
Dr. Luca Aresu

Citometria a flusso



3-IMMUNOFENOTIPIZZAZIONE
DIAGNOSI DI NEOPLASIA

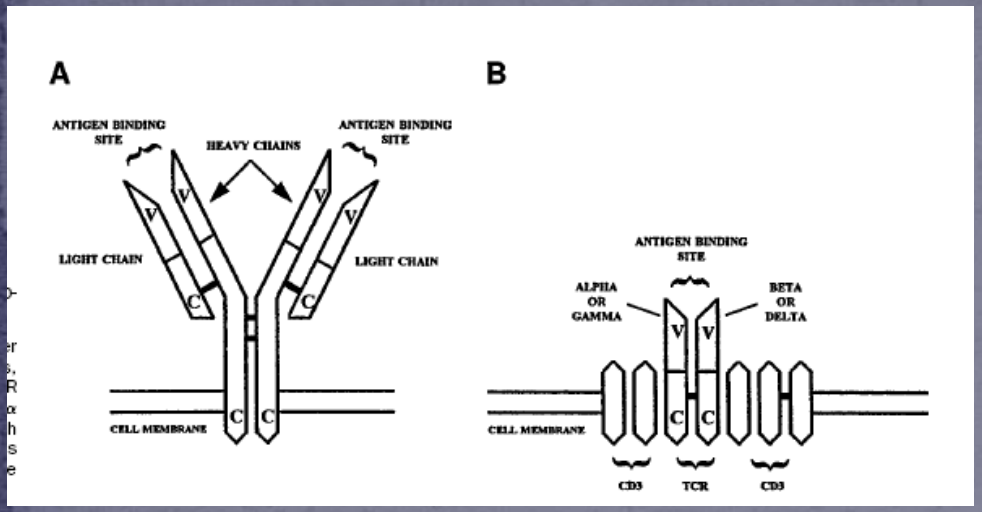
-POPOLAZIONE CLONALE

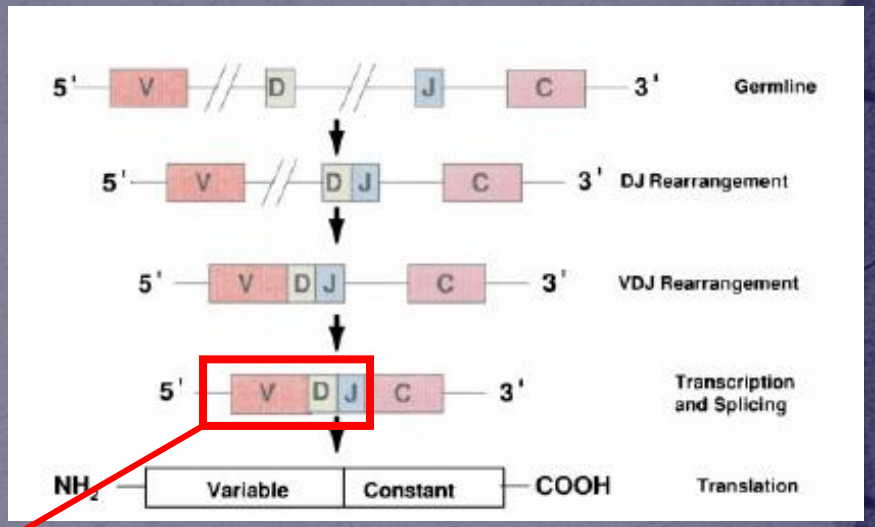
"LINEAGE INFEDELITY" (espressioni aberranti):
-diminuita o assente espressione di marker panleucocitari
-perdita di espressione di marker tipici della linea cellulare in esame,
-la presenza contemporanea di antigeni appartenenti a
-linee cellulari diversi
-stadi maturati diversi

3-IMMUNOFENOTIPIZZAZIONE
STADIO MATURATIVO

Positività a marker dei precursori ematopoietici : CD34
Positività a marker degli stadi immaturi (marker citoplasmatic vs marker di membrana): CD79a-CD21

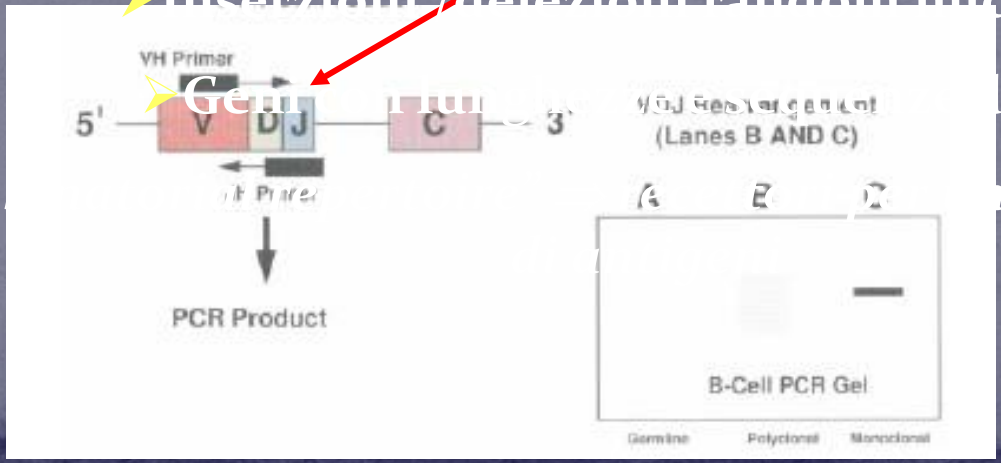
4-PARR (PCR for Antigen Receptor Rearrangements)
DIAGNOSI DI NEOPLASIA





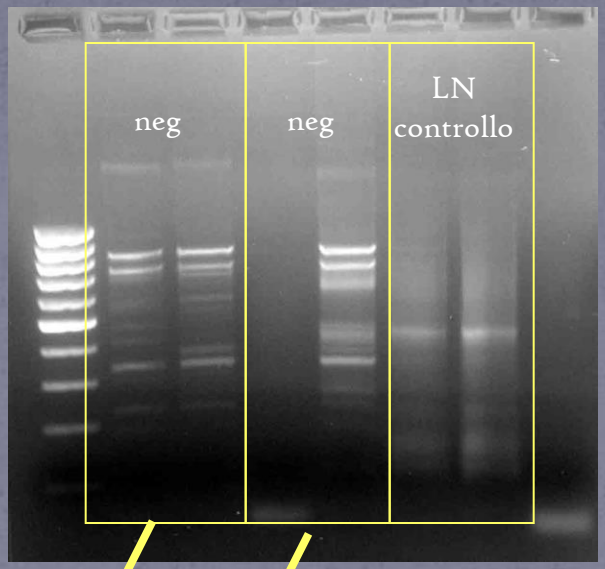
Geni che codificano per la regione che lega l'antigene

- **Inserzioni /delezioni random nucleotidi**
- **Geni [illegible] diverse**
- ***"com[illegible] numero "infinito"***



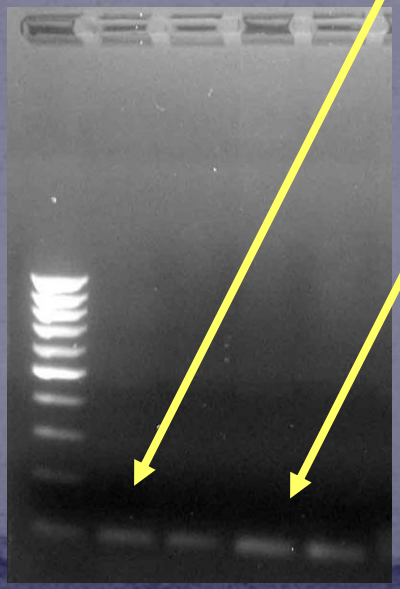
4-PARR (PCR for Antigen Receptor Rearrangements)
DIAGNOSI DI NEOPLASIA

negativo

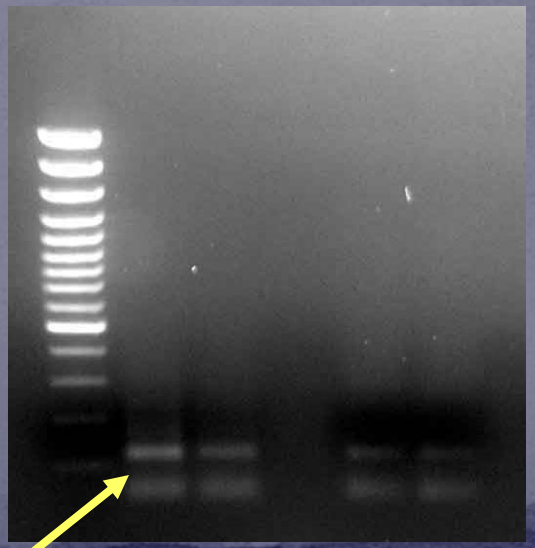


IgH

positivo



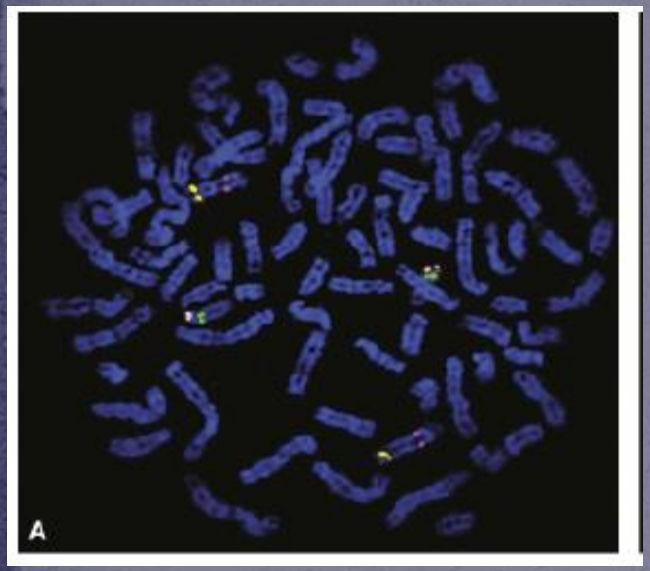
TCR



IgH

FUTURO.....PROSSIMO

ANALISI GENETICA
-IDENTIFICARE TRANSLOCAZIONI CROMOSOMICHE
-IDENTIFICARE MUTAZIONI SPECIFICHE



Identification of JAK2 mutations in canine primary polycythemia

Stephanie Beurlet[a,b,c], Patricia Krief[a,b], Arnaud Sansonetti[a,b], Alexandra Briend-Marchal[d], Jean-Jacques Kiladjian[e], Rose Ann Padua[a,b], Christine Chomienne[a,b,f], and Bruno Cassinat[a,b,f]

[a]Unité INSERM UMR-S 940, Hôpital Saint-Louis, Paris, France; [b]Institut Universitaire d'Hematologie, Universite Paris Diderot, Hôpital Saint-Louis, Paris, France; [c]CHV Fregis, Arcueil, France; [d]Laboratoire Vébiotel, Arcueil, France; [e]AP-HP, Centre d'Investigation Clinique, Hôpital Saint-Louis, Paris, France; [f]AP-HP, Unité de Biologie Cellulaire, Hôpital Saint-Louis, Paris, France

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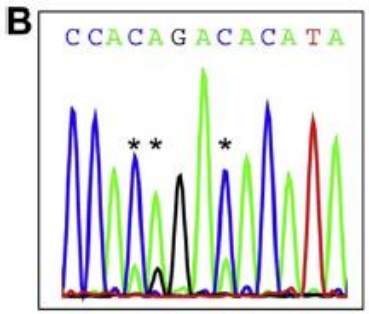


CASE REPORT

BCR-ABL translocation in a dog with chronic monocytic leukemia

Janice A. Cruz Cardona[1], Rowan Milner[2], A. Rick Alleman[1], Christina Williams[3], William Vernau[4], Matthew Breen[3,5], Mary Tompkins[6]

Departments of [1]Physiological Sciences and [2]Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA; [3]Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA; [4]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California–Davis, Davis, CA, USA; [5]Center for Comparative Medicine and Translational Research, North Carolina State University, Raleigh, NC, USA; and [6]Clinical Immunology Laboratory, North Carolina State University, Raleigh, NC, USA

SISTEMI CLASSIFICATIVI

Non considerare la pluralità se non è necessario
Guglielmo di Occam

PERCHE'?

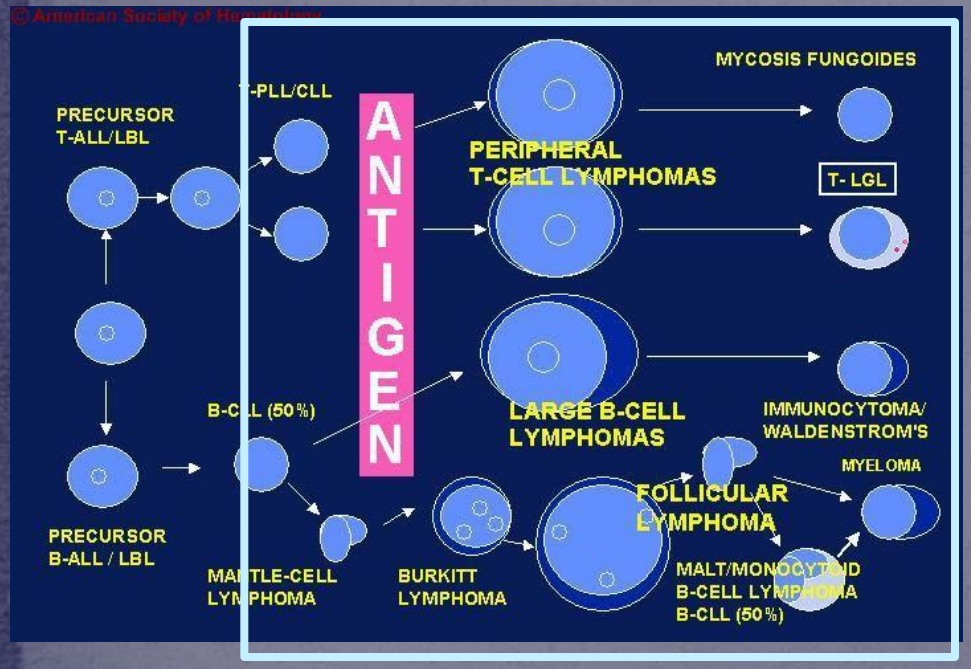
- **Dare un nome e cognome ad un'entità per predirne il comportamento biologico**
  - **Prognosi**
  - **Terapia**

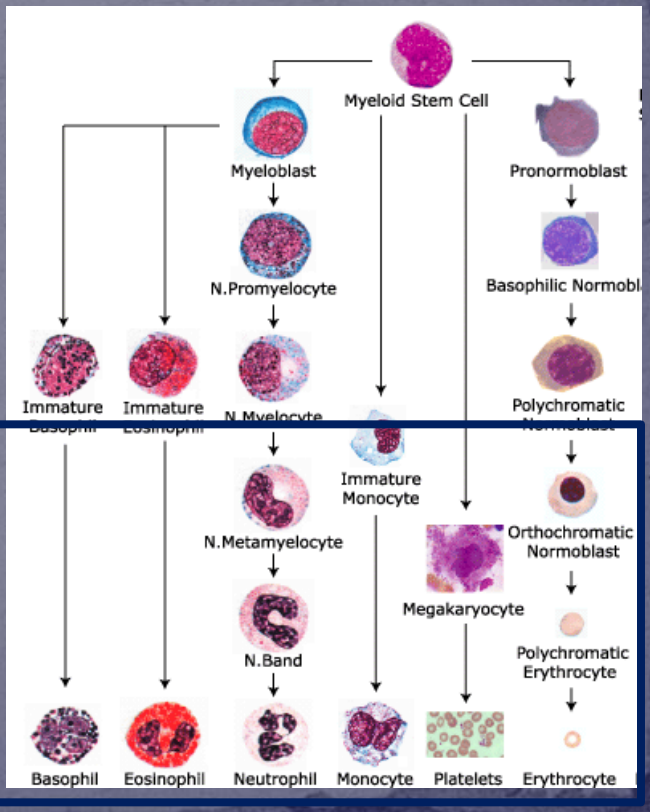
COME?

Analogia cane-uomo: adattamento degli schemi classificativi

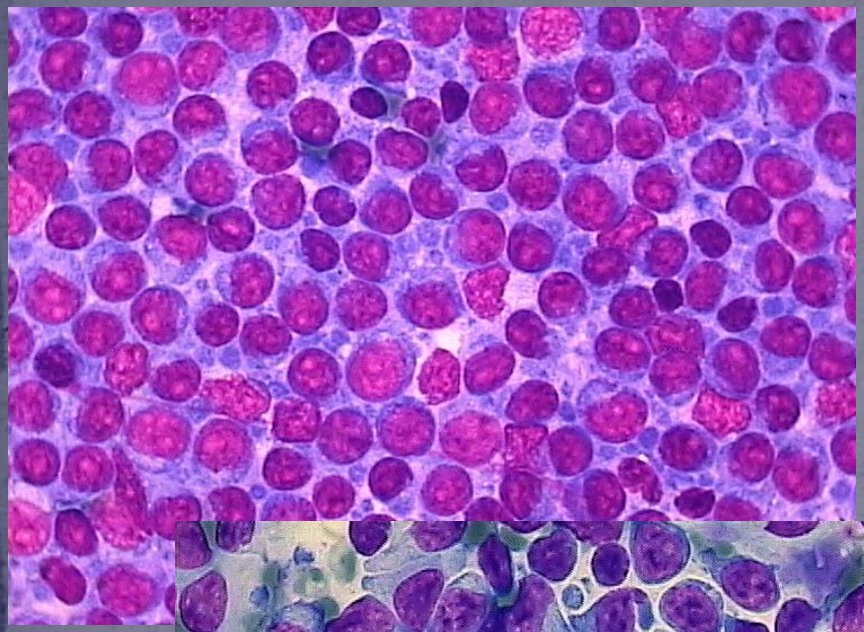
- **Presentazione clinica**
- **Criteri anatomici**
- **Criteri citomorfologici**
- **Criteri fenotipici**

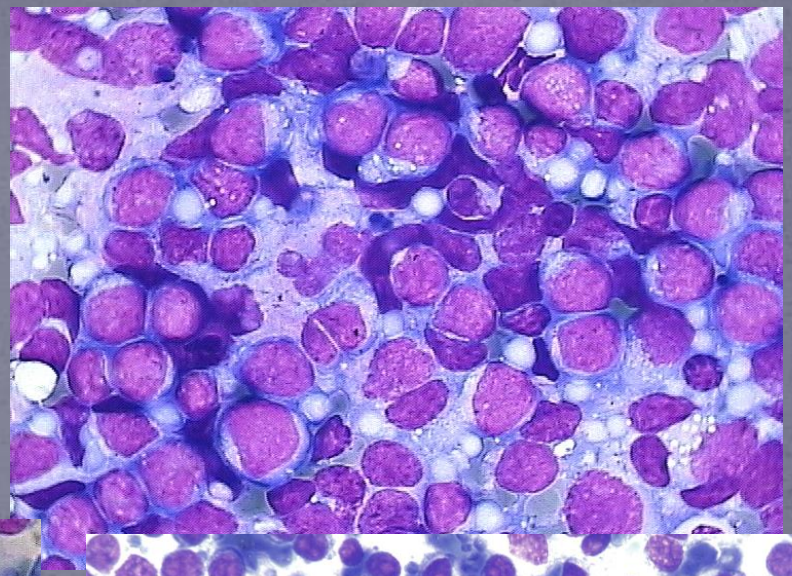
NEOPLASIE DELLE CELLULE MATURE

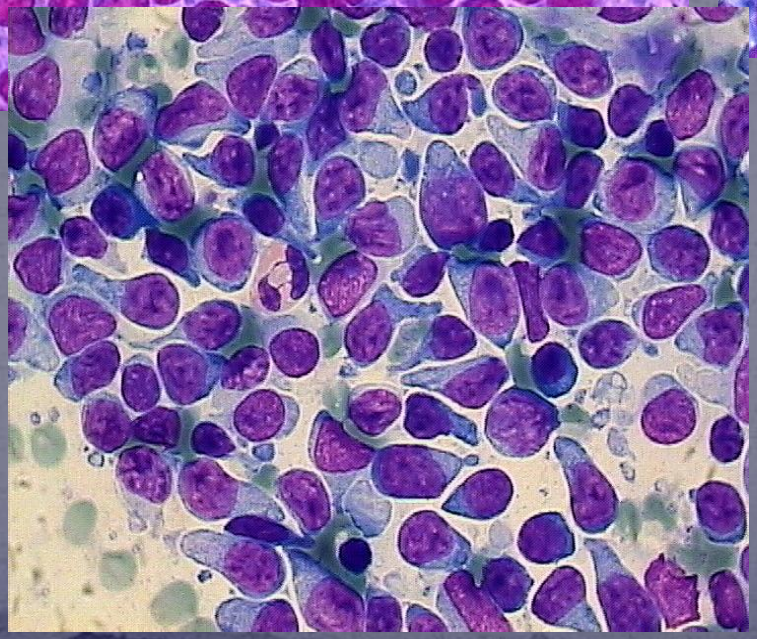


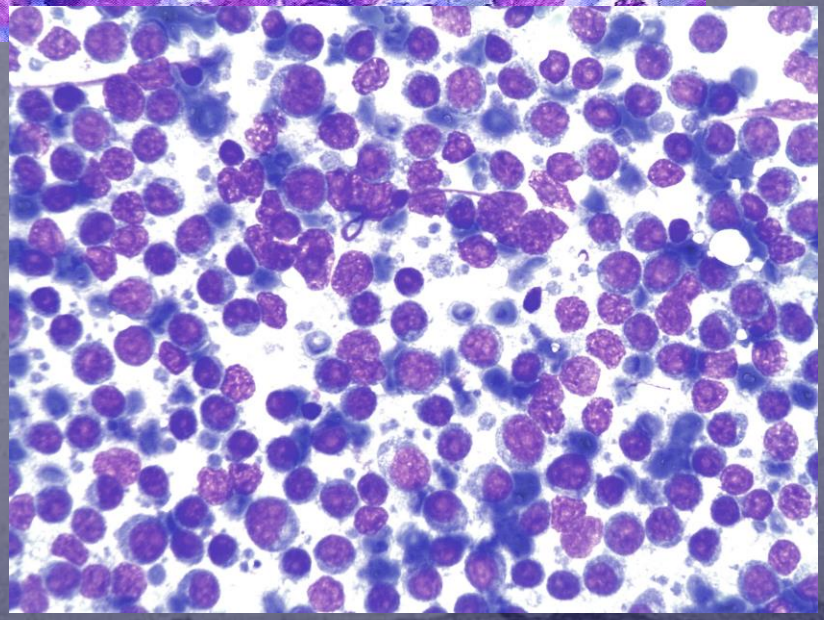


Il linfoma non-hodgkin









SISTEMI CLASSIFICATIVI

- Rappaport
- Lukes & Collins,
- Kiel, Kiel up-date
- Working formulation del National Cancer Institute
- Classificazione World Health Organization (WHO)

Inquadramento della patologia:
TUTTE LE INFORMAZIONI POSSIBILI

- ✓**Clinica**
- ✓**Ematologia**
- ✓**Morfologia (cito-isto)**
- ✓**Quadro fenotipico**

CLASSIFICAZIONE WHO

NEOPLASIE DEI PRECURSORI B-T

- LEUCEMIA LINFOBLASTICA ACUTA/LINFOMA LINFOBLASTICO

NEOPLASIE DELLE CELLULE MATURE B-T

Alto grado

Basso grado

- **CELLULE B**
  - Diffuse large B-cell lymphoma (centroblastico pleomorfo) (immunoblastico) (anaplastico)
- **CELLULE T**
  - Pheriperal T-cell lymphoma (pleomorfo misto) (immunoblastico) (anaplastico)

LINFOMI HIGH GRADE

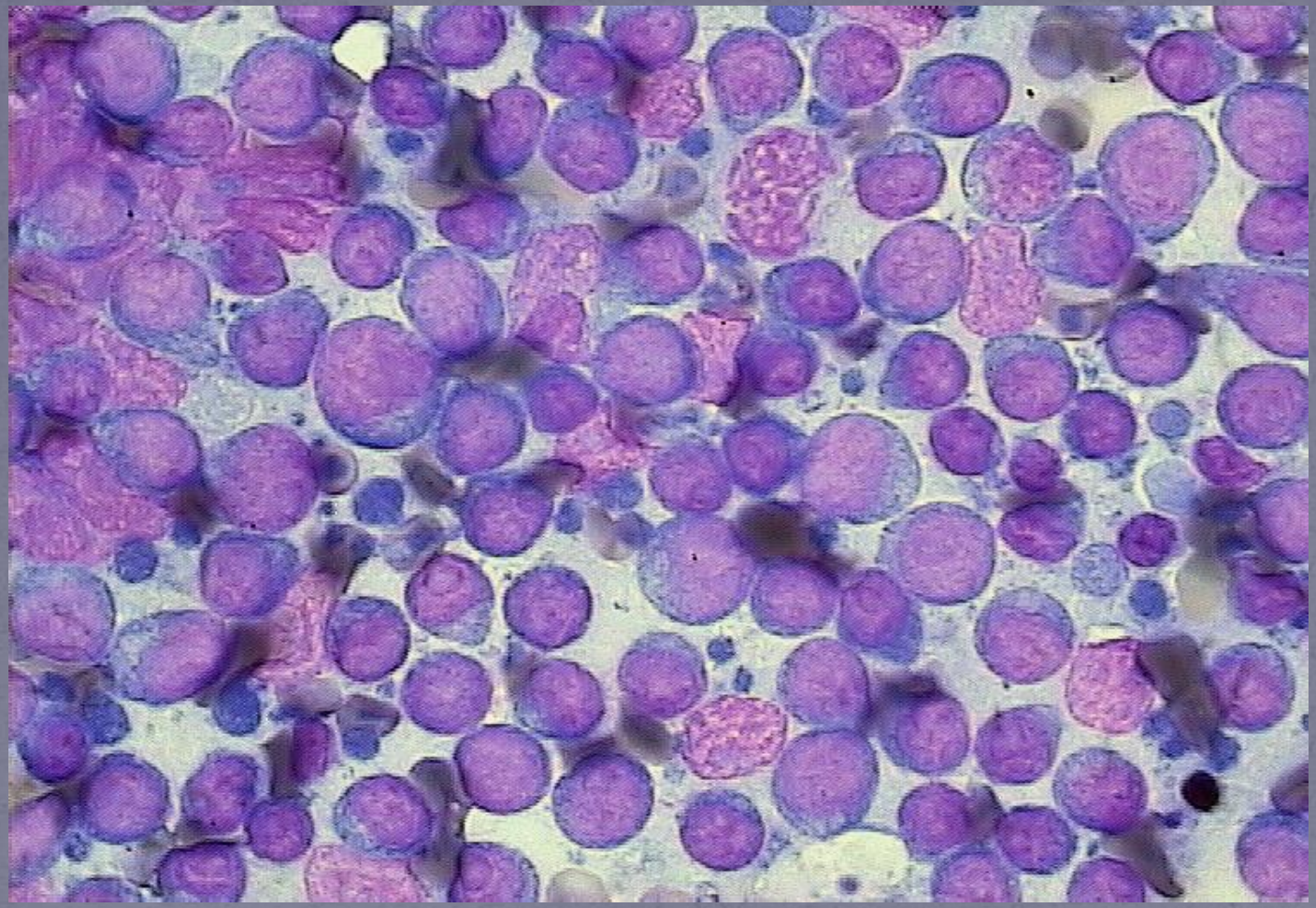
Citologia linfonodale

- Forme ad alto grado
- Interessamento linfonodale diffuso
- Caratteristiche citologiche peculiari

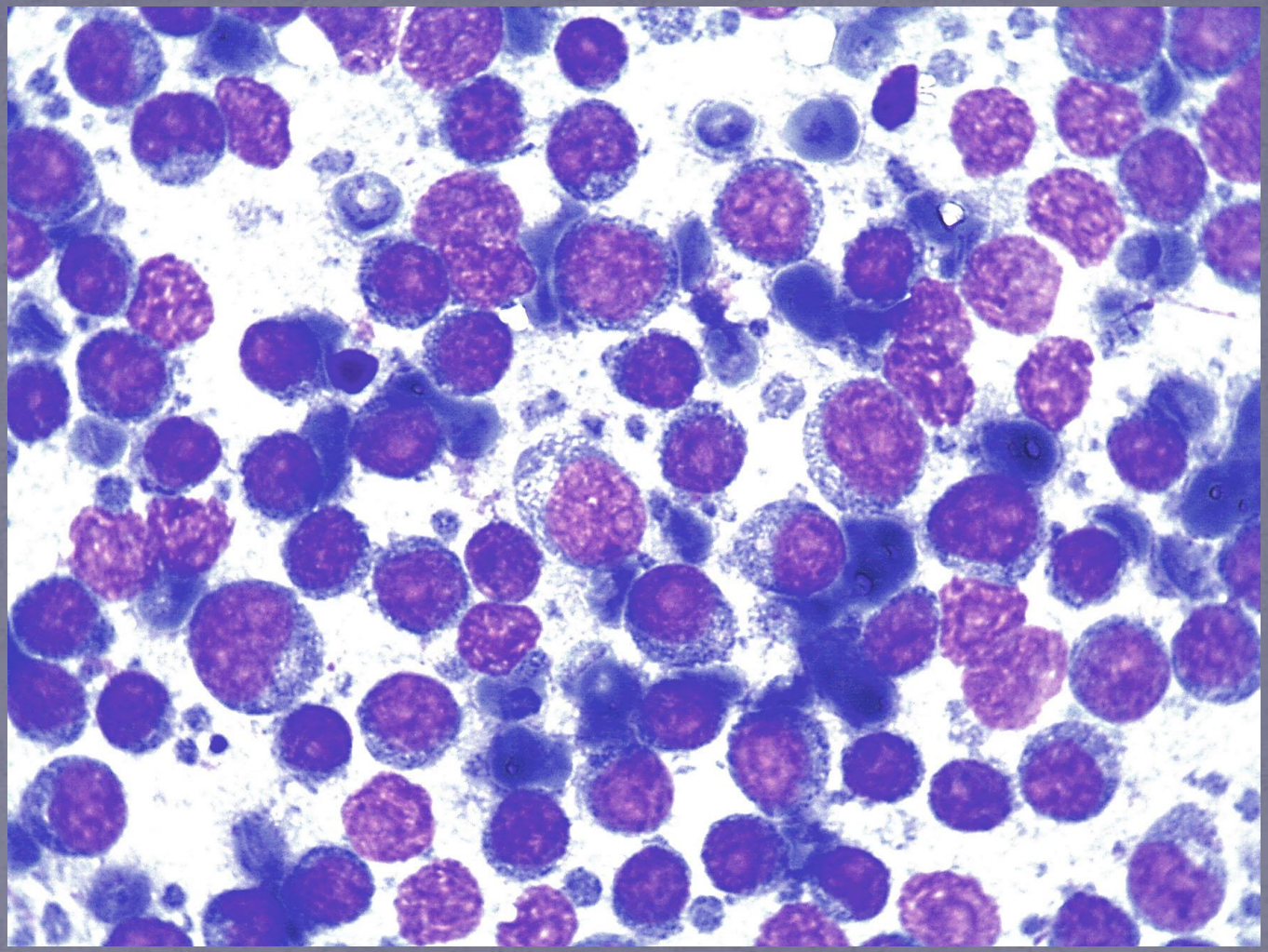
Caratteristiche citologiche

- Dimensioni delle cellule (grandi; medio-grandi)
- Quantità, distribuzione, aspetto del citoplasma
- Forma , localizzazione, cromatian del nucleo
- Evidenza, numero dei nucleoli
- Mitosi, altre cellule

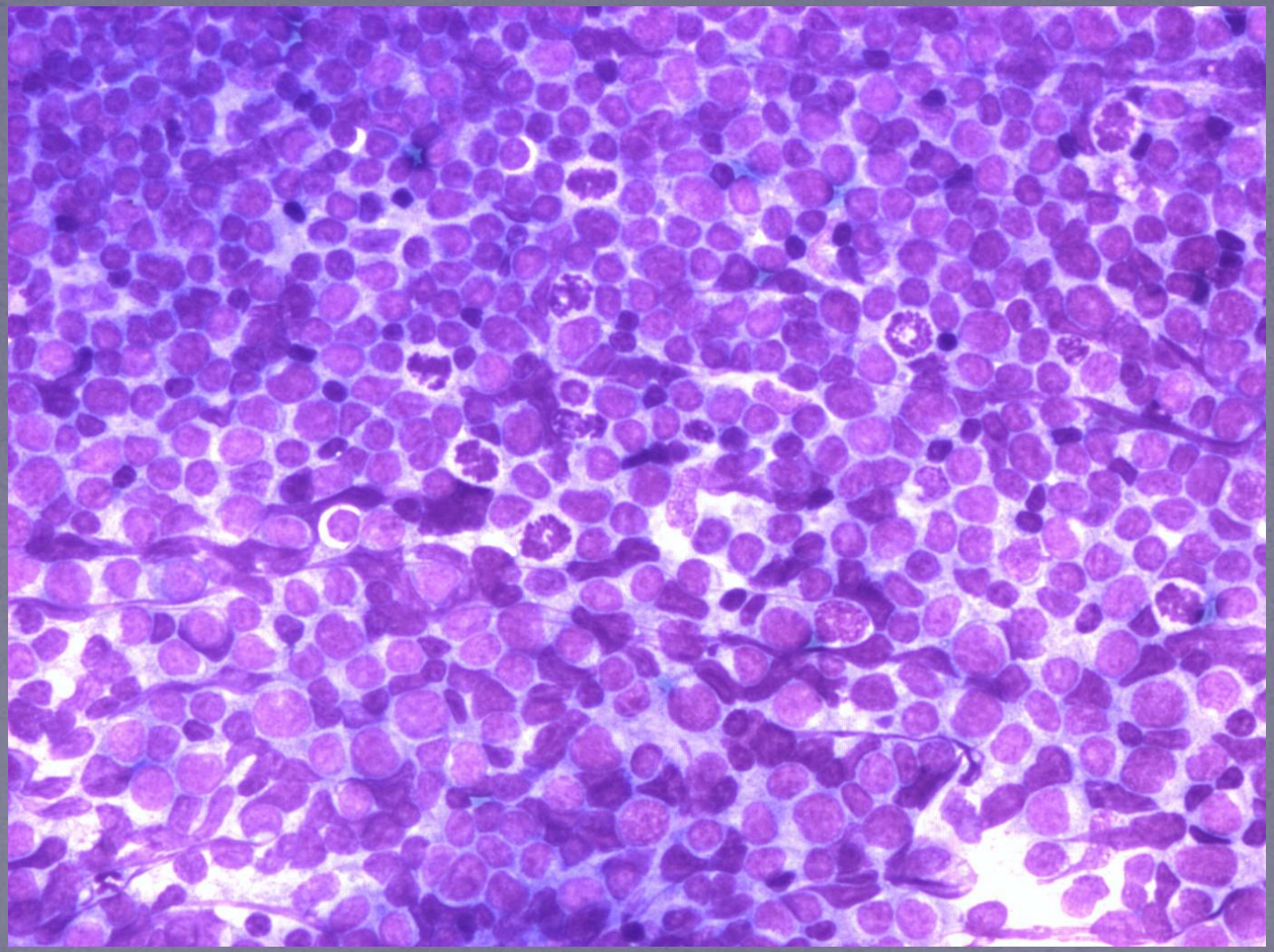
Linfoma centroblastico polimorfo B

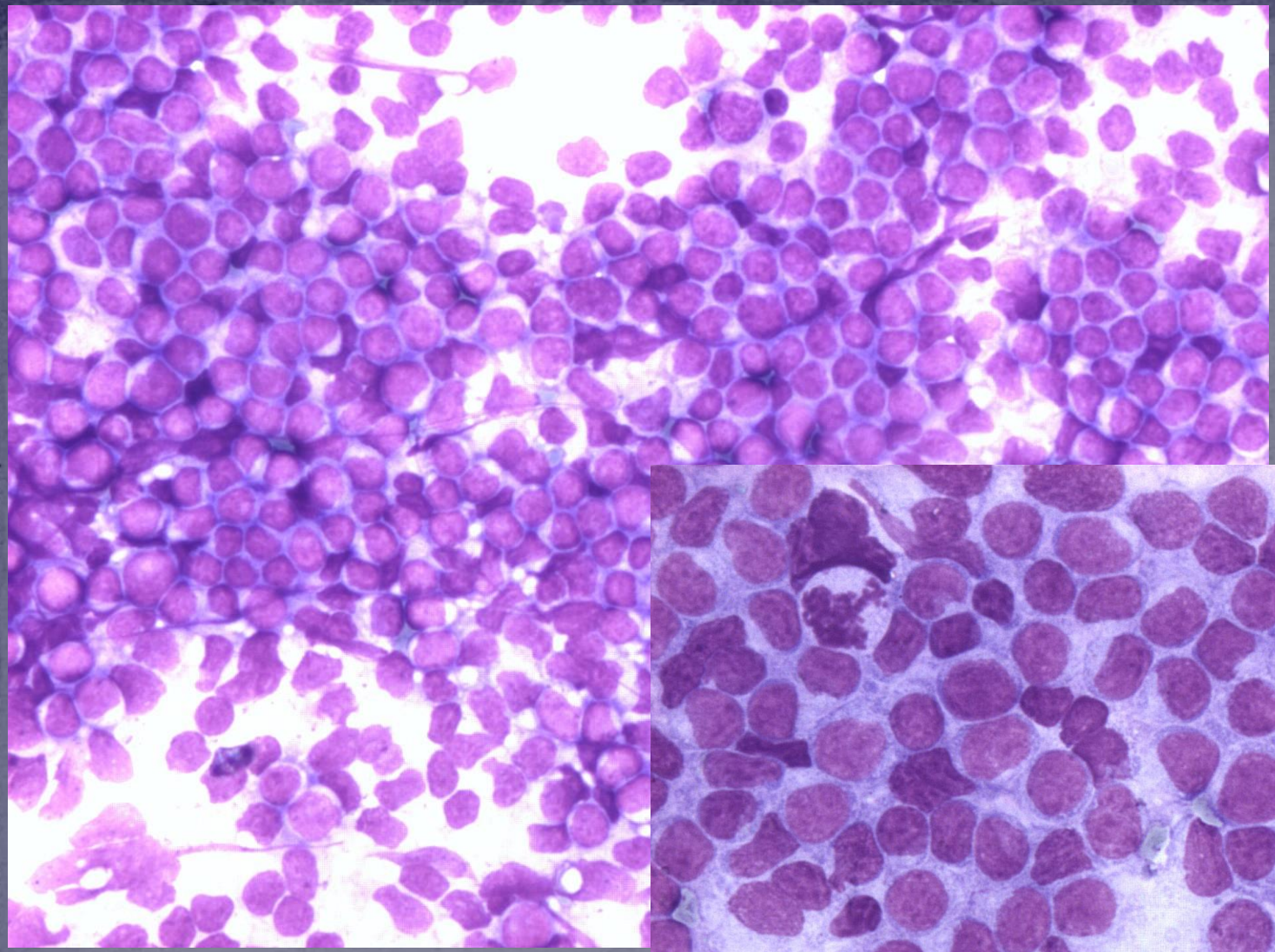


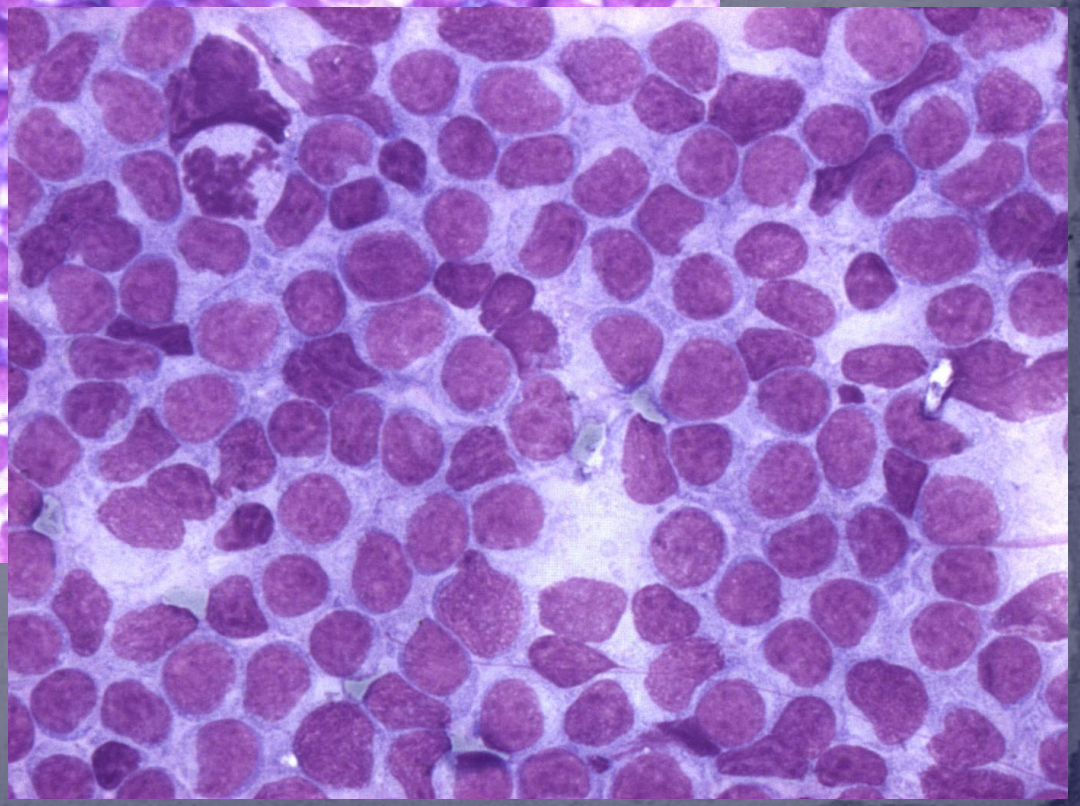
Linfoma centroblastico polimorfo B



Linfoma pleomorfo misto T







Linfoma pleomorfo misto T

LINFOMI HIGH GRADE

Citologia linfonodale

- Forme ad alto grado
- Interessamento linfonodale diffuso
- Caratteristiche citologiche peculiari

Test aggiuntivi

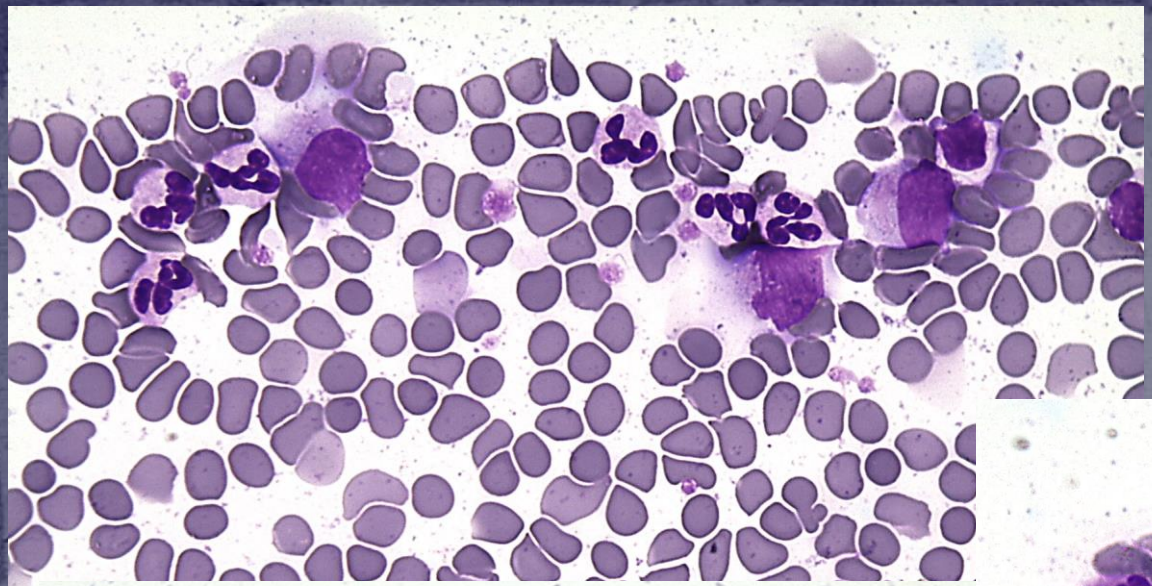
- Immunofenotipizzazione:
  - Definire il fenotipo
  - Definire il quadro antigenico complessivo
    - Staging
      - Ricerca di cellule con le stesse caratteristiche in altri organi

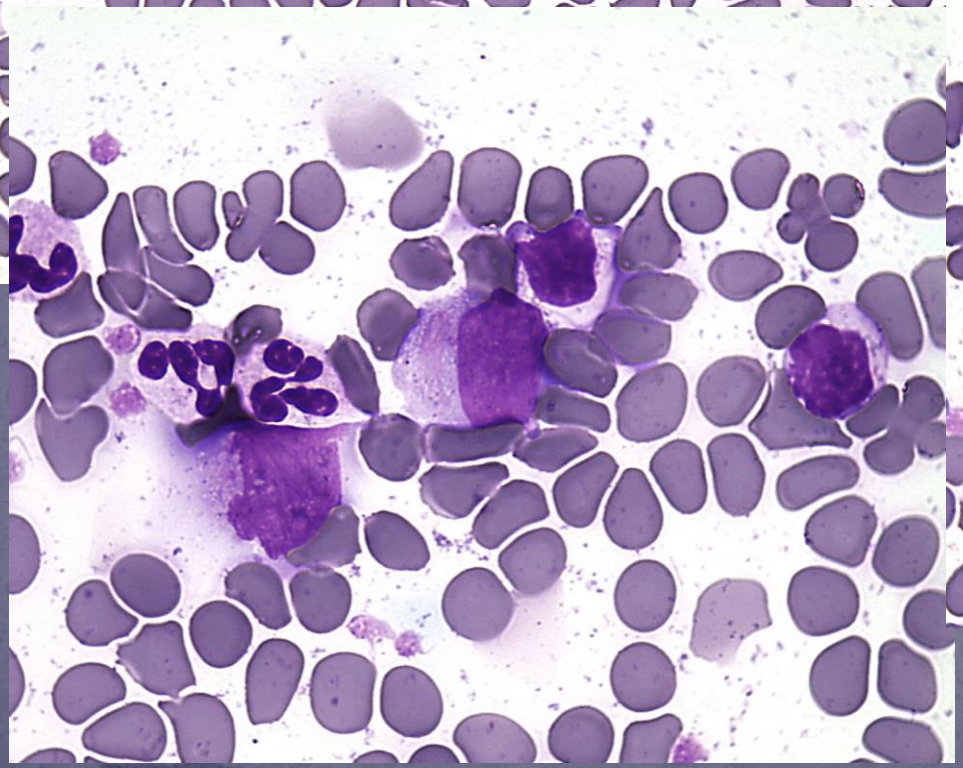
LINFOMI HIGH GRADE

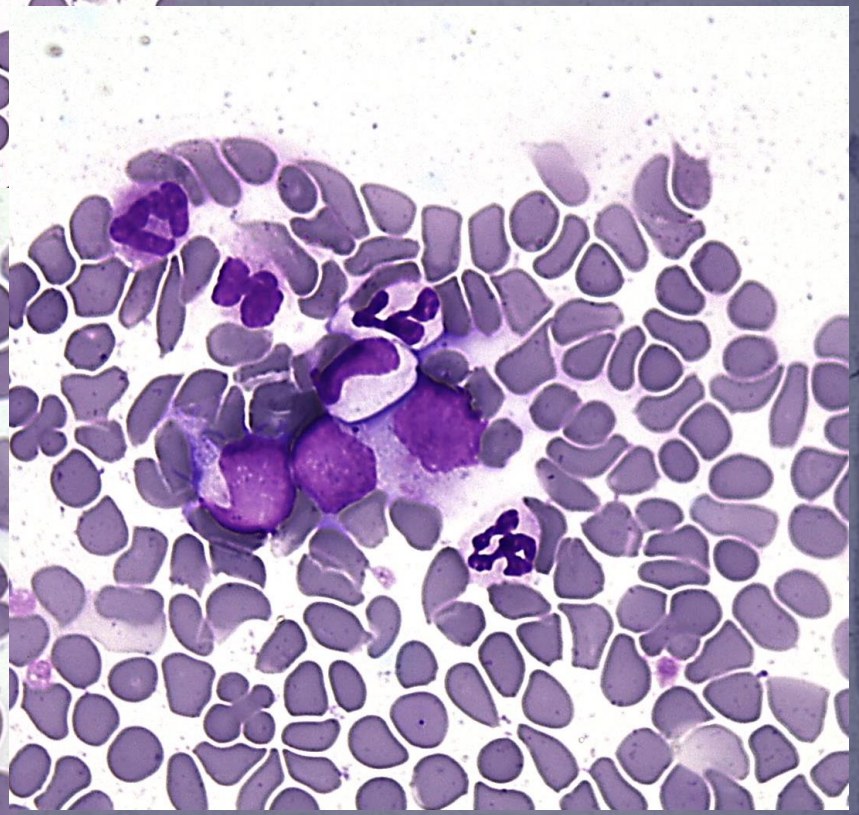
- DATI CLINICI
- DATI CLINICO PATOLOGICI
  - ✓CITOLOGIA LINFONODALE
  - ✓EMATOLOGIA
  - ✓CITOLOGIA MIDOLLARE
  - ✓IMMUNOFENOTIPIZZAZIONE SU
    - ❖AGO ASPIRATO LINFONODALE
    - ❖SANGUE PERIFERICO
    - ❖MIDOLLO

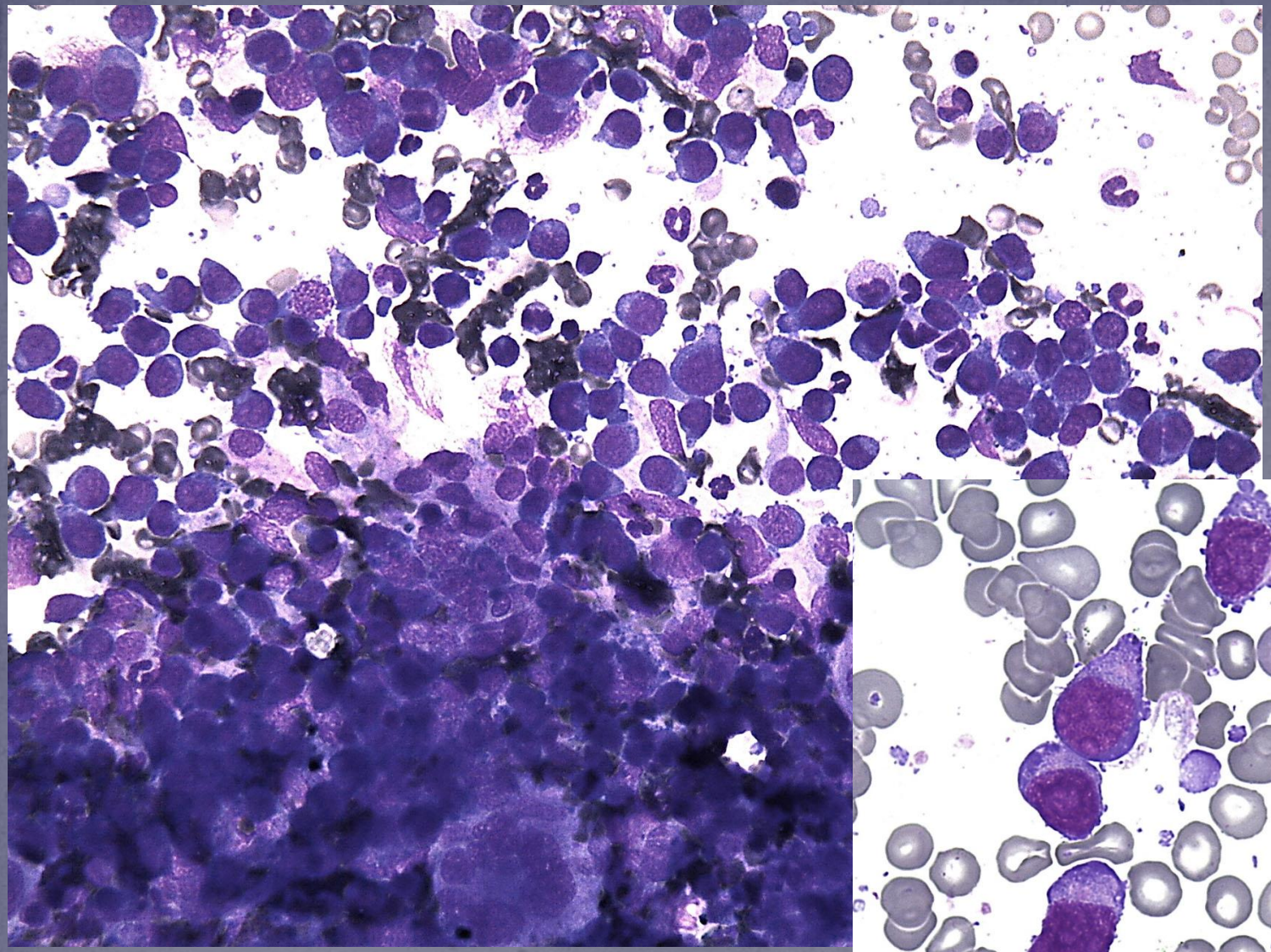
- IDENTIFICARE IL TIPO DI LINFOMA
- FENOTIPIZZARE
- STADIARE
  - ✓VALUTAZIONE DI FATTORI PROGNOSTICI (es. infiltrazione midollare, anemia)

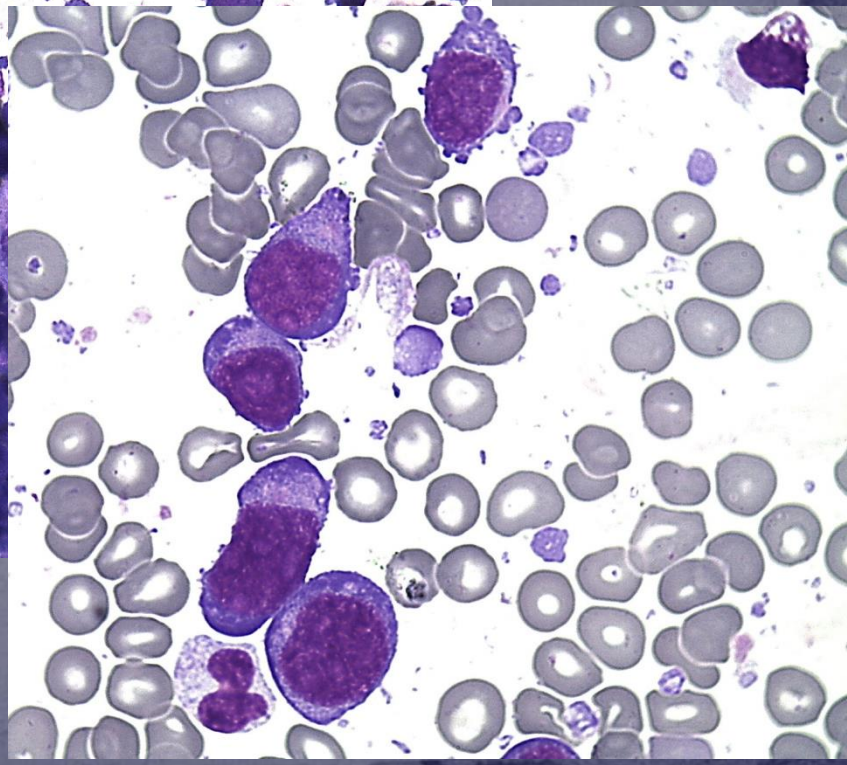
Sangue periferico











Midollo osseo

CLASSIFICAZIONE WHO

NEOPLASIE DEI PRECURSORI B-T

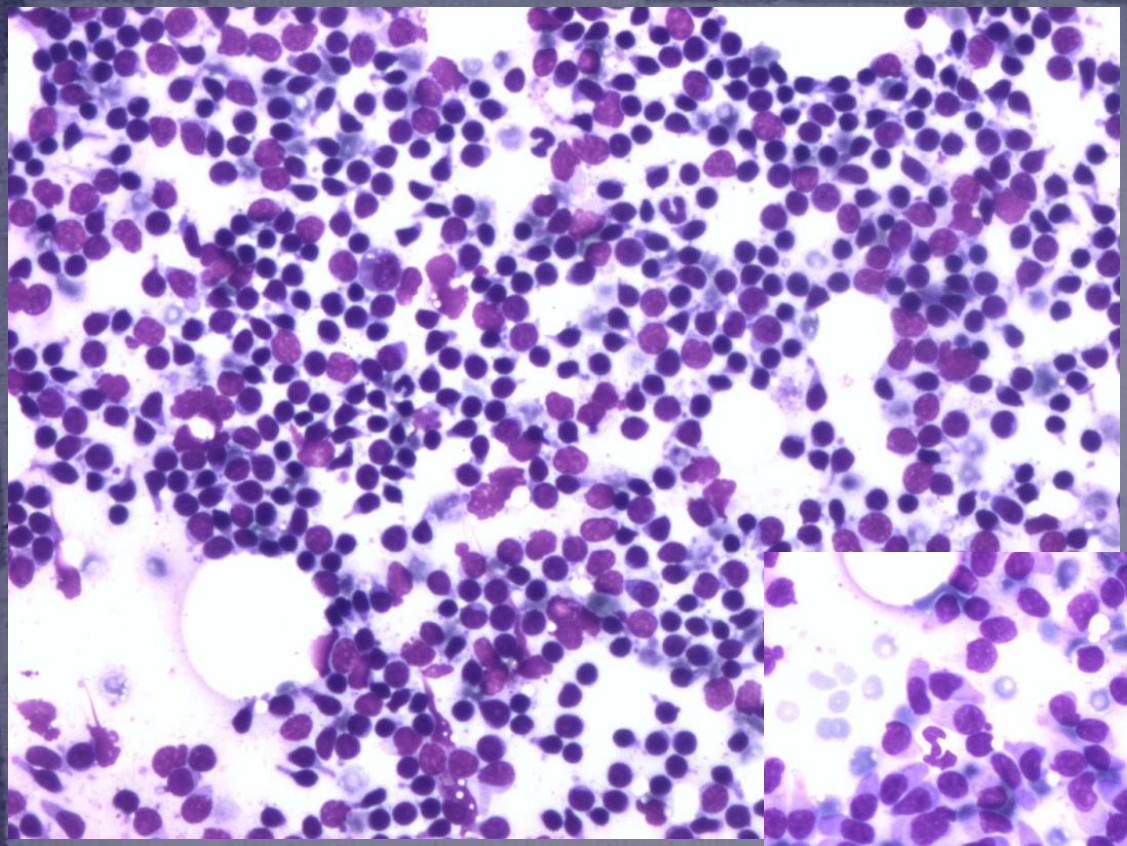
- LEUCEMIA LINFOBLASTICA ACUTA/LINFOMA LINFOBLASTICO

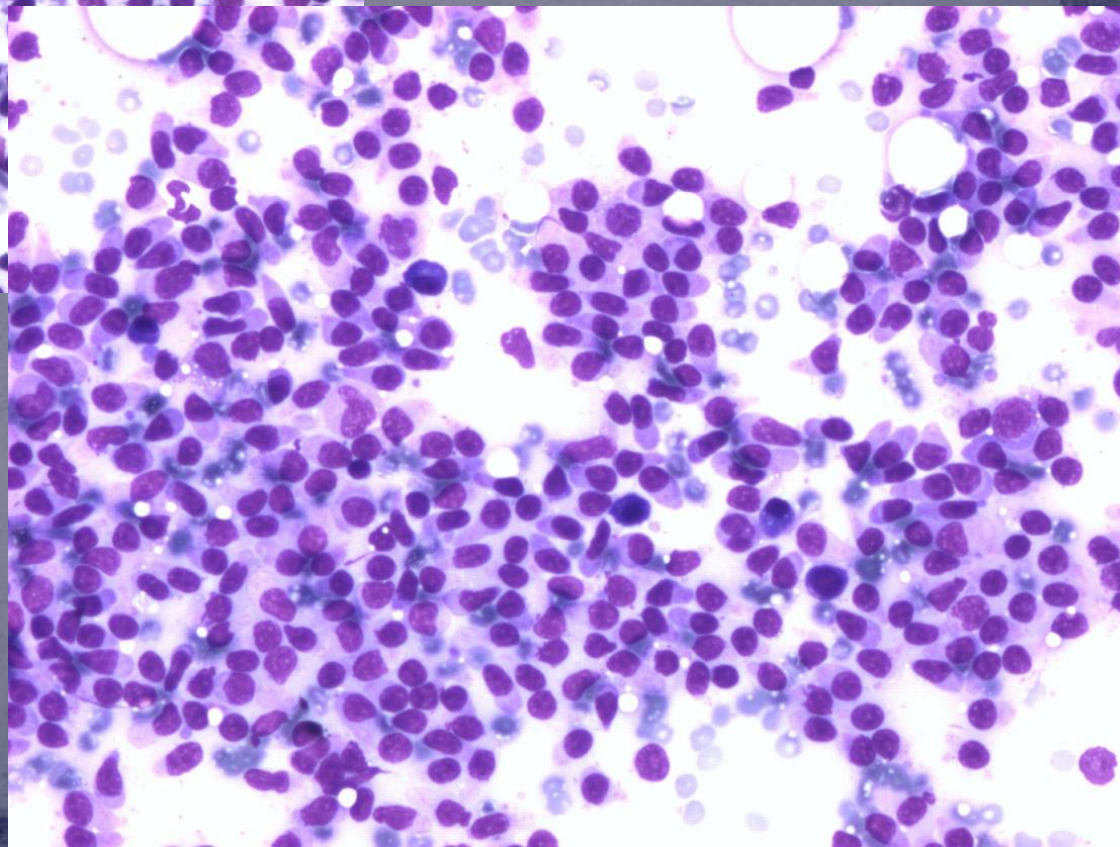
NEOPLASIE DELLE CELLULE MATURE B-T

Alto grado

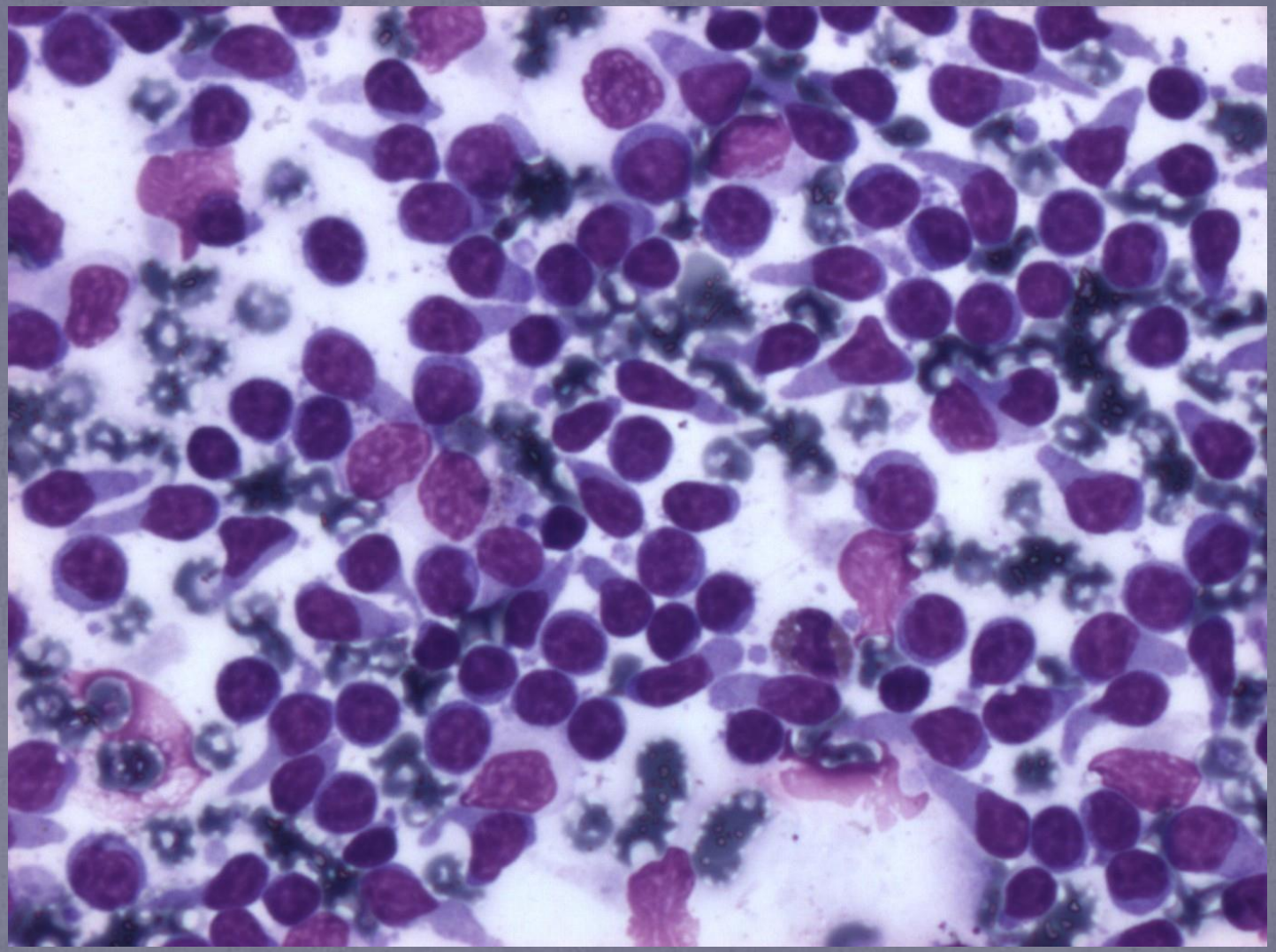
Basso grado

- **CELLULE B**
  - CLL-B/linfoma linfocitico
  - Linfomi marginali (MMC)
  - Linfomi follicolari (centroblastico-centrocitico)
- **CELLULE T**
  - CLL-T/linfoma linfocitico o prolinfocitico
  - Pheriperal T-cell lymphoma (linfomi a piccole cellule, small clear)

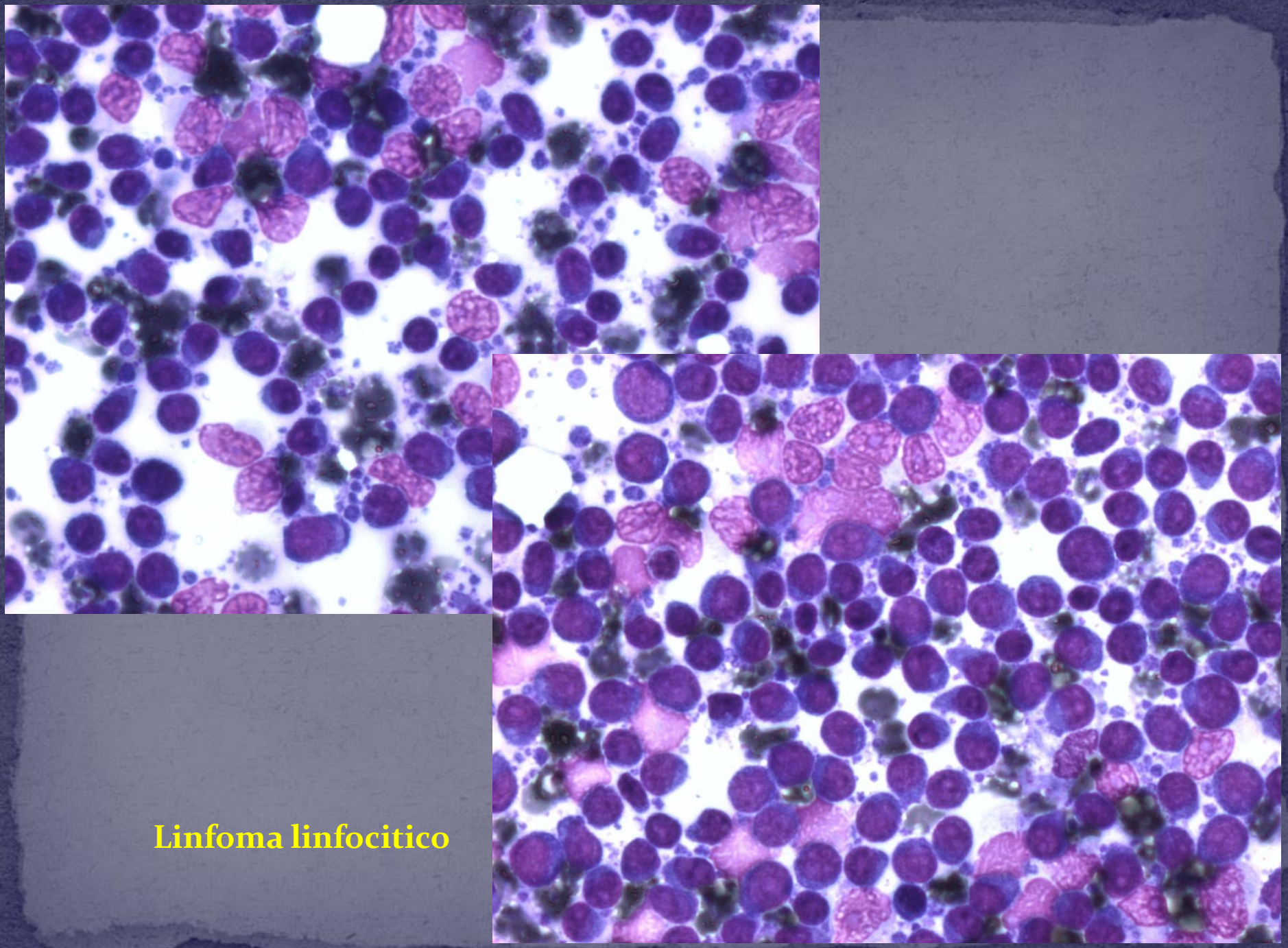




Linfoma small clear



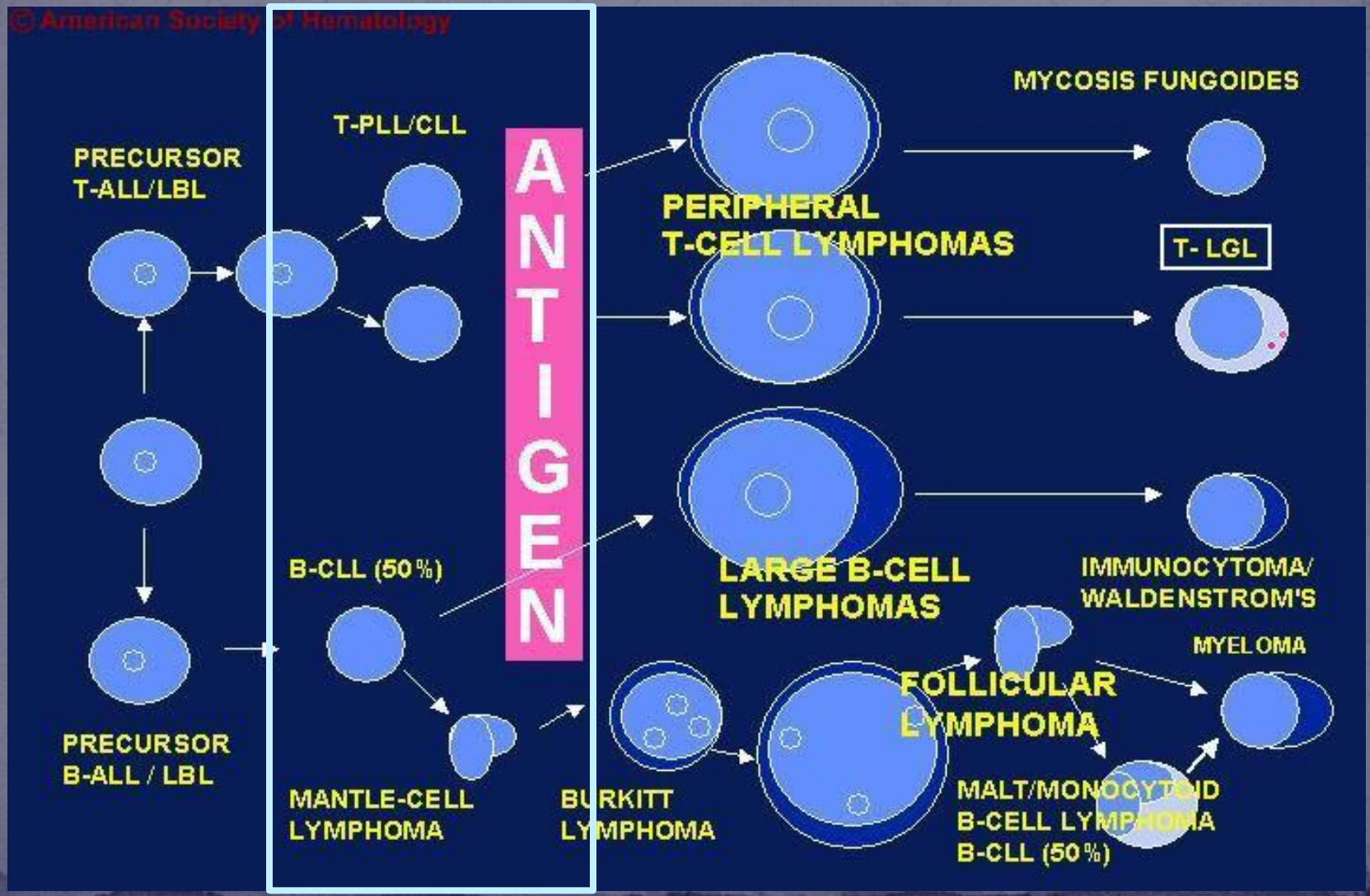
Linfoma small clear



Linfoma linfocitico

Neoplasie linfoidi

Leucemie linfocitiche croniche
Linfomi



Leucemia linfocitica cronica

Proliferazione clonale di linfociti neoplastici maturi. Diminuzione fenomeni di apoptosi e scarsa proliferazione

Cane: soggetti adulti o anziani

Sintomatologia: sintomi vaghi, aspecifici, a volte non presenti.

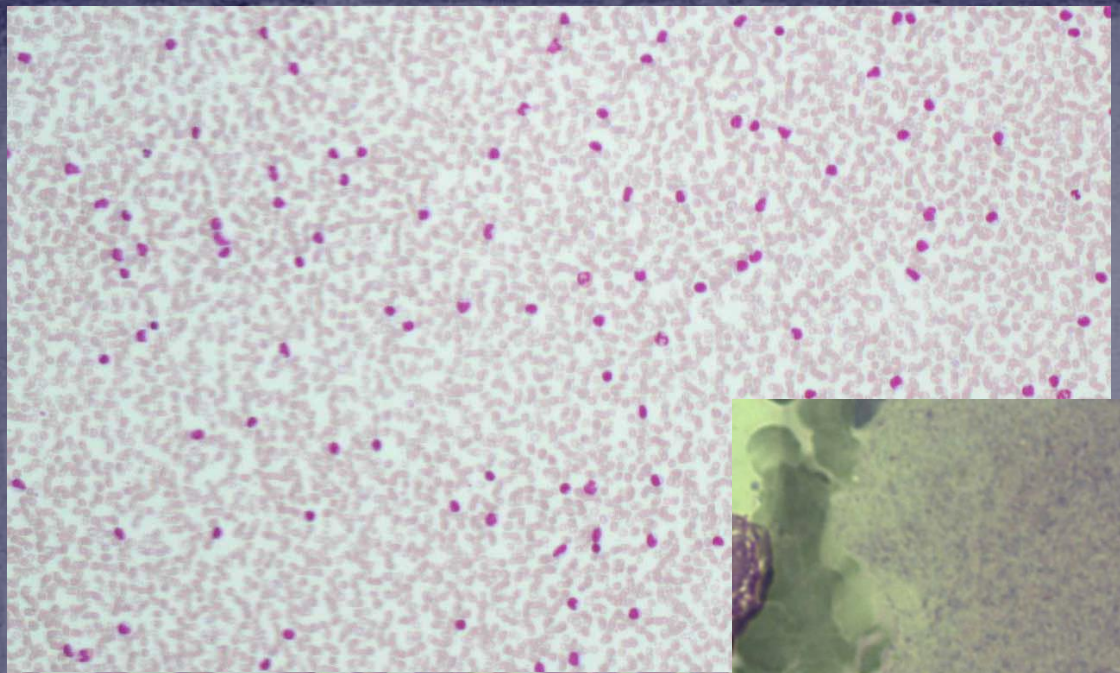
Segni clinici: in base al grado di linfocitosi, coinvolgimento di midollo e altri organi

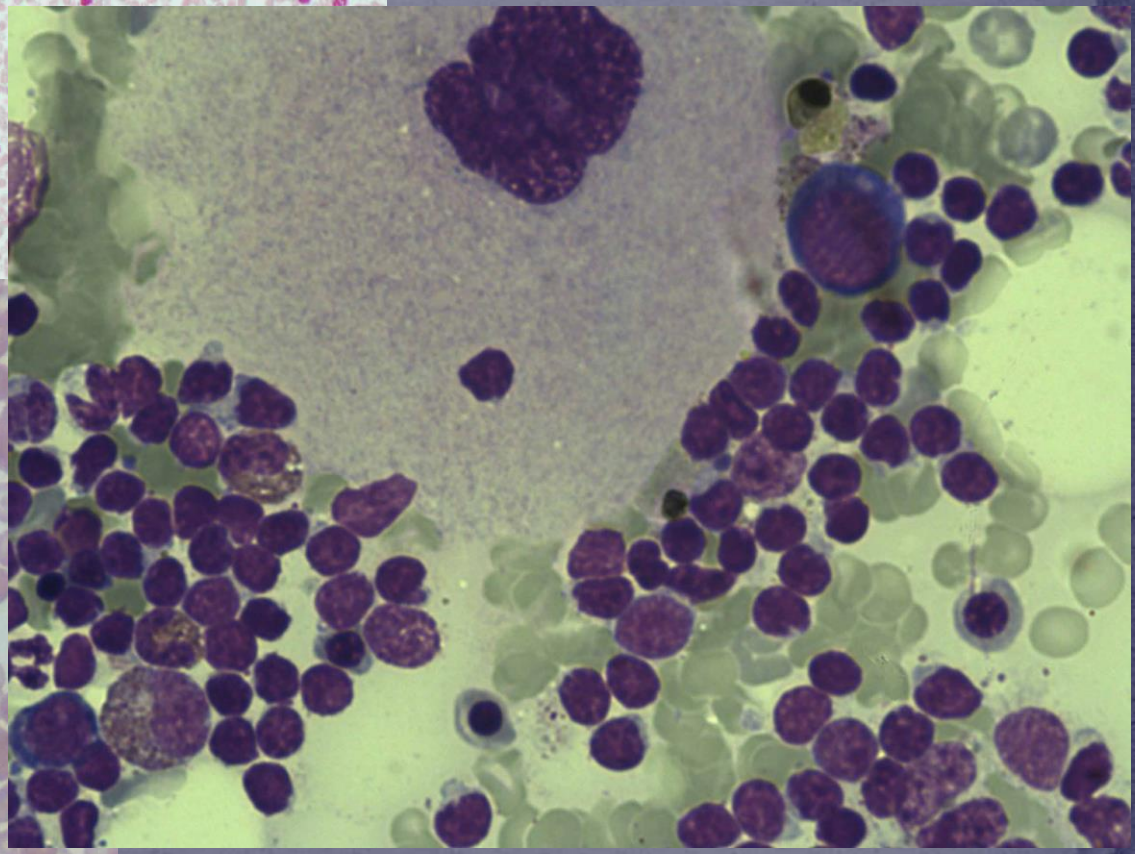
splenomegalia, linfoadenopatia ed epatomegalia

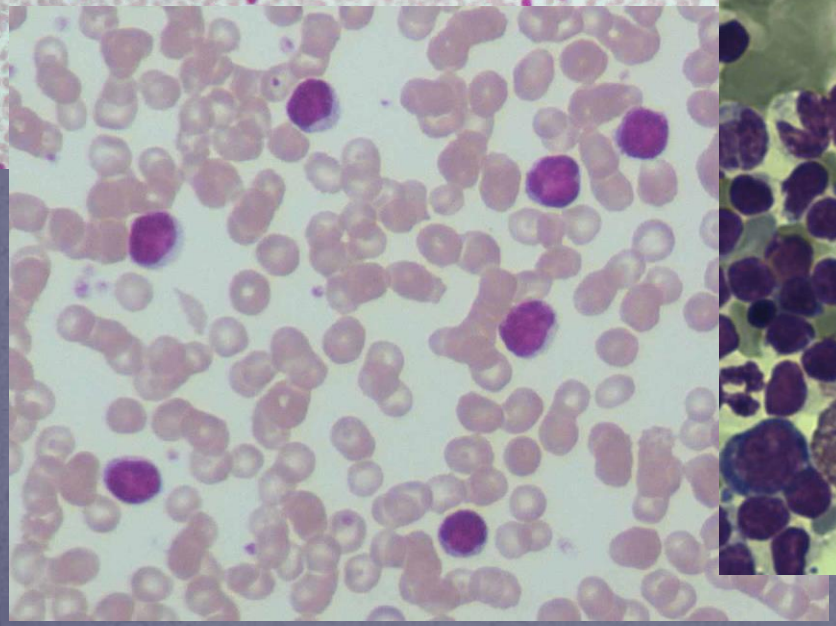
- Anemia da lieve a moderata, normocitica normocromica, non rigenerativa
- Rara trombocitopenia
- Aumento dei blasti neoplastici in corso di trasformazione blastica
- Possibile iperglobulinemia o gammopatia monoclonale

Rilievi ematologici:

- Leucocitosi estrema (>50.000, spesso >100.000) con linfocitosi assoluta (6000-100.000 linfociti/µl) (rari blasti neoplastici <5%.)
- Midollo: >30% piccoli linfociti
- linfocitosi persistente (> 3 mesi)

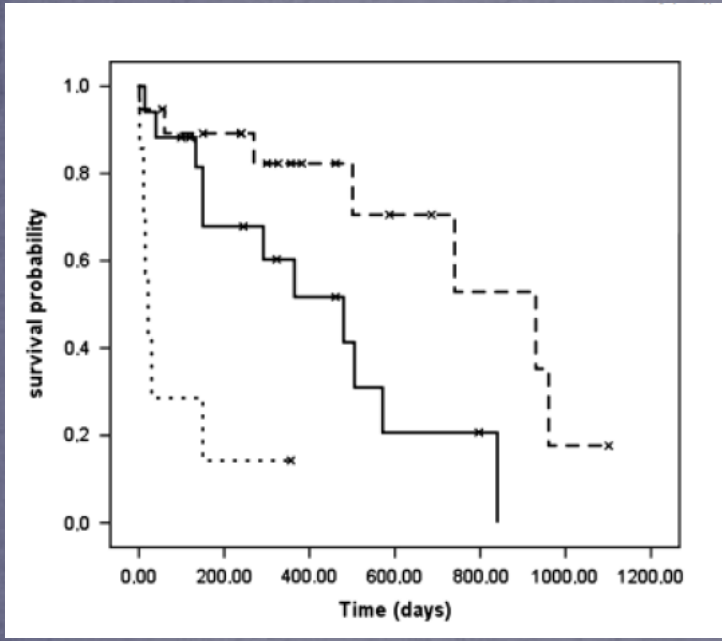






Leucemia linfocitica cronica

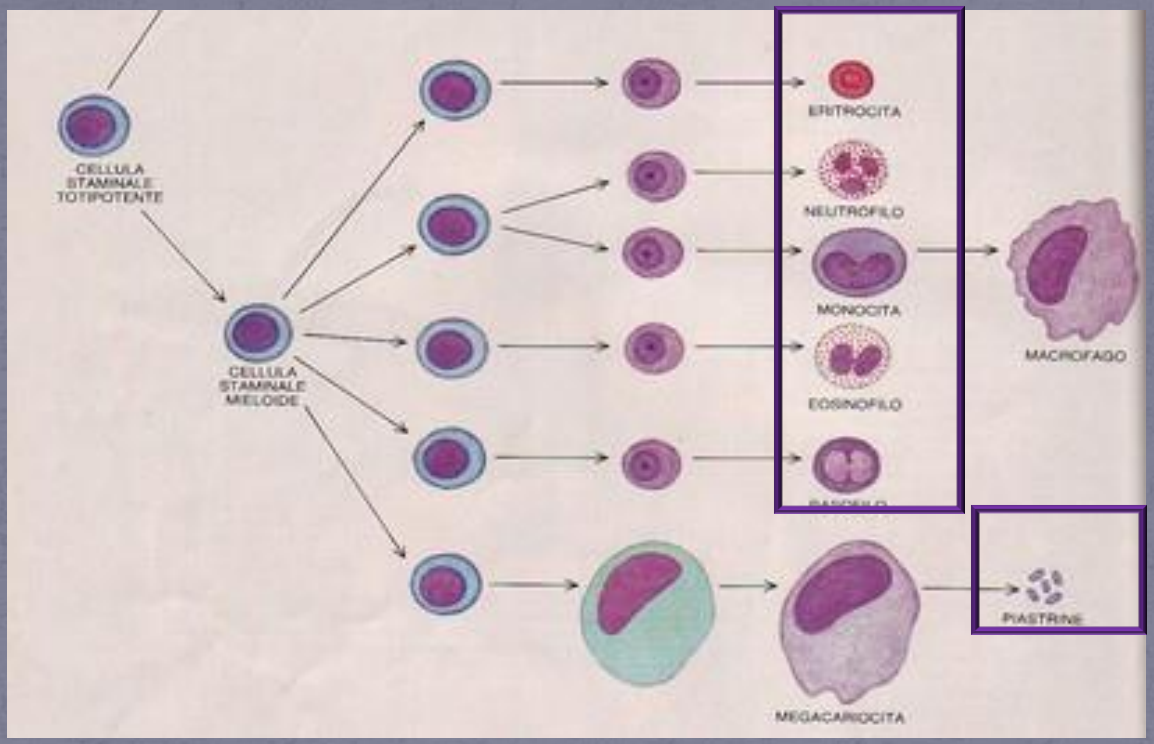
medicina umana: 95% delle CLL sono forme B,
medicina veterinaria : 70% delle CLL è di origine T, (LGL di origine splenica)
CLL che presentano fenotipo aberrante
CD3+CD21+,
CD4+CD8+,
CD4-CD8-



tempi di sopravvivenza : minor fenotipo B,
cani < 8 anni e fenotipi aberranti,

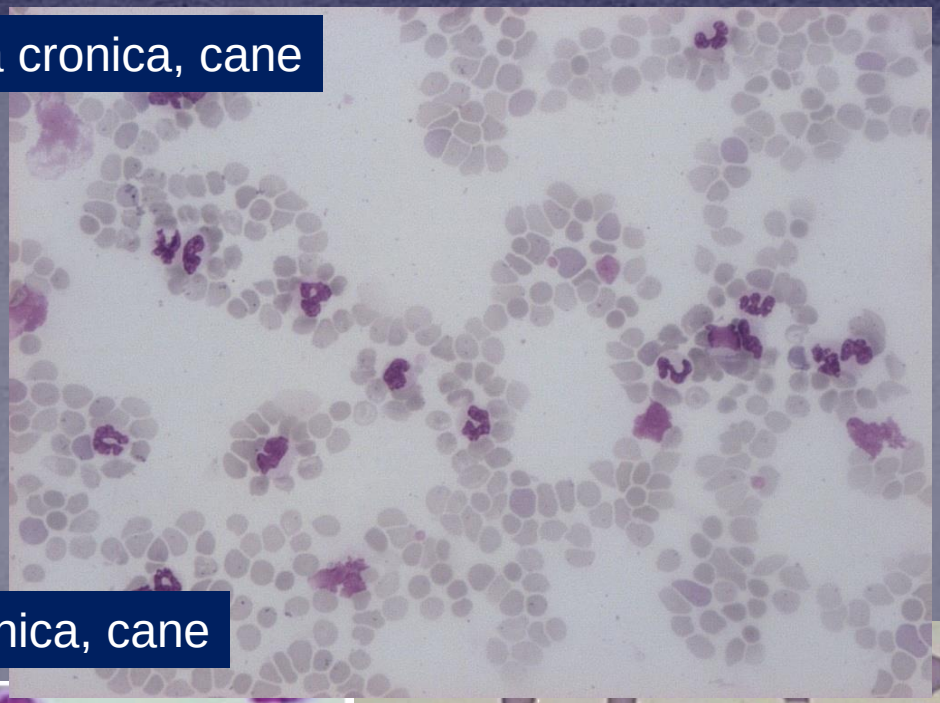
LECEUMIE MIELOIDI CRONICHE

Disordini clonali
Midollo osseo: Eterogenee

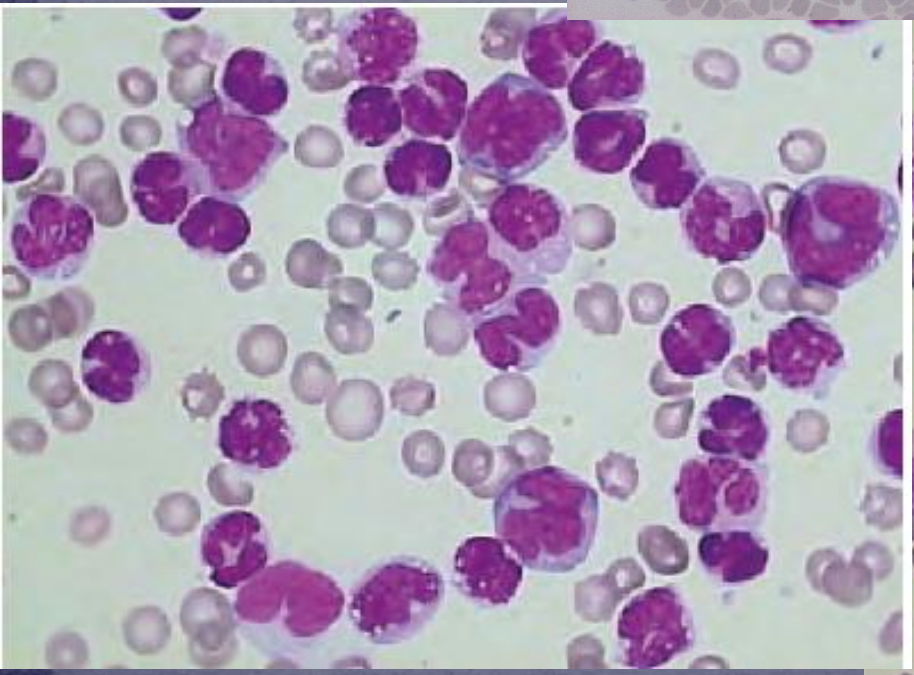


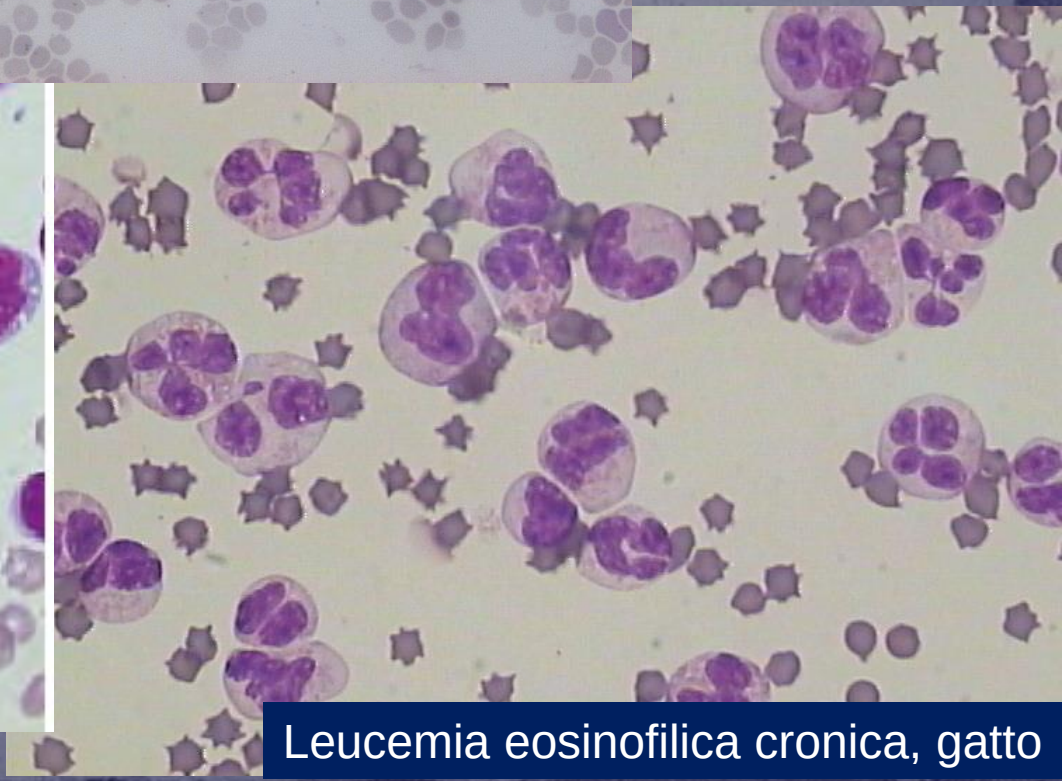
Animali anziani
Andamento indolente
Sintomi vaghi e aspecifici

Leucemia granulocitica cronica, cane



Leucemia monocitica cronica, cane





Leucemia eosinofilica cronica, gatto

CLASSIFICAZIONE WHO medicina umana

I. caratteristiche clinico-patologiche,
II. morfologia,
III. citochimica,
IV. immunofenotipiche
V. genetiche

Tabella 1 Classificazione WHO delle neoplasie mieloidi croniche in medicina umana (2008)

Neoplasie mieloproliferative (Myeloproliferative neoplasms (MPN)

Leucemia Mieloide cronica BCR–ABL-1-positiva (Chronic myelogenous leukemia, CML)

Lecemia cronica neutrofilia (Chronic neutrophilic leukemia, CNL)

Policitemia vera (PV)

Trombocitosi essenziale (ET)

Mielofibrosi primaria

Leucemia eosinofilica cronica (Chronic eosinophilic leukemia, CEL)

Mastocitosi

Neoplasie mieloproliferative non ulteriormente classificabili (Myeloproliferative neoplasms, unclassifiable, MNU)

Neoplasie mielodi e linfoidi associate con eosinofilia e anomalie di PDGFRA, PDGRRB o FGFR1

Cosa possiamo fare in medicina veterinaria?

I. Caratteristiche clinico-patologiche

-emocromocitometrico:
neutrofilia da grave ad estrema (oltre 50.000/microlitro)
Anemia e trombocitopenia raramente presenti , lieve entità.

II. morfologia

neutrofili maturi differenziati,
neutrofili a banda, metamielociti e mielociti: percentuale minore.
Un aiuto: disgranulopoiesi (neutrofili con nuclei ipersegmentati, nuclei ad anello, neutrofili a banda, metamielociti e mielociti giganti,

midollo osseo:

ipercellulare, rapporto M:E aumentato

III. Citochimica

sovrapponibili a quelle della controparte normale

IV. Immunofenotipo

sovrapponibili a quelle della controparte normale
Un aiuto: fenotipo aberrante.

Indagini cliniche: escludere le differenziali

LE DIAGNOSI DIFFERENZIALI

Neutrofilie estreme:

processi reattivi :

flogosi

Necrosi (tumori)

lesioni piogranulomatose

monocitosi>1,4 x 10^3 /microlitro:

processi reattivi :

flogosi (sia acute sia croniche),

necrosi,

lesioni piogranulomatose,

in alcuni casi batteriemia, infezioni protozoarie

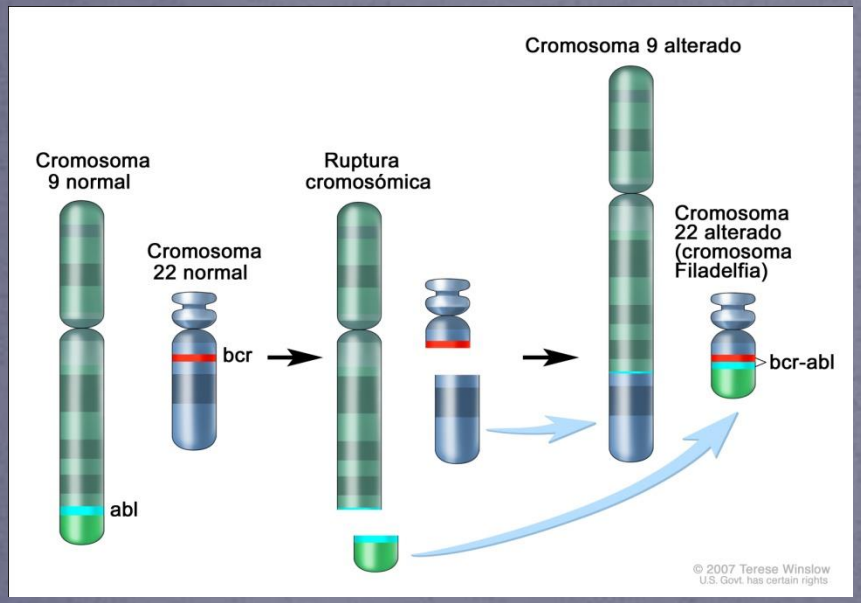
processi non flogistici:

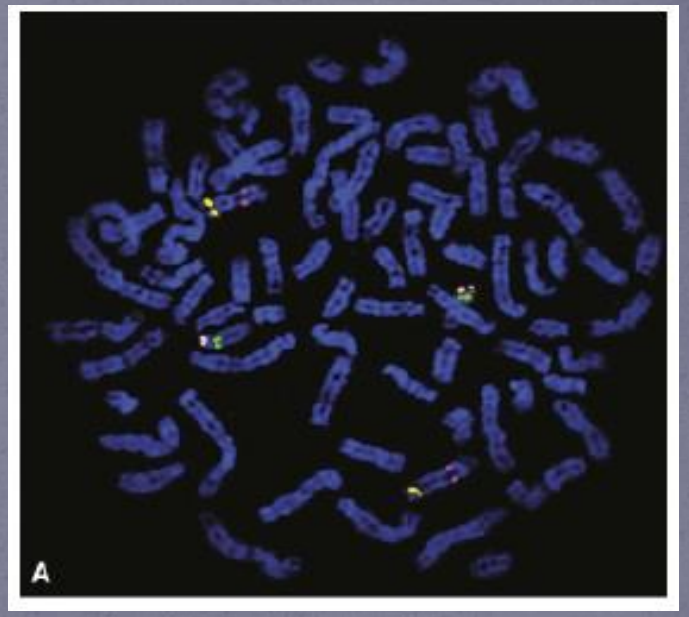
emorragie interne, traumi, emolisi o patologie immunomediate.

⇒monocitosi non persistente e raramente arriva a conte di oltre 50 x 10^3 monociti/microlitri,

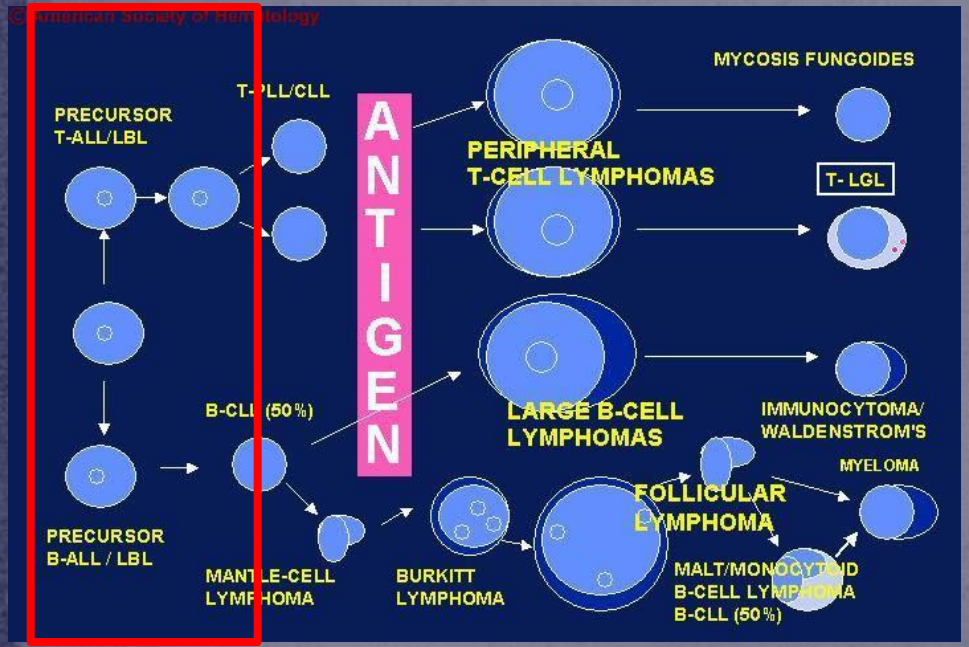
V. genetica
Citogenetica-FISH

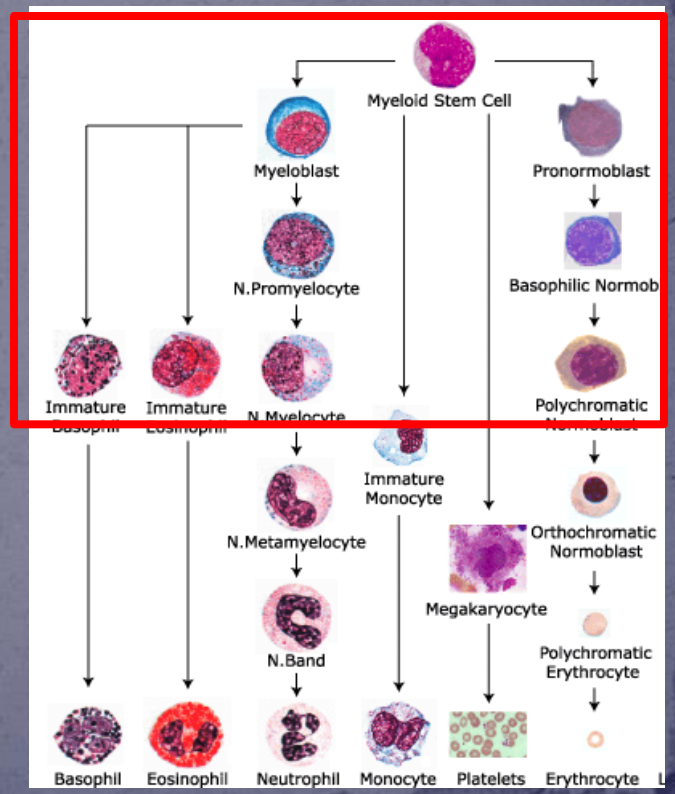
Patogenesi: in alcuni casi di CML canina è stata identificata la lesione genetica equivalente alla traslocazione BCR-ABL.
-tecniche diagnostiche ancora sperimentali, non praticabili nella ruotine diagnostica.





NEOPLASIE DEI PRECURSORI





Leucemie linfoidi acute (ALL)

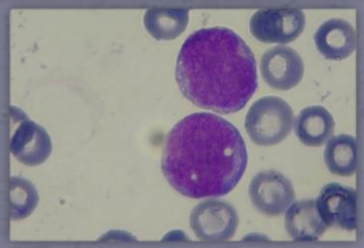
- proliferazione clonale nel midollo osseo di progenitori linfoidi maligni scarsamente differenziati.
- CANE: forme neoplastiche relativamente infrequenti (vs neoplasie delle cellule linfoidi mature),

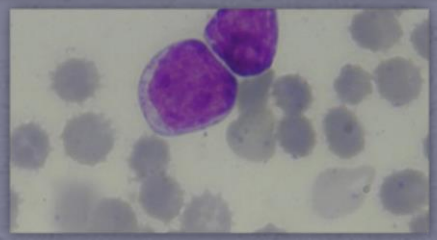
Ematologia:

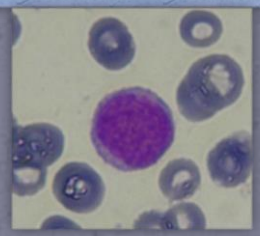
anemia, spesso da moderata a grave,
trombocitopenia e neutropenia
5% dei leucociti si presenta con caratteristiche morfologiche atipiche

Valutazione morfologica:

dimensioni medie-grandi con citoplasma basofilo, da moderato ad abbondante, nuclei rotondi con cromatina omogenea e nucleoli evidenti

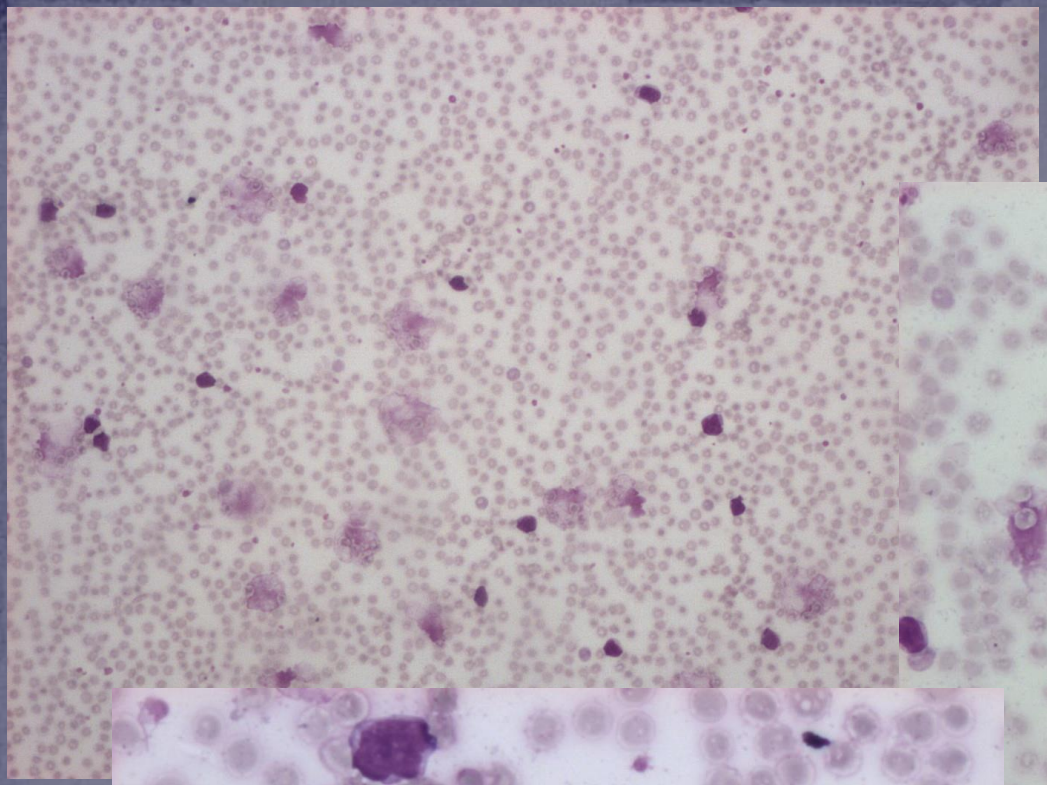


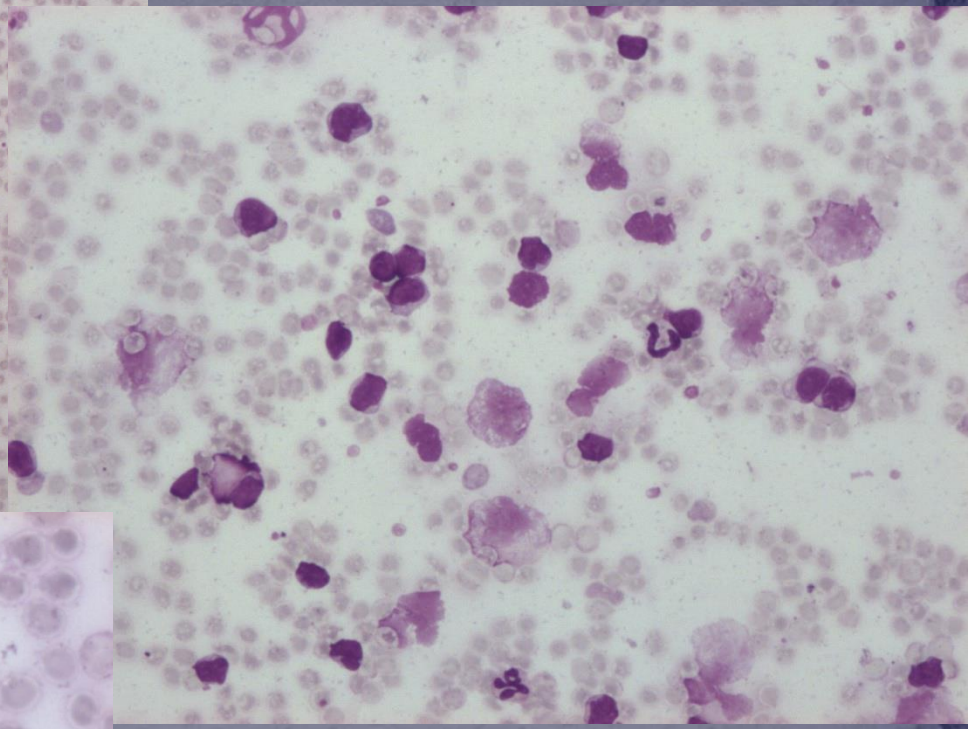


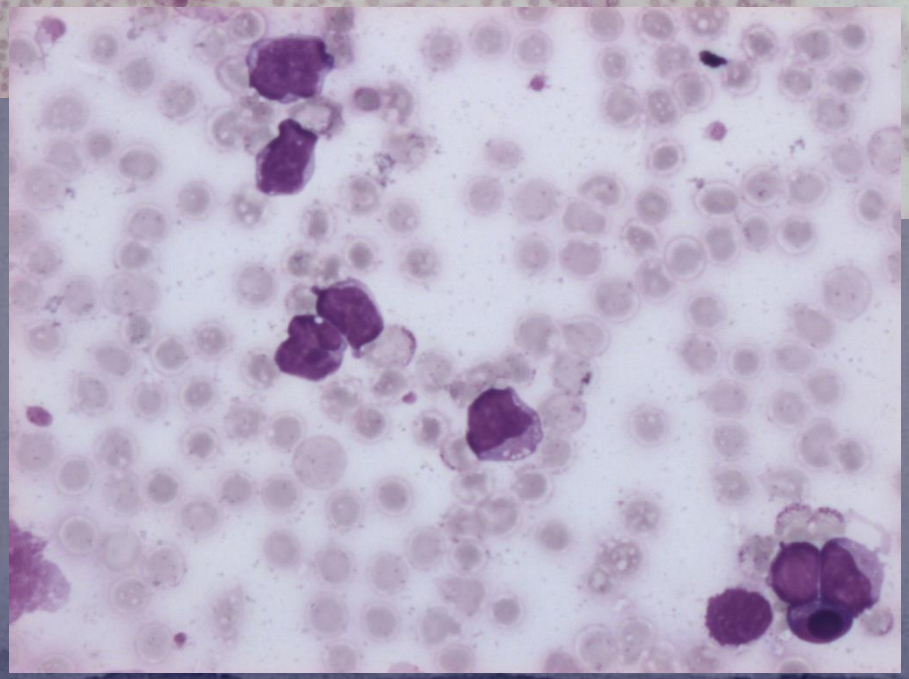


Esame del midollo osseo

del 20% delle cellule nucleate è costituita da blasti.



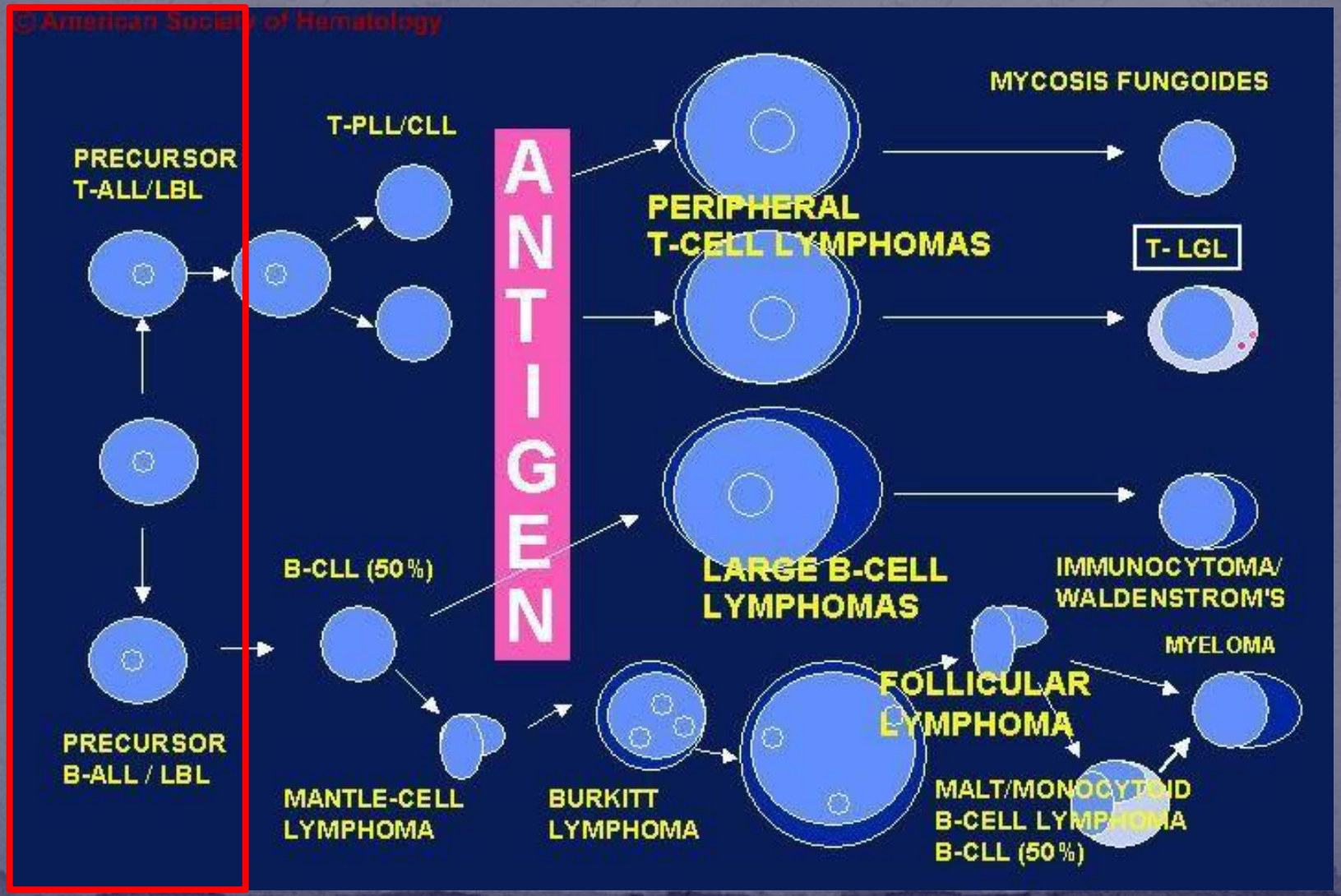


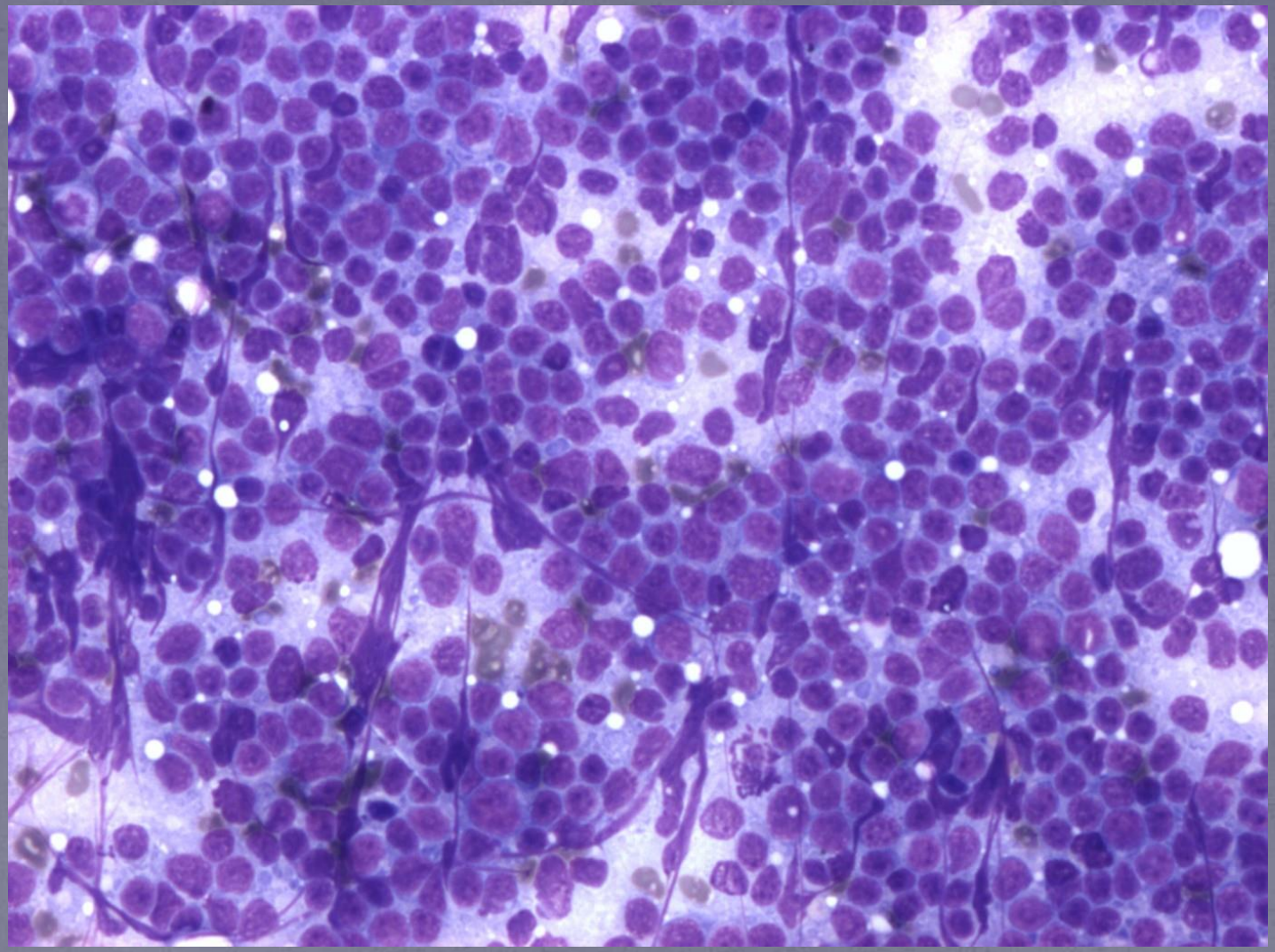


Leucemia linfoblastica acuta

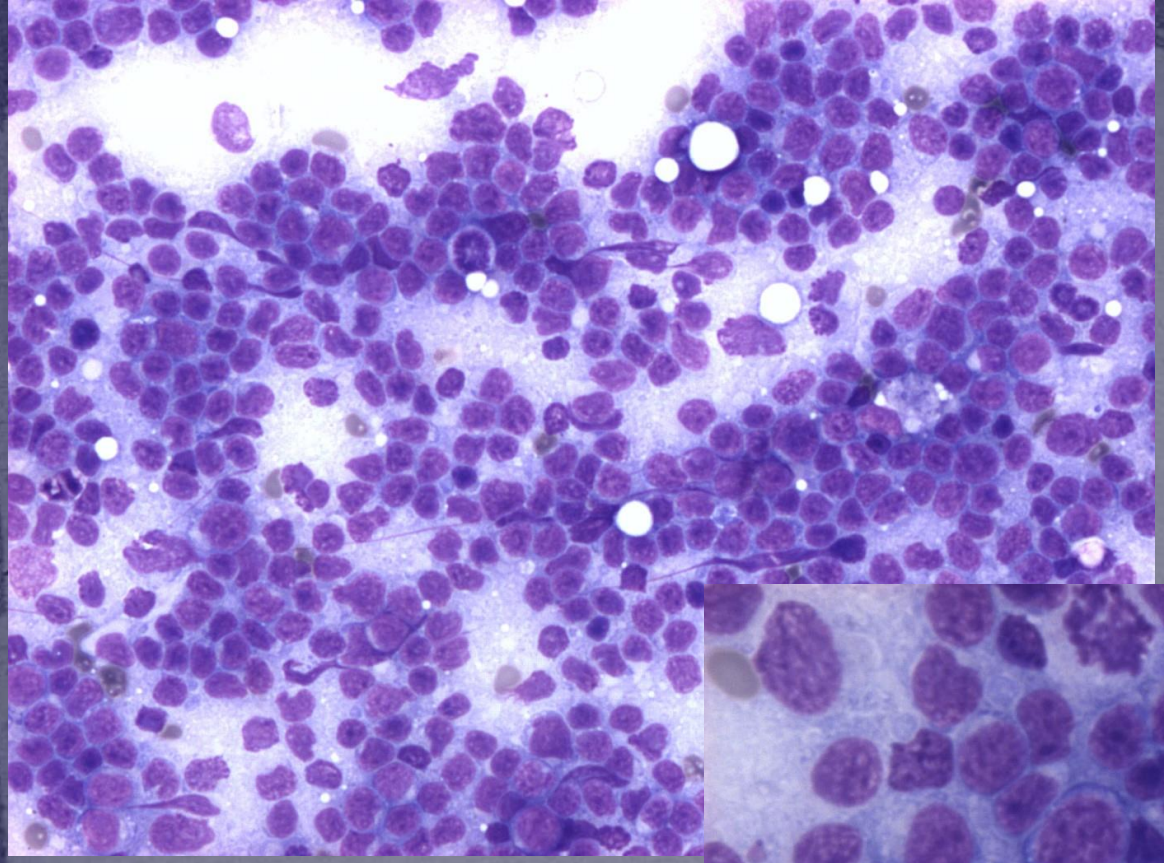
Neoplasie linfoidi

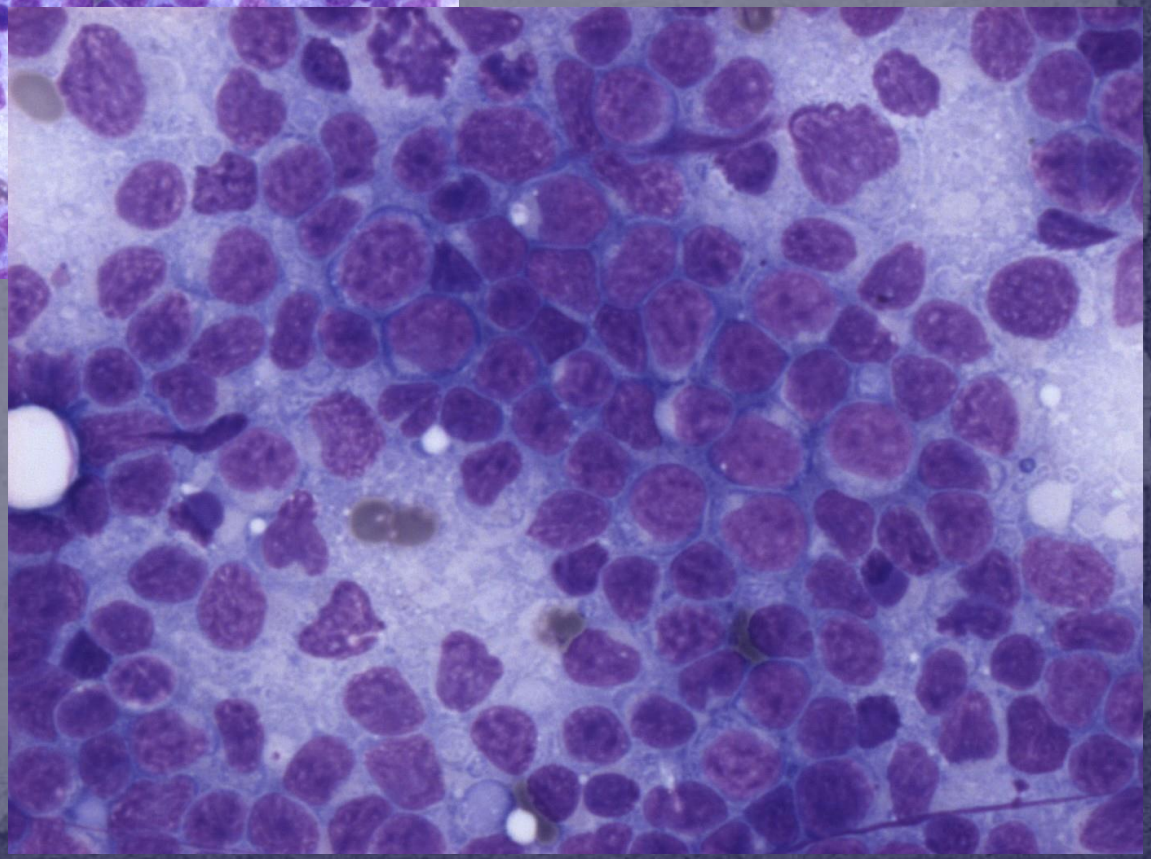
Leucemie linfoidi acute
Linfomi linfoblastici





linfoma linfoblastico





linfoma linfoblastico

Leucemie mielodi acute (AML)

Proliferazione cellule immature nel midollo

- moltiplicazione neoplastica di precursori della linea granulocitica, monocito-macrofagica, eritrocitica o piastrinica
- Decorso molto rapido, prognosi infausta

Ematologia:

anemia, spesso da moderata a grave,
trombocitopenia e neutropenia
5% dei leucociti si presenta con caratteristiche morfologiche atipiche

Valutazione morfologica:

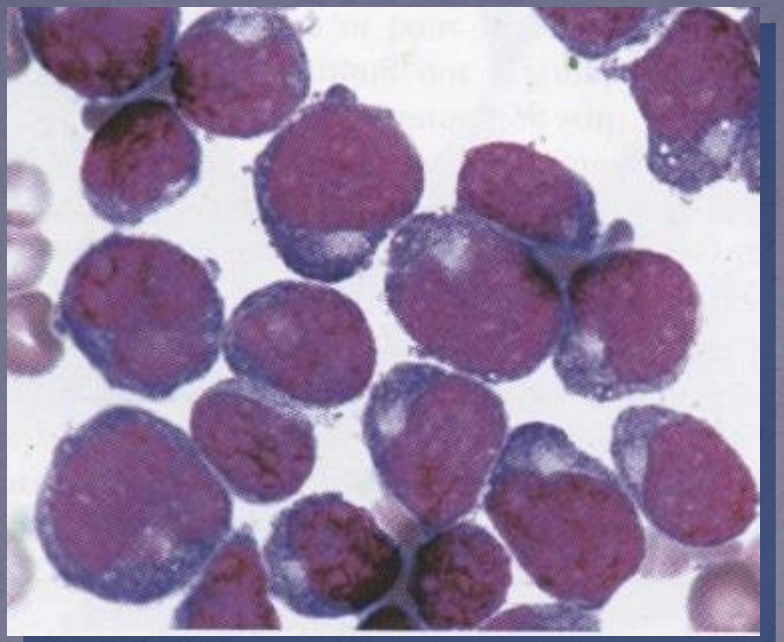
- blasti mieloidi: cellule di grosse dimensioni
- ampio citoplasma da moderatamente (mieloblasti e monoblasti) ad intensamente (rubriblasti) basofilo,
- spesso con vacuolizzazioni
- scarse granulazioni azzurrofile, nucleo rotondo o indentato con cromatina finemente dispersa e uno o più nucleoli visibili

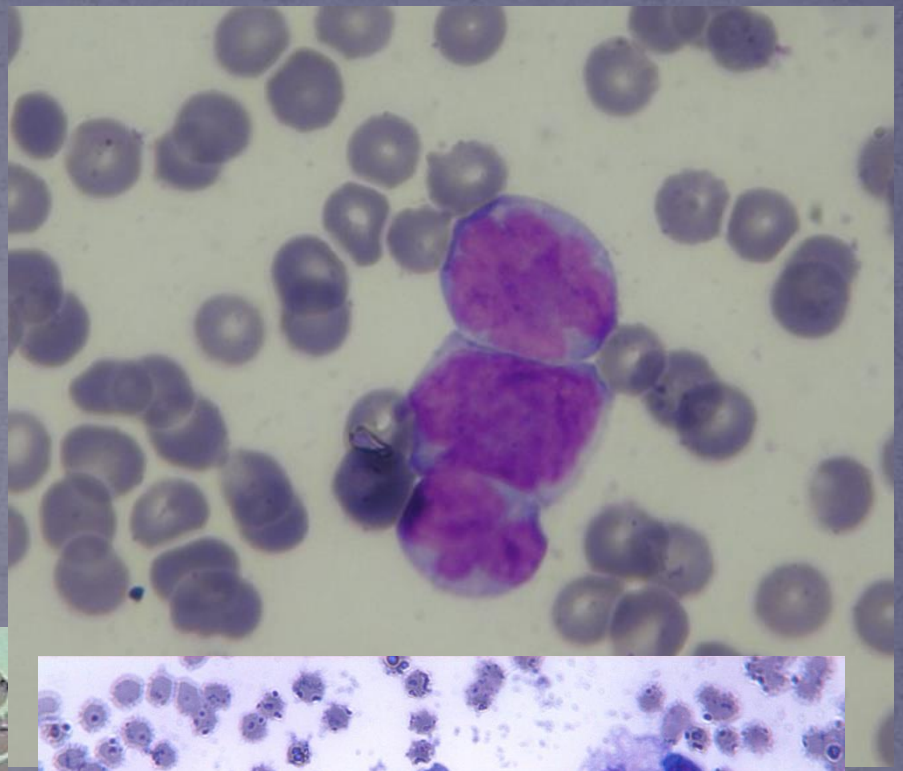
Esame del midollo osseo

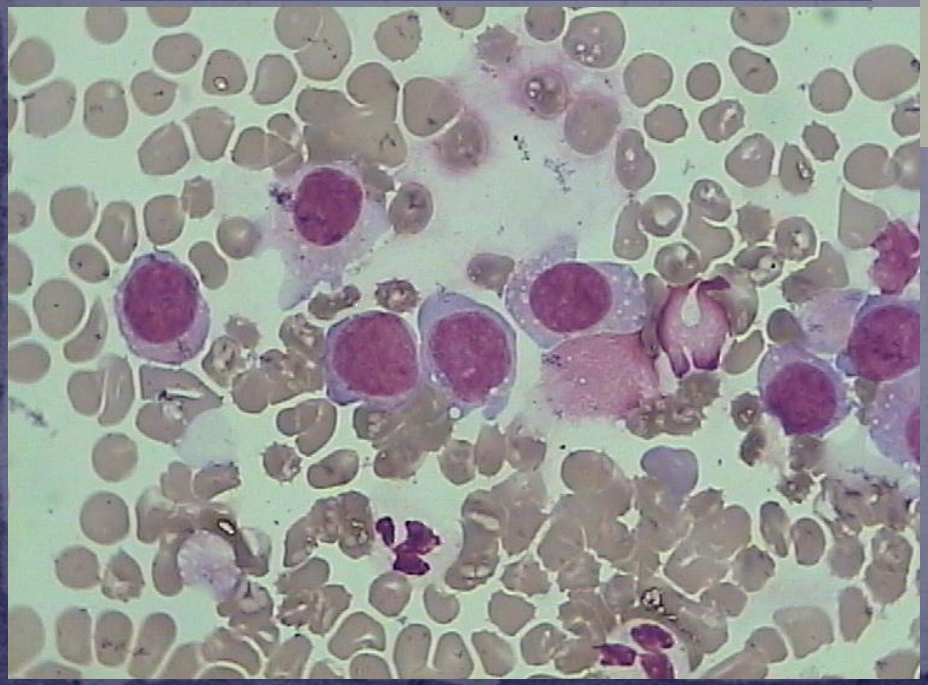
del 20% delle cellule nucleate è costituita da blasti.

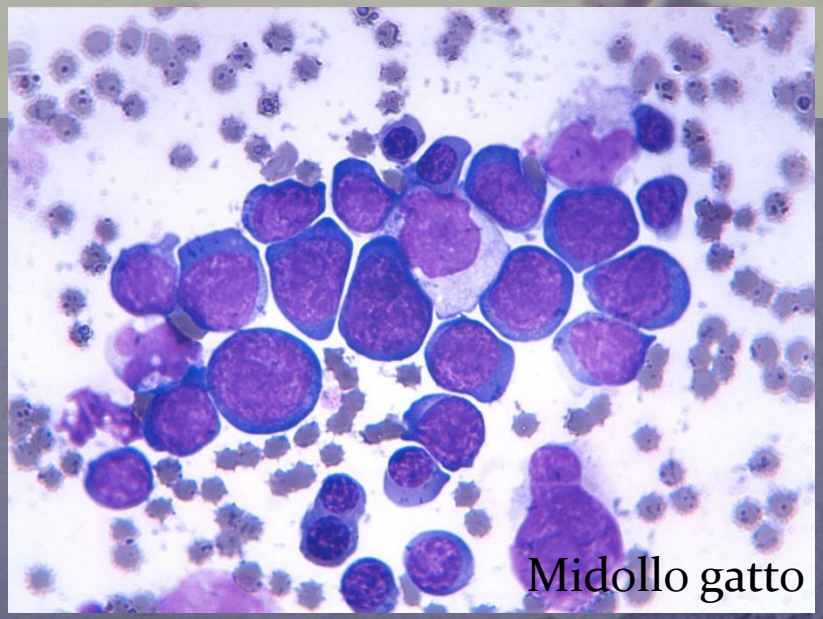
CLASSIFICAZIONE FAB

Sottotipo	Descrizione
AML-M1	Leucemia mieloblastica, senza differenziazione Mieloblasti ≥30% (ANC) Mieloblasti ≥90% (NEC) Granulociti e monociti <10% (NEC)
AML-M2	Leucemia mieloblastica, con differenziazione Mieloblasti >30% (ANC) Promielociti ed elementi più maturi >10% (NEC)
AML-M3	Leucemia promielocitica (non descritta negli animali)
AML-M4	Leucemia mielomonoblastica Mieloblasti e monoblasti >30% (ANC) Monociti e precursori >20% (NEC) Neutrofili e precursori >20% (NEC)
AML-M5a	Leucemia monoblastica, senza differenziazione Mieloblasti e monoblasti >30% (ANC) Monoblasti >80% (NEC) Cellule di origine monocitica >80% (NEC)
AML-M5b	Leucemia monocitica, con differenziazione monocitica Mieloblasti e monoblasti >30% (ANC) Monoblasti <80% (NEC) Cellule di origine monocitica >80% (NEC)
AML-M6	Eritroleucemia M:E< 1:1 Blasti mieloidi e monocitari>30% (NEC)
AML-M6Er	Mielosi eritremica M:E<1:1 Rubriblasti, prorubrociti + blasti altre linee >30% Blasti di altre linee <30% (NEC)
AML-M7	Leucemia megacarioblastica Megacarioblasti >30% (ANC)
AUL	Leucemia acuta indifferenziata Blasti ~100%








Midollo gatto