



# Practical problems in bone marrow diagnosis

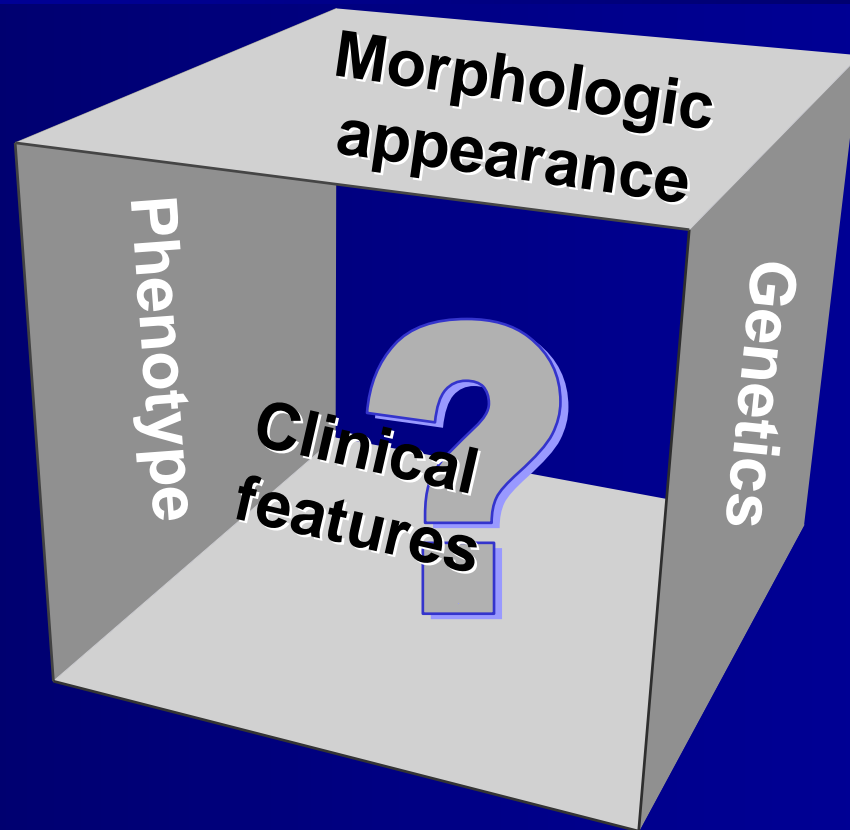
**Robert P Hasserjian, MD**

**Assistant Pathologist,  
Massachusetts General Hospital  
Assistant Professor, Harvard  
Medical School**

# Outline of lecture

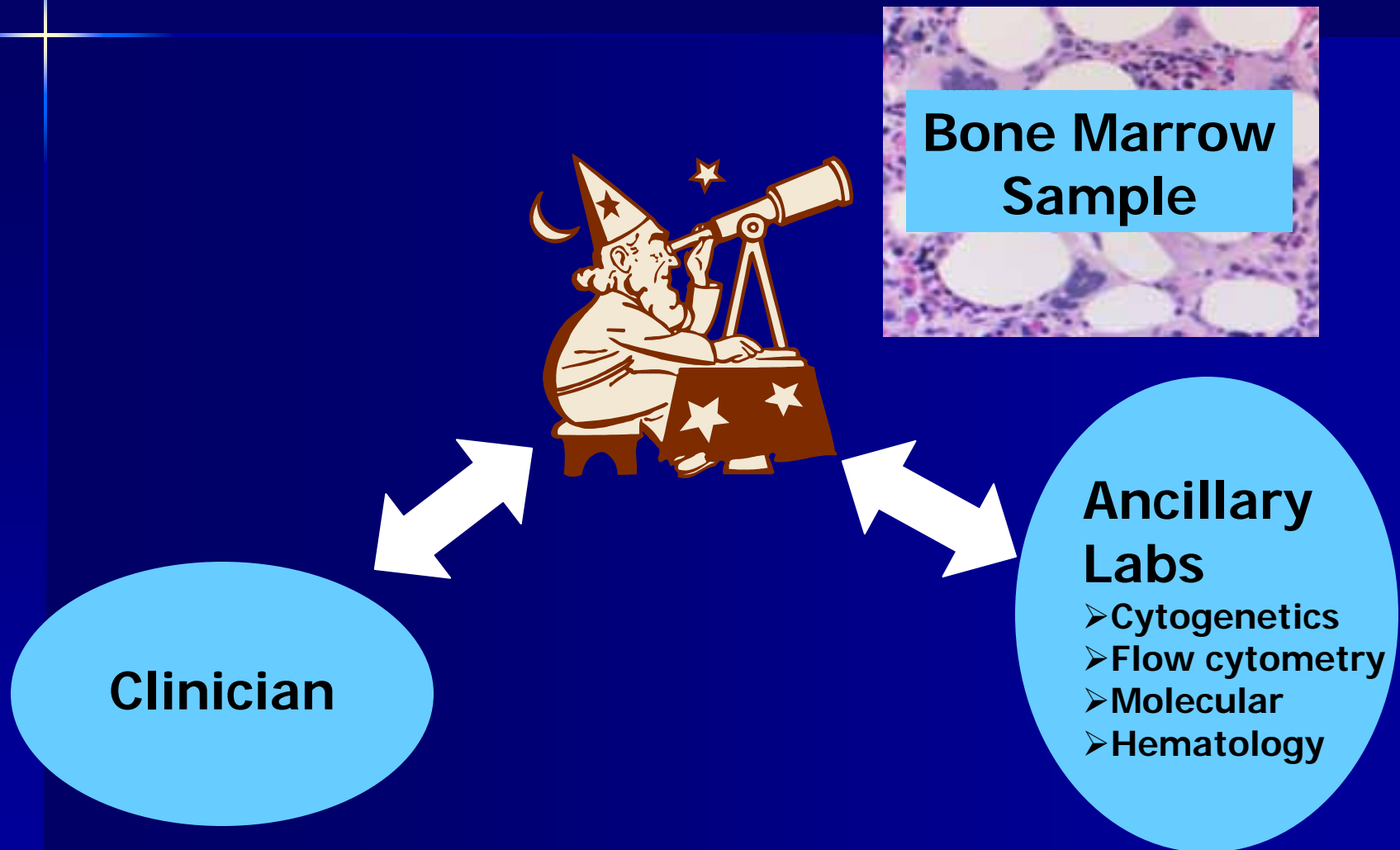
- Review the overall role of the pathologist in diagnosing diseases involving the bone marrow
- Discuss the differential diagnoses in specific common clinical scenarios

**Current concepts of hematologic disease  
necessitate a multi-modality approach. . .**



**. . . which increases the complexity of  
bone marrow interpretation**

# Pathologist assumes a central role in work-up



# Reasons for bone marrow

|                 | Clinical indication        | Primary | Tertiary |
|-----------------|----------------------------|---------|----------|
| Primary disease | Anemia                     | 28%     | 14%      |
|                 | Paraprotein                | 19%     | 8%       |
|                 | Elevated peripheral counts | 9%      | 2%       |
|                 | Thrombocytopenia           | 5%      | 3%       |
|                 | New diagnosis of leukemia  | 4%      | 4%       |
|                 | Leukopenia or pancytopenia | 1%      | 3%       |
|                 | Other primary indications  | 1%      | 4%       |
| Follow-up       | Lymphoma staging/follow-up | 22%     | 26%      |
|                 | Leukemia follow-up         | 10%     | 30%      |
|                 | Other follow-up (MDS, MM)  | 1%      | 7%       |

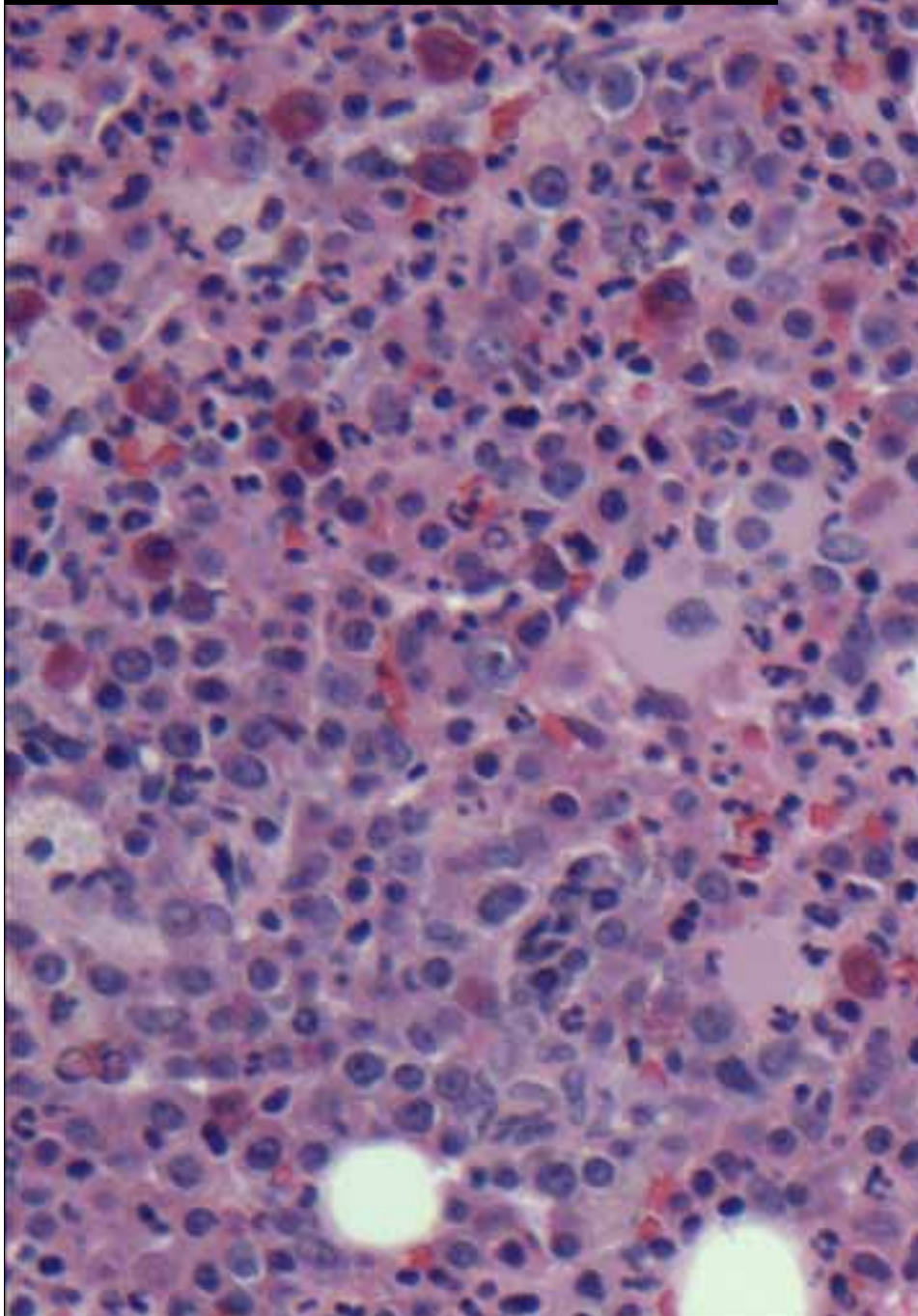
# Bone marrow sampling in anemic patients

- Anemia is the most frequent indication for bone marrow examination
- Hematologist has (hopefully) excluded toxic, metabolic, and peripheral causes
  - Anemia degree and duration and the extent of screening vary among different hematologists
  - Usually anemia has been already shown to be refractory to iron, Vitamin B<sub>12</sub> and folate therapy
- Main reason for bone marrow sampling is to evaluate for a myelodysplastic syndrome (MDS)

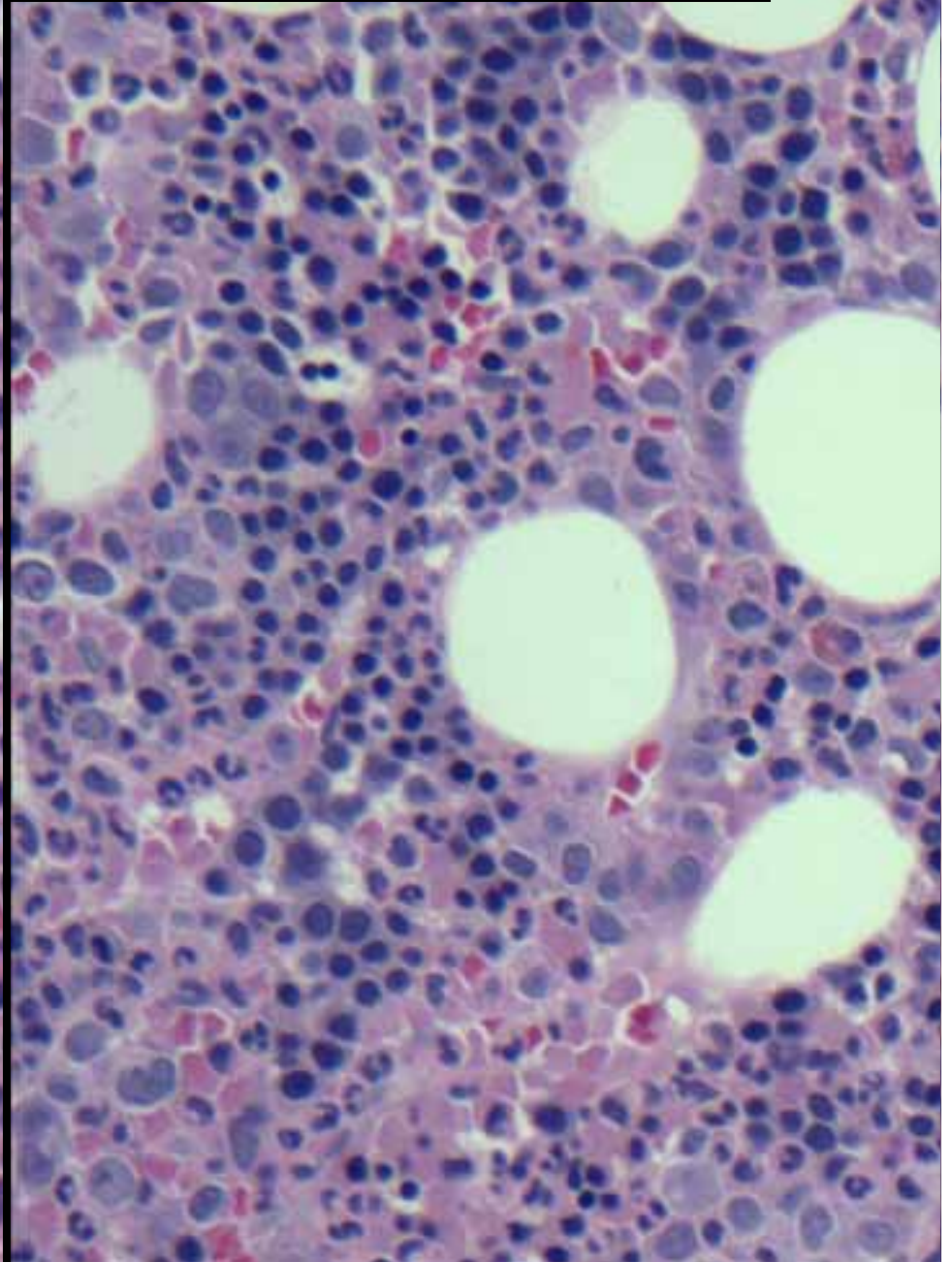
# MDS: Low power morphologic abnormalities

- Hypercellular marrow (80% of cases)
- Disorganization of hematopoiesis
  - Immature myeloid elements occur away from bone trabeculae
  - Erythroid elements fail to form well-defined clusters
- Often many small, hypolobated megakaryocytes

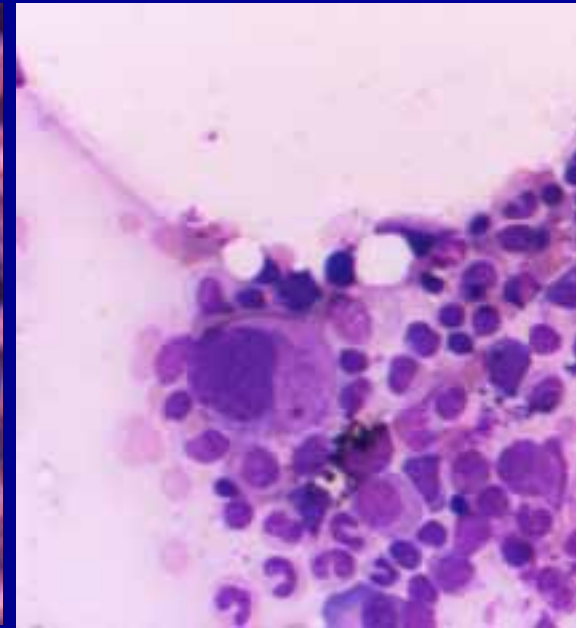
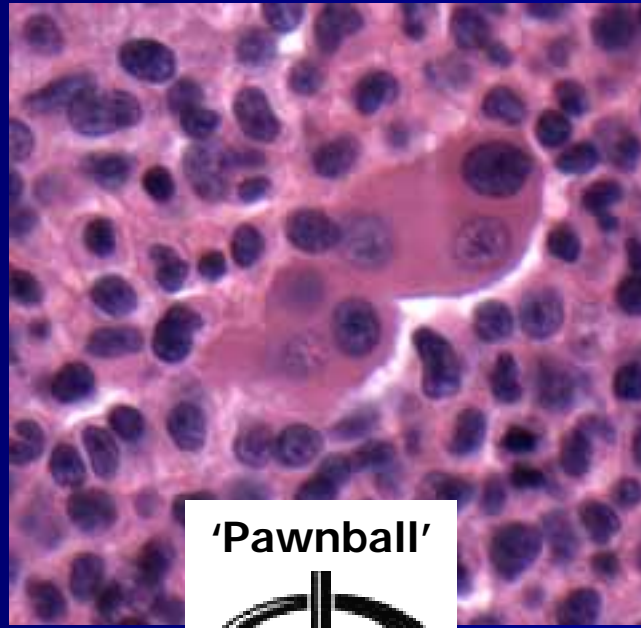
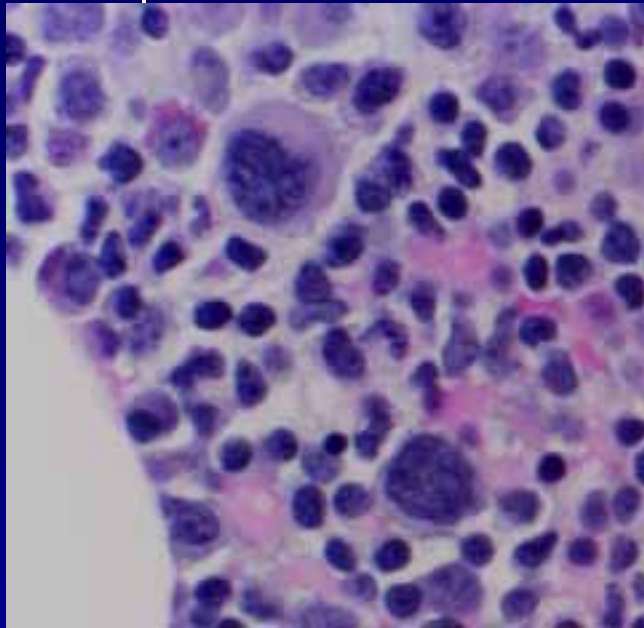
Architectural disorganization in MDS



Maintained architecture in reactive marrow hyperplasia (AIHA)

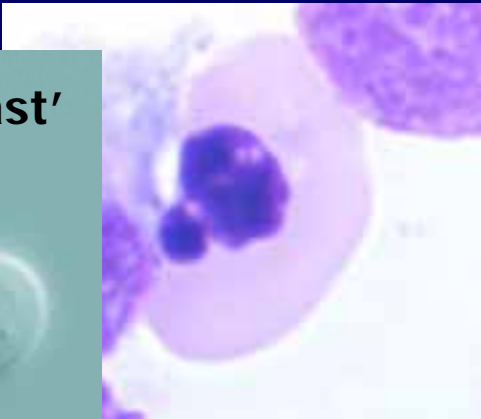
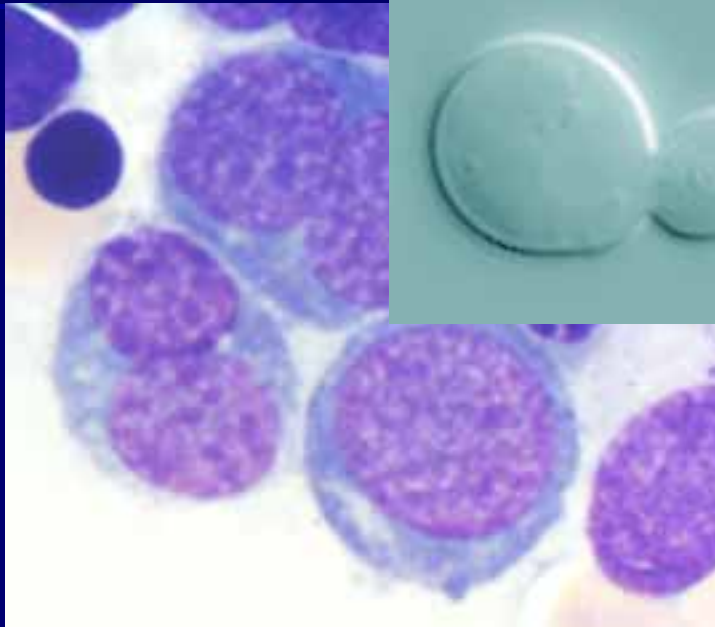
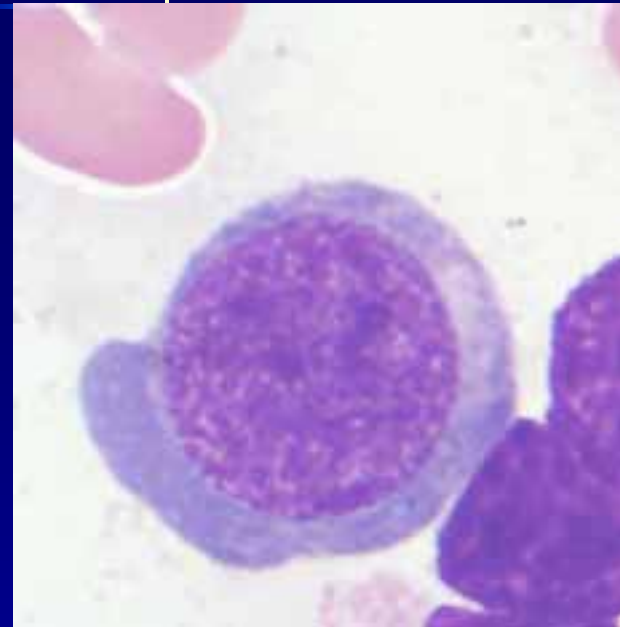


# Megakaryocyte dysplasia



- Small size
- Nuclear simplification
- Separated nuclear lobes

# Erythroid lineage dysplasia



- Megaloblastoid change (nuclear:cytoplasmic dyssynchrony)
- Binucleation
- Nuclear budding, nuclear irregularities

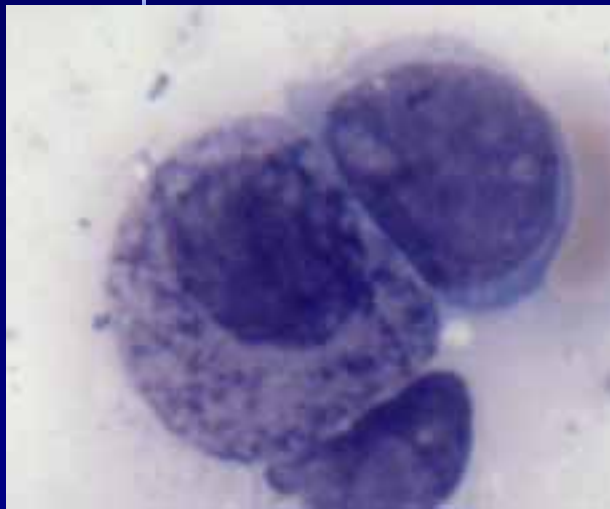
# Myeloid lineage dysplasia



'Pince-nez'

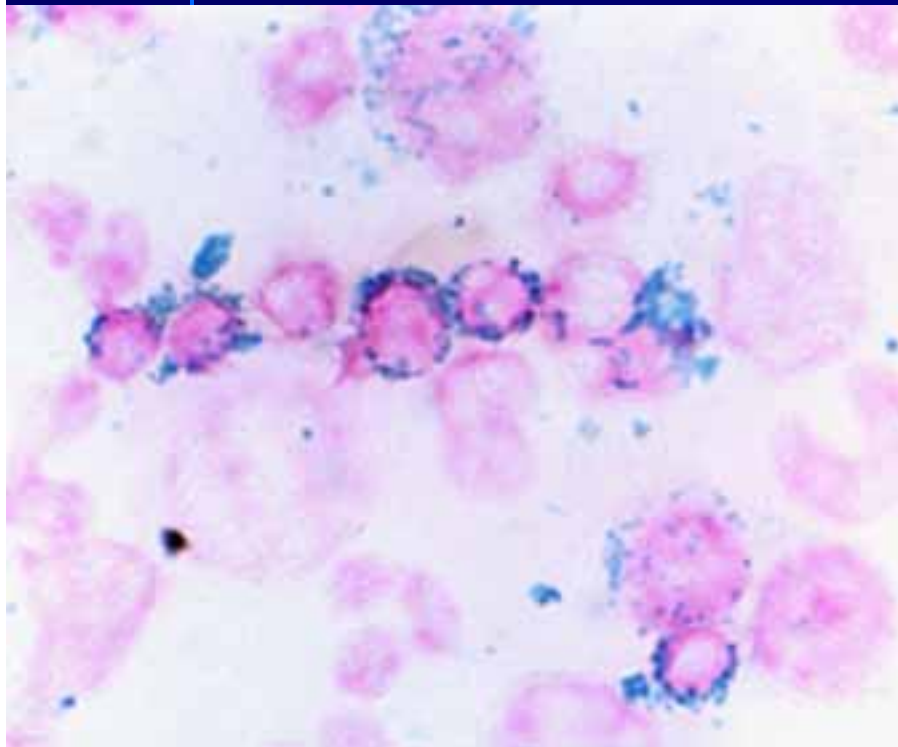


Normal poly



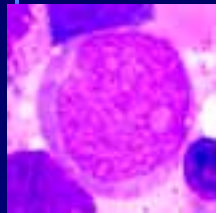
- Pseudo Pelger-Huet nucleus (bilobed)
- Nuclear hypersegmentation or abnormal nuclear shape
- Cytoplasmic hypogranulation or abnormal granulation

# Ringed sideroblasts



- Refractory anemia with ringed sideroblasts (RARS)
  - >15% of erythroids are ringed sideroblasts
  - Anemia and dysplasia in erythroid lineage only
  - Good prognosis MDS
- Ringed sideroblasts (<15%) are common in all MDS types
- Differential diagnosis
  - MPD/MDS overlap diseases
  - Alcohol
  - Chronic lead poisoning
  - Drugs
  - Hereditary sideroblastic anemia

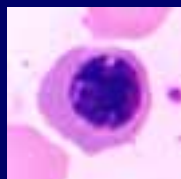
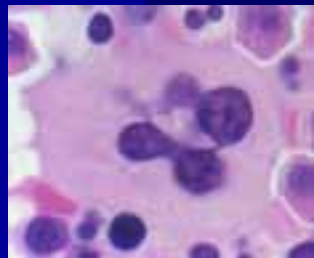
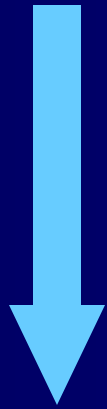
# Neoplastic versus 'reactive' dysplasia



Normal stem cell

**AND**

Normal  
microenvironment



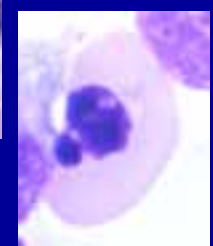
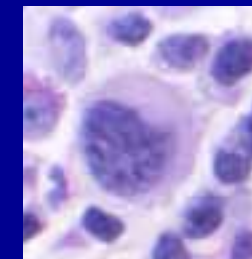
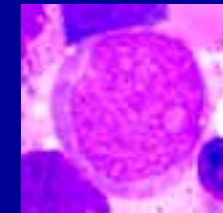
**YIELDS**

Normal  
progeny

Normal stem cell

**AND**

Abnormal  
microenvironment



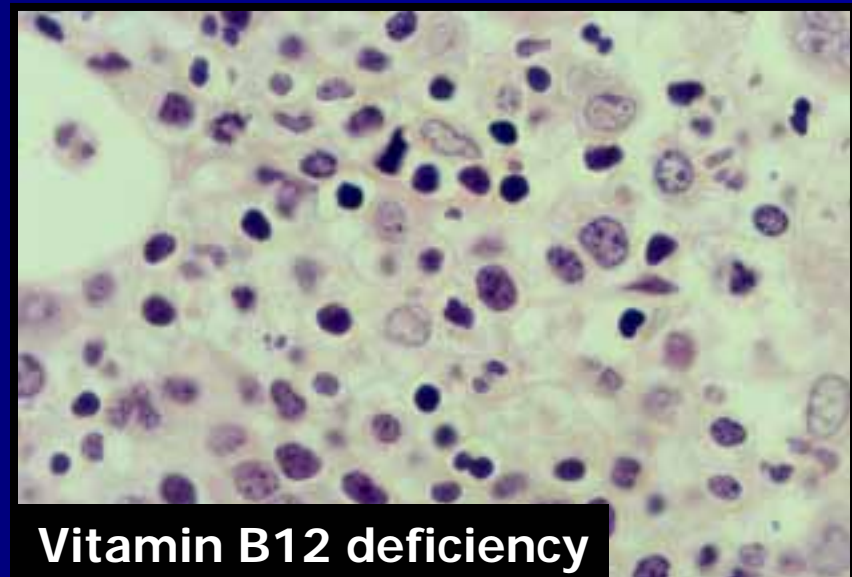
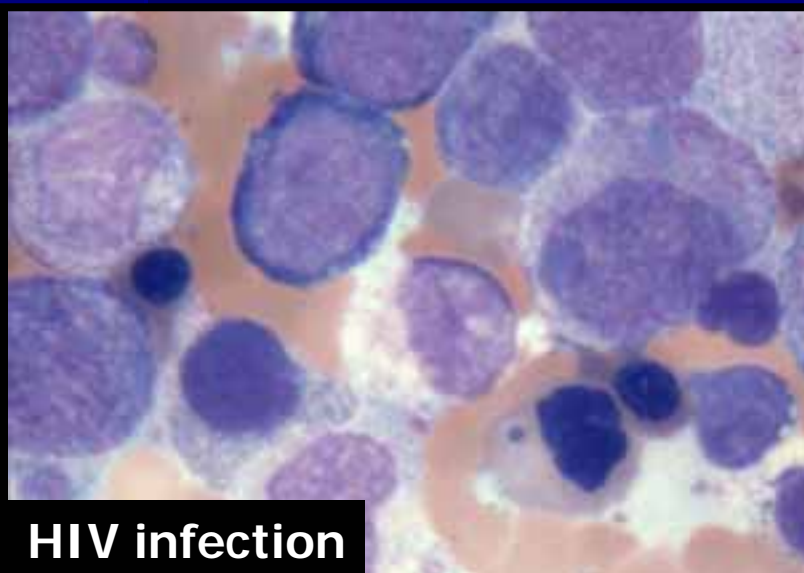
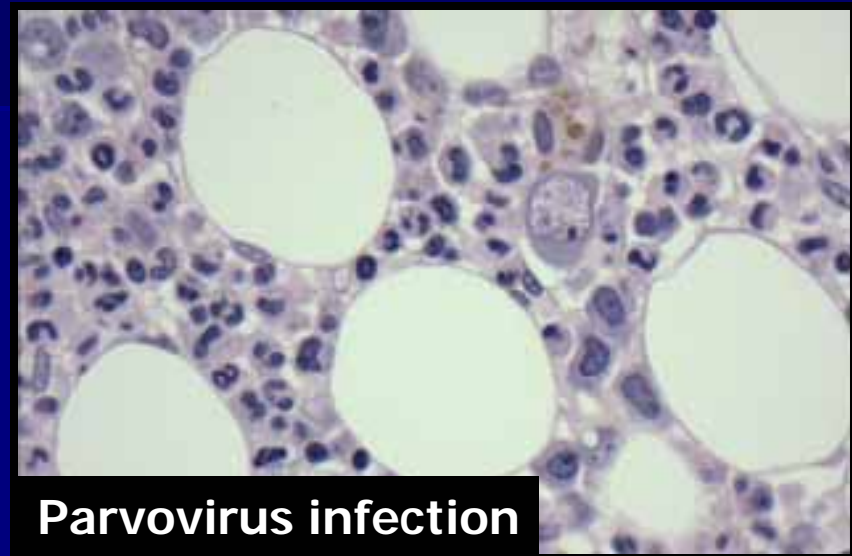
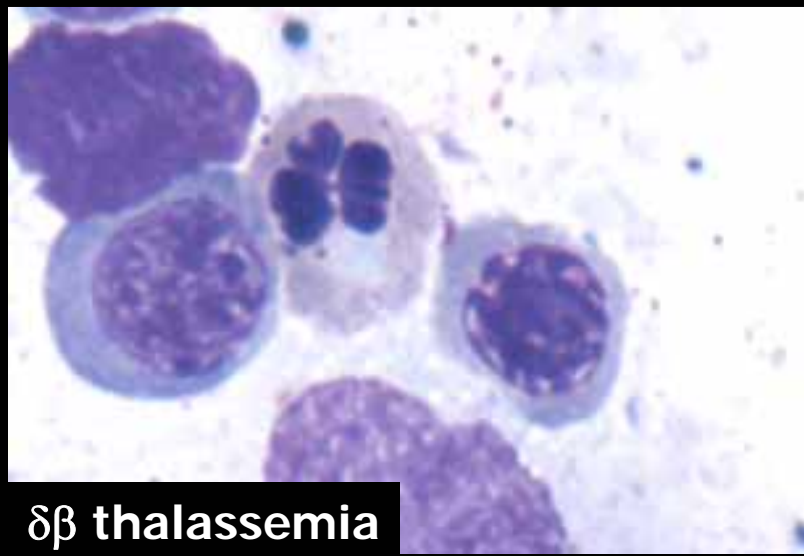
**YIELDS**

Dysplastic  
progeny

# Situations to avoid MDS diagnosis

- Drugs/toxins
  - Recent (<6 months) chemotherapy
  - Alcoholism
- Vitamin B12/folate deficiency
- Hemoglobinopathies
- Infections
  - AIDS
- Autoimmune diseases
- Neoplasms
  - Bone marrow infiltrate (especially myeloma, hairy cell leukemia)
  - (Rarely) 'paraneoplastic' dysplasia from remote solid tumor

# Reactive mimics of MDS



# Cytogenetics in MDS

- Abnormal cytogenetics result confirms a diagnosis of neoplasia
  - May pick up MDS cases with subtle morphologic changes
- But. . . negative cytogenetics result does not necessarily exclude MDS
  - 50% of MDS cases have normal karyotype

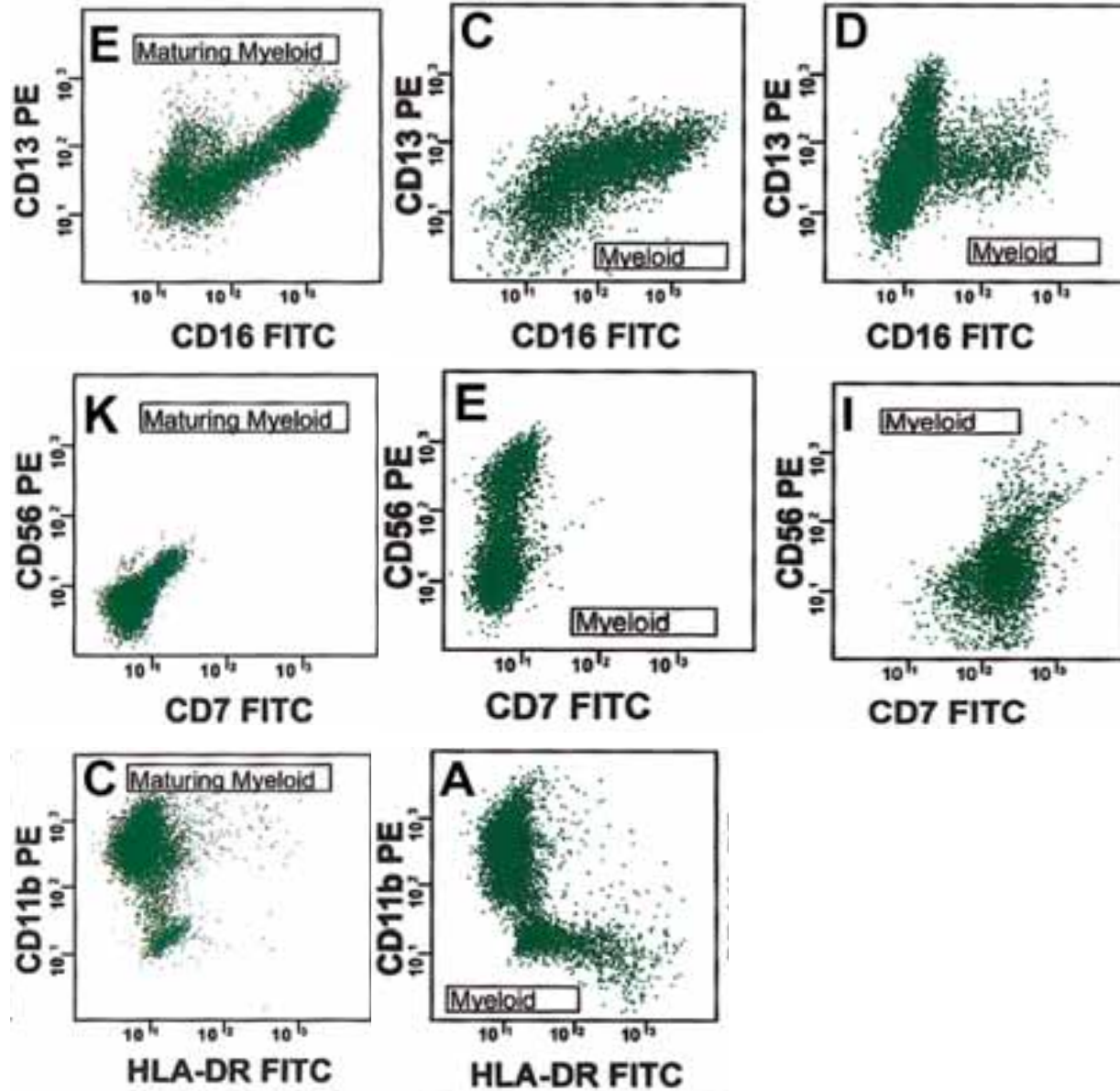
# Flow cytometry in evaluation of MDS

- Bone marrow and peripheral blood cell populations in MDS often show aberrant antigen expression by flow cytometry
  - Myeloid, monocytic, blast, and erythroid
- Most specificity in distinguishing MDS from reactive cases is achieved by relying on multiple aberrations

Wells et al. Blood 2003; Stetler-Stevenson et al. Blood 2001;  
Cherian et al. Cytometry 2005

Normal pattern

MDS patterns



Abnormal relationship of CD13 and CD16

Abnormal expression of CD56

Abnormal relationship of CD11b and HLA-DR

blood

JOURNAL OF  
THE AMERICAN  
SOCIETY OF  
HEMATOLOGY

Wells, D. A. et al. *Blood* 2003;102:394-403

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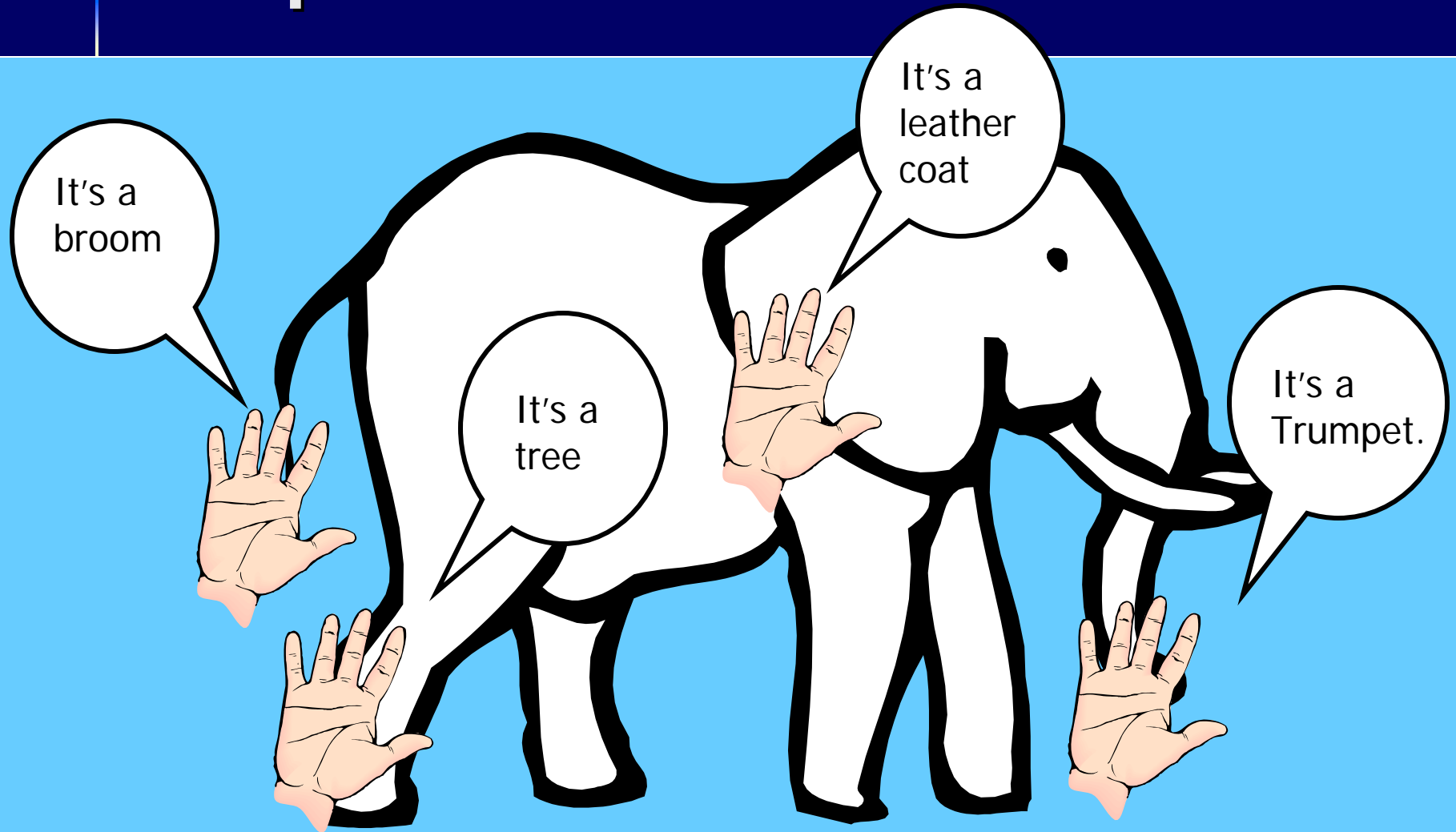
# Morphologic dysplasia versus 'true' MDS

- 'Reactive' dysplasia
- Avoid overcalling MDS by
  - Finding possible explanation for dysplasia
  - Beware of putting too much emphasis on single lineage dysplasia



- 'Phantom' dysplasia
- Clues to avoid missing MDS diagnosis
  - Typical cytogenetic abnormality (e.g. 20q-)
  - Abnormal flow cytometry
  - Excess blasts
  - Strong clinical evidence for MDS

# Parable of four blind men feeling an elephant. . .

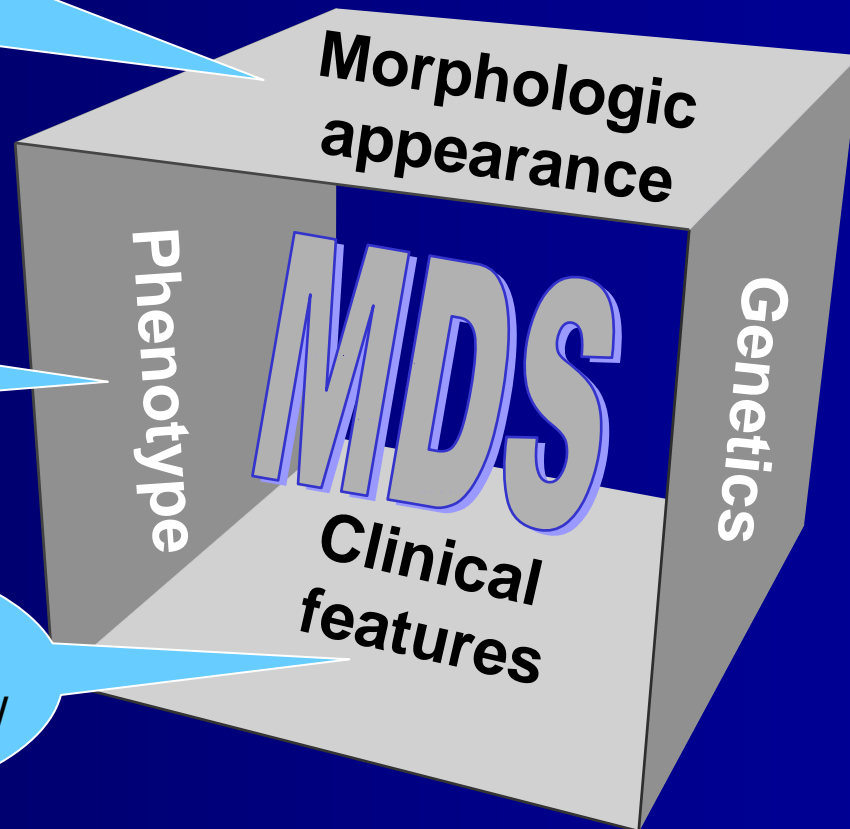


# ... like four non-communicative professionals trying to diagnose MDS!

Megaloblastic changes  
?real dysplasia or reactive

Normal  
flow cytometry  
Not lymphoma

Anemia  
refractory to therapy



Trisomy 8  
consistent  
with a  
myeloid  
neoplasm

# Findings in Bone Marrow Biopsy for Anemia\*

| Pathologic findings       | #         | Percent    |
|---------------------------|-----------|------------|
| <b>Non-specific</b>       | <b>61</b> | <b>66%</b> |
| Myelodysplastic syndromes | 20        | 22%        |
| Infections (HIV, other)   | 6         | 6%         |
| Aplastic anemia           | 4         | 4%         |
| Non-Hodgkin's lymphoma    | 4         | 4%         |
| Gaucher's                 | 1         | 1%         |
| Megaloblastic anemia      | 1         | 1%         |

\*Baystate Medical Center, Springfield, MA, USA (9 months)

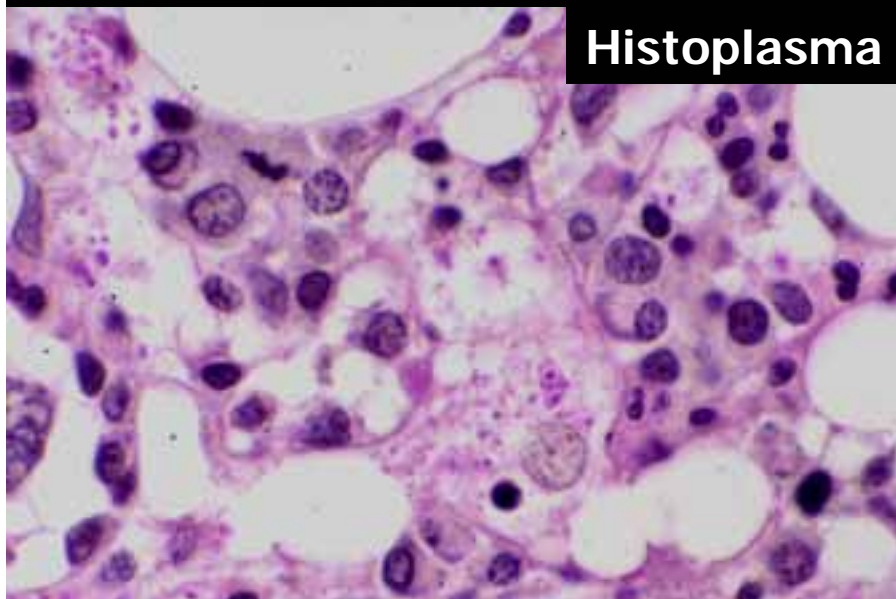
# No specific diagnosis?

- A common diagnosis in this setting!
- Anemia of chronic disease
  - Often increased iron in marrow histiocytes
- Reactive causes which become evident after bone marrow sample is taken
  - 'Test of time': transient cause resolves
- Early MDS cases which are not well-developed enough for definitive diagnosis
  - 'Test of time': anemia is refractory or worsen
  - OK to hedge on initial marrow in these situations

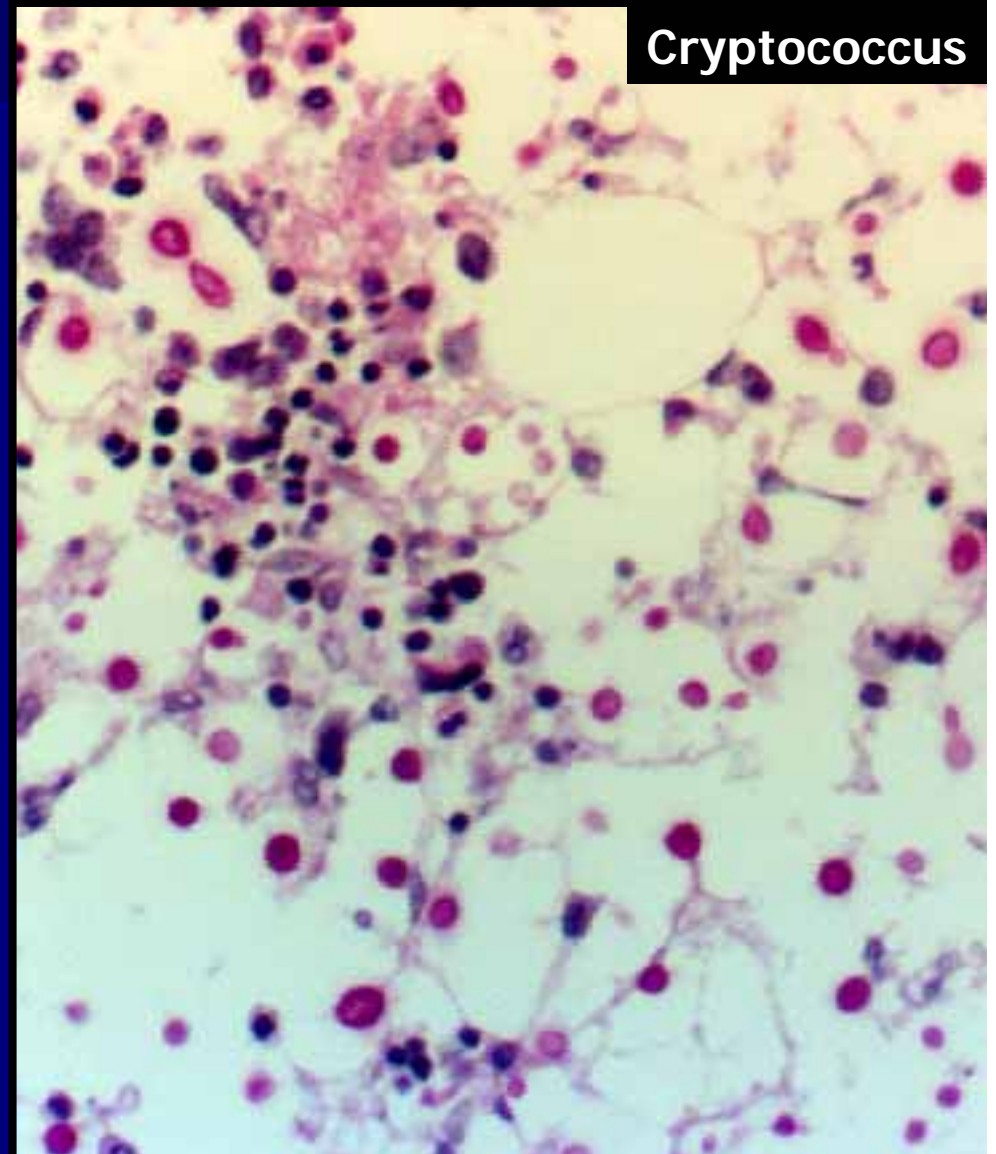
# Bone marrow sampling in the cytopenic HIV+ patient

- Rule out infection
- Rule out neoplasm
  - Diffuse large B-cell lymphoma
  - Hodgkin lymphoma
- Other bone marrow abnormalities related to HIV infection may mimic MDS
  - Primary myeloid neoplasm (MDS, AML) uncommon in this setting

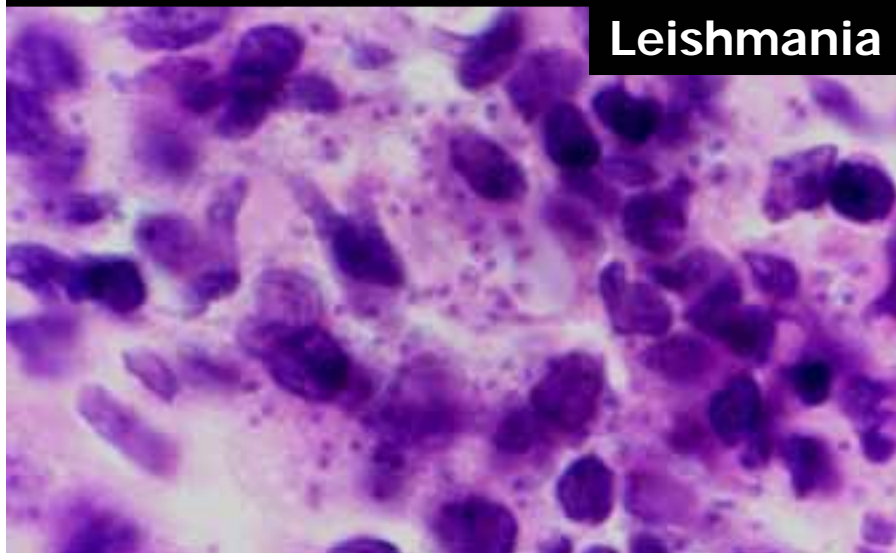
# Infections in HIV+ patients



**Histoplasma**



**Cryptococcus**



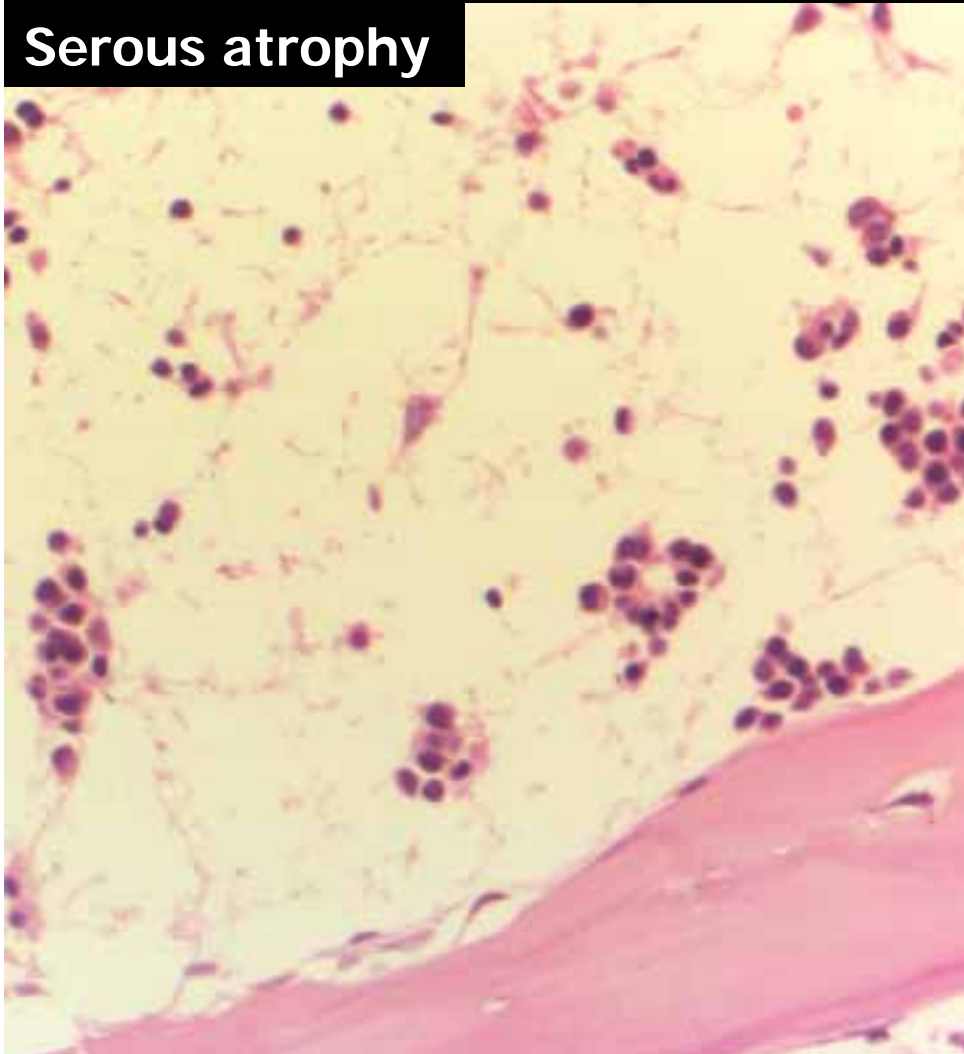
**Leishmania**

# Non-specific findings in HIV or autoimmune diseases

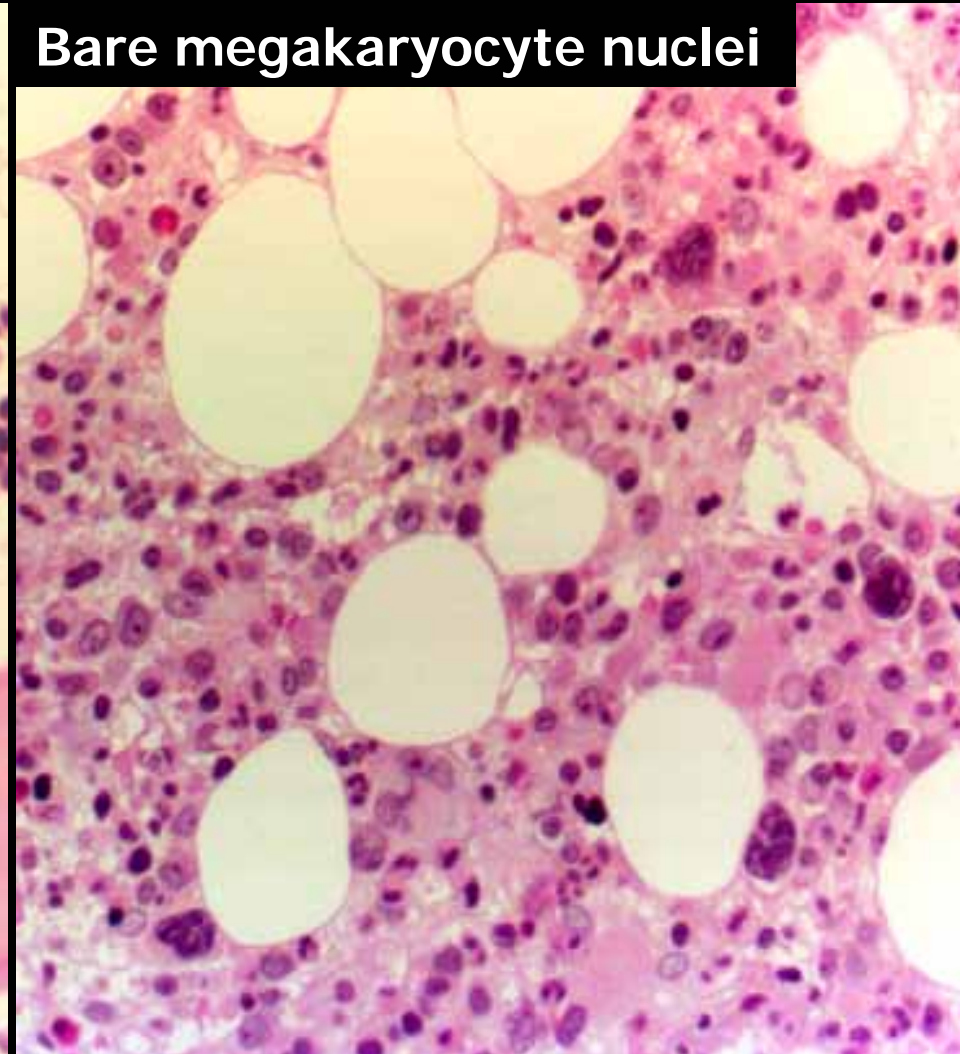
- Hyper- or hypocellularity
  - Serous atrophy (gelatinous transformation)
- Morphologic 'dysplasia'
  - Megakaryocytes and erythroid, less commonly myeloid lineage
- Plasmacytosis
- Lymphoid aggregates
- Reticulin fibrosis

# Other findings in HIV+ patients' bone marrow

Serous atrophy



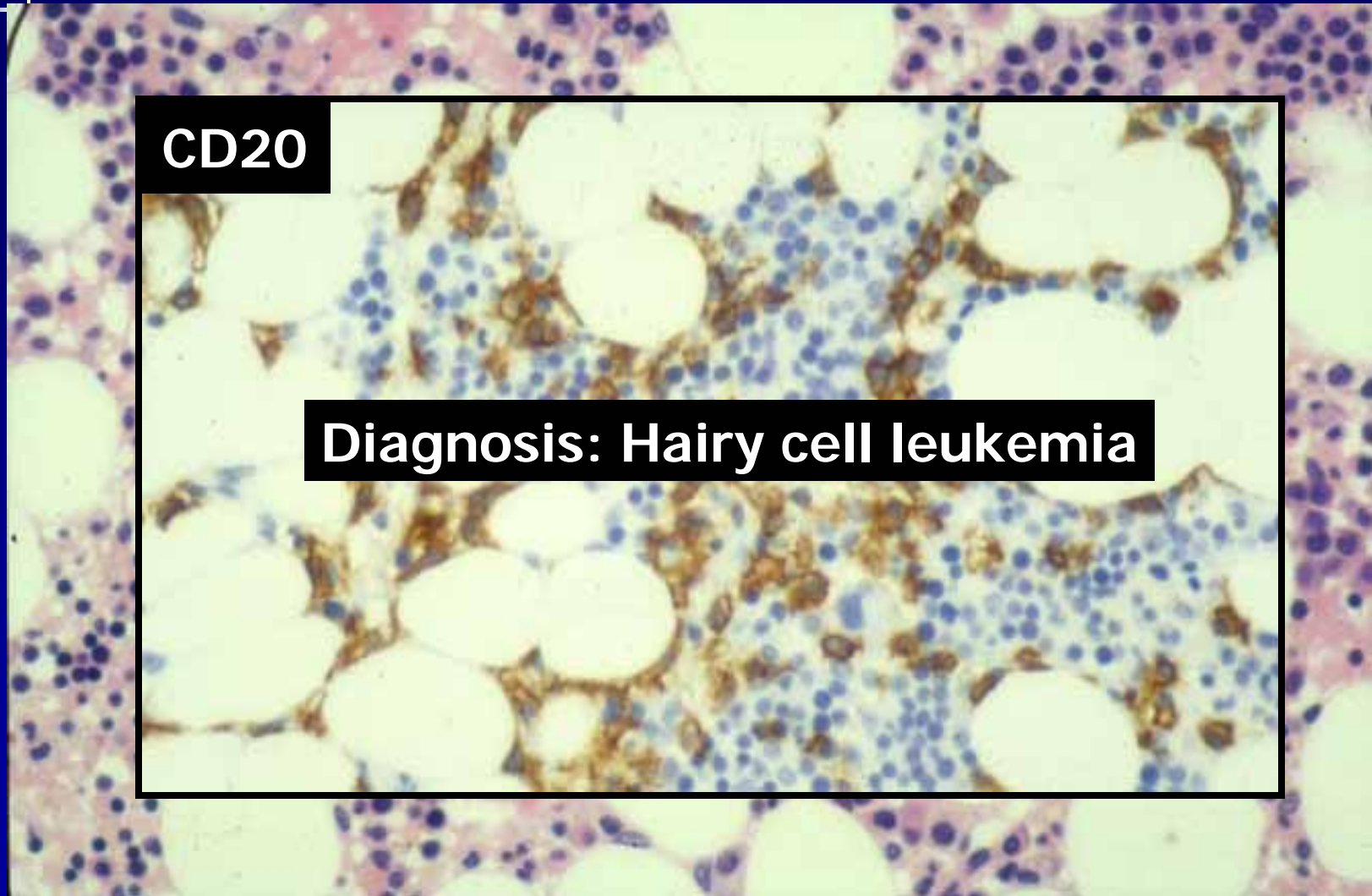
Bare megakaryocyte nuclei



# NHL discovered in marrow of anemic patients

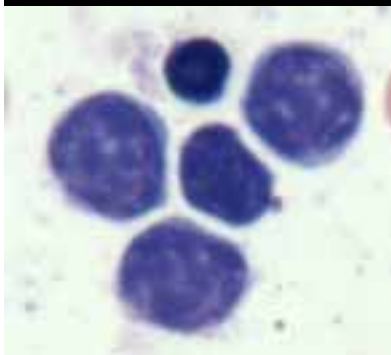
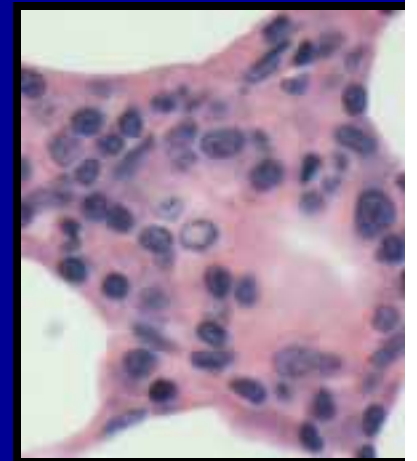
- Hairy cell leukemia
  - Cell morphology and interstitial infiltration pattern helpful in diagnosis
    - Immunohistochemistry (CD20, DBA.44) often discloses subtle infiltrate
    - Peripheral blood may yield sufficient cells for flow cytometry if dry tap
- Lymphoplasmacytic lymphoma

# Bone marrow in anemic patient ?MDS



# Clues to diagnosis of lymphoplasmacytic lymphoma

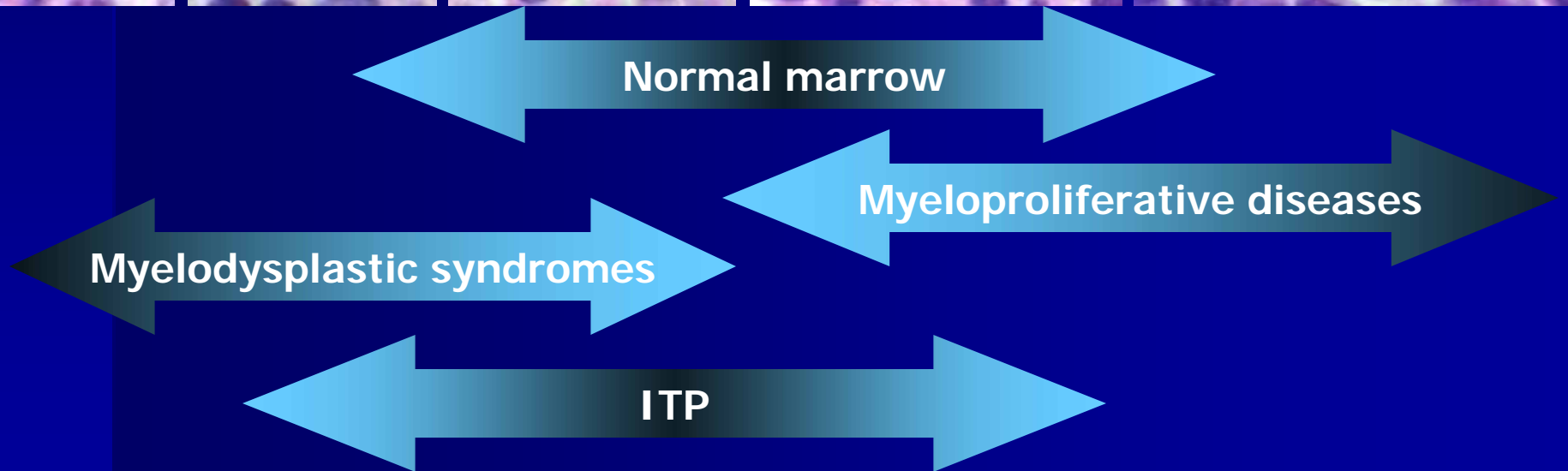
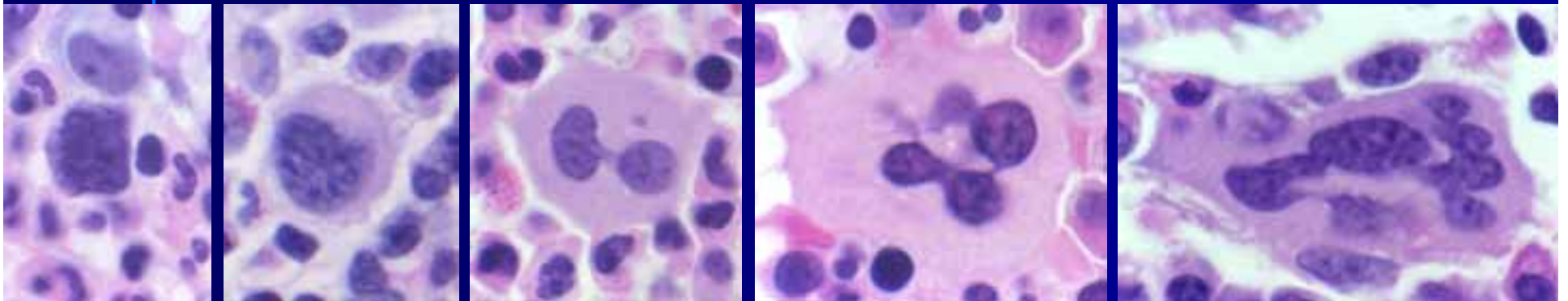
- Nodular lymphoid aggregates with admixed plasma cells
- IgM paraprotein and IgM positive plasma cells
- Lymphoplasmacytoid cells, Dutcher bodies



# Thrombocytopenia evaluation by bone marrow

- Myelosuppression due to drug or toxin
  - Decreased marrow megakaryocytes
- Peripheral platelet consumption
  - Normal to increased marrow megakaryocytes
  - Autoimmune (ITP) most common (>90%)
    - May have an increase in early (small) megas mimicking MDS
    - MDS only rarely presents with isolated thrombocytopenia

# Spectrum of megakaryocyte morphology

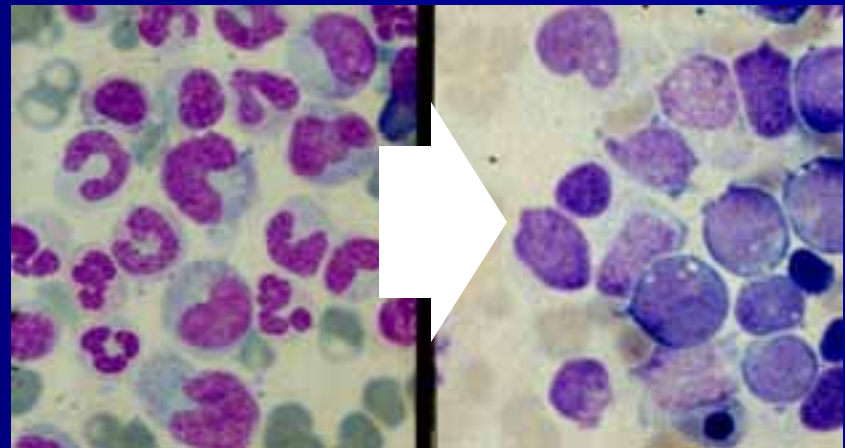


# Marrow evaluation for elevated peripheral counts

- Leukocytosis → CML *versus* reactive
- Erythrocytosis → PCV *versus* reactive
- Thrombocytosis
  - Presents a broad differential diagnosis of various myeloid neoplasms and reactive thrombocytosis
  - Bone marrow evaluation of megakaryocyte morphology helpful

# Diagnosis of CML in the era of imatinib (Gleevec)

- Diagnosis of CML must be confirmed by proving *bcr/abl* translocation
- If morphology suggests CML and cytogenetics is negative, pursue further tests to look for *bcr/abl* (FISH and/or RT-PCR)
- Gleevec therapy most effective early in the course of disease



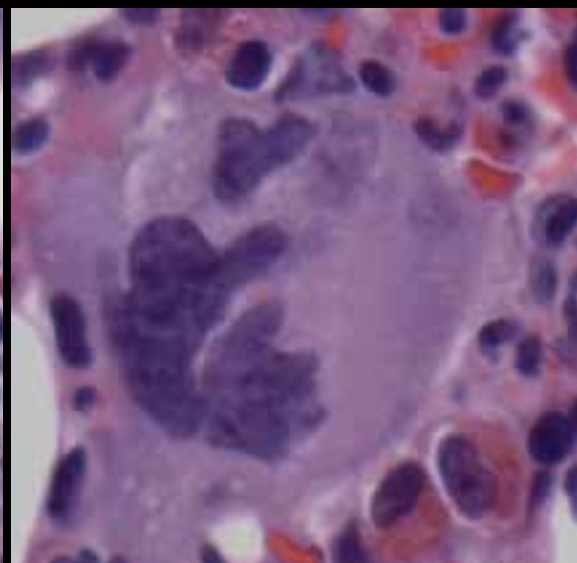
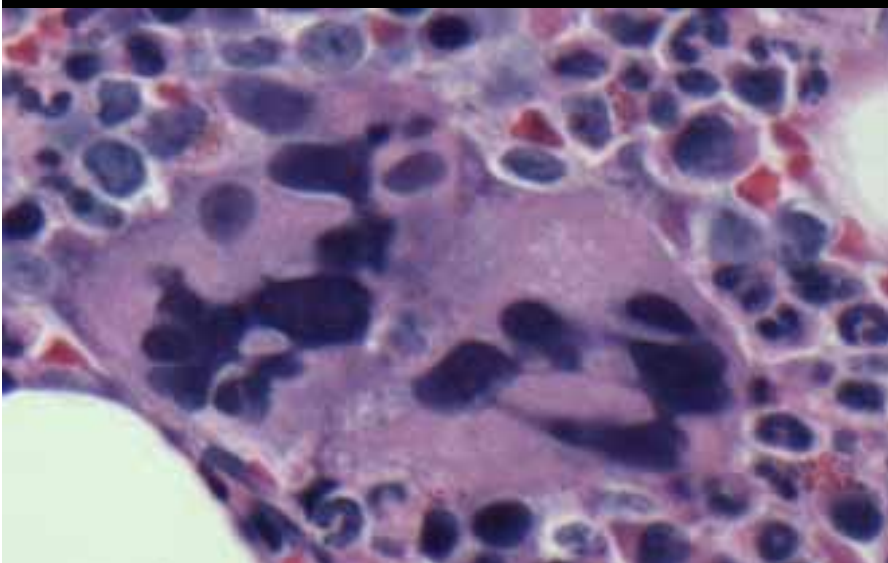
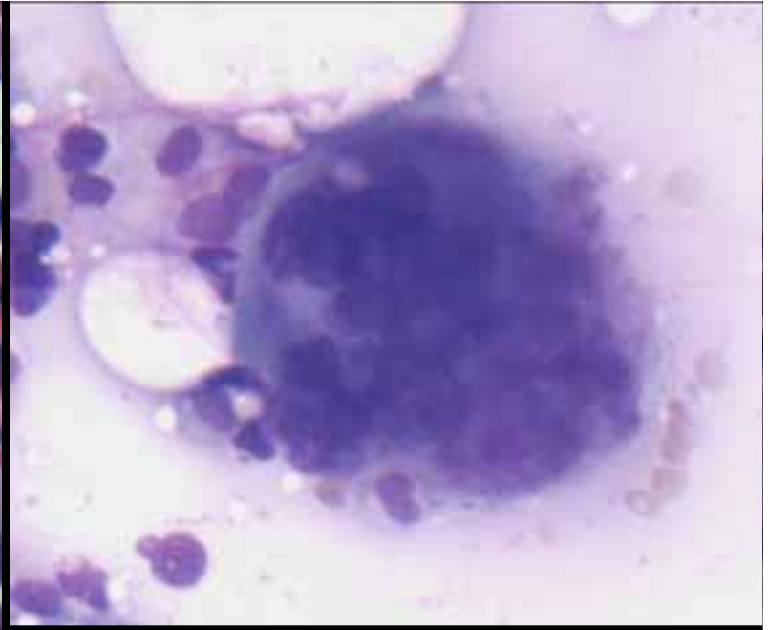
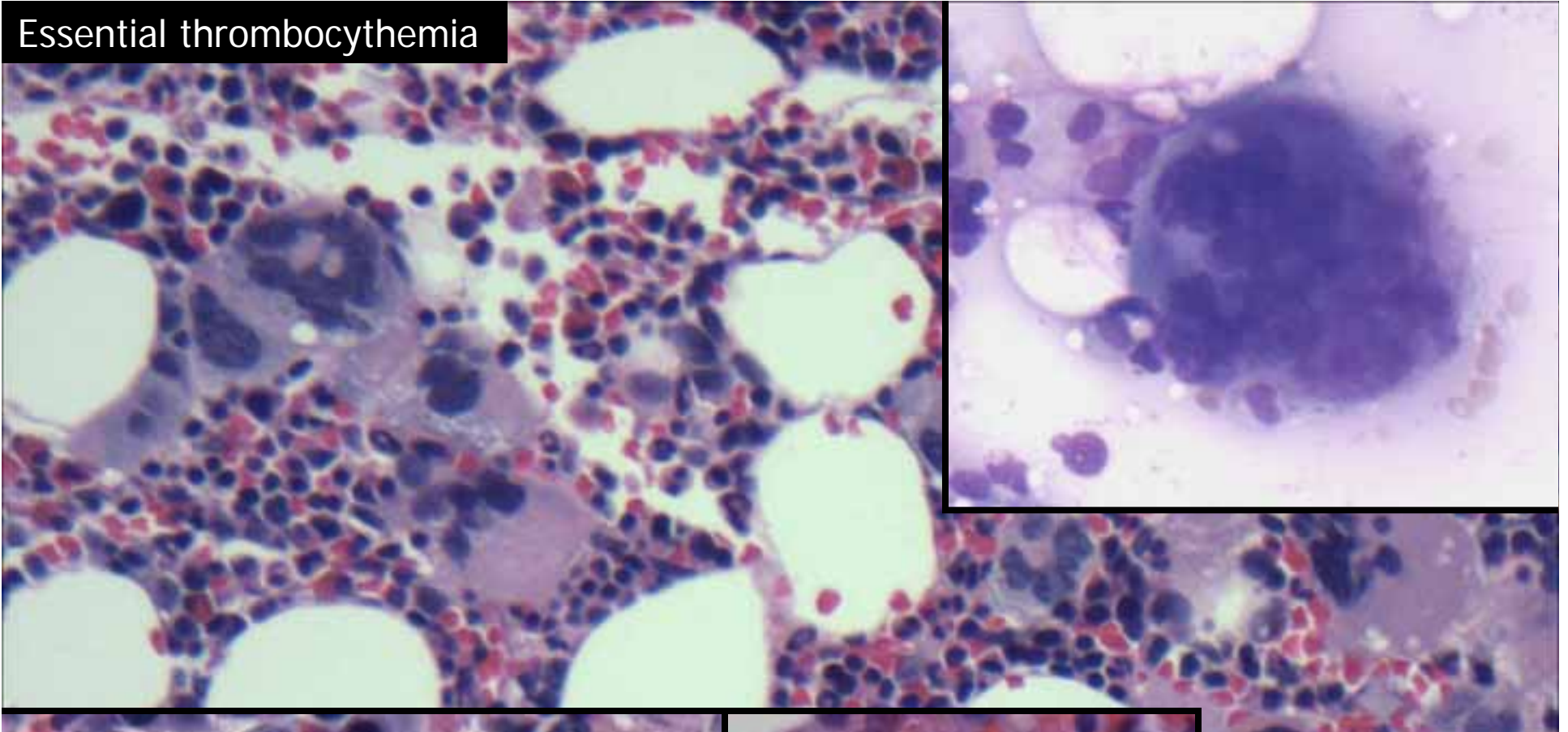
# Consider atypical presentations of CML!

- Myelofibrotic
- With marked eosinophilia
- With prominent thrombocytosis
- With relative erythroid hyperplasia
  - Patients with hemoglobinopathies
- With blunted leukocytosis and macrocytosis due to folate deficiency
- In blast crisis mimicking AML or ALL

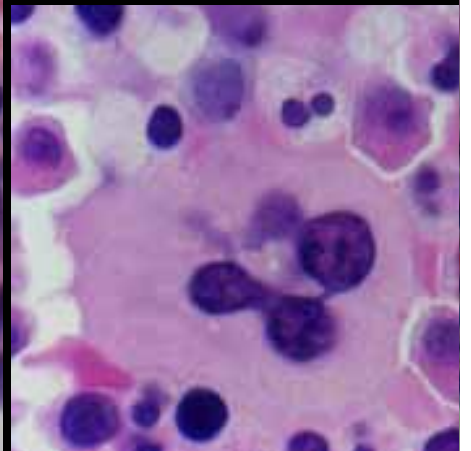
# Diagnosis and classification of other MPDs

- Morphology
  - No significant dysplasia
  - Abnormal megakaryocyte morphology
  - Often bone marrow reticulin fibrosis
- Cytogenetics usually normal
- These diseases are mainly classified based on their clinical manifestations

Essential thrombocythemia



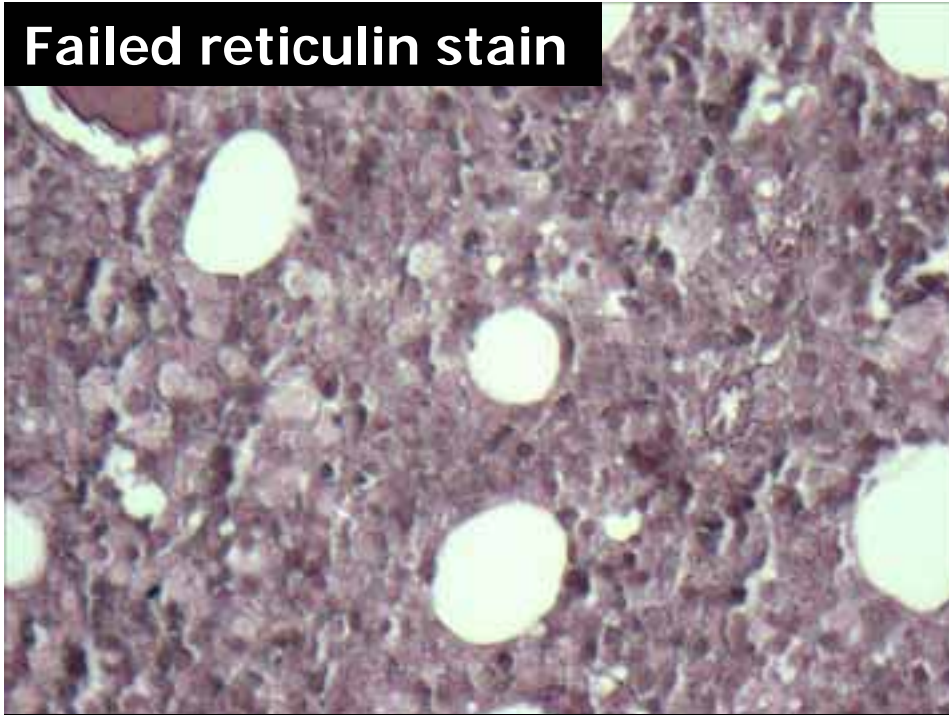
Normal mega



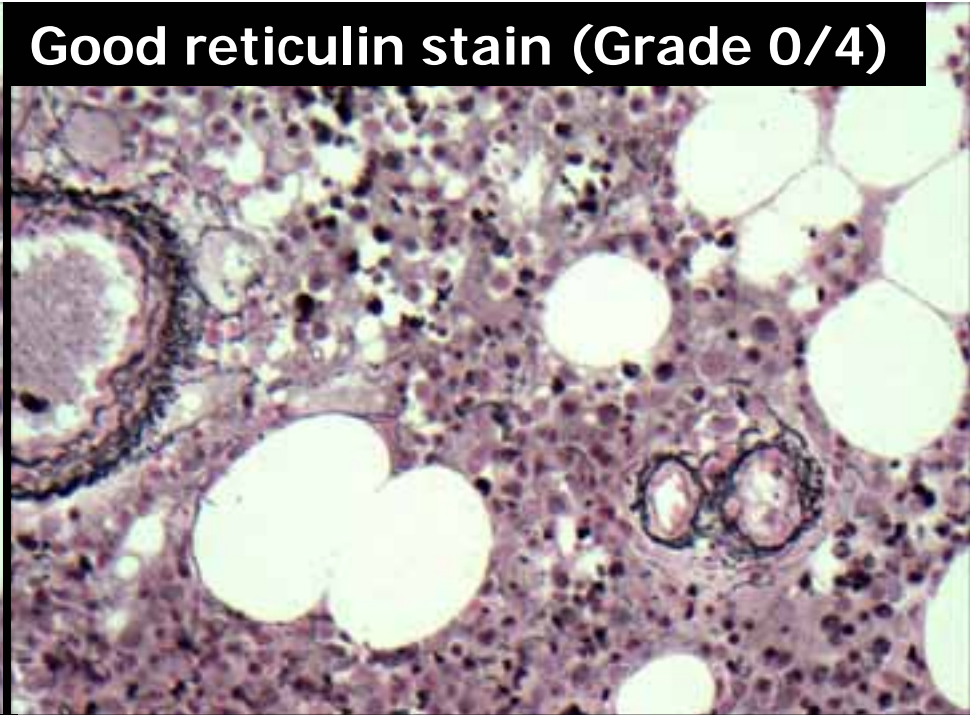
# 'Myelofibrosis'

- Descriptive term meaning increased collagen deposition in the marrow
  - Measured by reticulin and trichrome stains
- Broad differential diagnosis
  - Any myeloid neoplasm (always exclude CML!)
  - Lymphomas, especially hairy cell leukemia
  - Metastatic tumors
  - Autoimmune diseases and infections, especially HIV
  - Chronic idiopathic myelofibrosis

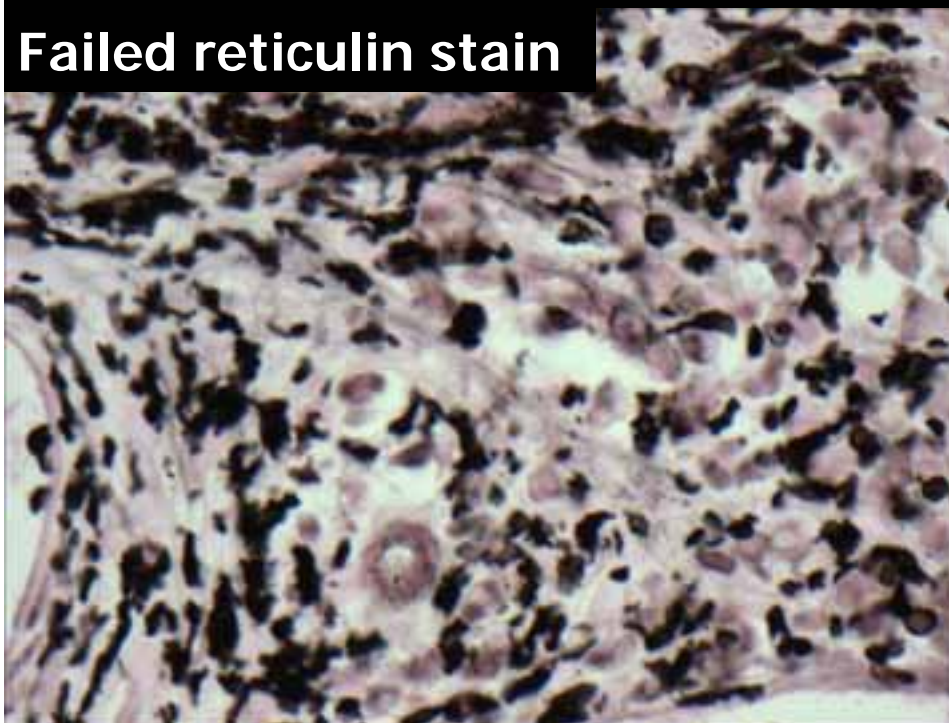
**Failed reticulin stain**



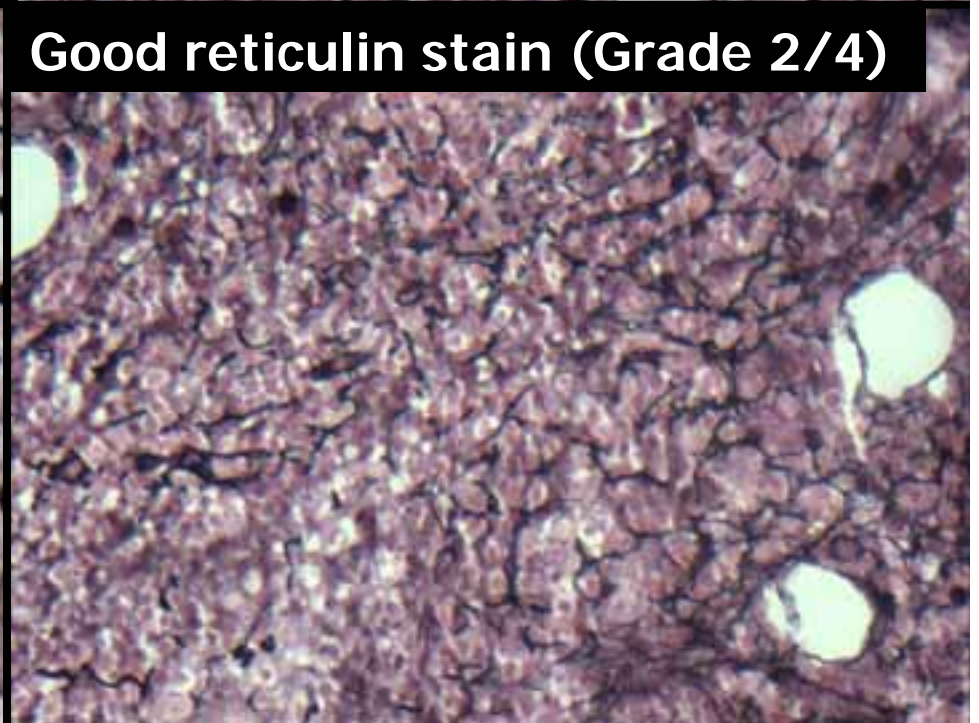
**Good reticulin stain (Grade 0/4)**



**Failed reticulin stain**



**Good reticulin stain (Grade 2/4)**



# JAK2 mutations in MPDs

- Acc
- tyro
- U
- M
- Imp
- S
- r
- F
- M

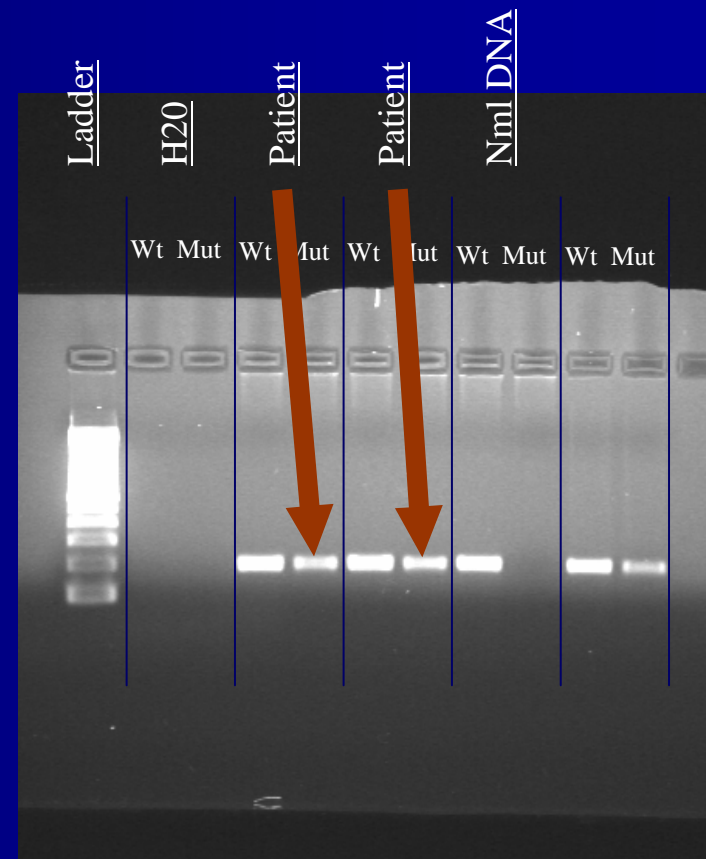


K2  
S  
MF  
  
by

James C et al. Nature 2005; Baxter EJ et al. Lancet 2005; Levine RL et al. Cancer Cell 2005; Kralovics R et al. NEJM 2005

# JAK2 V617F point mutation in MPD

- PCR performed on blood or bone marrow sample
- Allele-specific PCR distinguishes mutated JAK2 from wild-type JAK2
  - G>T mutation at position 1849



# Findings in Bone Marrow Biopsy for Paraprotein

| <b>Pathologic findings</b>                          | <b>Percent</b> |
|---|----------------|
| <b>Plasma cell myeloma</b>                          | <b>33%</b>     |
| <b>NHL</b>  | <b>10%</b>     |
| <b>Not diagnostic of plasma cell myeloma (MGUS)</b> | <b>57%</b>     |

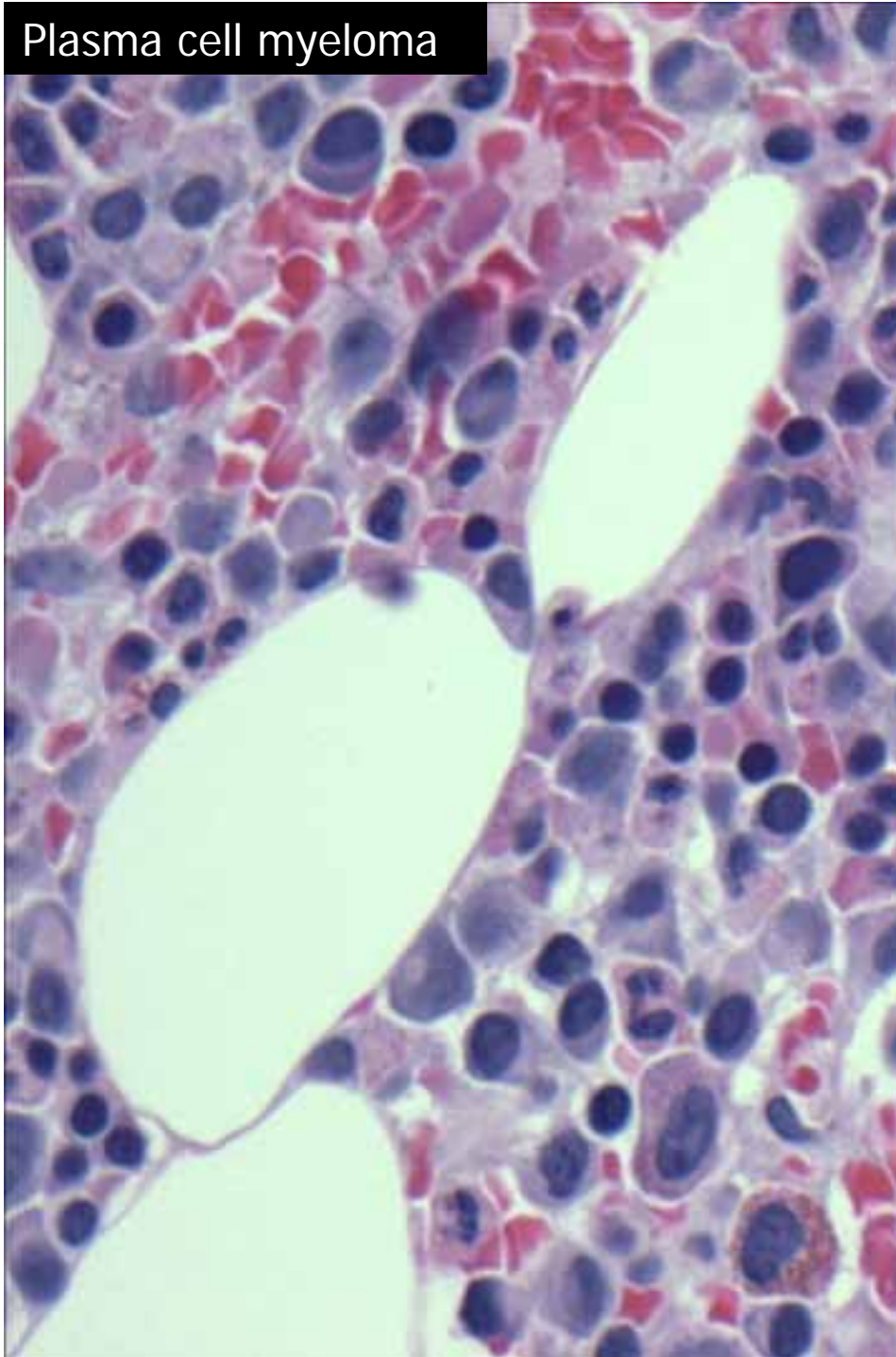
# Paraprotein type and level provide helpful clues

- Higher level of paraprotein makes positive marrow finding more likely
- Type of paraprotein correlates with disease
  - IgM paraprotein
    - Lymphoplasmacytic or other B-cell lymphoma, only rarely plasma cell myeloma
  - IgG, IgA, IgD paraprotein
    - Plasma cell myeloma or B-cell lymphoma, only rarely lymphoplasmacytic lymphoma

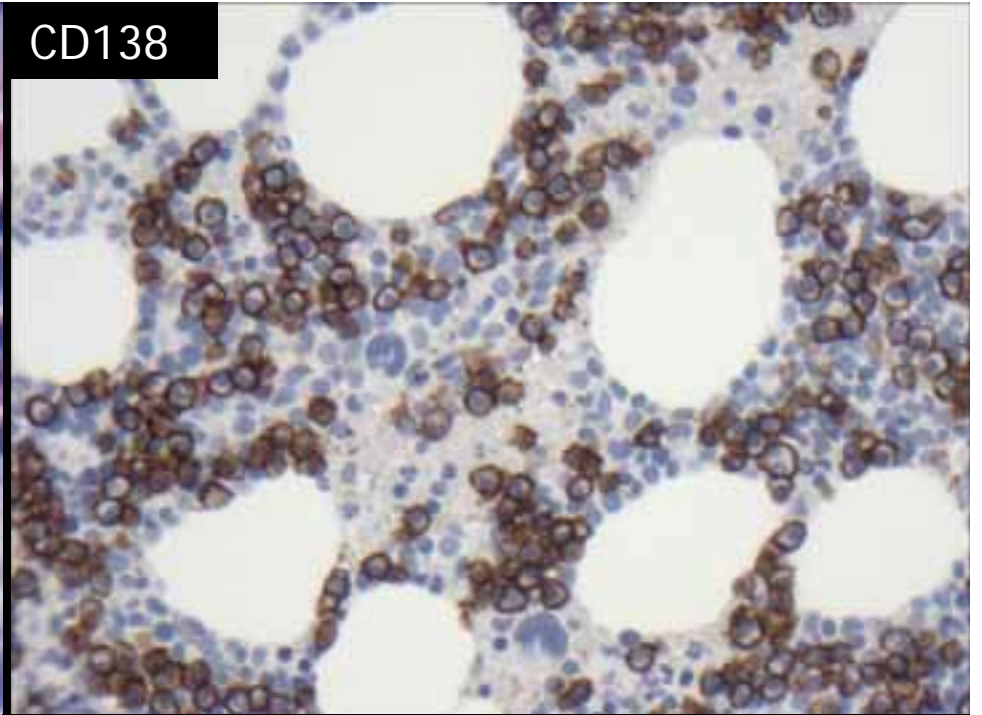
# Other clues to plasma cell malignancy

- Pattern in core biopsy
  - Very large clusters (>10) of plasma cells
  - Plasma cell clusters away from blood vessels
- Abnormal plasma cell morphology
  - However, significant overlap between reactive, non-neoplastic plasma cells and myeloma cells
- Prudent to perform CD138, kappa/lambda staining in all biopsies done to evaluate a paraprotein

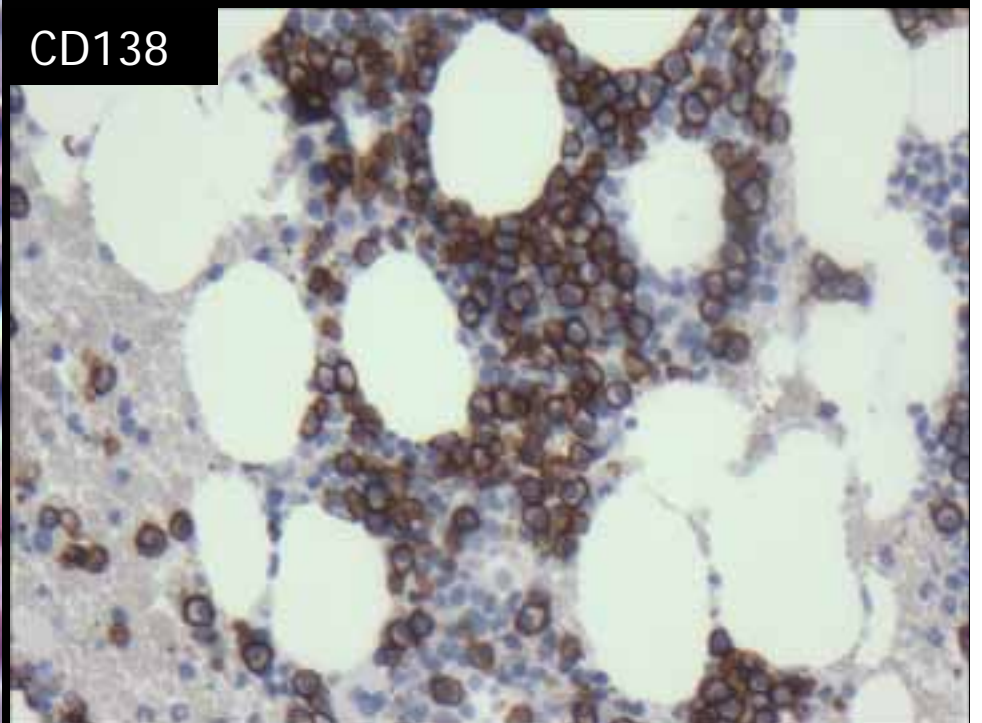
Plasma cell myeloma



CD138

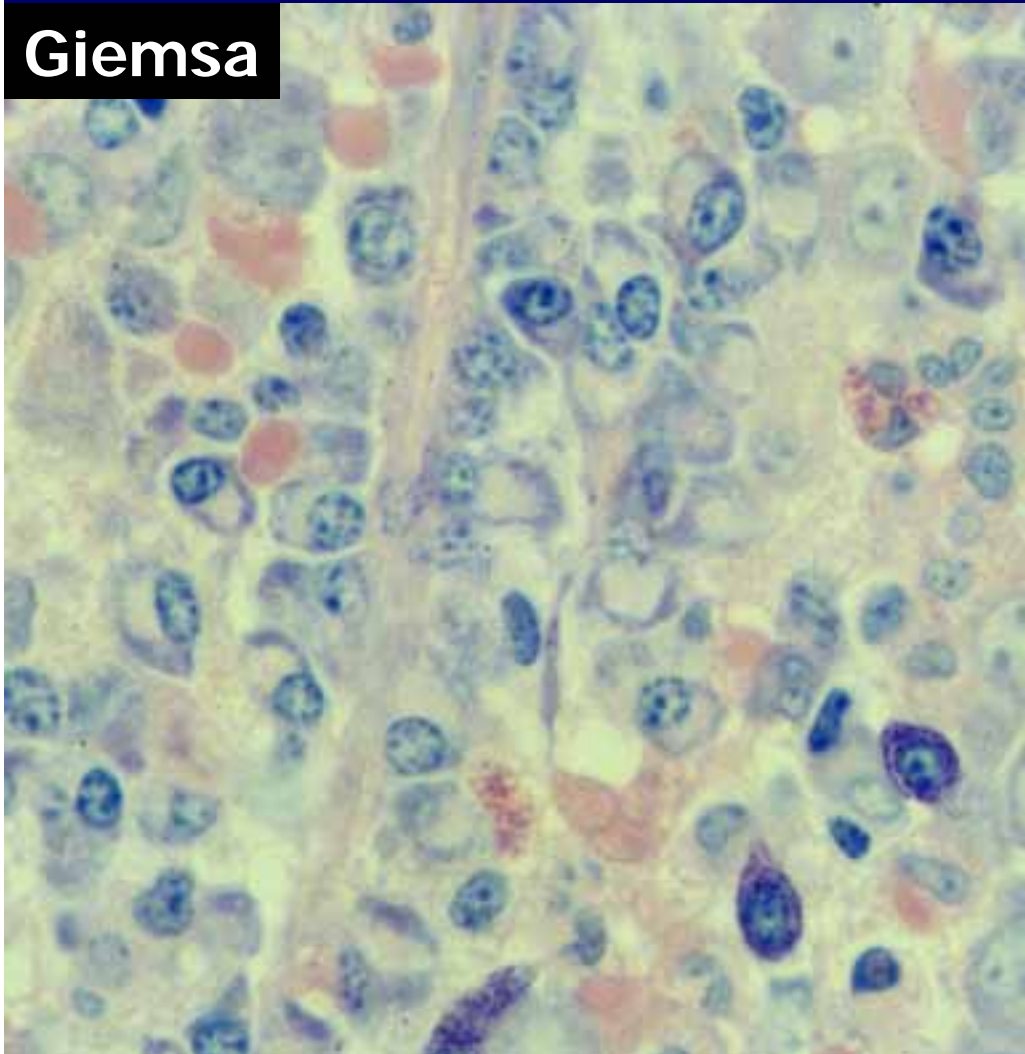


CD138

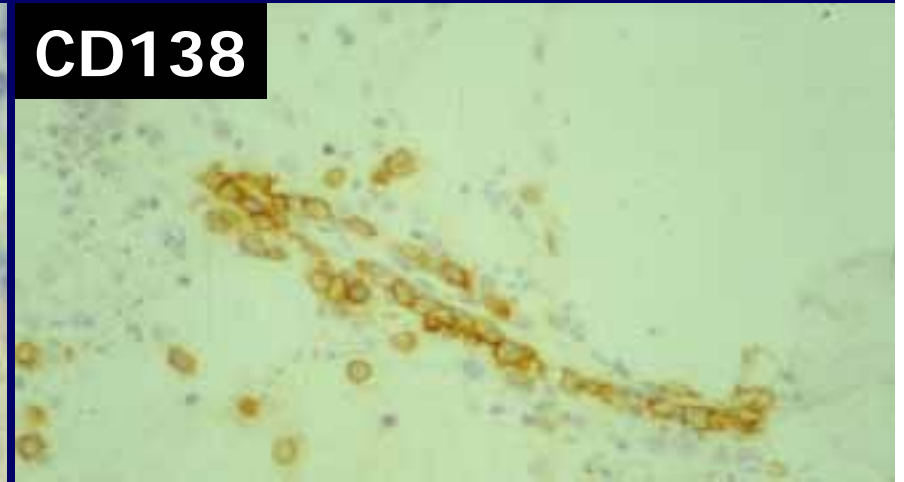


# Reactive plasmacytosis

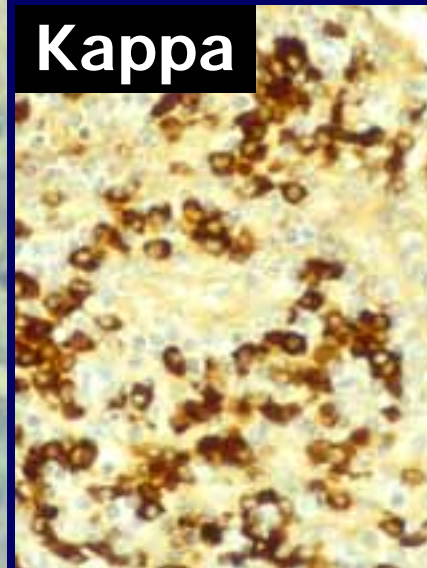
**Giemsa**



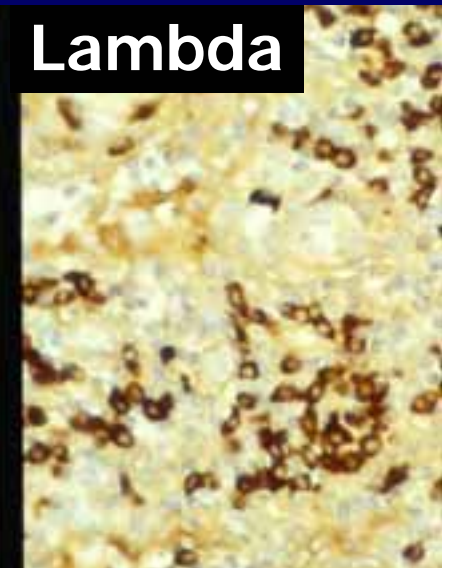
**CD138**



**Kappa**



**Lambda**



# Summary

- Distinction of neoplastic from reactive bone marrow processes poses special problems due to morphologic overlap of several diseases
- The pathologist often must supplement morphologic evaluation with ancillary studies to arrive at the correct diagnosis
- Knowledge of the clinical details and close communication with clinical colleagues and ancillary laboratory staff enhance our diagnostic accuracy