Cytogenetics for the Rest of Us: A Primer

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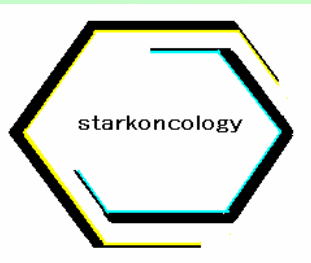
Case #1

- 78 y.o. lady seen in August 2005 for abnormal blood counts:
  - Hct 44
  - WBC 30,000 with a few promyelocytes and myelocytes
  - Platelet count 820,000
- Recent night sweats
- PE: barely palpable spleen

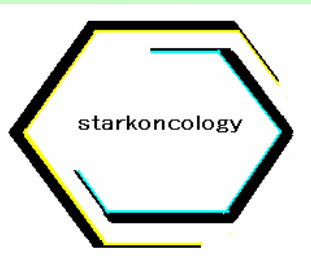


Case #1, continued

- Underwent bone-marrow biopsy to evaluate further
  - Morphology
  - Cytogenetics:
    - 46,XX,t(9;22)(q34;q11.2)[20] – indicating chronic myelogenous leukemia with presence of “Philadelphia chromosome”
- Pending cytogenetics results started on Hydroxyurea to lower platelet count

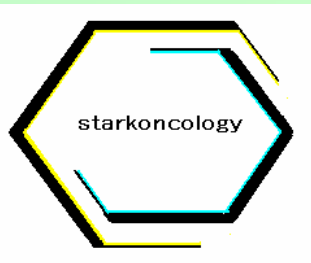
Case #1, continued

- Switched to Gleevec upon receipt of chromosome report
- Has since felt better with loss of night sweats
- Blood counts normalized quickly and have stayed normal; Gleevec held intermittently, then restarted at full dose
- Gleevec toxicity (ankle and periorbital edema) mild and well tolerated



Case #2

- 61 y.o. man seen in December for rapidly rising platelet count
- Severe comorbid diabetes with ASCVD, peripheral neuropathy
- 3-month history of increasing fatigue and night sweats

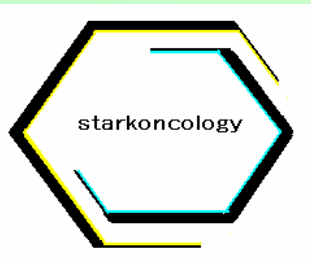


Case #2, continued

- Hct 37
- WBC 14,700 with 3% basophils, occasional myelocyte and metamyelocyte
- Platelets rose from 898,000 in August to 1,300,000 in December
- Underwent immediate bone-marrow biopsy and started on Hydrea….

Case #2, continued

- Bone-marrow morphology…
- Cytogenetics: 46, XY,t(9;22)(q34;q11.2)[20]
- Started on Gleevec as soon as report became available
- Shortly after starting Gleevec admitted to SNGH with ischemic foot
- WBC and platelet count fell to very low levels while there, have since recovered
- Foot saved, better with lower counts

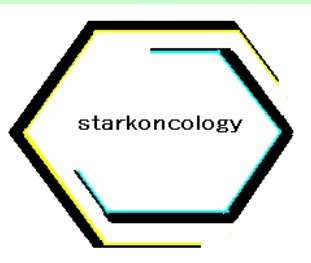


What these two cases have in common

- Atypical presentation of CML dominated by presence of thrombocytosis
- WBC elevations modest but with immaturity
- Out of the “usual” age range for classic CML – i.e., fifth decade
- Cytogenetics provided clue to real diagnosis, allowed for disease-altering therapy to be started

Case #3

- 66 y.o. man with CML first presented in 1996 with hyperleukocytosis and peripheral blood immaturity
  - Bone marrow c/w CML
  - Cytogenetics showed classic 9/22 translocation
  - Treated initially with hydrea with good success until switched to Gleevec after it became commercially available

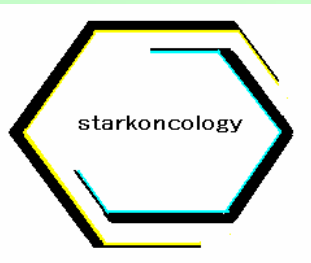


Case #3, continued

- Very sensitive to Gleevec; required frequent interruptions in therapy
- 2.5 years ago began to show evidence of Gleevec resistance with increasing thrombocytopenia and anemia
- Repeat bone marrow biopsy…
- Switched to hydrea and alpha interferon with stabilization of disease
- Further difficulty with maintenance; referred to transplant service at MCV

Case #3, continued

- Initially responded again to Gleevec at 400 mg/day but had to have dose increased to 600 mg after re-emergence of resistance
- Was stabilized on this dose for a few months but developed progressive hyperleukocytosis and thrombocytopenia precluding further treatment with Gleevec
- Developed culture-negative fever for several weeks
- Repeat bone-marrow biopsy with cytogenetics….

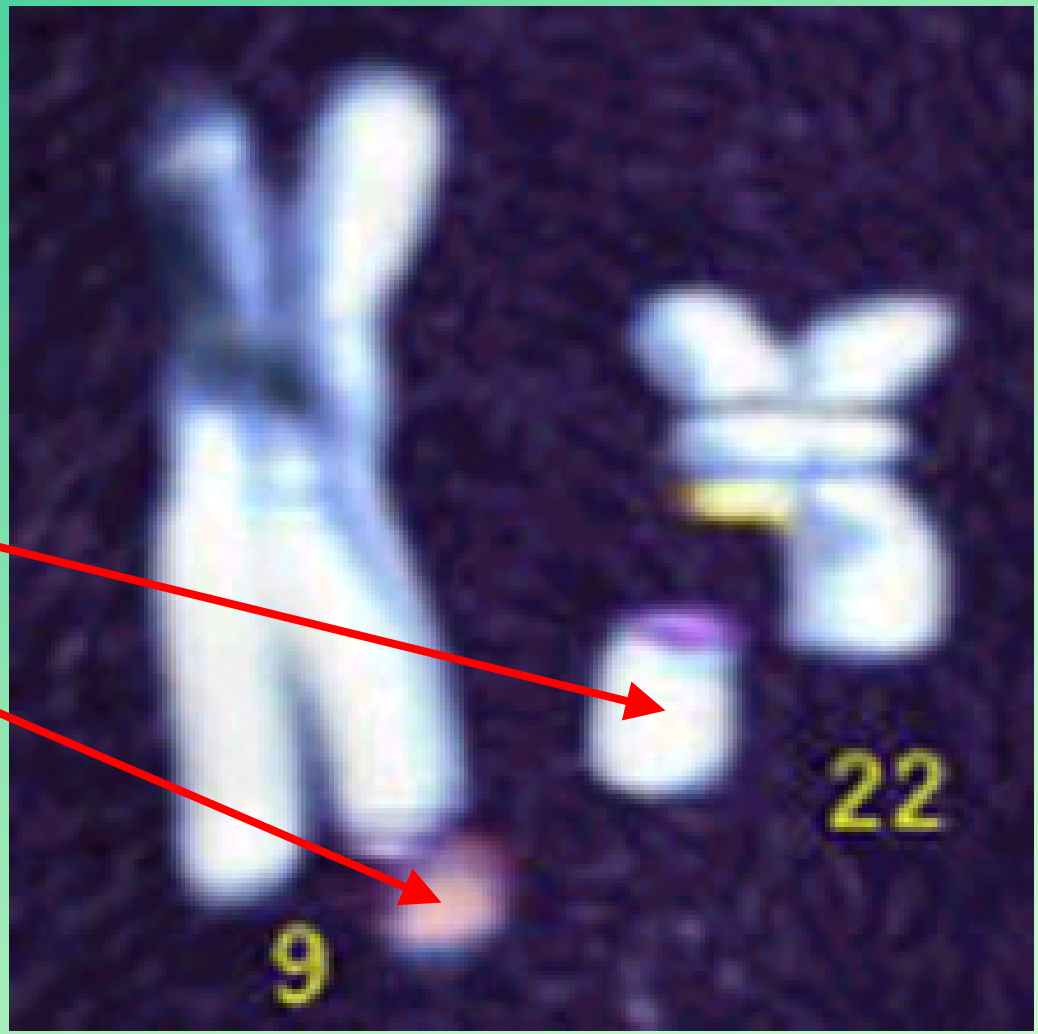


Case #3, continued

- Cytogenetics shows persistence of 9/22 translocation plus…
  - Further rearrangement of new chromosome 9
  - Complex translocation involving chromosomes 11, 19 and 20
- Patient now in the hospital undergoing remission induction chemotherapy with “FLAG” + idarubicin

A Word About Gleevec

Chromosome break products



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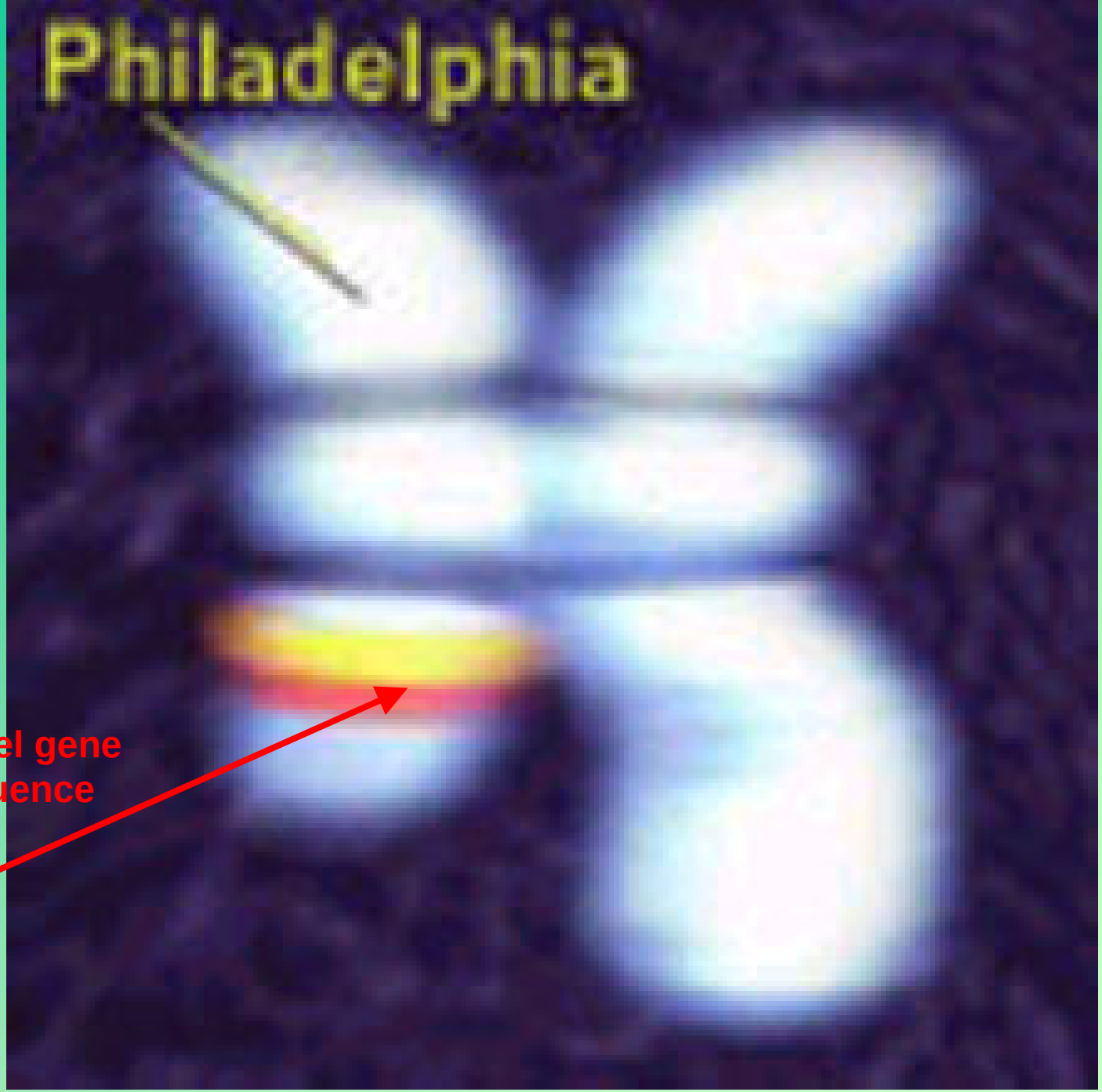

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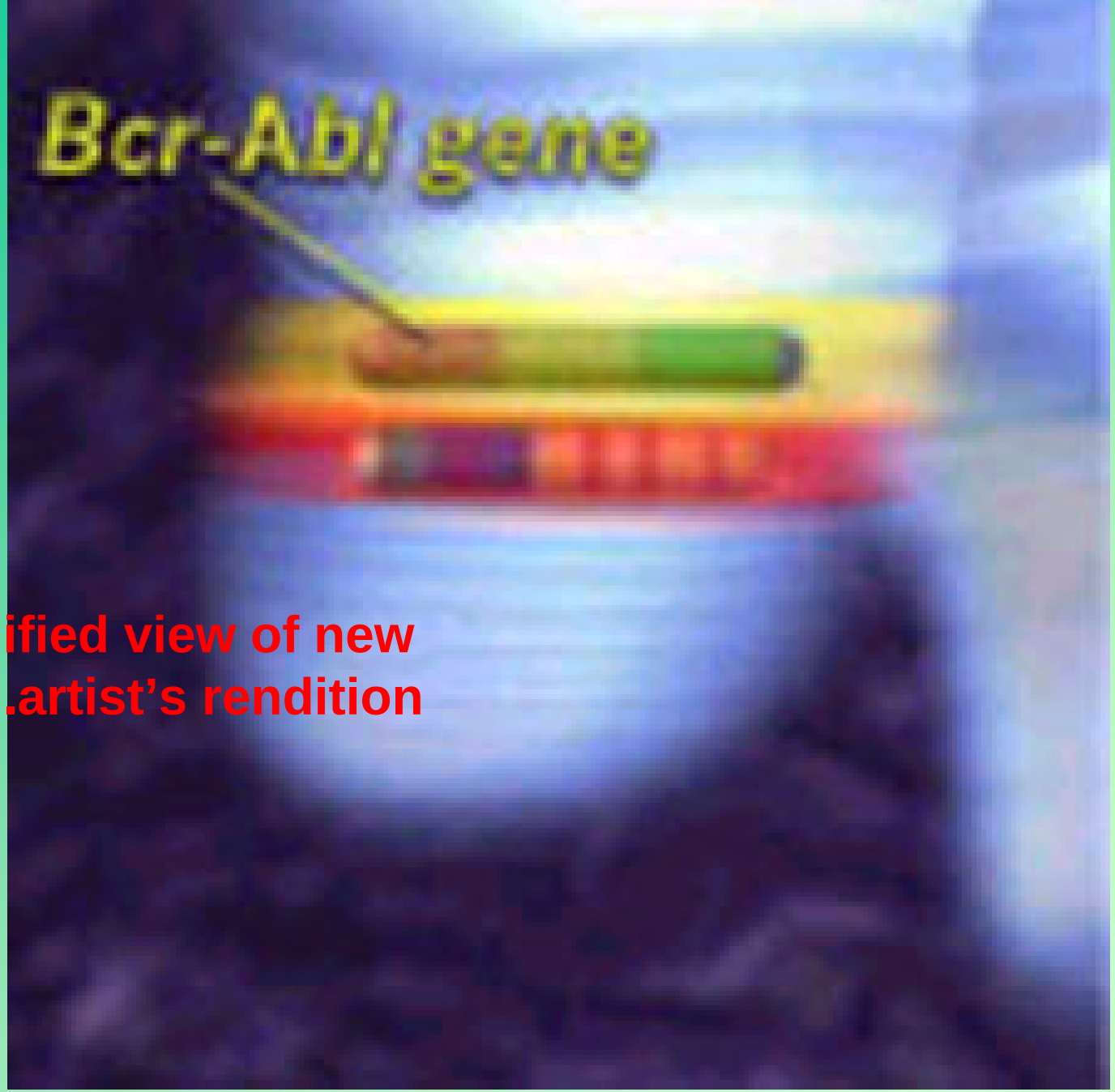

Magnified view of new gene..artist's rendition

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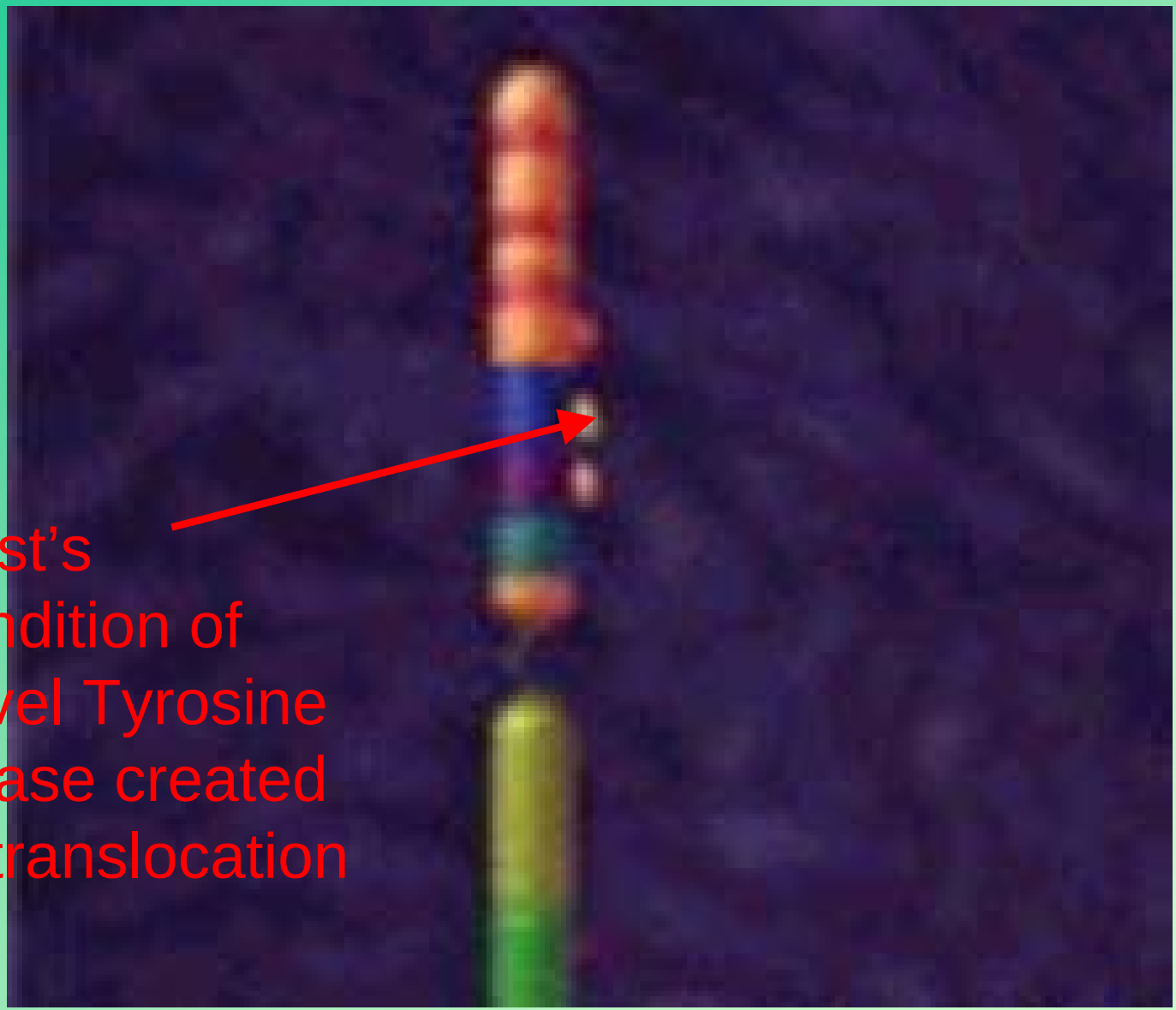

Artist's Rendition of Novel Tyrosine Kinase created by translocation

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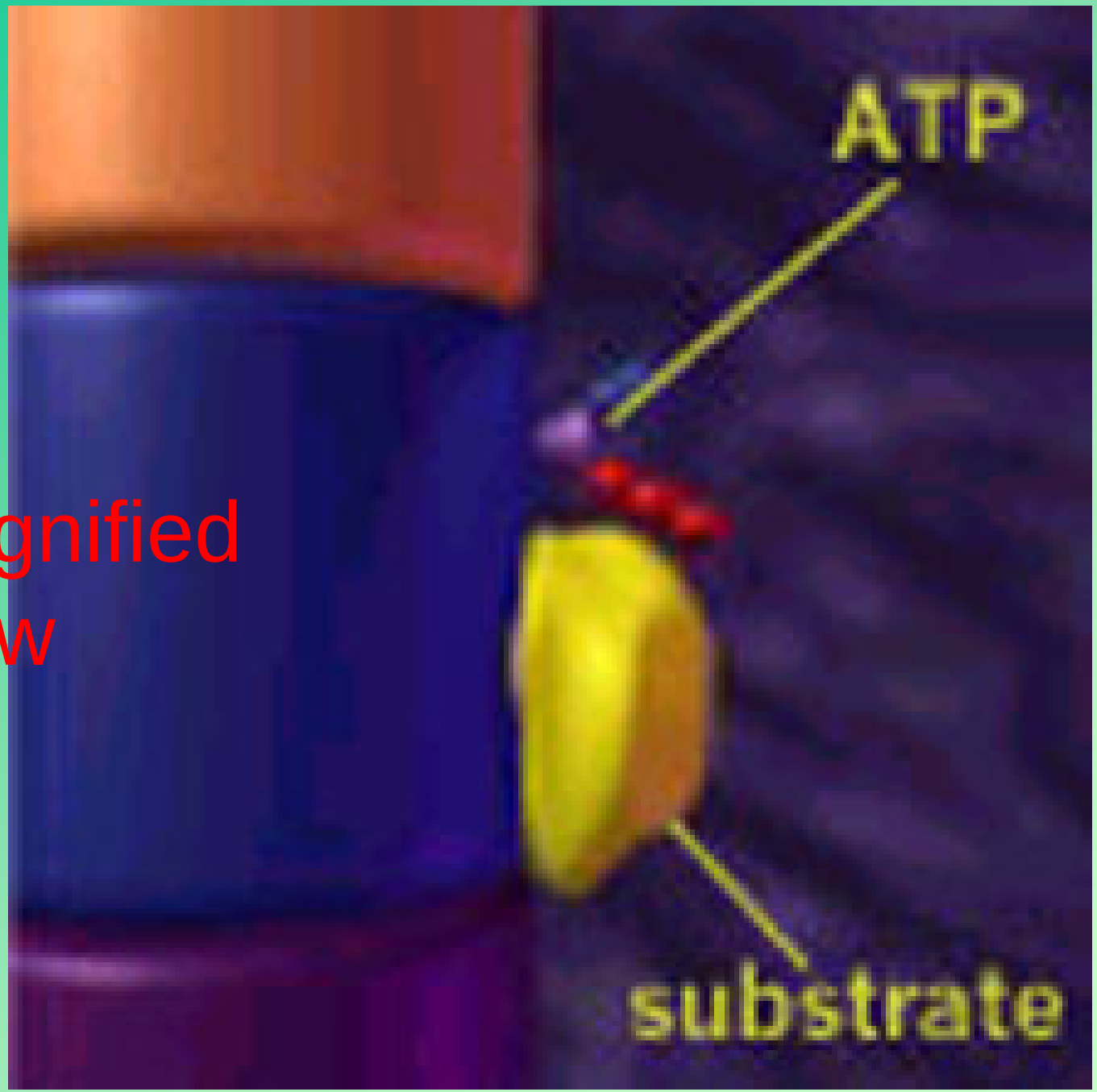
Magnified View



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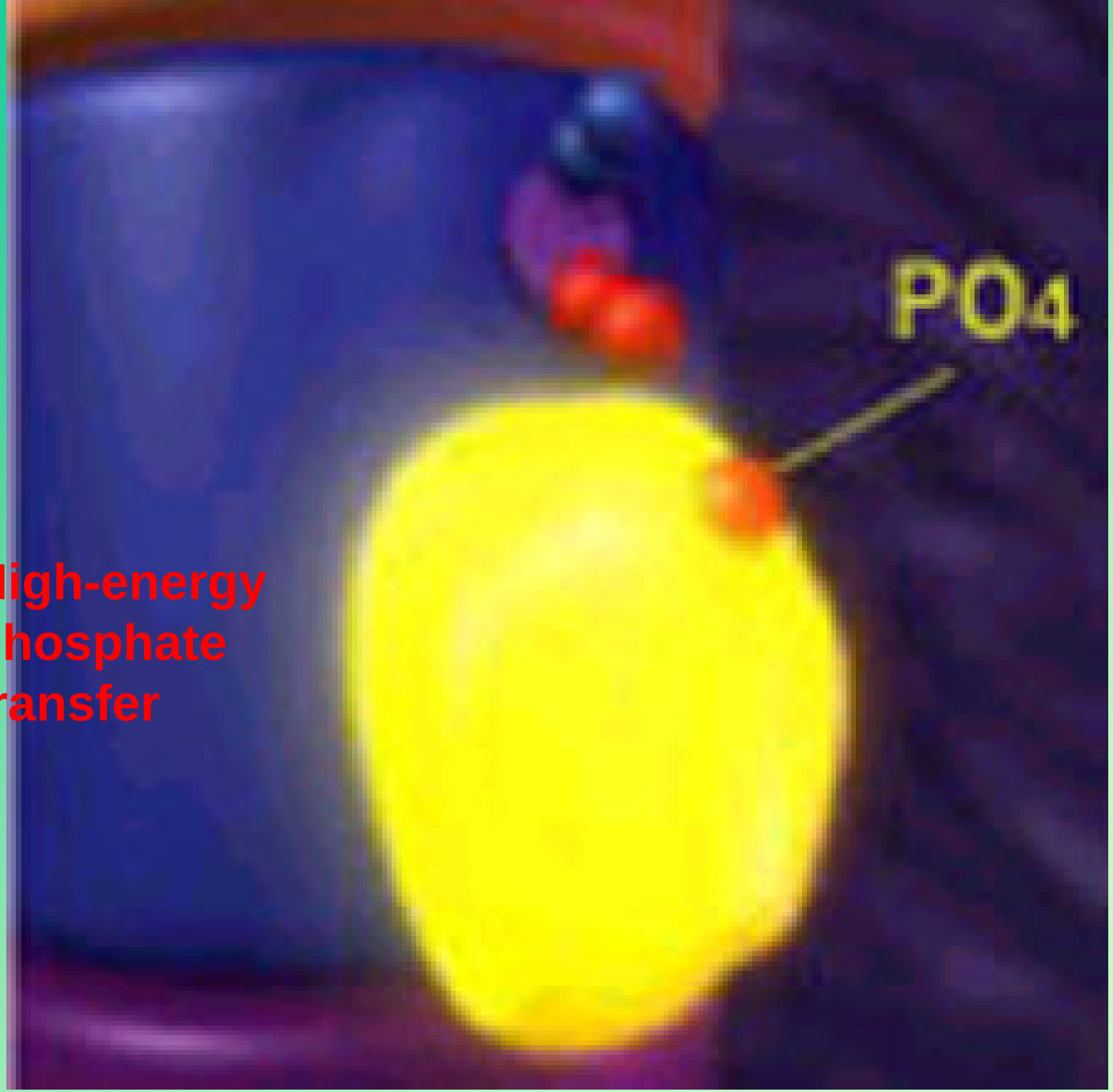

High-energy phosphate transfer

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Results of Initial Clinical Trials

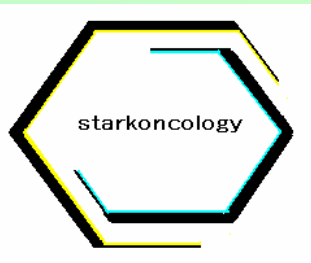
	Chronic Phase IFN Failure (n=532) 400 mg	Accelerated Phase (n=235) 600 mg n=158 400 mg n=77	Myeloid Blast Crisis (n=260) 600 mg n=223 400 mg n=37
		% of patients ($CI_{95\%}$)	
Hematologic Response[1]	**88% (84.9-90.6)**	**63% (56.5-69.2)**	**26% (20.9-31.9)**
Complete hematologic response (CHR)	88%	28%	4%
No evidence of leukemia (NEL)	Not applicable	11%	3%
Return to chronic phase (RTC)	Not applicable	24%	19%
Major Cytogenetic Response[2]	**49% (45.1-53.8)**	**21% (16.2-27.1)**	**13.5% (9.6-18.2)**
Complete (confirmed[3])	30% (16%)	14% (4%)	5% (1%)

Role of Cytogenetics in Diagnosis and Treatment of Hematologic Malignancies

Presented by Dr. Diane Maia,

Hematopathologist

Bon Secours Hampton Roads



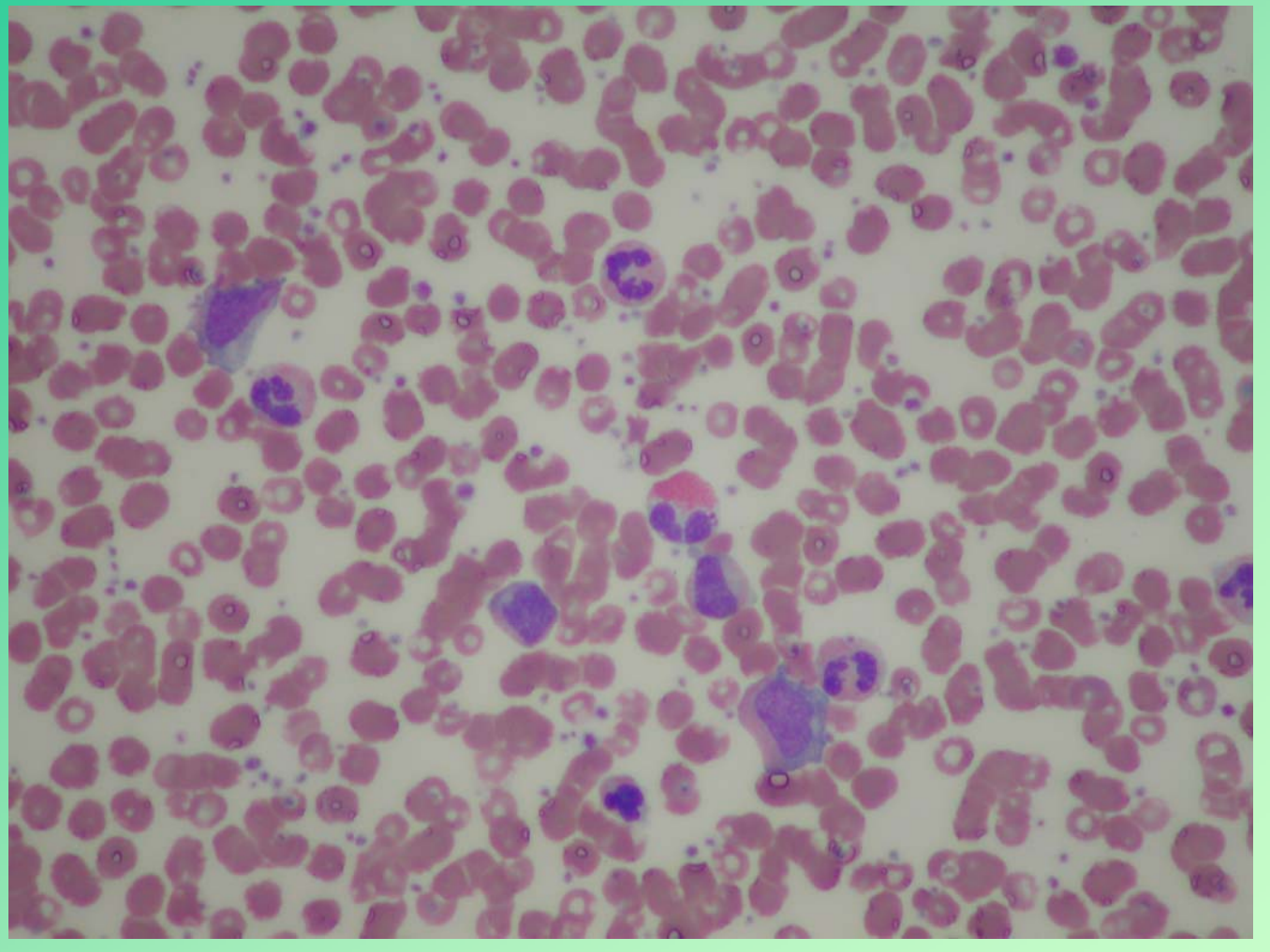
Cytogenetic and Molecular Genetic Studies in Myeloproliferative Disorders

Diane Maia, MD

Maryview Med. Center

March 3, 2006

M.R. peripheral blood



Hgb 14.4

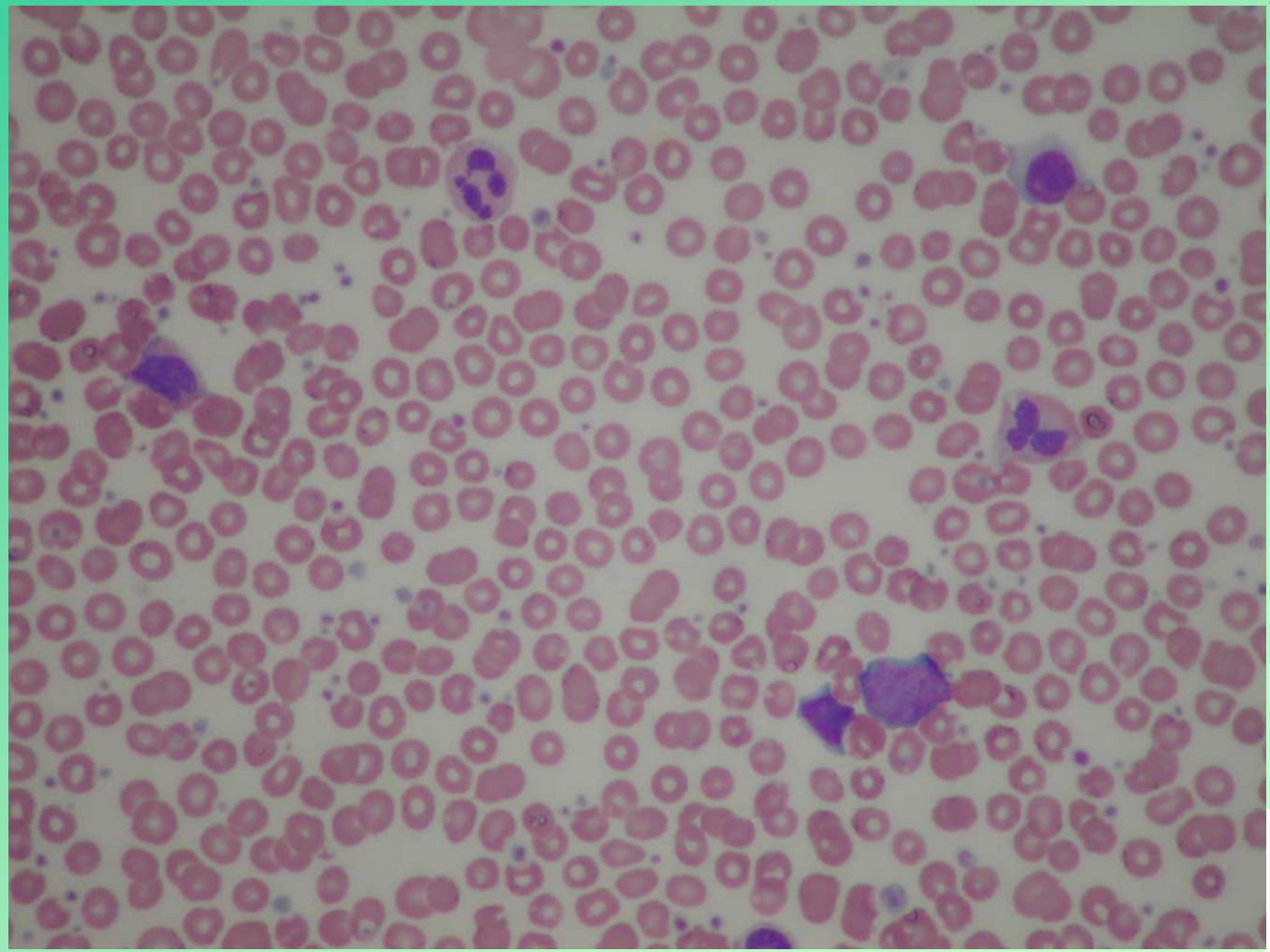
Hct 42.8

WBC 40.6

- neut 21.1
- band 7.3
- meta 3.2
- myelo 0.8
- pro 0.4
- blast 0.4 (1%)
- mono 1.2
- eo 1.2
- baso 1.6
- lymph 3.2

Plt 819

M.R. peripheral blood



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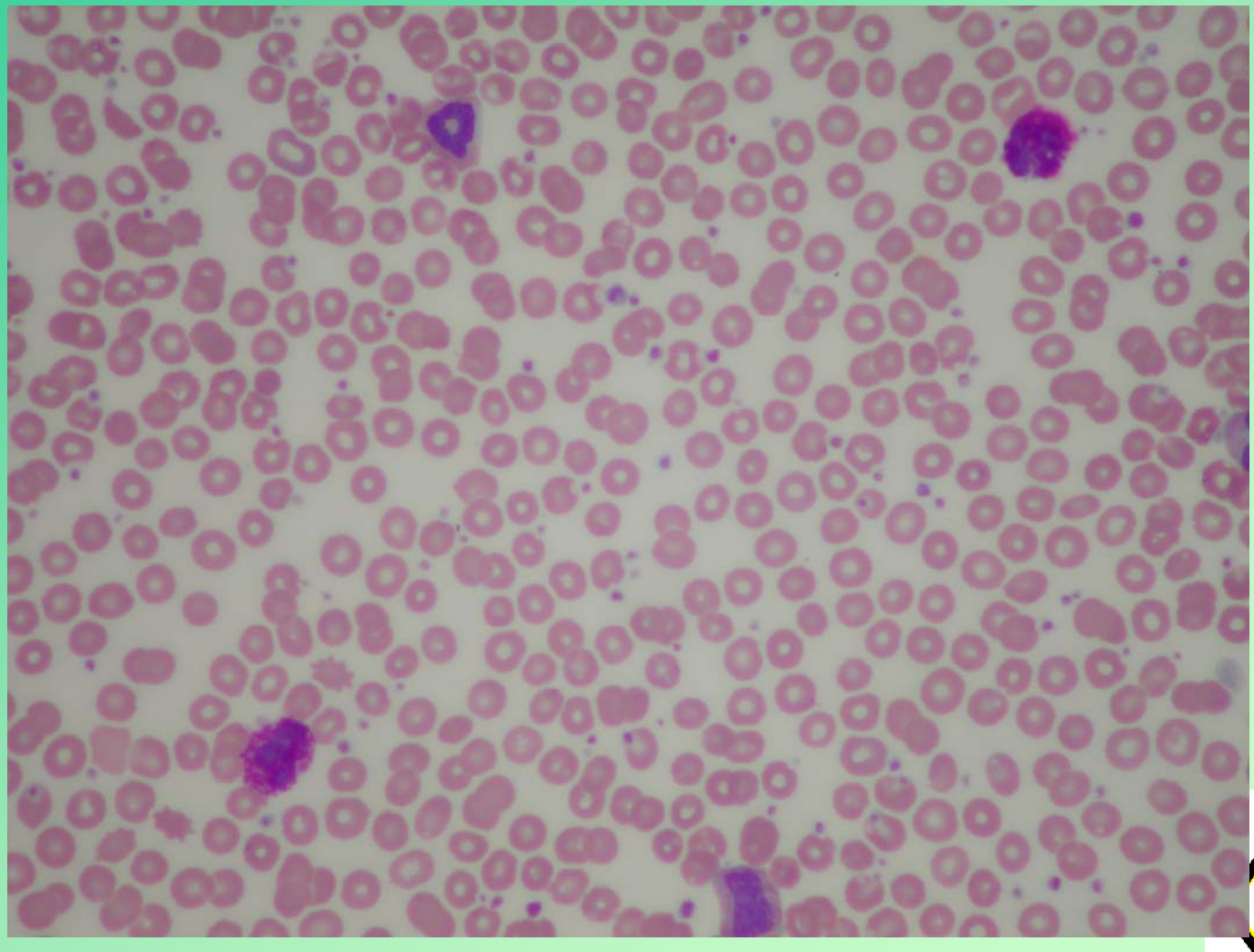
M.R. peripheral blood



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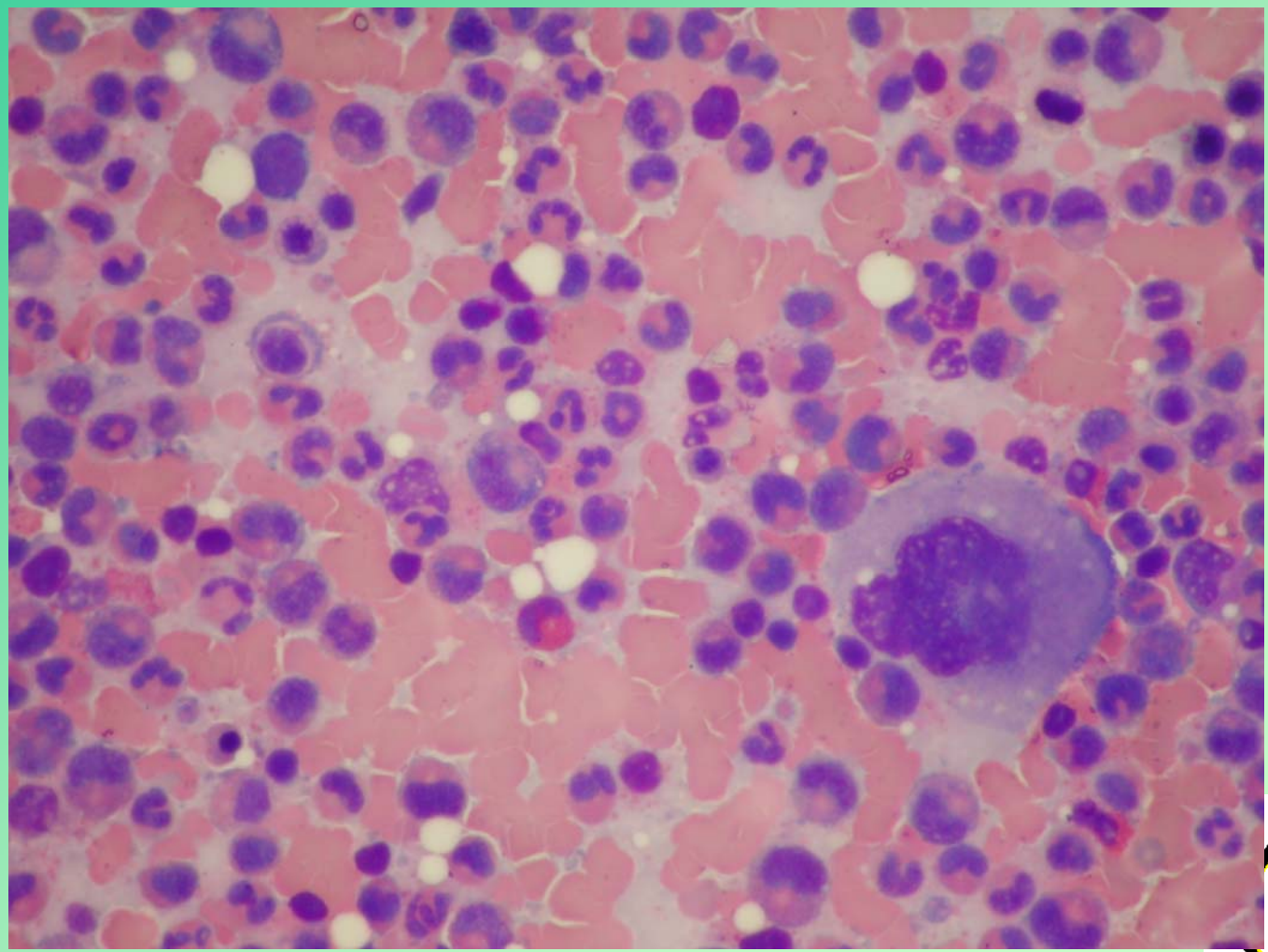
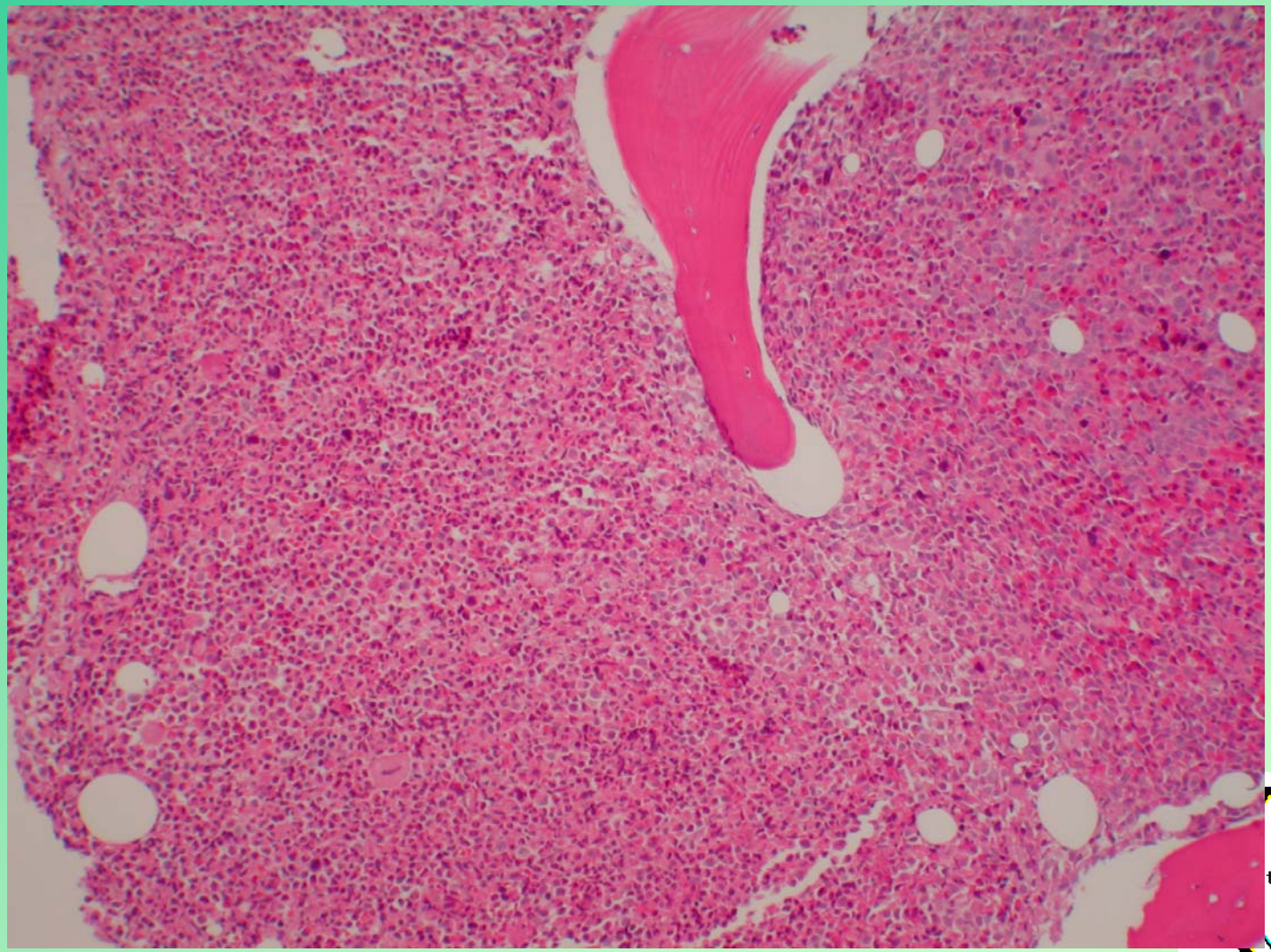
M.R. BM aspirate



M.R. BM biopsy



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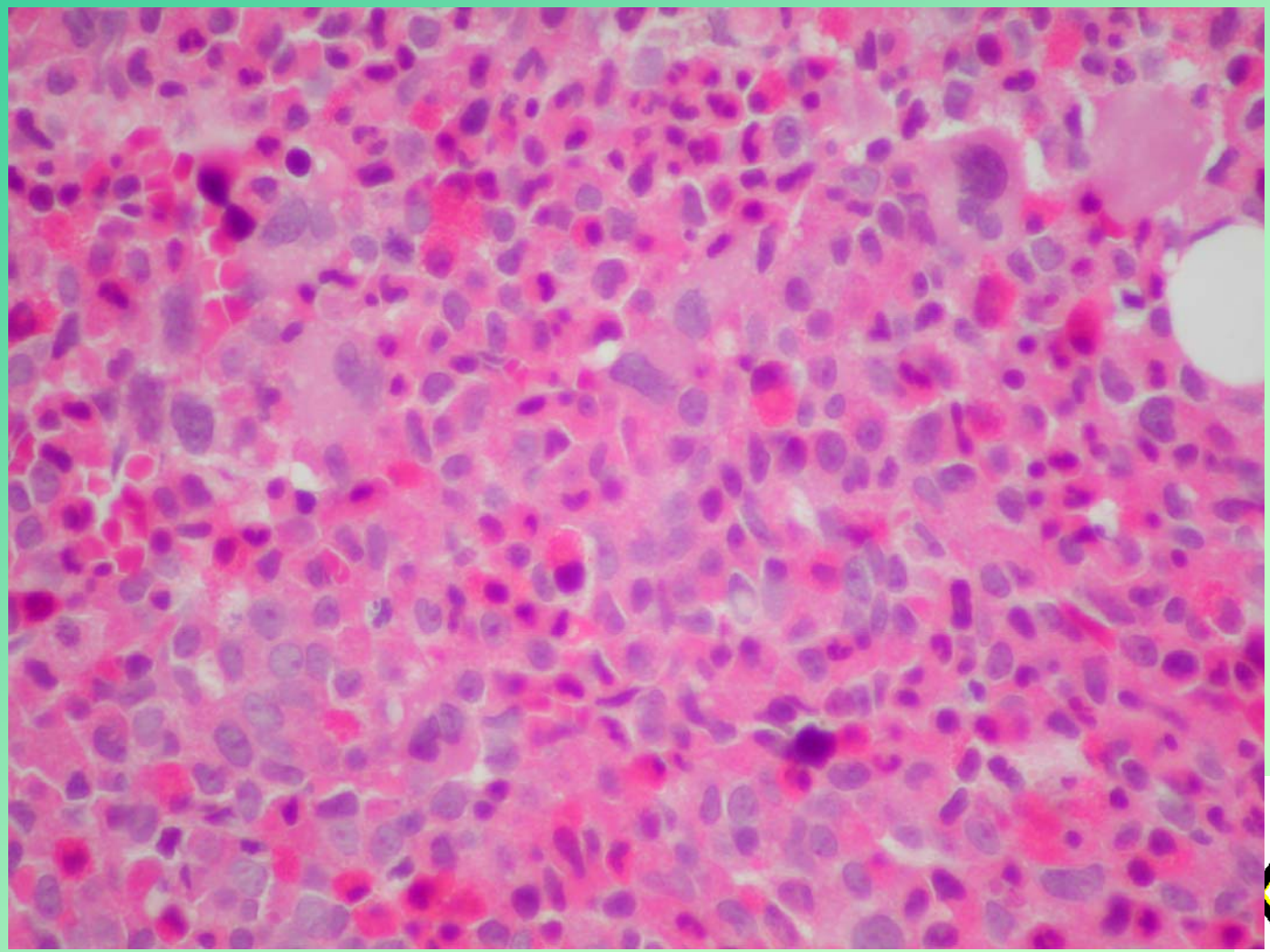
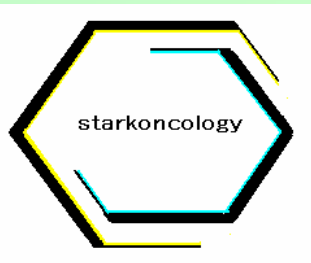
M.R. BM biopsy



Philadelphia Chromosome t(9;22)(q34;q11)

- BCR gene on chromosome 22 opposed to ABL protooncogene on chromosome 9 (90-95% of cases)
- remaining 5-10% of cases;
  - variant translocations involving 3rd or 4th chromosome
  - “cryptic” translocations at traditional breakpoints with translocated fragments too small to identify on banding studies



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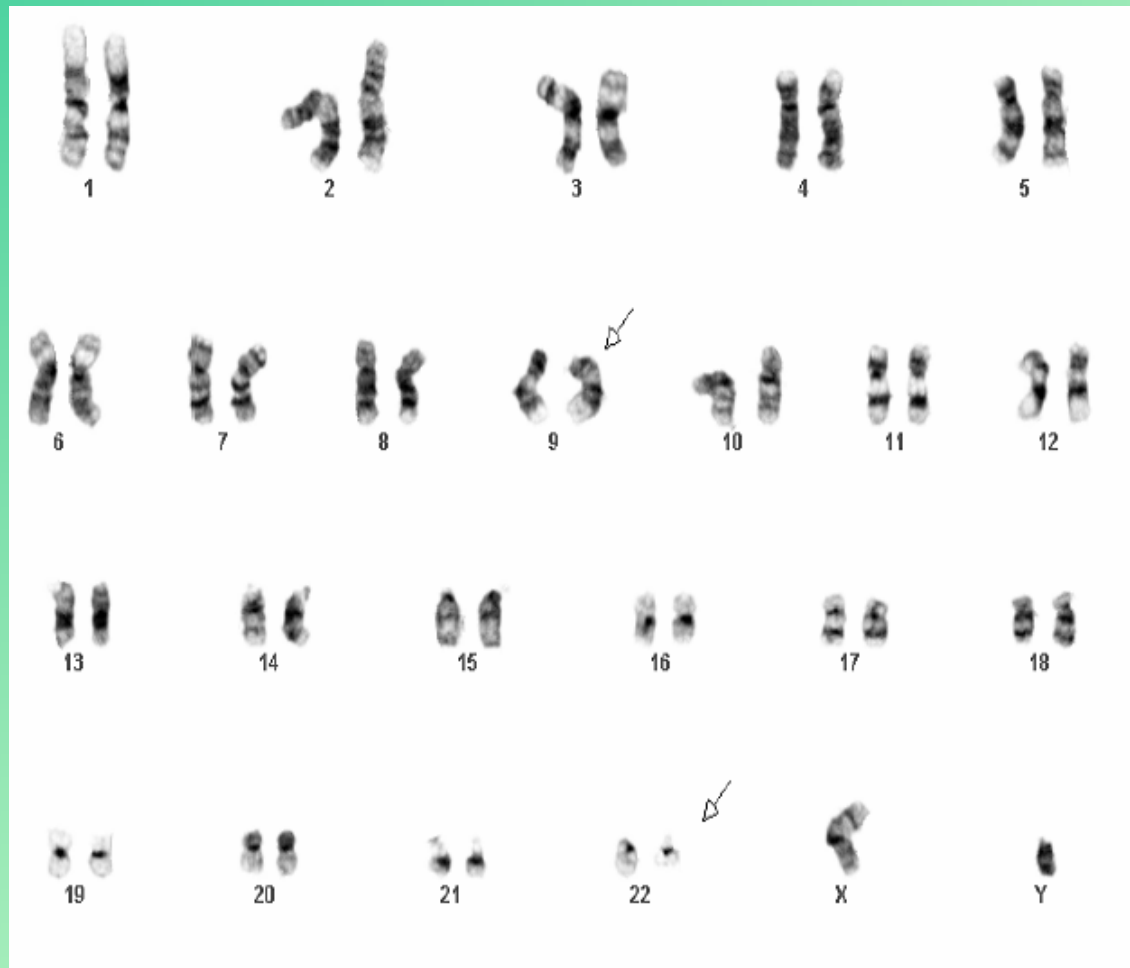

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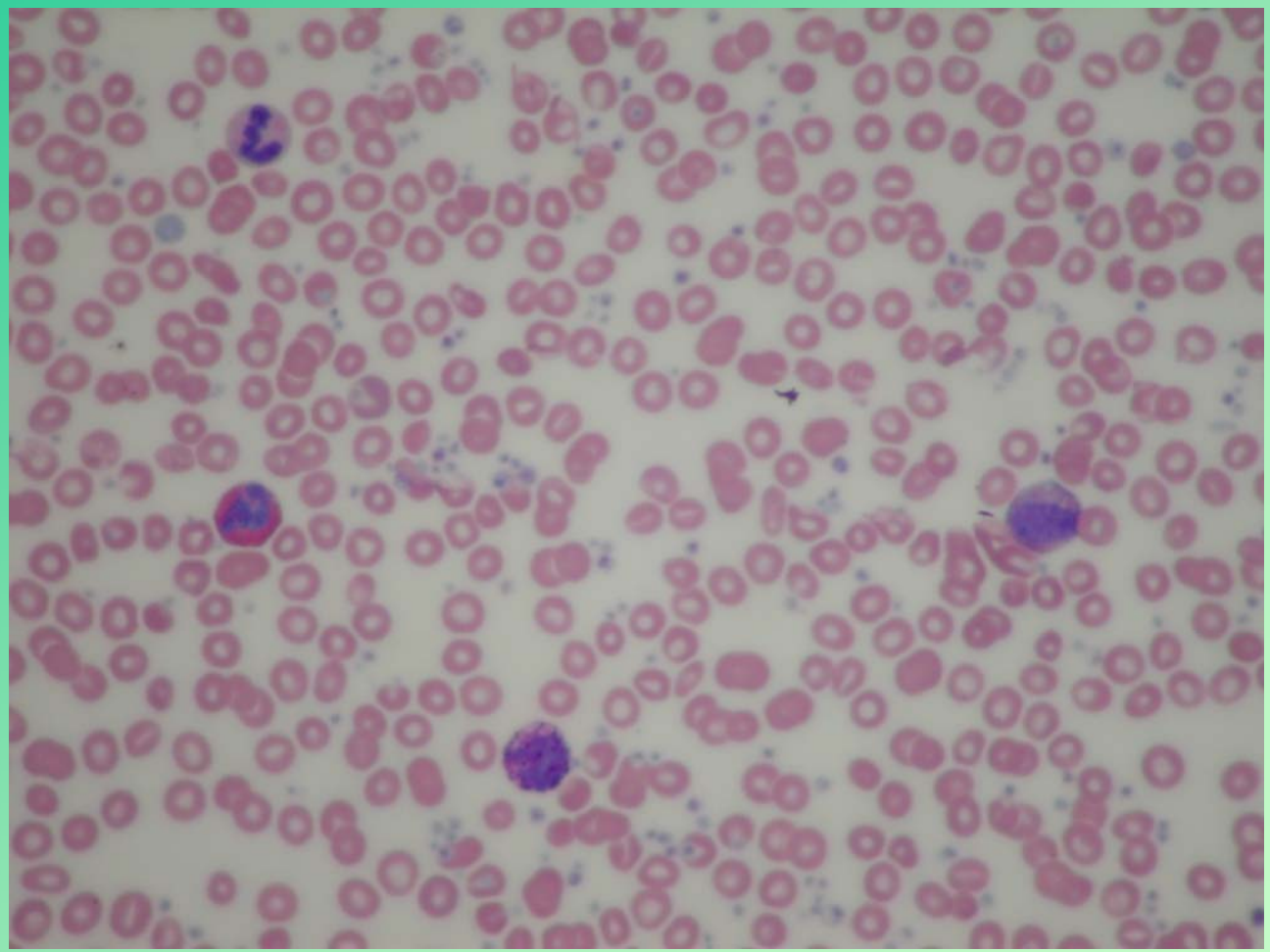
R.S. peripheral blood



Hgb 11.8

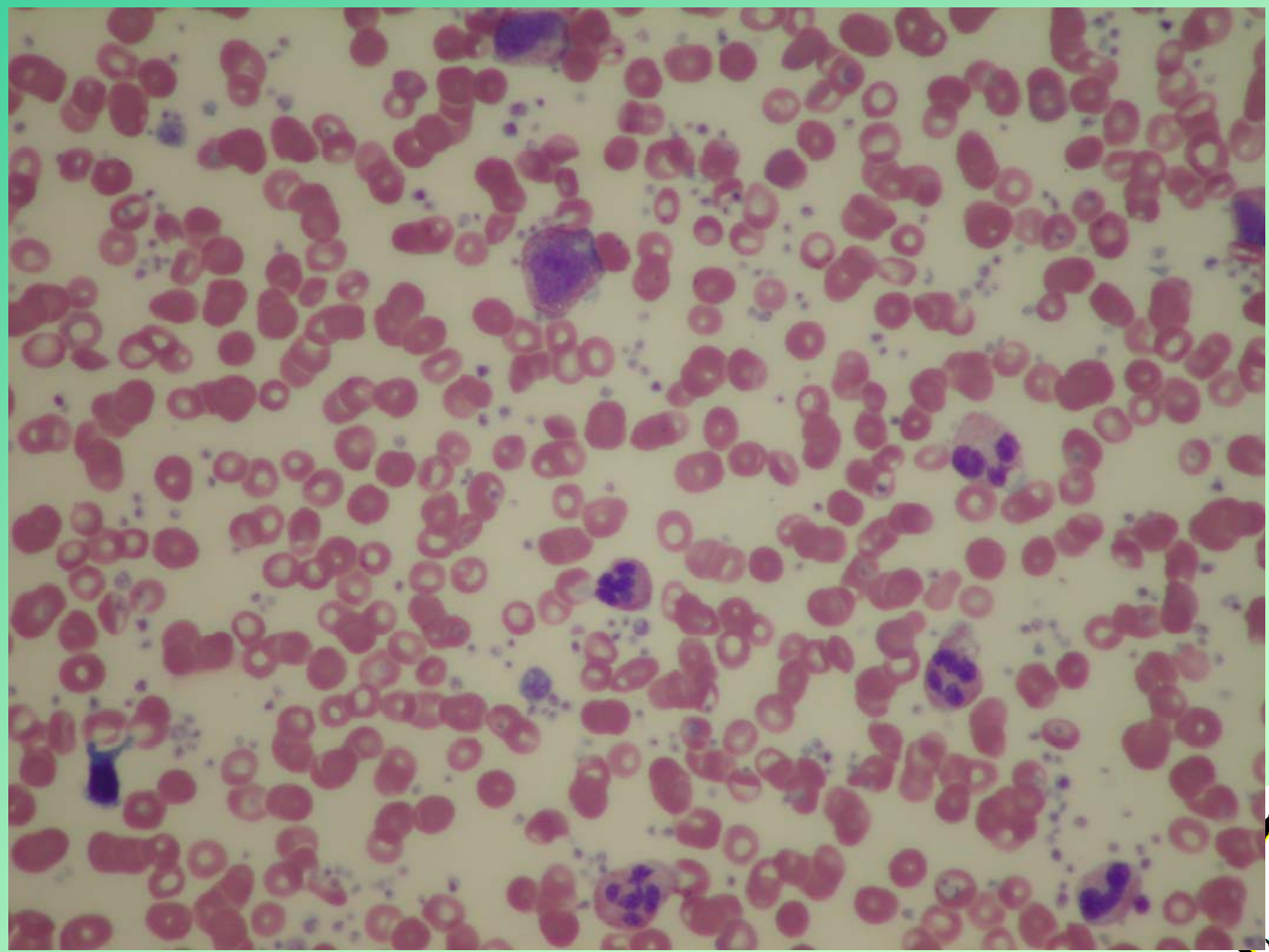
Hct 35.5

WBC 23.6

- neut 16.0
- band 1.7
- meta 1.0
- myelo 1.0
- mono 0.7
- eo 1.4
- lymph 1.9

Plt 1053

R.S. peripheral blood



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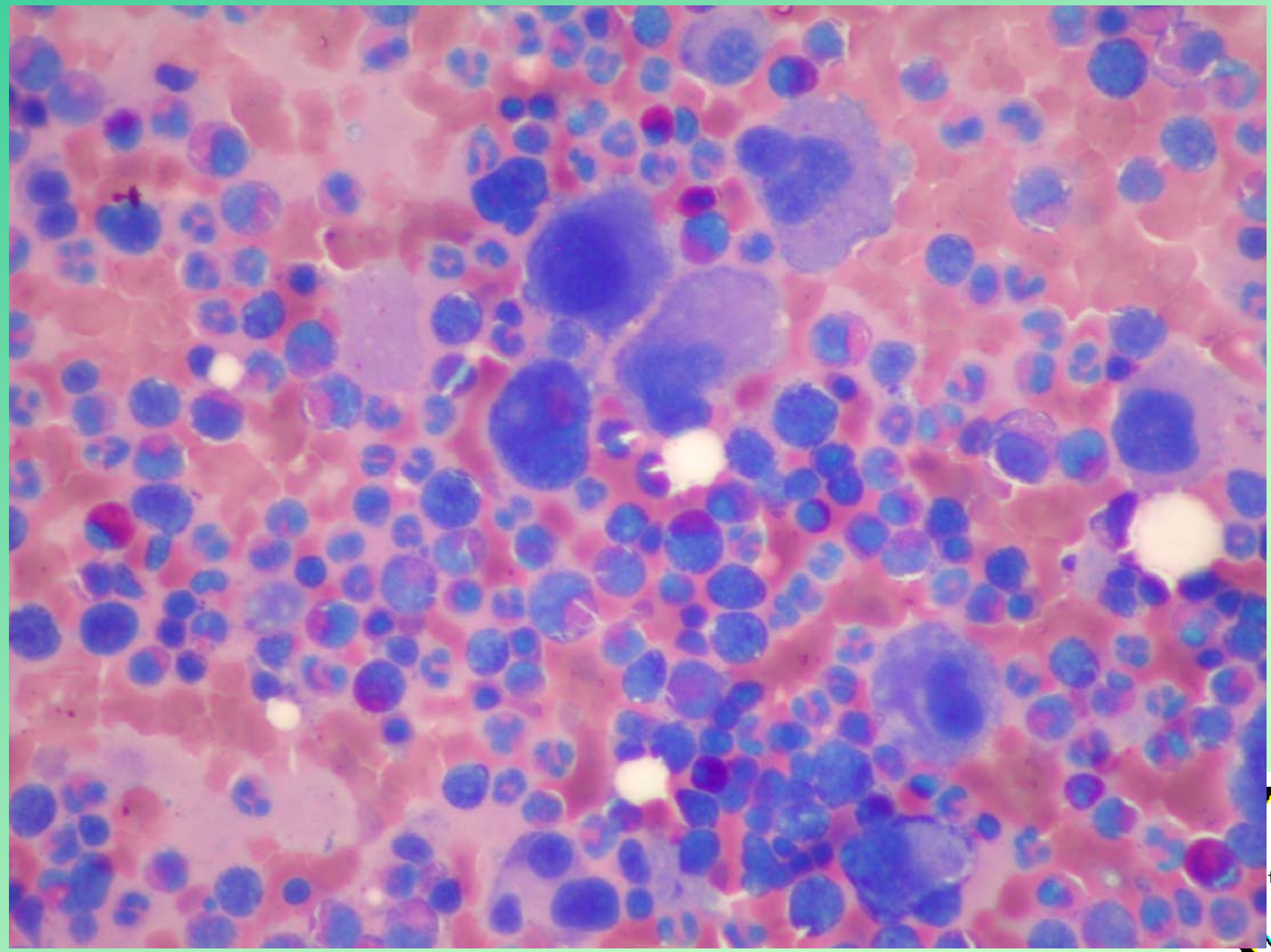

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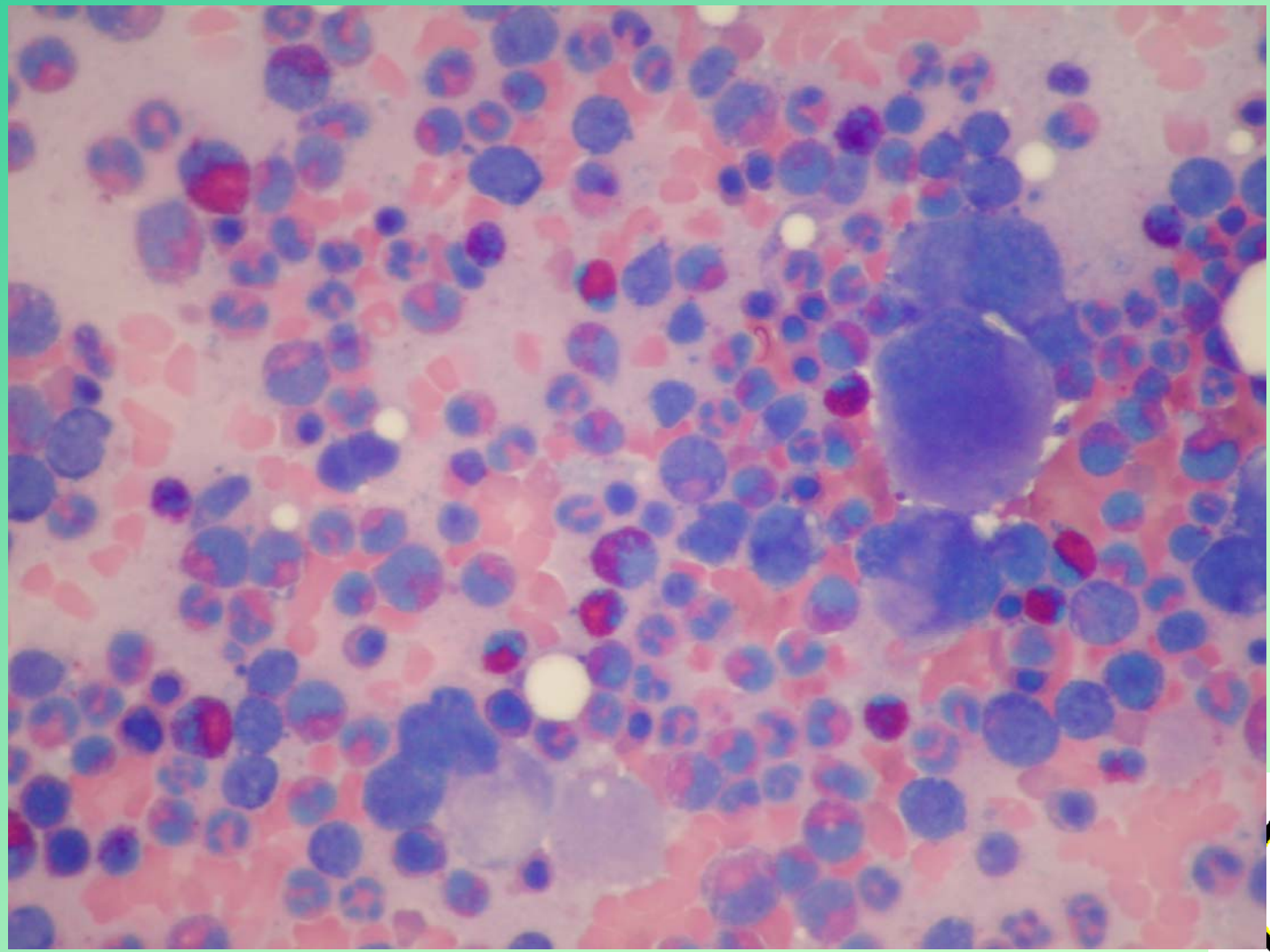

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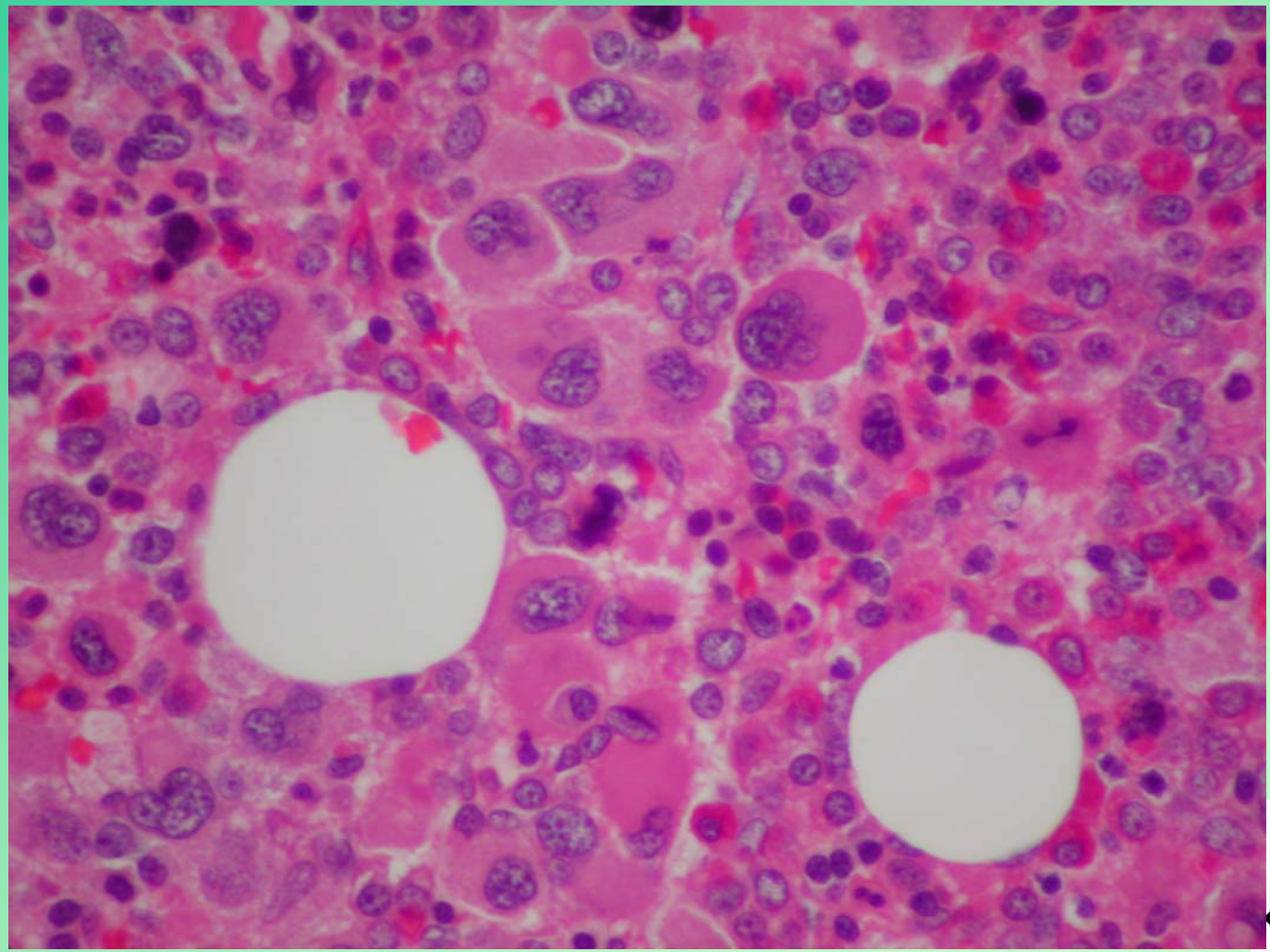
R.S. BM particle



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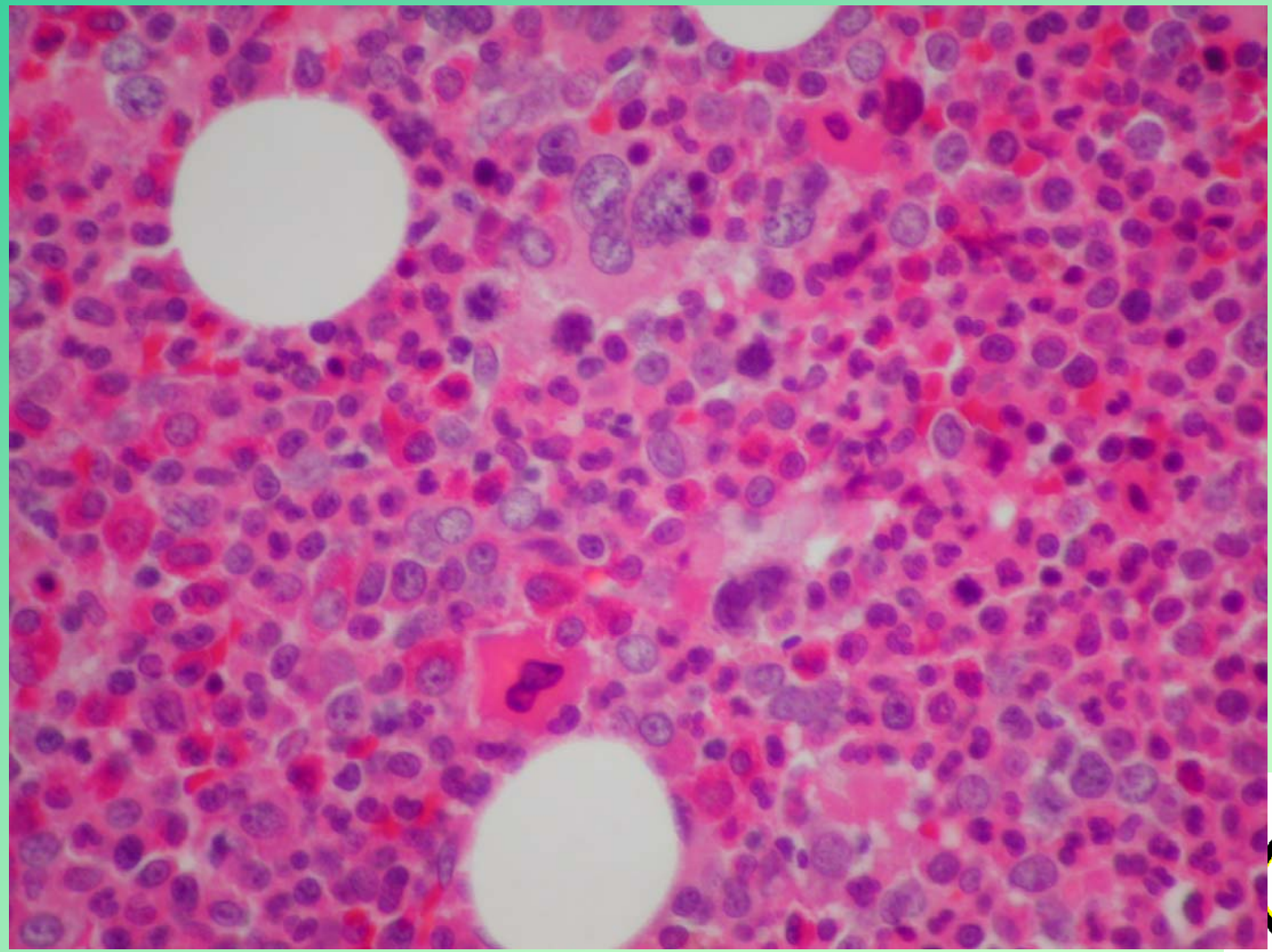
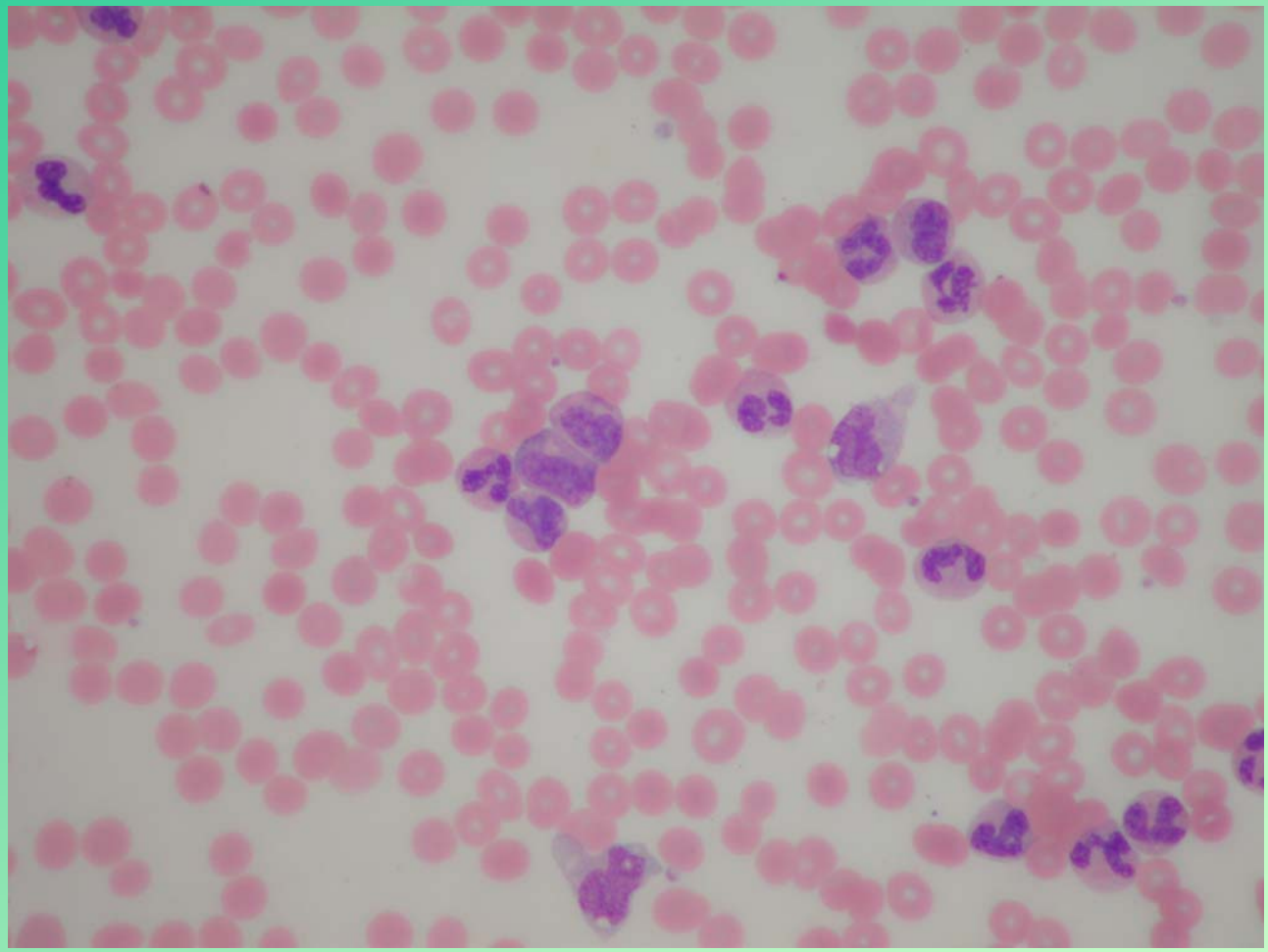


D.T.2004 blood



Hgb 11.7

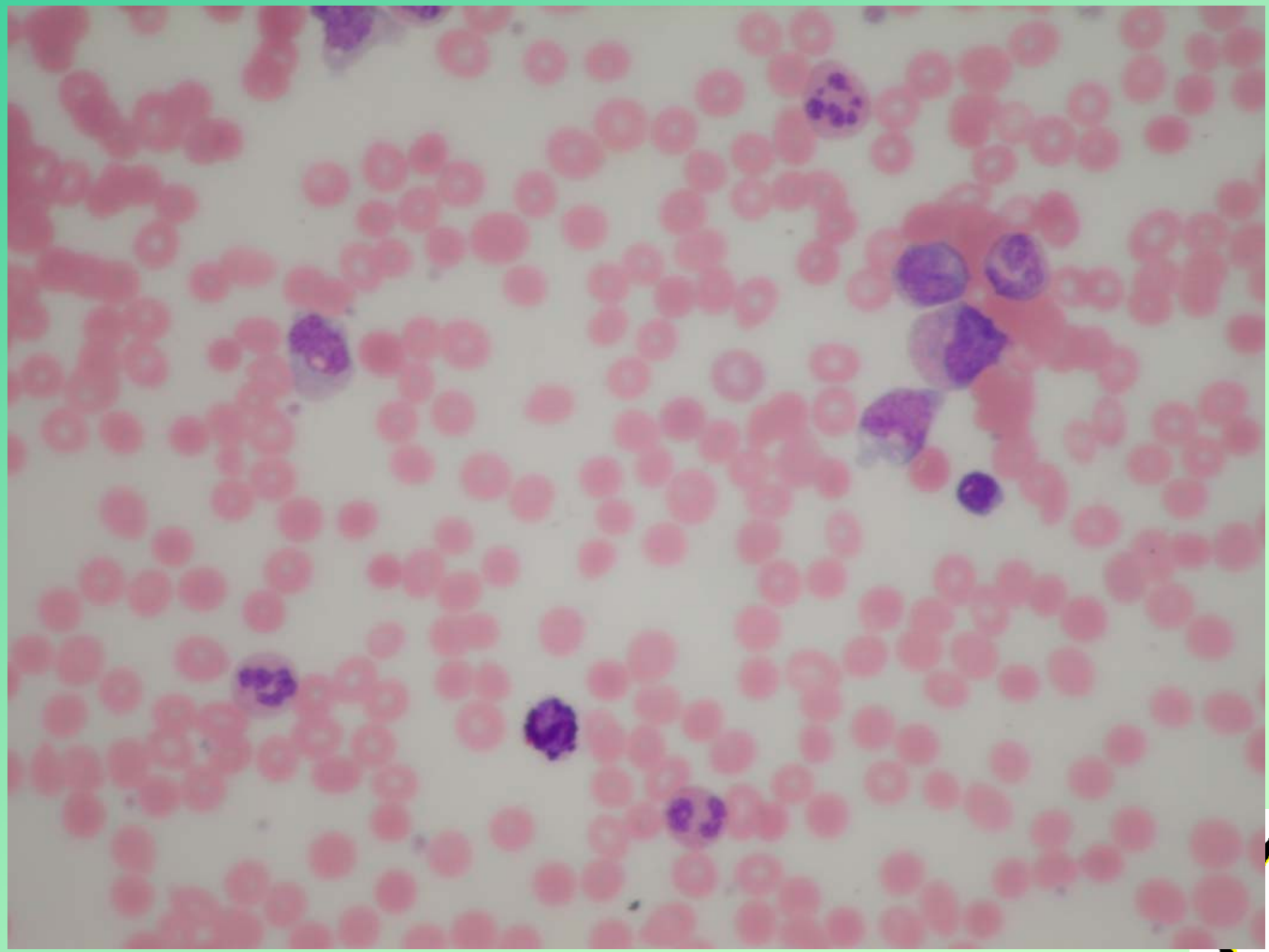
Hct 34.8

WBC 120.6

blasts 2%

Plt 116

D.T.2004 blood



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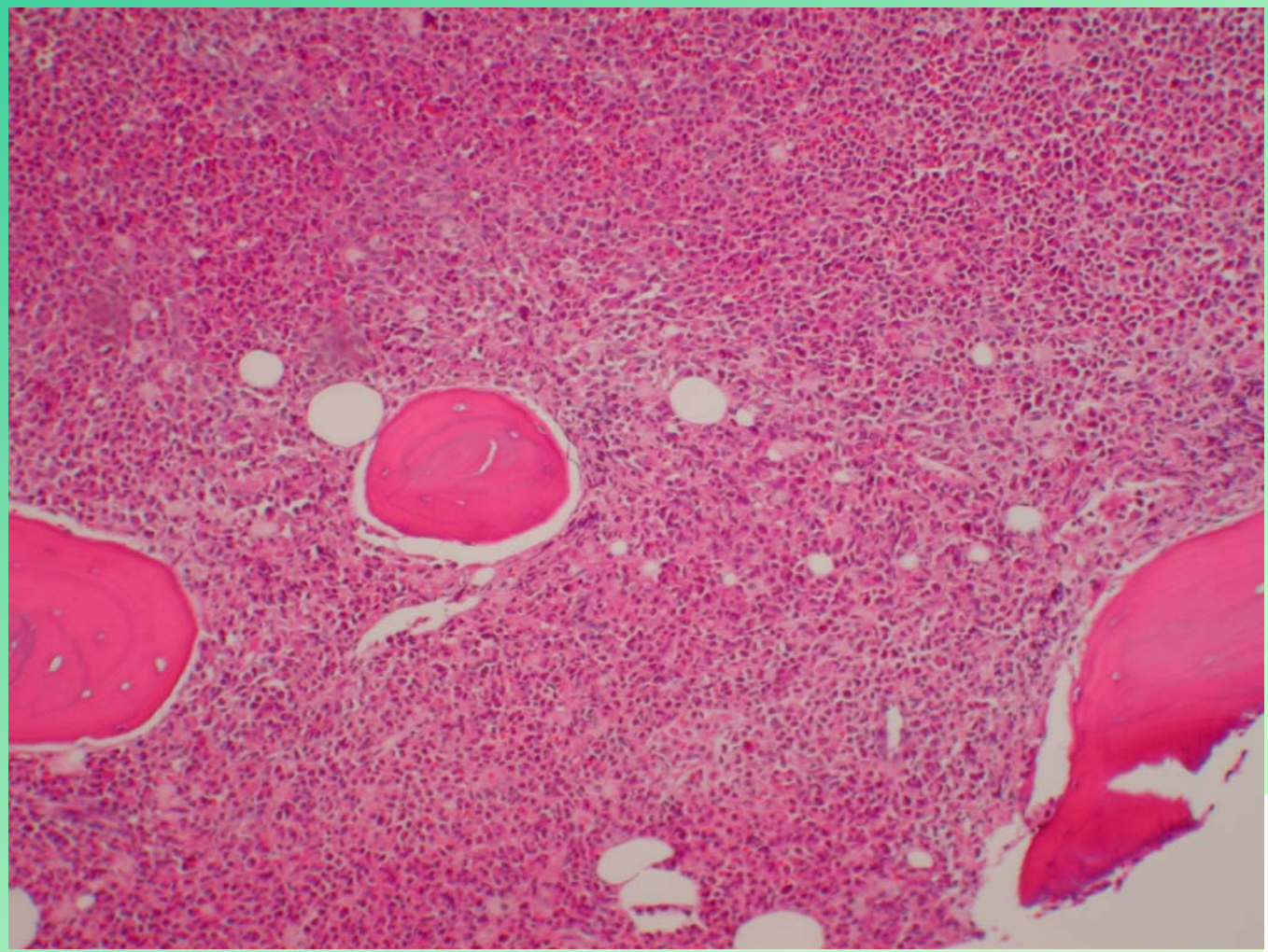
D.T. 2004 BM biopsy



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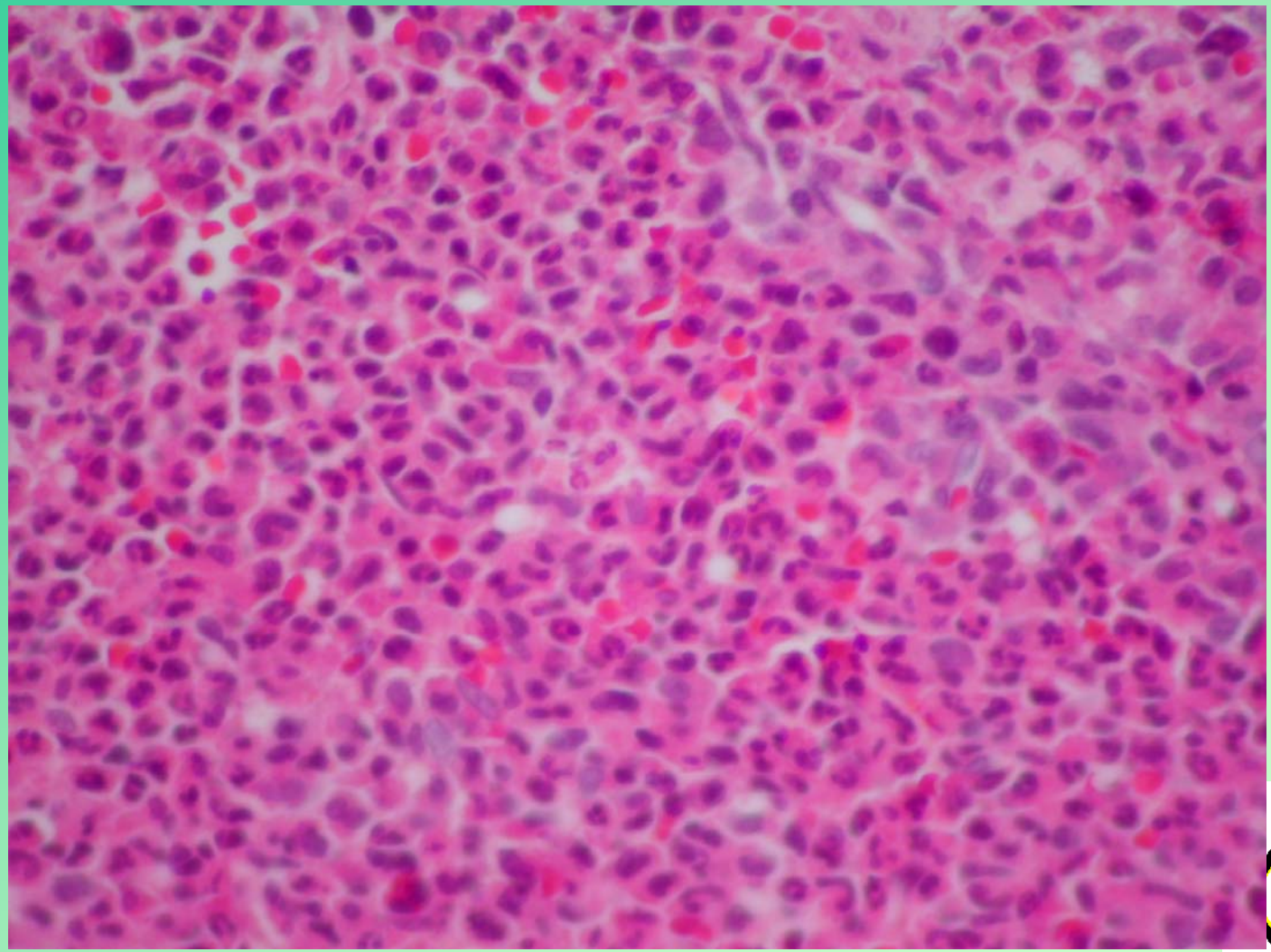

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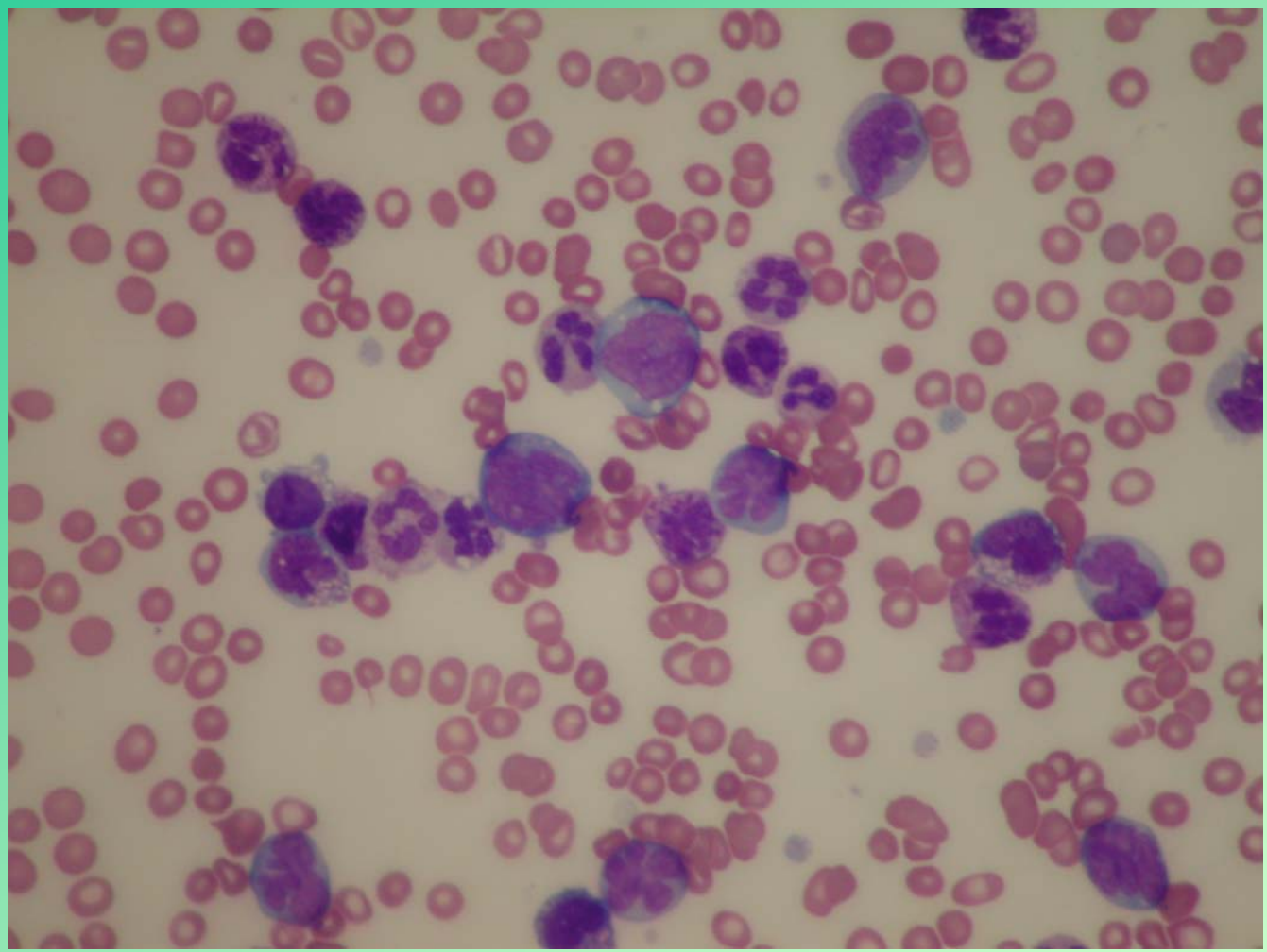
D.T.2006 blood



Hgb 8.7

Hct 26.2

WBC 184.3

blasts 13%

Plt 53

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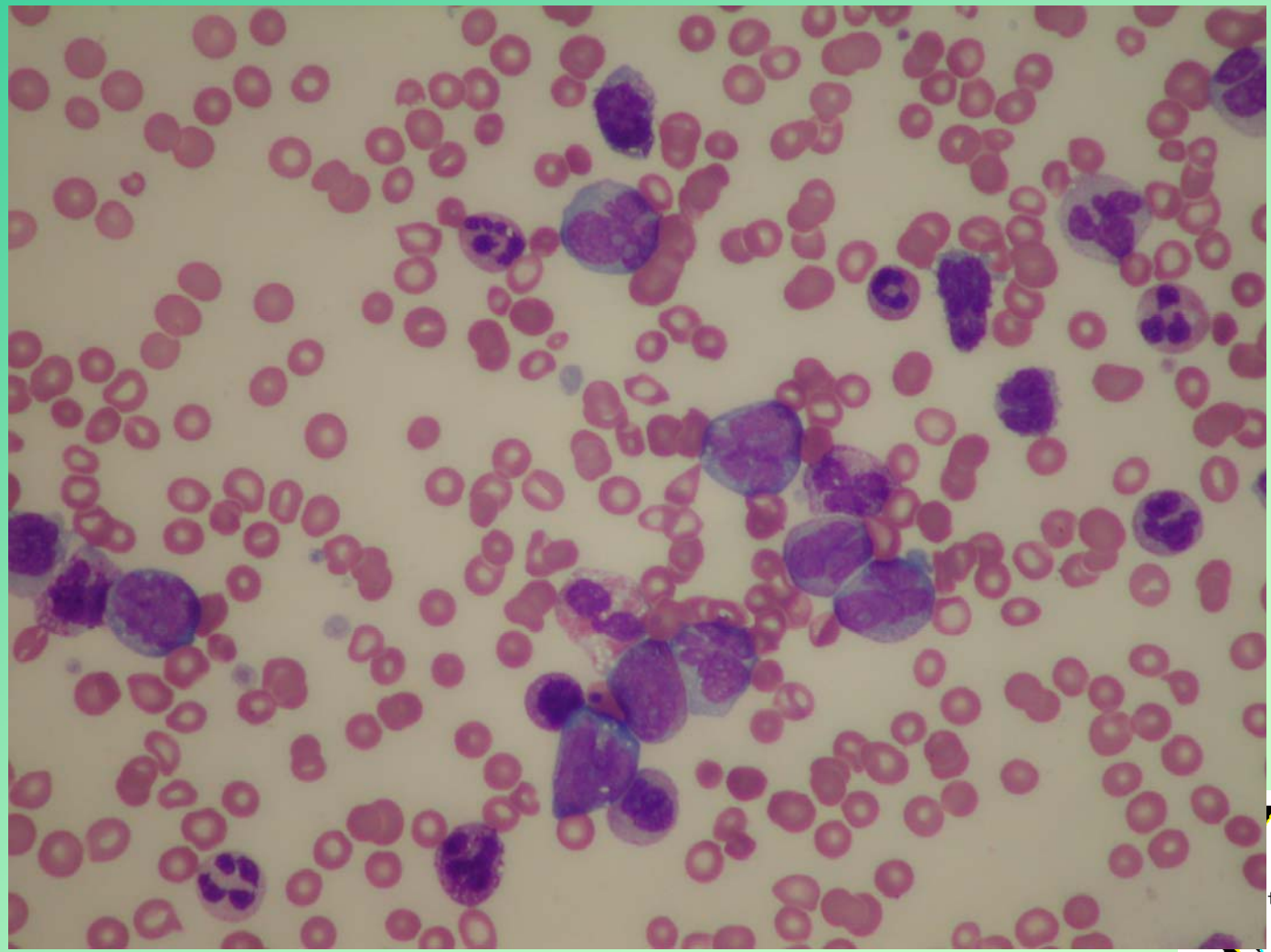
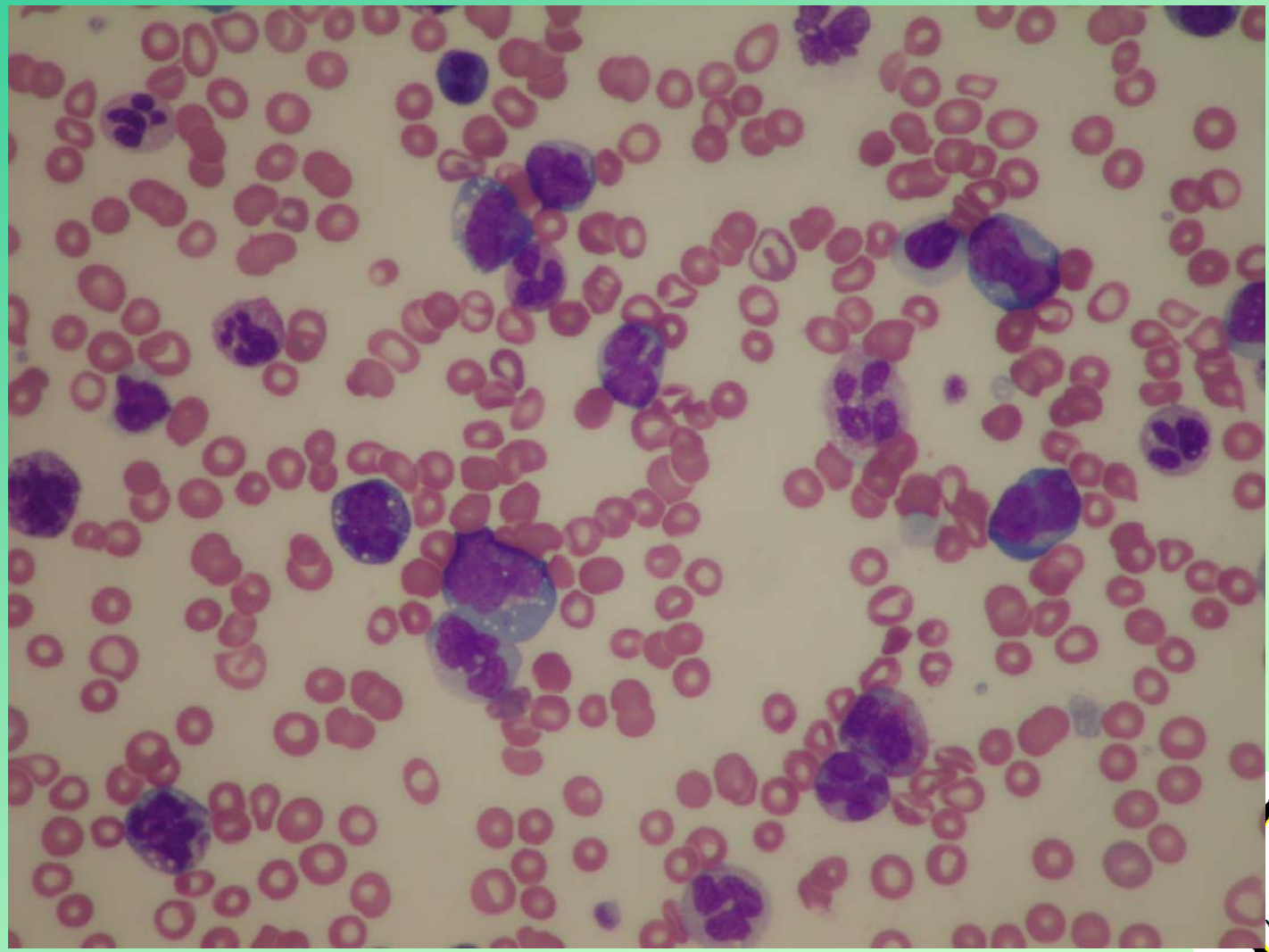
D.T.2006 blood



D.T.2006 blood



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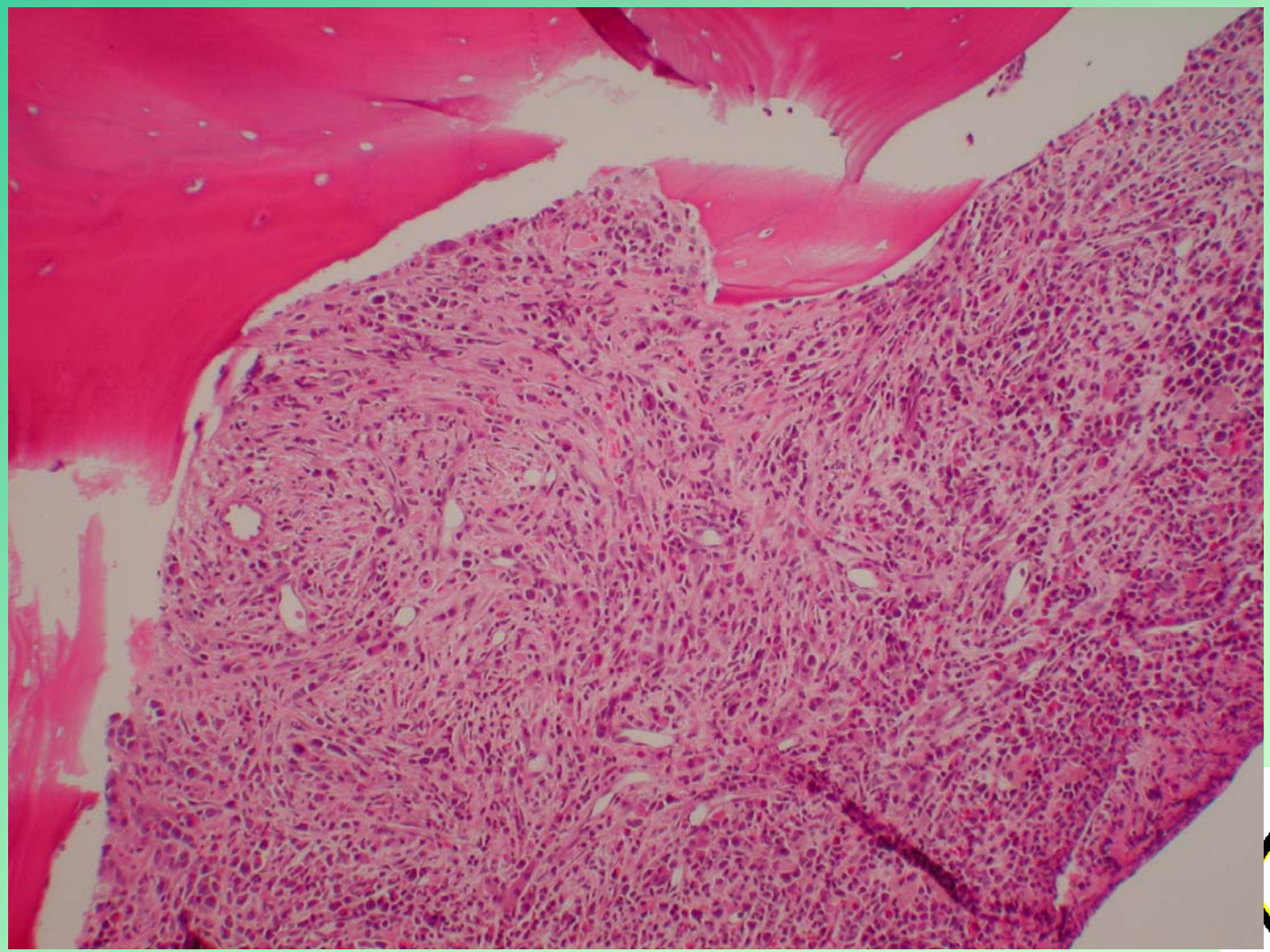
D.T. 2006 BM biopsy



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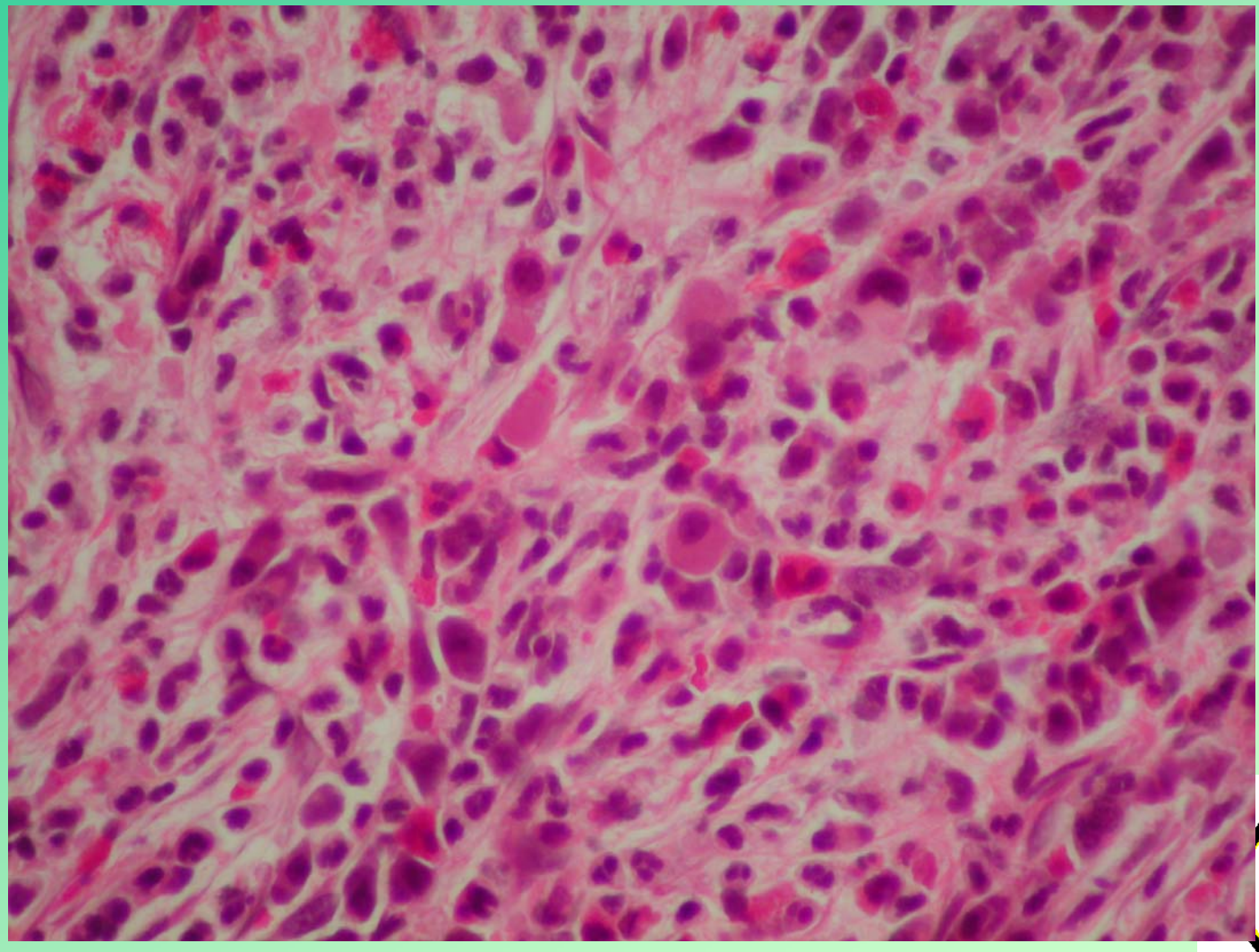
D.T. 2006 BM biopsy



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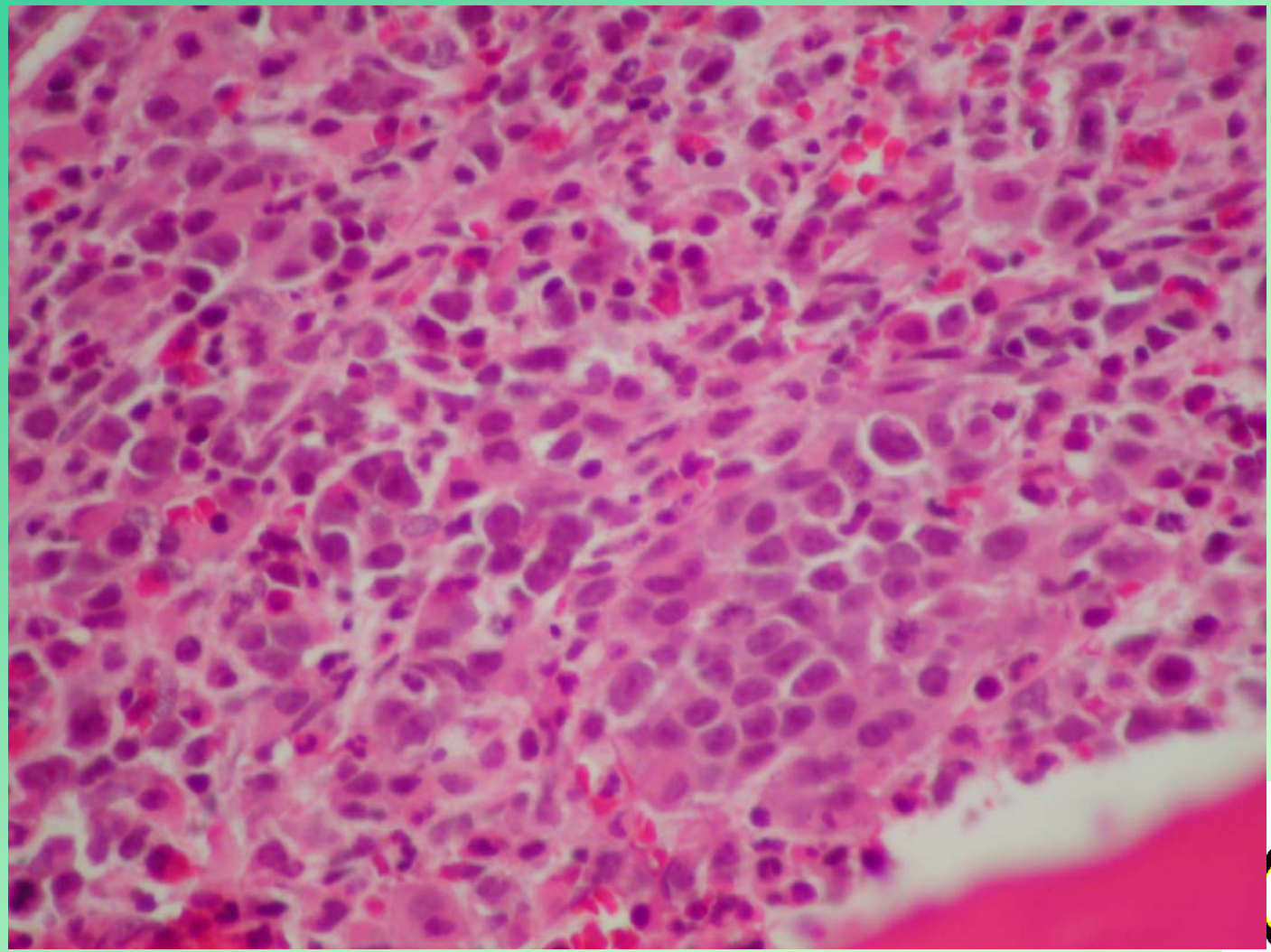

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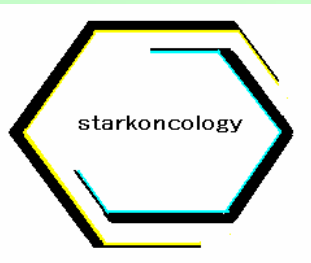
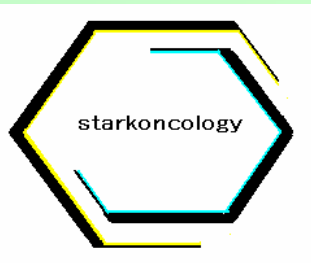
CML Accelerated Phase (2001 WHO criteria)

- 10-19% blasts in blood or marrow
- peripheral blood basophils > or = 20%
- thrombocytopenia (<100) unrelated to therapy, or thrombocytosis (>1000) unresponsive to therapy
- increasing spleen size and WBC unresponsive to therapy
- cytogenetic clonal evolution

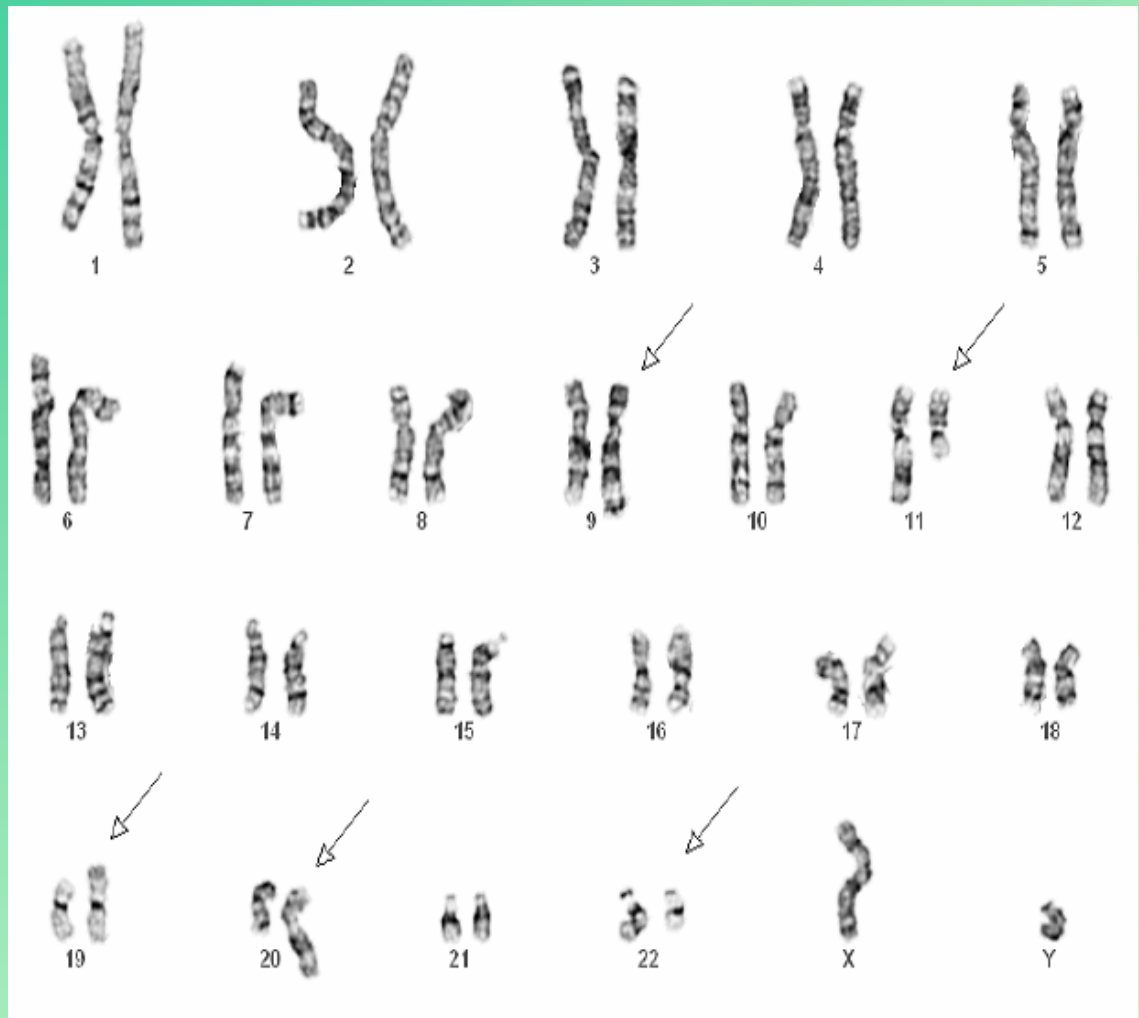


Cytogenetic Evolution

- Definition - acquisition of new genetic abnormalities in tumor cells
- Commonly seen at time of blast crisis in CML (sometimes included in definition of accelerated phase)
- Frequently seen following chemotherapy in aggressive neoplasms
- Almost always associated with poorer prognosis, and frequently with treatment resistance



D.T. 2006



D.T. 2004

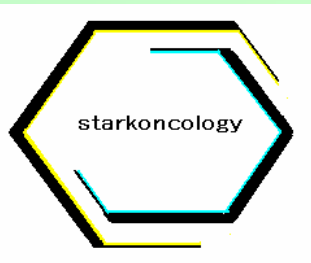
46,XY,t(9;22;19;11;17;20)(q34;q11.2;p13.3;q13;q21;q13.1)[20]

Molecular Diagnosis - The Big 3

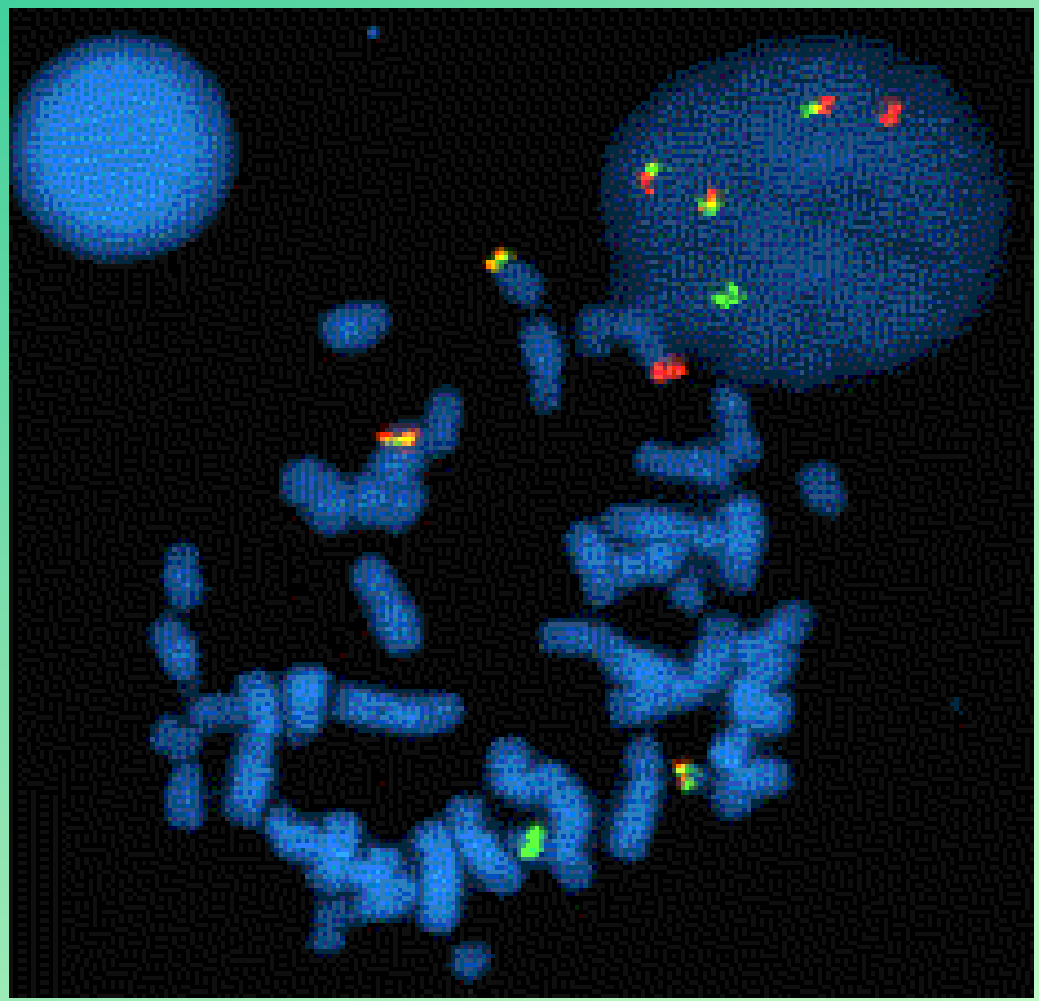
- Chromosomal banding studies
- In Situ Hybridization
  - fluorescence in situ hybridization (FISH)
- PCR based testing

FISH

- Uses short pieces of DNA which are complementary to a genetic sequence of interest (a probe)
- Probe binds specifically to target DNA sequence
- Probe is linked to a fluorescent compound for visualization
- 200 cells typically scored



Metaphase and Interphase FISH



- BCR green
- ABL orange
- Fusion signal yellow

PCR-based Tests

- DNA (or RNA) amplified
- Able to detect up to single base abnormalities in specific sequences using probes or direct sequencing
- Faster and more cost effective way to detect genetic abnormalities in small subsets of cells

JAK2 Analysis

- Single base pair substitution in JAK2 gene present in:
  - 97% of polycythemia vera
  - 57% of essential thrombocythemia
  - 50% of idiopathic myelofibrosis
  - Occasional cases of AML, MDS, CMML, JMML, other myeloid disorders
- Never (to date) seen in:
  - reactive polycythemia or thrombocytosis
  - Philadelphia chromosome + CML

JAK2 Gene

- Janus kinase 2 , a tyrosine kinase
- Mutation appears to lead to JAK2 hyperactivation, resulting in erythropoeitin hypersensitivity in cell culture
- Exact role in disease process unknown

JAK2 Mutational Analysis

- Uses automated real-time PCR (sensitivity typically 5% mutant alleles)
- Specimen: blood, the mutation is present in neutrophils
- Fast TAT
- Very expensive equipment

Chromosomal Banding	FISH	PCR-based
•Culture necessary to produce metaphases – increases expense and TAT •“Low power screen” of entire genome •Very laborious to quantitate	•Can be performed on interphase (non-dividing) or metaphase cells •Limited probes available •Yes or no answer on specific sequences •Can quantitate	•Can be performed on non-dividing cells •Limited probes and primers available •Highest sensitivity for very small sequence abnormalities •Yes or no answer on specific sequences •Most sensitive quantitative monitor of MRD