Differential Diagnosis and Testing for Hematologic Malignancies

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THE ANNUAL APSHO MEETING

Learning Objective

1. Interpret advancements in diagnostic tests used in risk stratification for hematologic malignancies including next-generation sequencing (NGS) for the discovery of novel molecular abnormalities as defined by the World Health Organization (WHO) and European Leukemia Network (ELN)

Financial Disclosure

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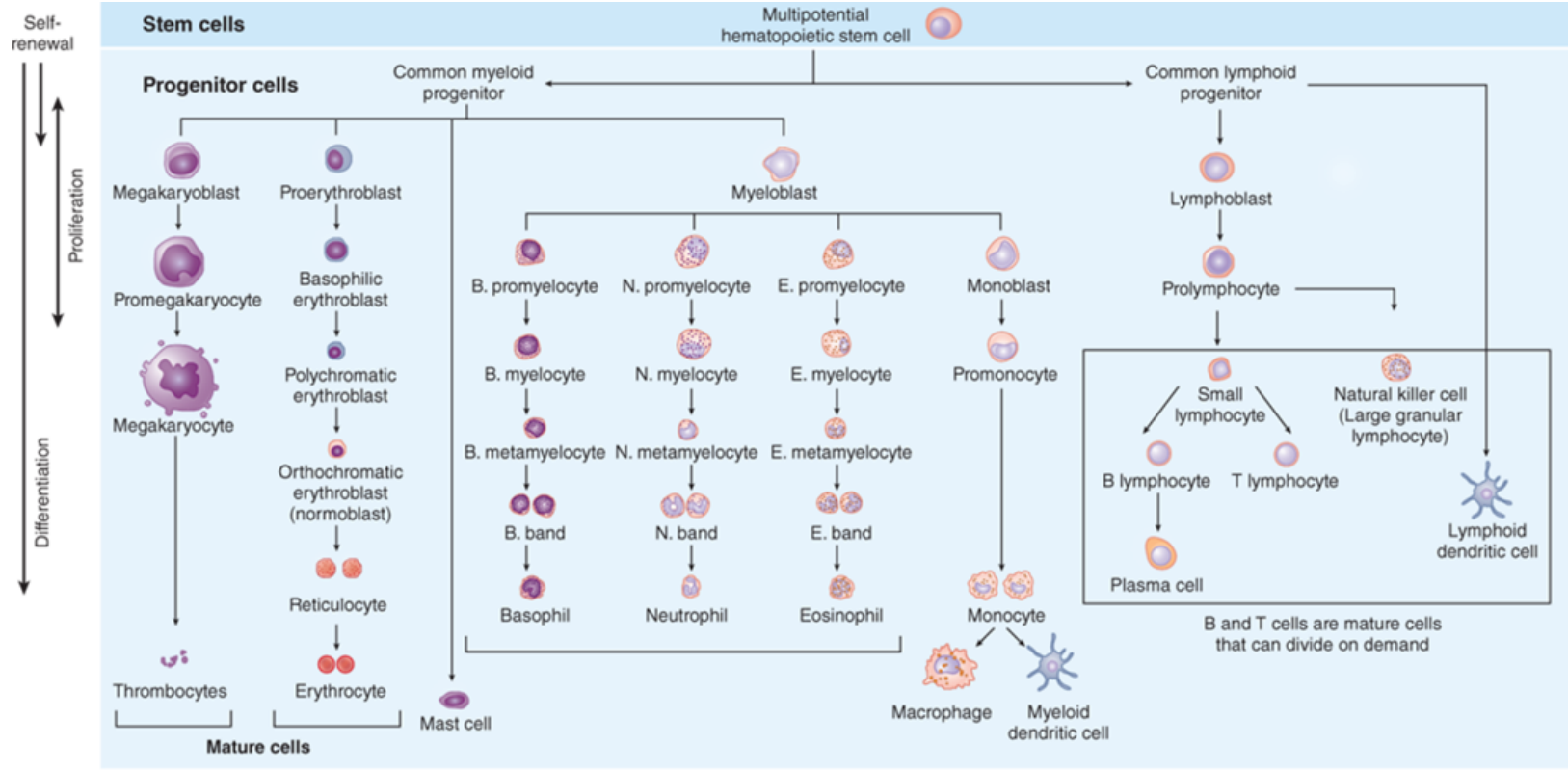
- Dr. Knight has nothing to disclose.

Precision Hematology: Keeping Up With the Science

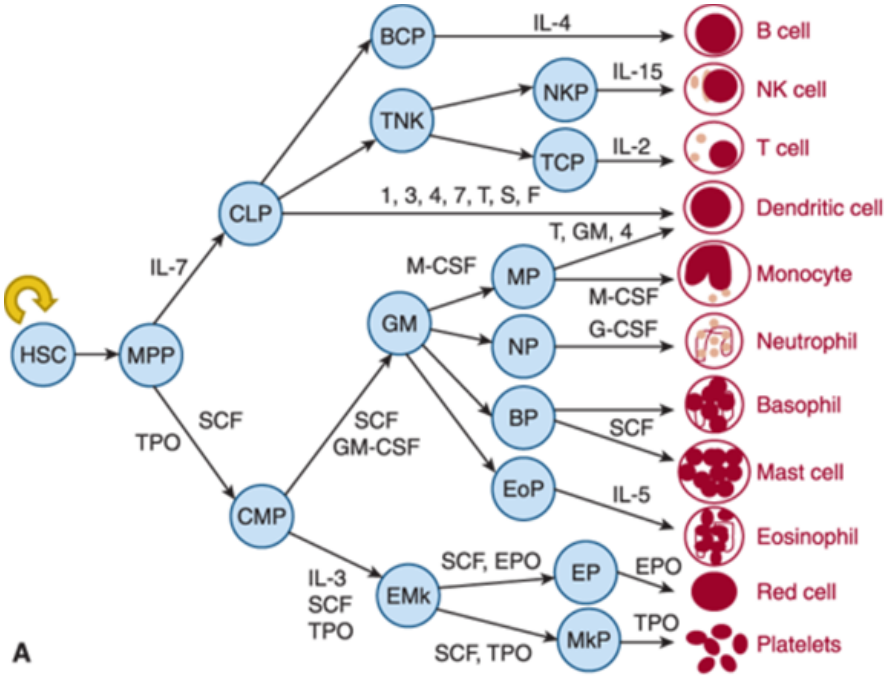
- Heterogeneity of the majority of hematologic malignancies
  - Variable clinical presentation
  - Variable survival
  - Rapidly evolving characterization
- Evolution of numerous targeted therapies across most hematologic malignancies
  - Pathways and targets being characterized
  - Exploiting targets for therapeutic benefit

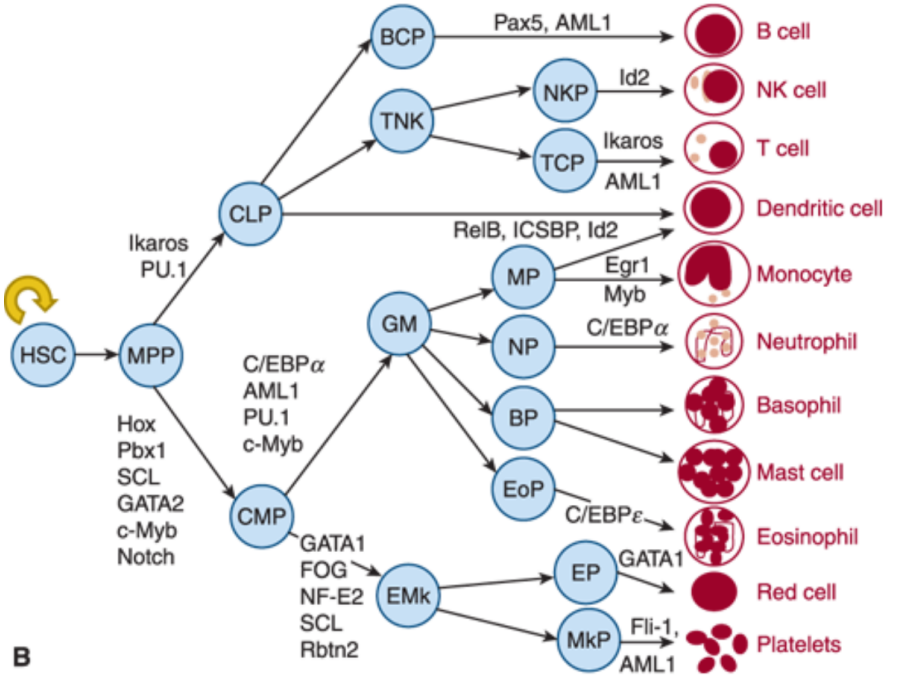
Hematopoiesis Revisited



Hematopoiesis, Aster JC, Bunn H. *Pathophysiology of Blood Disorders, 2e;* 2016. https://accessmedicine.mhmedical.com/content.aspx?bookid=1900&sectionid=137394642

Hematopoiesis Revisited: Growth Factors and Transcriptional Factors





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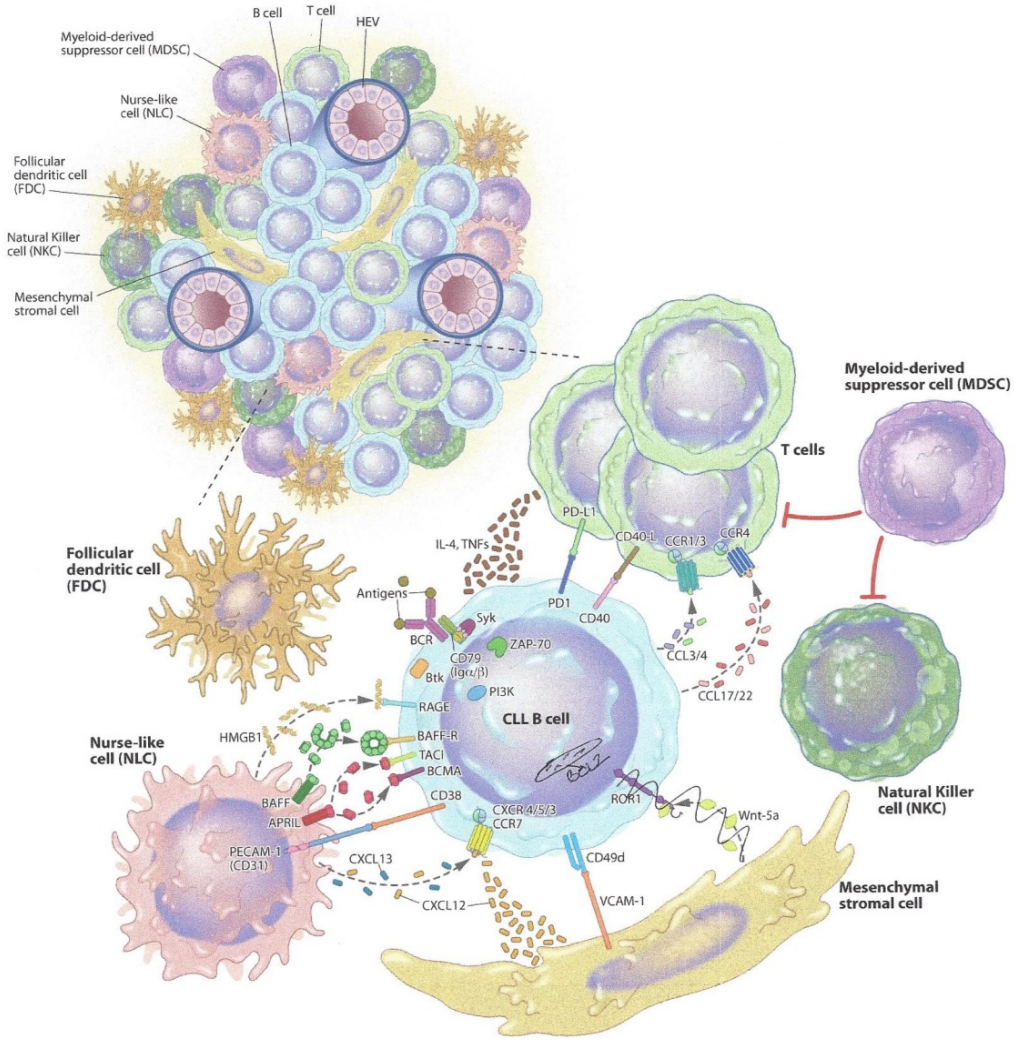


Pathways and Targets

Opportunities that require optimization of the diagnostic process

Used with permission from DNA Illustrations.

Tissue Sampling for Hematologic Malignancies

- Plan ahead
  - What questions to ask for suspected diagnosis or evaluation of response to treatment or progression
  - How many samples? What type of tube?
- Get a good sample
  - Excisional biopsy
  - Core biopsies
  - Fine needle aspirate

WHO Criteria for the Diagnosis of PV

Diagnosis requires meeting either all 3 major criteria or the first 2 major criteria and the minor criterion

	Major criteria	Minor criterion
1.	Elevation in red blood cell count Men: Hgb >16.5 or Hct > 49% Women: Hgb > 16 or Hct > 48%	Decrease in erythropoietin levels in blood
2.	Hypercellular bone marrow	
3.	Identification of a molecular mutation JAK2 V617F (95%) or JAK2 Exon 12 (3%)	

Key changes:

↓ Hct threshold to increase diagnosis of "masked PV"

Included requirement for bone marrow biopsy for differential diagnosis of PV vs. JAK2 mutated ET

Hgb = hemoglobin; Hct = hematocrit.

Arber DA, Orazi A, Hasserjian R, et al. *Blood.* 2016;127(20):2391-2405.

Driver Mutations in Ph- MPNs

- **JAK2 V617F**
  - Present in ~90% of PV
  - Present in ~50% of ET and PMF
  - Activates erythropoietin receptor, thrombopoietin receptor, and G-CSF receptor
  - Janus kinase (JAK)2 V617f mutation was first discovered in 2005
- **JAK2 exon 12**
  - A variant of the JAK2 mutation
  - JAK2 V617F, exon 12 mutations are not seen in ET or MF
- **MPLW515l** and **MPLW515K**
  - Receptor for thrombopoietin which regulates megakaryopoiesis
  - Present in 3%–4% of ET and 6%–7% of PMF
  - MPL mutations are absent in PV
  - More common with older age, higher platelet counts and low hemoglobin levels
  - In PMF, it occurs more commonly in females
  - MPL mutation was first discovered in 2006

ET = essential thrombocytopenia; PMF = primary myelofibrosis.

Schischlik F, Kralovics R. *Expert Rev Hematol*. 2017;10(11):961-973.
O'Sullivan JM, Harrison CN. *Molecular and Cellular Endocrinology*. 2017;451:71-79

Driver Mutations in Ph- MPNs (cont.)

- **CALR exon 9**
  - Calreticulin gene (CALR) mutations are present in 20%–35% of patients with JAK2 and MPL negative ET or PMF
  - Binds to the thrombopoietin receptor (MPL)
  - Type I (insertion associated with lower DIPSS risk) and type II (deletion associated with poor risk)
  - CALR is not found in PV
  - The CALR mutation was first discovered in 2013
- **Triple negative**
  - 5%–12% of MF, 5%–15% of ET
  - Very poor prognosis, higher risk of leukemic transformation, associated with a higher Dynamic International Prognostic Scoring System (DIPSS) risk
- Other associated mutations with prognostic importance
  - Epigenetics: TET2, ASXL1
  - Other: IDH1/2

Schischlik F, Kralovics R. *Expert Rev Hematol.* 2017;10(11):961-973.
O'Sullivan JM, Harrison CN. *Molecular and Cellular Endocrinology*. 2017;451:71-79

Bone Marrow Aspirate

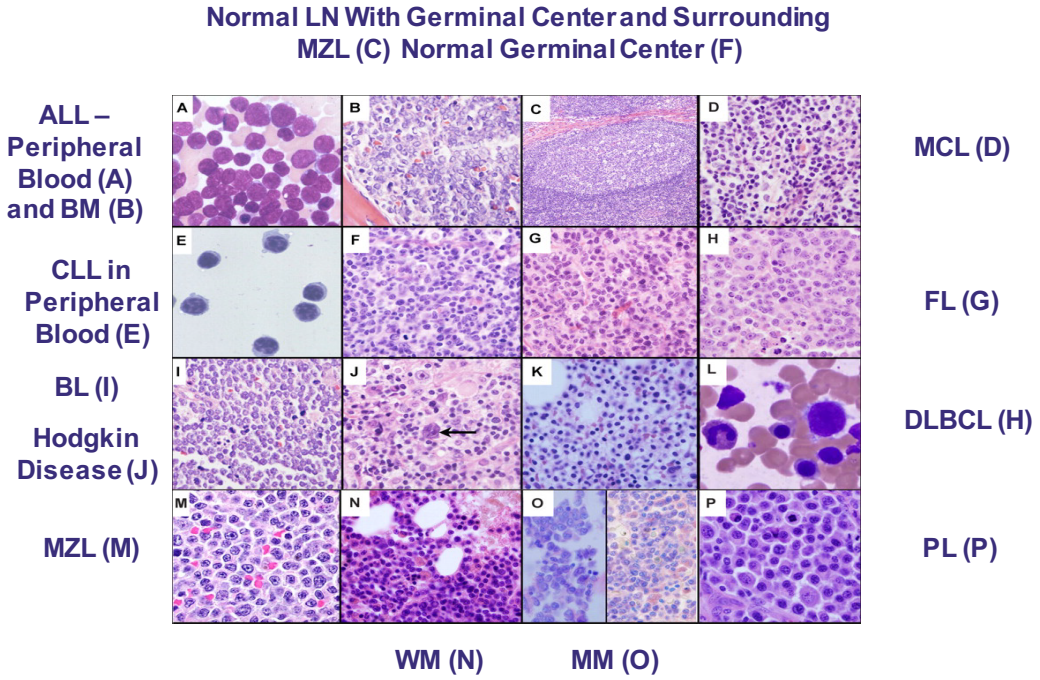
- Evaluation of cytoplasmic and nuclear morphology
  - Maturation: Including blasts %
  - Auer rods and other TdT analysis ringed sideroblasts and iron stores
  - Morphology: Dyserythropoiesis, dysmyelopoiesis
  - Flow cytometry for cell surface antigens
  - Cytogenetics
  - Polymerase chain reactions
  - Gene expression profiling
  - Chimerisms
- Clot section
  - Leftover aspirate material allowed to clot then processed for morphologic evaluation similar to the core but not decalcified (no bony component)

Bone Marrow Biopsy: Decalcification

- Touch preparations – imprints of the core biopsy made by touching or rolling it on a slide, looks similar to the aspirate
  - Take two cores if dry tap
- Cellularity
  - Normal ~100-age
- Architecture
  - M:E ratio, location of cell lines, presence of immature precursor cells (ALIP), iron stores
  - Fibrosis
  - Presence of malignant disease
    - Critical for lymphomas
  - Bone morphology

Immunohistochemistry (Heme-Path)

- Based on algorithm for application of antibodies
- Can correlate morphology with stain
- Requires hours, sometimes days
- Quantitative assessment is subjective – requires expertise

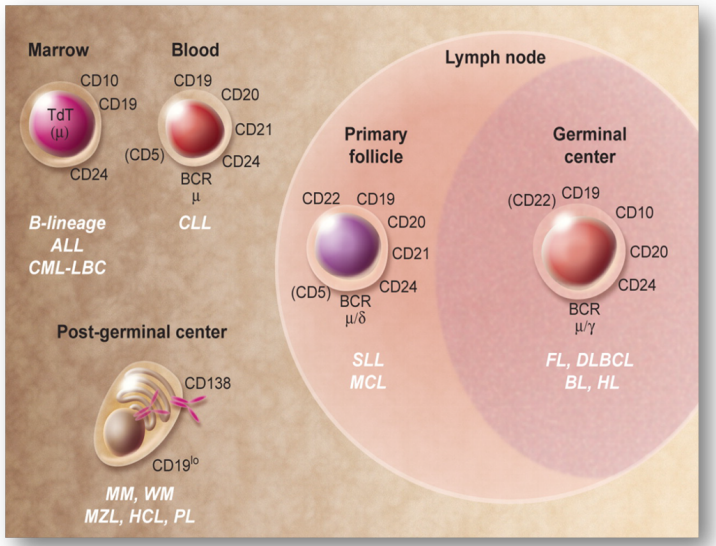


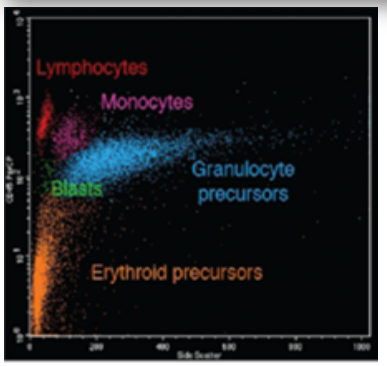
ALL = acute lymphoblastic leukemia; HCL = hairy cell leukemia; PL = plasmablastic lymphoma; MM = multiple myeloma.

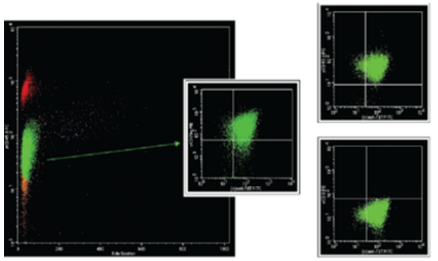
Lebien T & Tedder T (2008). Blood. 2008;112:157-580

Flow Cytometry (Flow)

- Uses large panels to characterize the neoplastic population:
  - Identify lineage/immunophenotype – "Zip Code for cell of origin"
  - Detect aberrant antigenic expression patterns (surface proteins and cytoplasmic expression)
  - May not be diagnostic alone
  - Useful for detecting blasts
- Can be performed on blood, body fluids, bone marrow aspirate, and lymphoid tissue – ***must be fresh***
- Useful in evaluating residual disease or progression for some disease
- Fast (hours) and quantitative method with evaluation of multiple antigens simultaneously



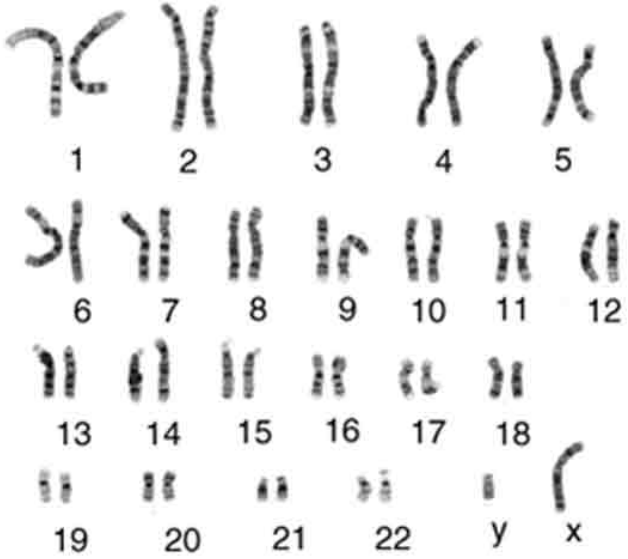




LeBien, T. W. and T. F. Tedder (2008). Blood 112(5):1570-1580.

Metaphase Cytogenetics (Cyto)

- Provides karyotype for individual patient
- Cells are arrested in metaphase and stained (typically 20 cells noted in brackets [ ])
  - Cells must be actively dividing
  - Requires several days
  - Labor intensive and requires expertise
- Relatively low sensitivity
- Requires bone marrow aspirate with a few exceptions



46,XY[20]
Normal Male Karyotype

Pittaluga, S., Barry, T.S., Raffeld, M. (2017) Hematopathology. Elsevier

Metaphase Cytogenetics (Cyto)

May detect numeric or structural abnormalities

- Numeric
  - Ploidy
    - Hyperdiploid: >46 chromosomes
    - Hypodiploid: <46 chromosomes
  - Trisomies – change in the number of individual chromosomes
- Structural
  - Deletions (del)
  - Duplications (+)
  - Translocations (t)
  - Inversions (inv)
  - Rings or markers

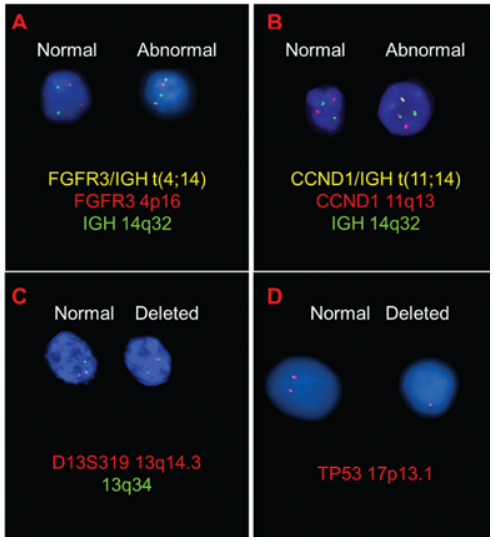
Cytogenetic abnormalities sufficient to diagnose AML with MRC when > PB or BM blasts and prior therapy have been excluded

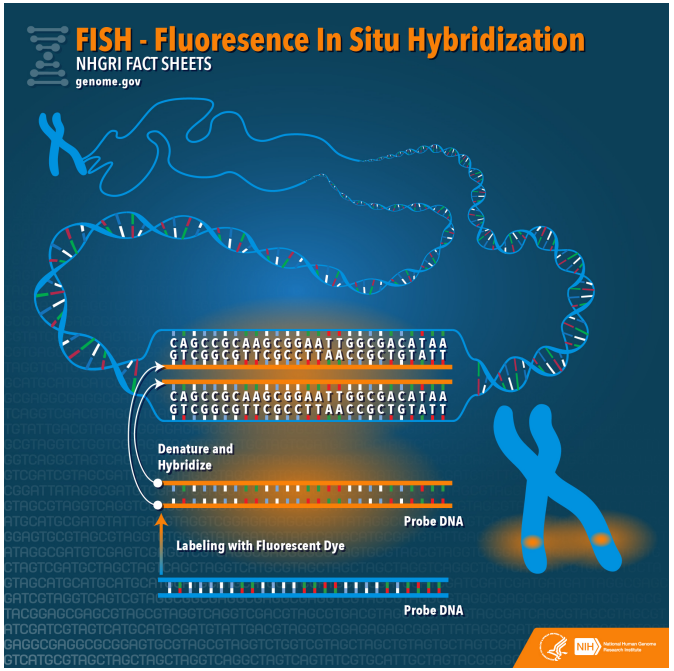
- Complex karyotype (3 or more abnormalities)
- Unbalanced abnormalities
  - 27/del(7q)
  - del(5q)/t(5q)
  - i(17q)/t(17p)
  - 213/del(13q)
  - del(11q)
  - del(12p)/t(12p)
  - idic(X)(q13)
- Balanced abnormalities
  - t(11;16)(q23.3;p13.3)
  - t(3;21)(q26.2;q22.1)
  - t(1;3)(p36.3;q21.2)
  - t(2;11)(p21;q23.3)
  - t(5;12)(q32;p13.2)
  - t(5;7)(q32;q11.2)
  - t(5;17)(q32;p13.2)
  - t(5;10)(q32;q21.2)
  - t(3;5)(q25.3;q35.1)

Arber, D. A., et al. (2016). *Blood, 127*(20), 2391-2405. doi:10.1182/blood-2016-03-643544; Pittaluga, S., Barry, T.S., Raffeld, M. (2017) Hematopathology. Elsevier

Fluorescence in situ Hybridization (FISH)

- A process that vividly paints chromosomes or portions of chromosomes with fluorescent molecules
  - Identifies chromosomal abnormalities often not detectable by metaphase cytogenetics
  - Aids in gene mapping
  - Analysis of chromosome structural aberrations
  - Ploidy determination





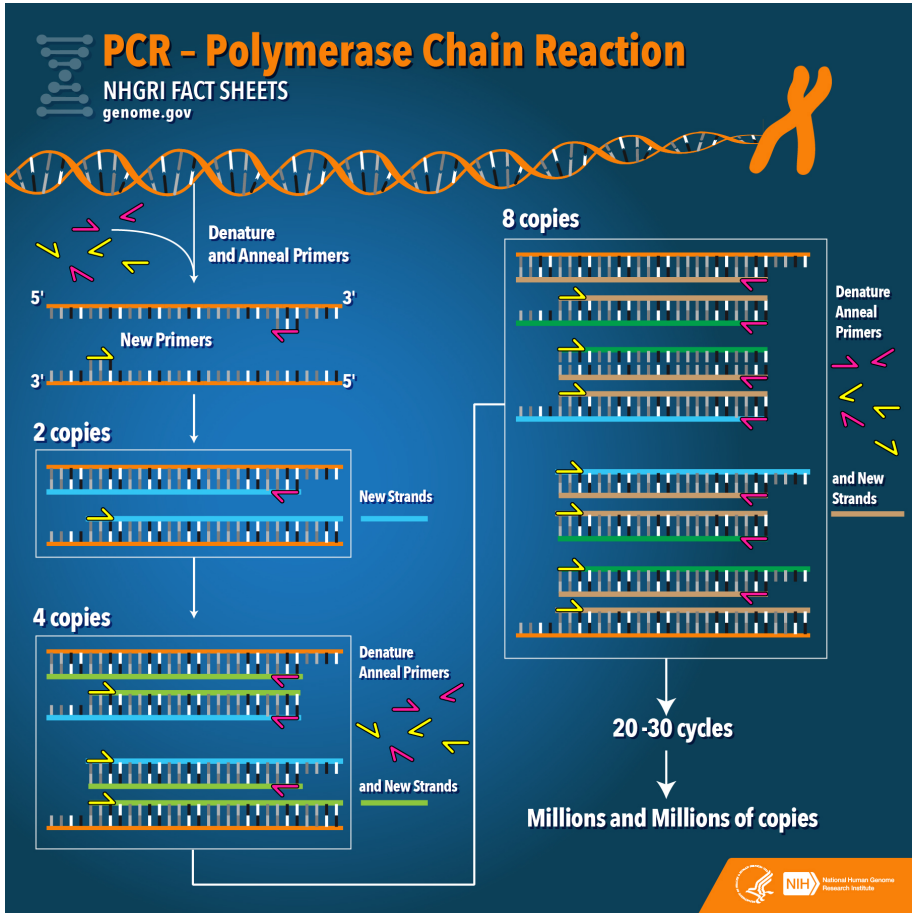
https://www.genome.gov/images/content/fish_factsheet.jpg

Fluorescence in situ Hybridization (FISH) (cont.)

- **Benefits**
  - Metaphase or interphase
  - Fresh tissue or FFPE (sometimes)
  - Higher sensitivity than karyotype
  - Good for following patients over time
  - Can detect smaller abnormalities than karyotype
- **Drawbacks/limitations**
  - Have to know what you are looking for
  - Still looking at fairly large structural abnormalities
  - Still relatively low sensitivity

PCR Testing

- Can produce many copies of a target segment of DNA
  - Amplify nucleic acid sequences
  - Can detect structural abnormalities as well as single base pair abnormalities
  - RT-PCR – Reverse Transcriptase
    - Uses RNA sequences



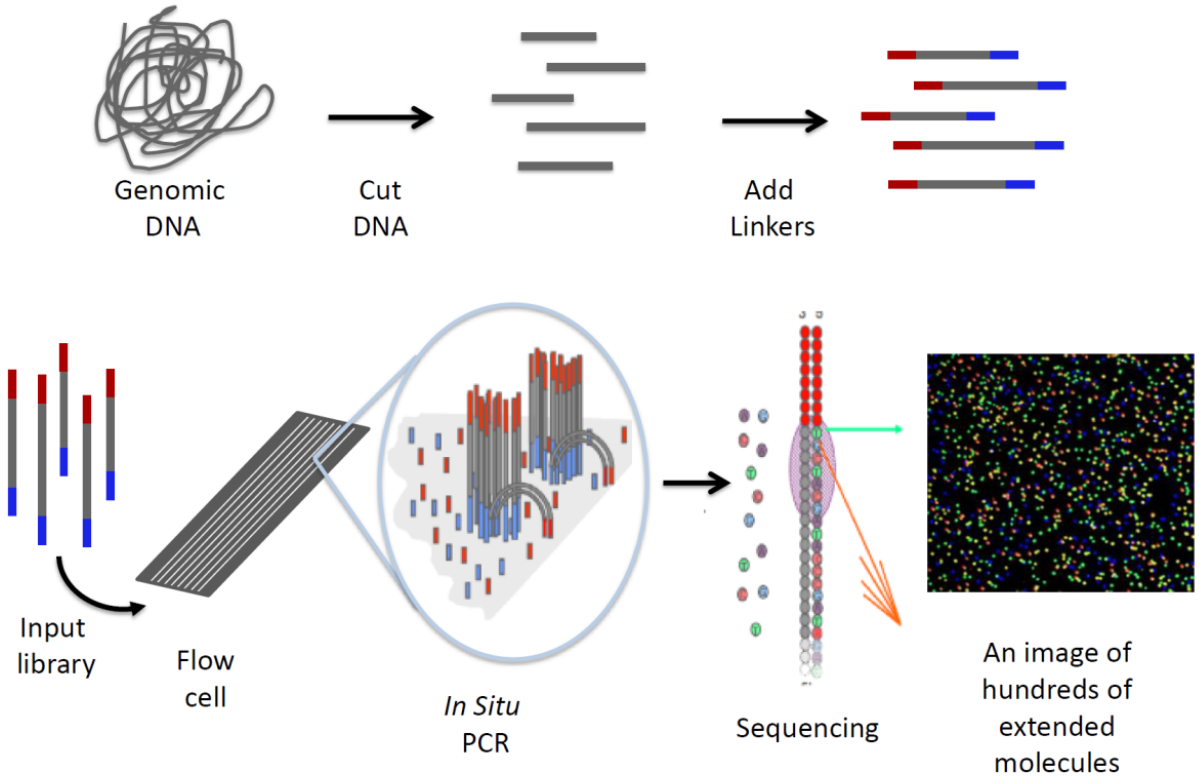
https://www.genome.gov/images/content/pcr_factsheet.jpg

Polymerase Chain Reaction (PCR)

- Benefits
  - Highly sensitive
  - Fast
  - Qualitative and quantitative
  - Good for following patients over time (e.g., BCR-ABL)
- Drawbacks/limitations
  - Have to know what you are looking for
  - Requires high skill level
  - Fresh tissue or formalin-fixed paraffin-embedded tissue (FFPE)

Next-Generation Sequencing (NGS)

- Fast, inexpensive, prepacked analysis software and reagents
- Can simultaneously sequence millions of DNA molecules in parallel
- Random, unselected or genetic regions of interest
- Upfront costs to develop technology and reagents (the library) are high
- Not all tests are covered



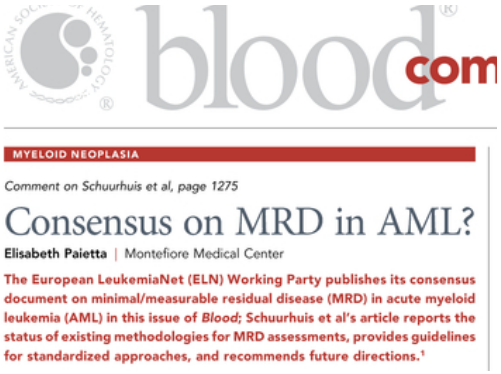
Kuo, F.C. et al. (2017). *Blood*, 130 (4), 433-439

Minimal Residual Disease Detection

LYMPHOID NEOPLASIA

Measurable residual disease detection by high-throughput sequencing improves risk stratification for pediatric B-ALL

Brent Wood,[1,*] David Wu,[1,*] Beryl Crossley,[2] Yunfeng Dai,[3] David Williamson,[2] Charles Gawad,[4] Michael J. Borowitz,[4] Meenakshi Devidas,[3] Kelly W. Maloney,[5] Eric Larsen,[6] Naomi Winick,[7] Elizabeth Raetz,[8] William L. Carroll,[9] Stephen P. Hunger,[10] Mignon L. Loh,[11] Harlan Robins,[2,12,†] and Ilan Kirsch[2,†]



Review

Minimal Residual Disease Detection by Flow Cytometry in Multiple Myeloma: Why and How?

Mikhail Roshal, MD, PhD*

Hematopathology Service, Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY

FDA Authorizes First Next-Generation Sequencing–Based Test to Detect Minimal Residual Disease in B-Cell ALL or Multiple Myeloma

By The ASCO Post

Posted: 10/3/2018 11:03:40 AM

Last Updated: 10/3/2018 11:03:40 AM

Tumor Board

Patient #1

- 65-year-old man referred to oncology for PET positive pancreatic mass and multiple lytic bone lesions, ? widely metastatic pancreatic carcinoma

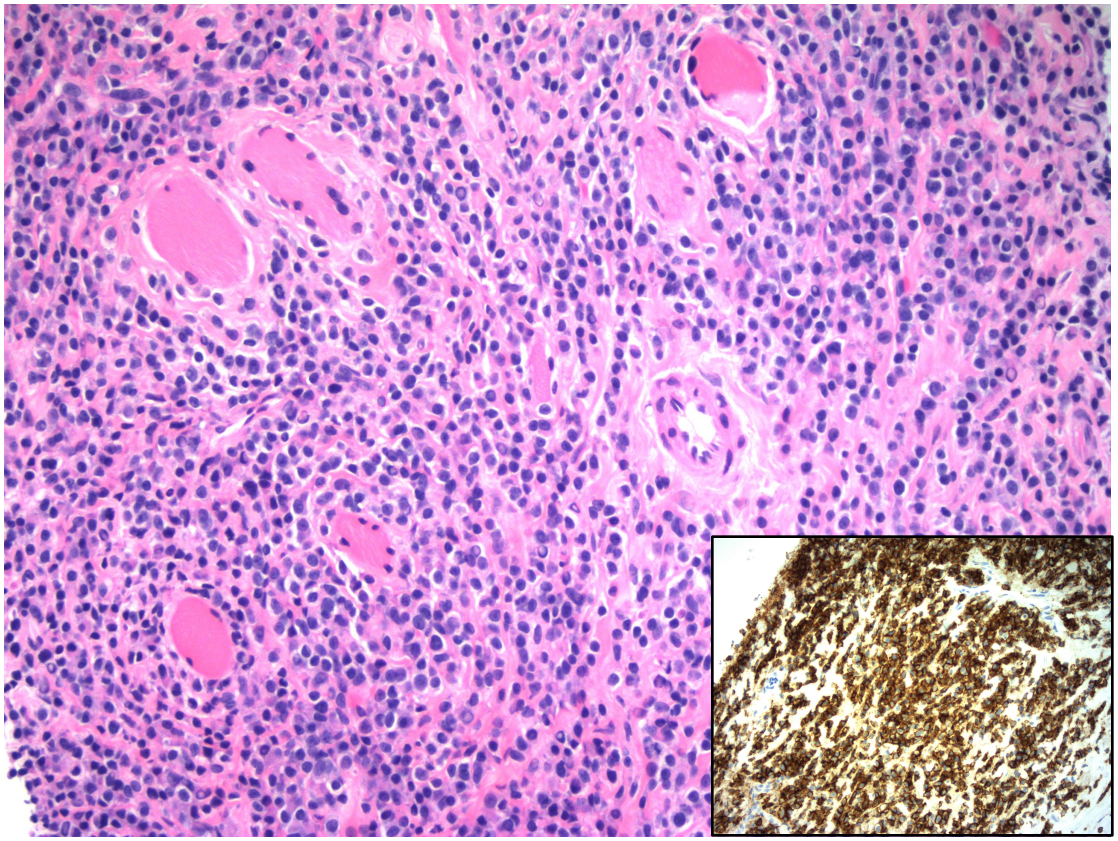
WBC	4.0 - 11.5 TH/mm3	5.9
RBC	4.50 - 5.90 Mil/mm3	1.78 ⌄
Hemoglobin	13.5 - 17.5 g/dL	6.2 ⌄⌄
Hematocrit	41.0 - 53.0 %	17.7 ⌄⌄
MCV	80.0 - 100.0 fL	99.4
MCH	26.0 - 34.0 pg	35.0 ⌃
MCHC	31.0 - 37.0 g/dL	35.2
RDW	12.0 - 16.0 %	15.8
Platelets	125 - 415 Th/mm3	100 ⌄
MPV	6.9 - 10.6 fL	8.4
Neutrophils, %	39 - 69 %	59
Lymphocytes, %	24 - 44 %	24
Monocytes, %	2 - 10 %	15 ⌃
Eosinophils, %	0 - 4 %	2

Sodium	133 - 144 mmol/L	141
Potassium	3.4 - 5.1 mmol/L	4.4
Chloride	101 - 111 mmol/L	106
CO2	20 - 30 mmol/L	21
Anion Gap	6 - 16 mmol/L	14
BUN	8 - 26 mg/dL	42 ^
Creatinine	0.72 - 1.25 mg/dL	2.63 ^
Glucose	82 - 99 mg/dL	84
Calcium	8.5 - 10.4 mg/dL	9.8
AST	5 - 51 U/L	27
ALT	1 - 55 U/L	17
Alkaline Phosphatase	32 - 125 U/L	43
Protein, Total	6.0 - 8.3 g/dL	10.7 ^
Albumin	3.4 - 4.8 g/dL	2.5 v
Bilirubin, Total	0.1 - 1.2 mg/dL	0.6

Patient #1

- Biopsy of one of the bone lesions: plasmacytoma
- FNA of pancreatic lesion: chronic pancreatitis

H&E with CD138 immunohistochemical stain in inset



FNA, fine needle aspiration

DEFINITION OF MULTIPLE MYELOMA (SMOLDERING AND ACTIVE)

Active (Symptomatic) Myeloma[2,3]

Clonal bone marrow plasma cells ≥10% or biopsy-proven bony or extramedullary plasmacytoma

And

Any one or more of the following myeloma-defining events:

- **Calcium >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL)**
- **Renal insufficiency (creatinine >2 mg/dL) [>177 µmol/L] or creatinine clearance <40 mL/min**
- **Anemia (hemoglobin <10 g/dL or hemoglobin >2 g/dL below the lower limit of normal)**
- **One or more osteolytic bone lesions on skeletal radiography, CT, or FDG PET/CT**
- **Clonal bone marrow plasma cells ≥60%**
- **Abnormal serum FLC ratio ≥100 (involved kappa) or ≤0.01 (involved lambda)**
- **>1 focal lesions on MRI studies ≥5 mm**

National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2019
Multiple Myeloma

NCCN Guidelines Index
Table of Contents
Discussion

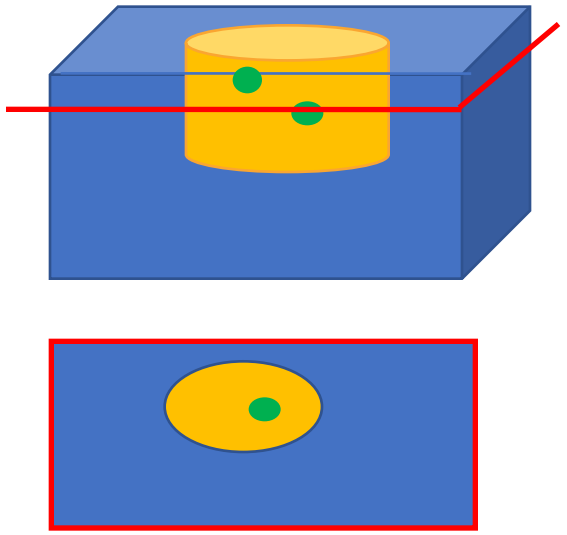
INITIAL DIAGNOSTIC WORKUP

- History and physical exam
- CBC, differential, platelet count
- Exam of peripheral blood smear
- Serum BUN/creatinine, electrolytes, albumin,[a] and calcium
- Creatinine clearance (calculated or measured directly)
- Serum uric acid
- Serum LDH[a] and beta-2 microglobulin[a]
- Serum quantitative immunoglobulins, serum protein electrophoresis (SPEP), serum immunofixation electrophoresis (SIFE)

- 24-h urine for total protein, urine protein electrophoresis (UPEP), urine immunofixation electrophoresis (UIFE)
- Serum free light chain (FLC) assay
- Skeletal survey or whole body low-dose CT scan[b,c]
- Unilateral bone marrow aspirate + biopsy, including bone marrow immunohistochemistry and/or bone marrow flow cytometry
- Metaphase cytogenetics on bone marrow
- Plasma cell FISH[a] [del 13, del 17p13, t(4;14), t(11;14), t(14;16), t(14:20), 1q21 amplification], 1p abnormality

Genetic Studies?

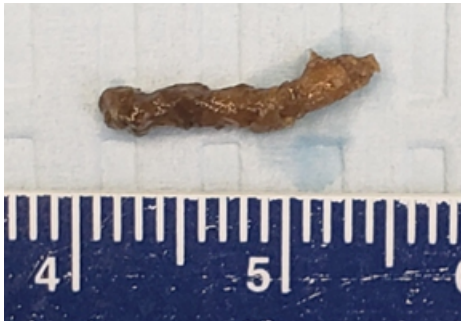
- Plasmacytoma biopsy – FFPE
  - Fresh tissue needed for karyotype
  - Myeloma FISH panel not validated on FFPE tissue
- Bone marrow biopsy performed… but is a dry tap
  - What do you do?



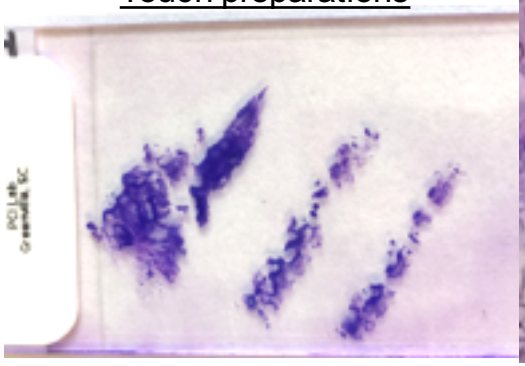
FFPE, formalin fixed paraffin embedded

Material Obtained From BM Biopsy

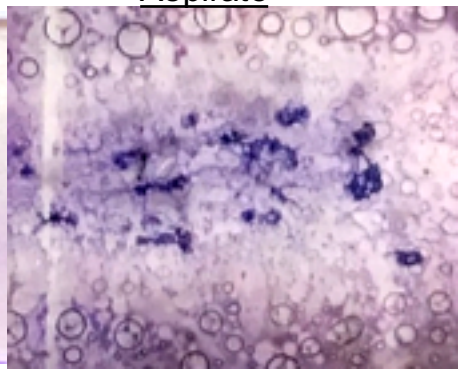
Core biopsy



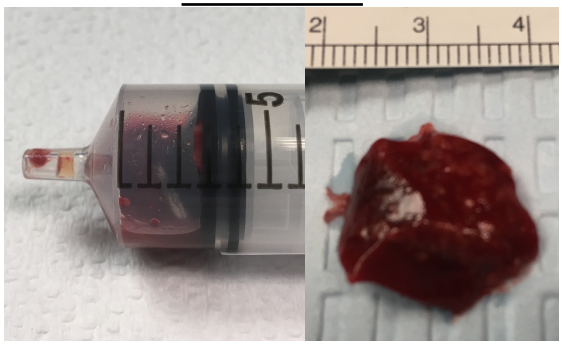
Touch preparations

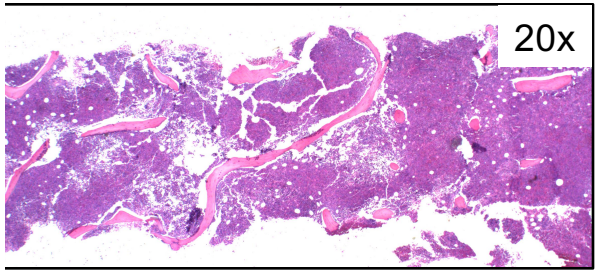


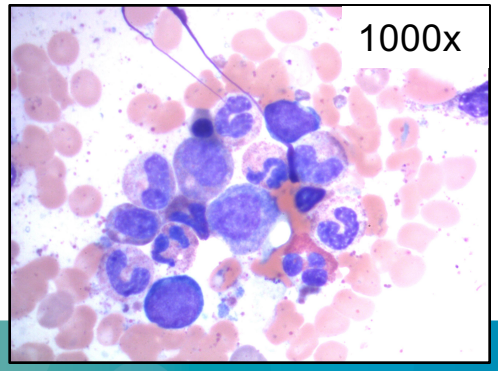
Aspirate

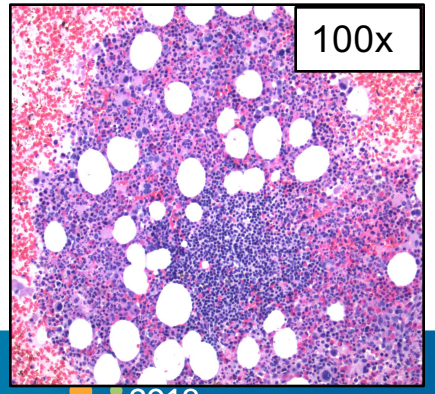


Clot section









Options to Get the Genetic Studies

- 2nd core fresh in RPMI (tissue culture media) or saline to disaggregate
  - Can be used for genetic studies…karyotype, FISH, and/or PCR
  - Could also can be used for flow cytometry
- Extra touch preparations
  - Can be used for FISH
- Peripheral blood sample if circulating plasma cells are present

Patient #2

Patient #2

- 81-year-old woman presented with SOB, weakness, and fatigue over the previous few weeks since returning from a trip to Italy, also had back/hip pain
- PMH includes osteoporosis and atrial fibrillation
- Medications: aspirin and clopidogrel

SOB = shortness of breath; PMH = past medical history.

Patient #2

- Sent for CTA – No PE but…
  - 1. Large retrocrural, retroperitoneal, and mesenteric lymph nodes, raising strong suspicion for lymphoma or unusual bulky metastatic adenopathy.
  - 2. Multiple hepatic lesions, likely malignant as well.
- X-ray of the right hip shows a pathologic fracture

CTA, computerized tomography angiogram; PE, pulmonary embolism

Patient #2: Plan

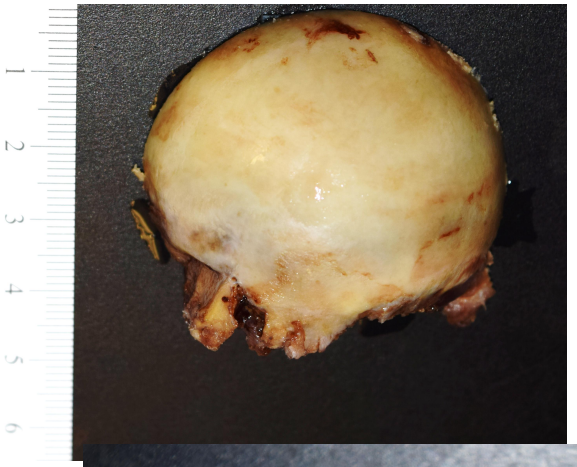
- Ortho: Hip arthroplasty and biopsy of a lymph node…but no lymph nodes in the operative field
- General surgery to biopsy one of the intra-abdominal nodes… but did not want to go into her abdomen if not necessary and suggested needle biopsy by interventional radiology (IR)
- IR will not do a biopsy because of the aspirin and clopidogrel

Patient #2: Diagnostic Material for Suspected Lymphoma?

- Oncologist calls pathology Friday afternoon to see if any diagnostic results would be available over the weekend in order to start treatment

Patient #2: Diagnostic Material for Suspected Lymphoma?

- Femoral head from the hip surgery performed that morning
  - Slides won't be available until Monday because of need for decalcification
  - Attempt flow cytometry from tissue dug out from the marrow

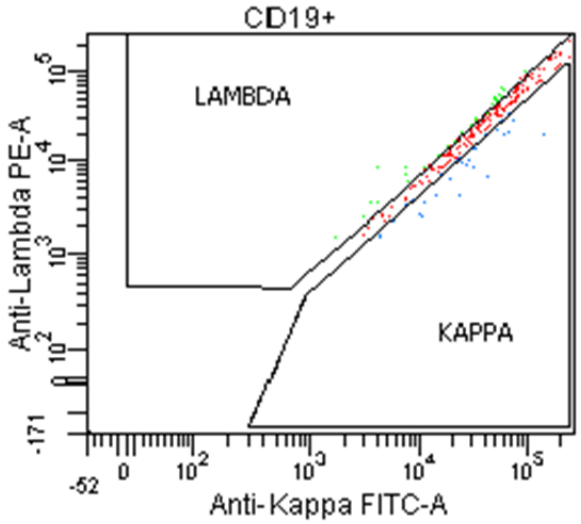


Patient #2: Diagnostic Material for Suspected Lymphoma?

- From peripheral blood flow cytometry
  - Yes, but unknown if peripheral blood is involved

CBC		
WBC	14.9	▲
RBC	4.43	
Hemoglobin	12.8	
Hematocrit	38.7	
MCV	87.4	
MCH	29.0	
MCHC	33.1	
RDW	13.3	
Platelets	448	▲
MPV	6.9	▼
Reticulocytes, %		
AUTO DIFF		
Neutrophils, %	74.8	▲
Lymphocytes, %	15.1	▼
Monocytes %	8.3	
Eosinophils %	0.9	
Basophils %	0.9	
Neutrophils, Abs	11.1	▲
Lymphocytes, Abs	2.3	
Monocytes Abs	1.2	▲
Eosinophils, Abs	0.1	
Basophils, Abs	0.1	

Patient #2: Femoral Head Flow Cytometry



FLOW CYTOMETRIC DIAGNOSIS:

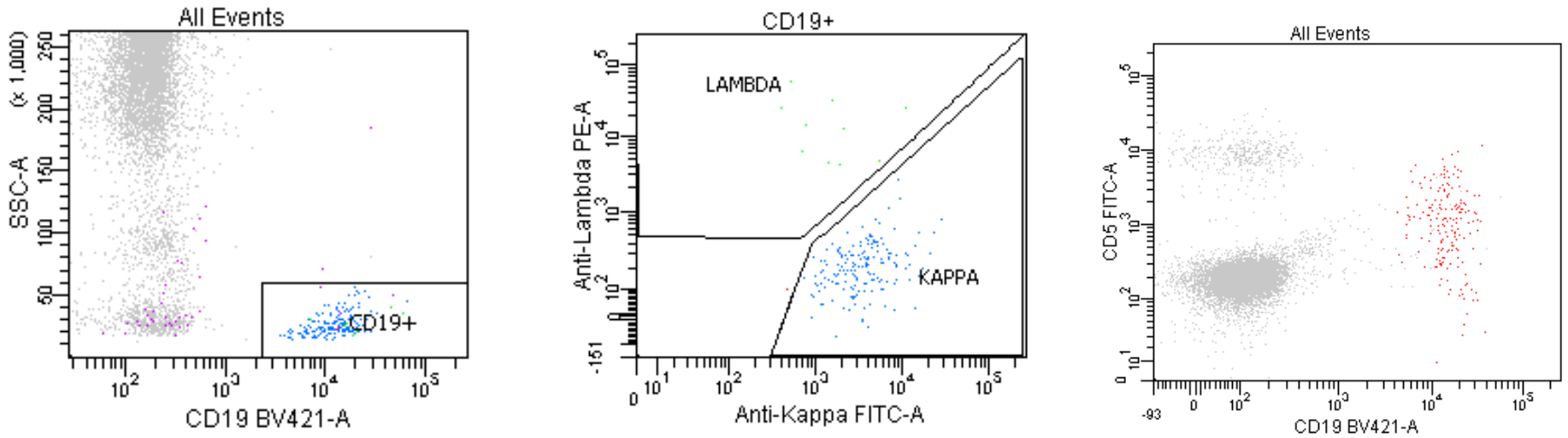
RIGHT FEMORAL HEAD, FLOW CYTOMETRY OF:

- **NO IMMUNOPHENOTYPICALLY ABNORMAL POPULATION DETECTED IN A LIMITED SPECIMEN.**
- **RARE ATYPICAL CELLS ON CYTOSPIN PREPARATION.**
- **SEE COMMENT.**

COMMENT:

No abnormal cells are detected by flow cytometric analysis. However, this absence does not exclude the possibility of lymphoma or malignancy. The analysis was limited by the bony nature of the specimen. Additionally, Hodgkin lymphoma, some large cell non-Hodgkin lymphomas, and non-hematopoietic malignancies are not analyzable by flow cytometry. Rare atypical cells are noted in the limited Cytospin preparation, the significance of which is uncertain, but correlation with the morphology is necessary.

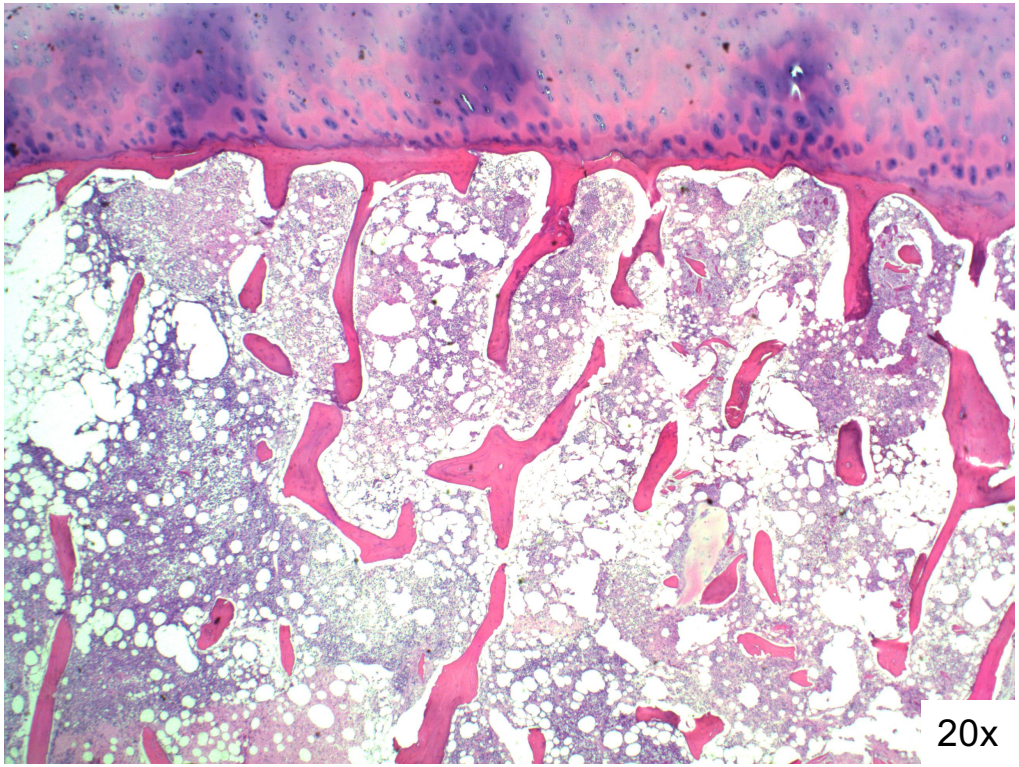
Patient #2: Peripheral Blood Flow Cytometry



1.8% small monoclonal B cells with CLL immunophenotype

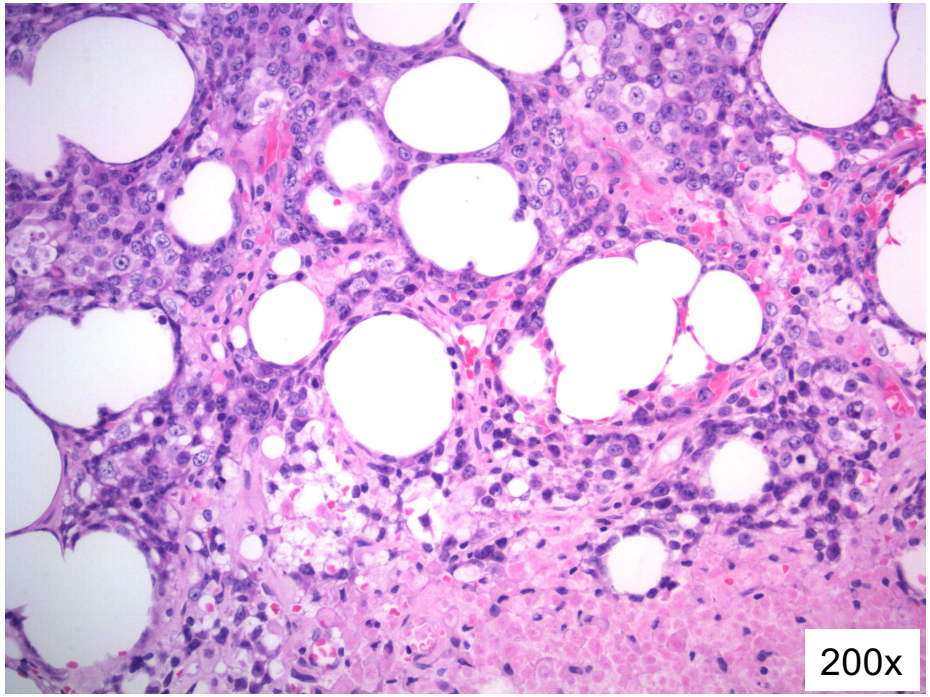
CLL, chronic lymphocytic leukemia

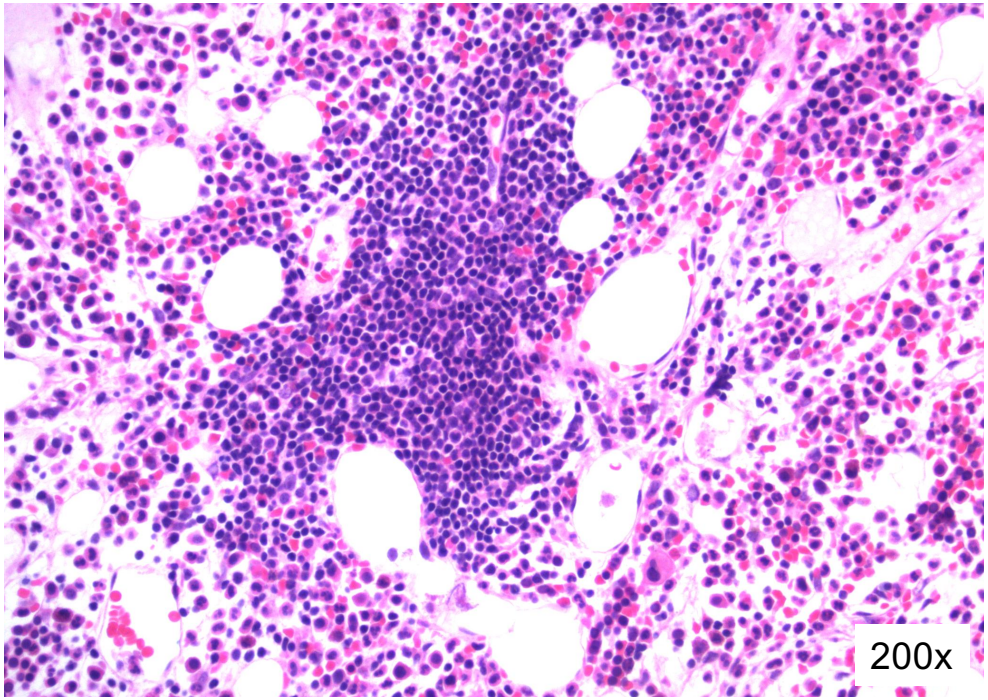
Patient #2: Femoral Head



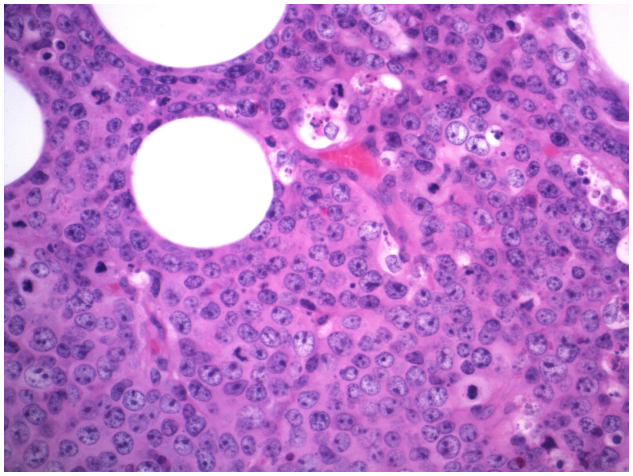
20x

Patient #2: Femoral Head Histology

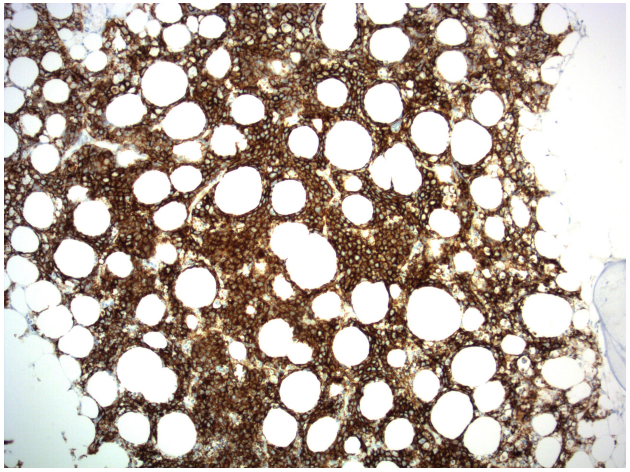




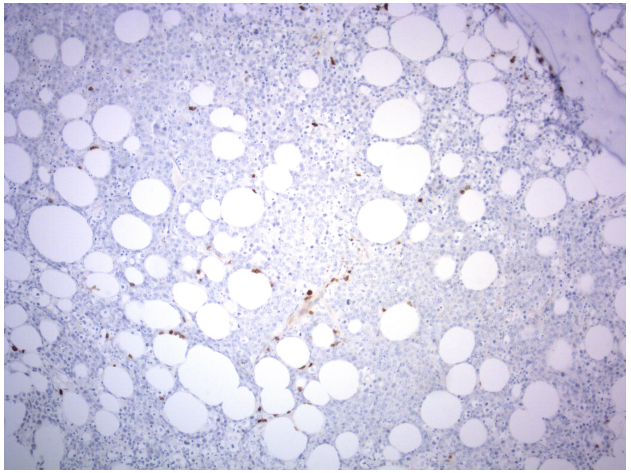
H&E 400x

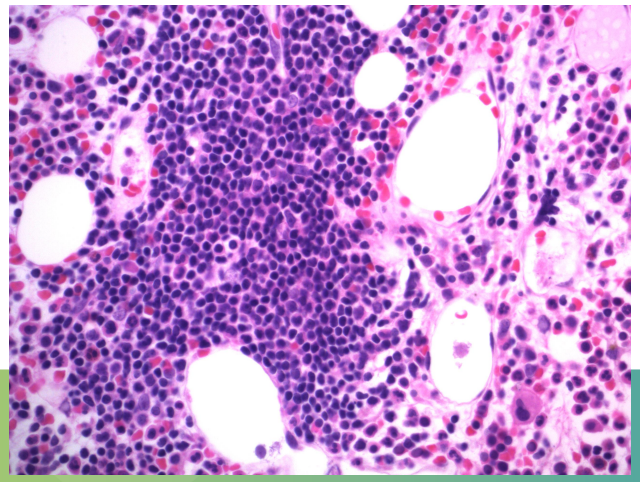


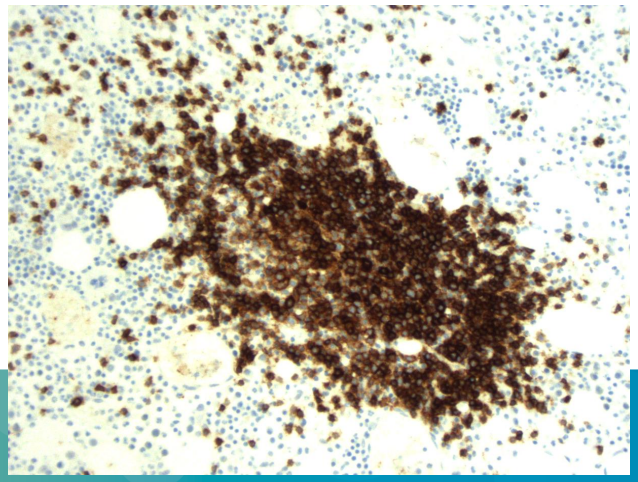
CD20

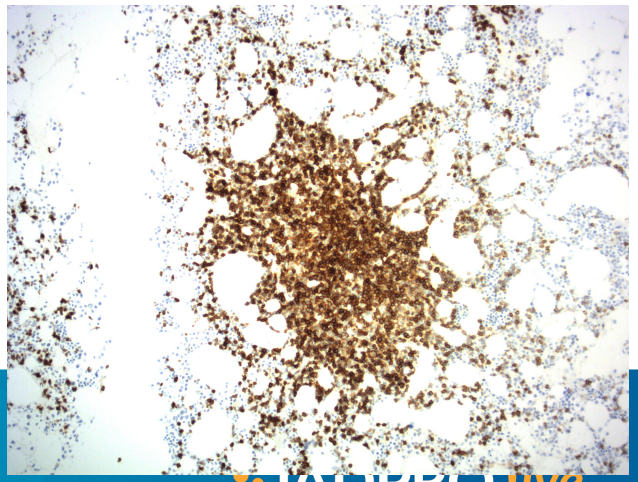


CD5









Patient #2

CLINICAL DATA/HISTORY:
Pre-op diagnosis: Pathologic fracture of femoral neck, right, initial encounter

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FINAL PATHOLOGIC DIAGNOSIS:
RIGHT FEMORAL HEAD, ARTHROPLASTY:

- **DIFFUSE LARGE B CELL LYMPHOMA, NON-GERMINAL CENTER TYPE (ACTIVATED B CELL TYPE).**
- **CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA.**

Approach to High-Grade B-Cell Lymphomas

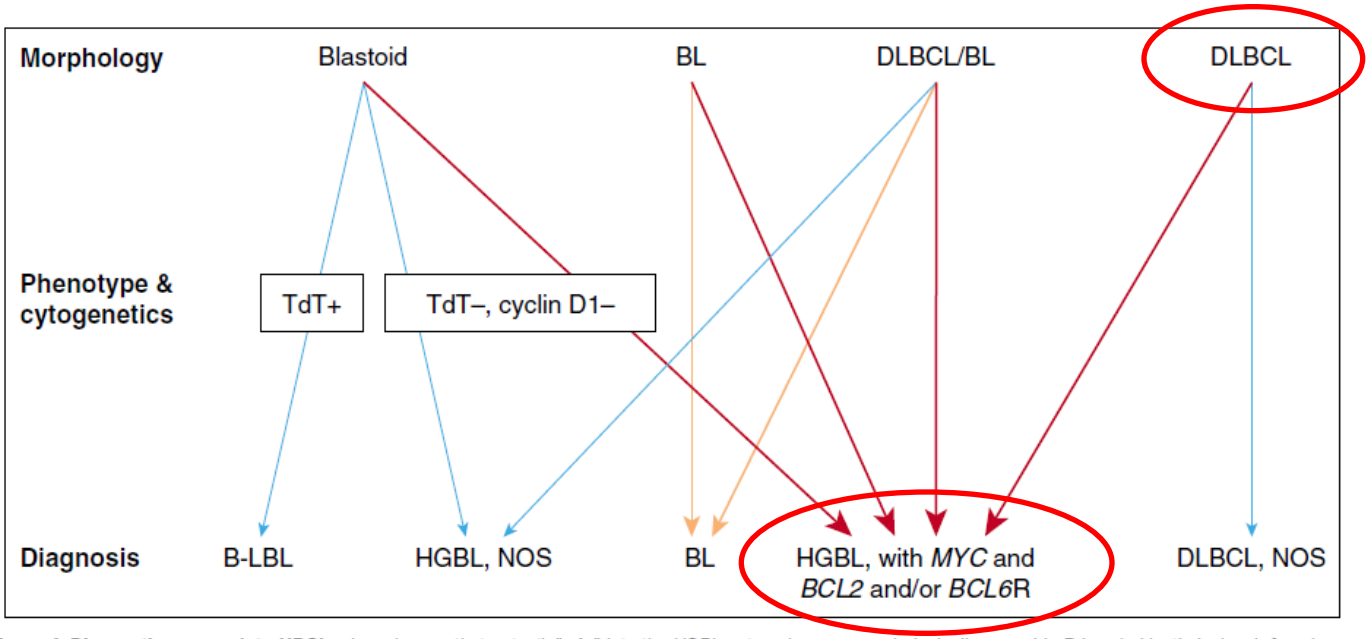
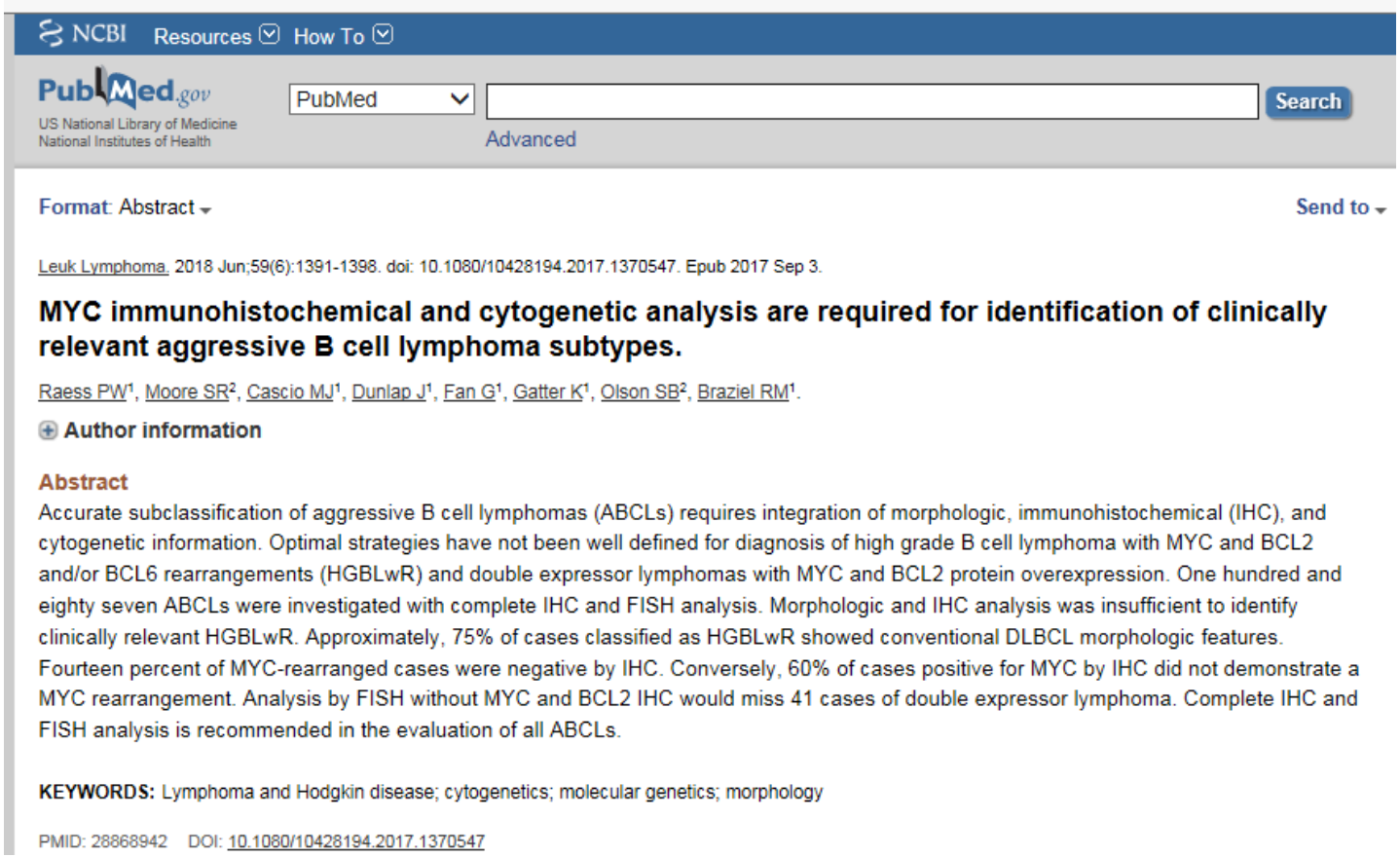


Figure 4. Diagnostic approach to HBCLs. Lymphomas that potentially fall into the HGBL categories can morphologically resemble B-lymphoblastic leukemia/lymphoma (B-LBL), BL, and DLBCL as well as lymphomas that are intermediate between DLBCL and BL (DLBCL/BL). These distinctions can be very subjective. The orange arrows indicate cases with a BL phenotype and a *MYC* rearrangement without *BCL2* or *BCL6* rearrangements ("single hit"). The red arrows indicate cases with *MYC* and *BCL2* and/or *BCL6* rearrangements ("double or triple hit"). Neither MCLs, subtypes of LBCLs, nor Burkitt-like lymphoma with 11q aberration are indicated in this diagram. Adapted from Kluin et al[89] with permission. Professional illustration by Patrick Lane, ScEYEnce Studios.

HBCL, high grade B cell lymphoma; B-LBL, B lymphoblastic lymphoma; BL, Burkitt lymphoma; DLBCL, diffuse large B cell lymphoma.



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Leuk Lymphoma. 2018 Jun;59(6):1391-1398. doi: 10.1080/10428194.2017.1370547. Epub 2017 Sep 3.

MYC immunohistochemical and cytogenetic analysis are required for identification of clinically relevant aggressive B cell lymphoma subtypes.

Raess PW[1], Moore SR[2], Cascio MJ[1], Dunlap J[1], Fan G[1], Gatter K[1], Olson SB[2], Braziel RM[1].

Author information

Abstract

Accurate subclassification of aggressive B cell lymphomas (ABCLs) requires integration of morphologic, immunohistochemical (IHC), and cytogenetic information. Optimal strategies have not been well defined for diagnosis of high grade B cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements (HGBLwR) and double expressor lymphomas with MYC and BCL2 protein overexpression. One hundred and eighty seven ABCLs were investigated with complete IHC and FISH analysis. Morphologic and IHC analysis was insufficient to identify clinically relevant HGBLwR. Approximately, 75% of cases classified as HGBLwR showed conventional DLBCL morphologic features. Fourteen percent of MYC-rearranged cases were negative by IHC. Conversely, 60% of cases positive for MYC by IHC did not demonstrate a MYC rearrangement. Analysis by FISH without MYC and BCL2 IHC would miss 41 cases of double expressor lymphoma. Complete IHC and FISH analysis is recommended in the evaluation of all ABCLs.

KEYWORDS: Lymphoma and Hodgkin disease; cytogenetics; molecular genetics; morphology

PMID: 28868942 DOI: 10.1080/10428194.2017.1370547

Patient #2: Options to Get the Genetic Studies

- Femoral head: FISH studies not possible as it was decalcified
  - Decalcification is an acid based process that denatures DNA
- Peripheral blood: Only involved by low level CLL
- Bone marrow biopsy
  - Send the aspirate or clot material for FISH studies (double/triple hit FISH is validated on FFPE)
  - May not be involved
- Lymph node open biopsy
- Options during hip surgery
  - Aspirate of the femoral head by orthopedic surgeon
  - Bone marrow biopsy while in surgery for staging for presumed lymphoma

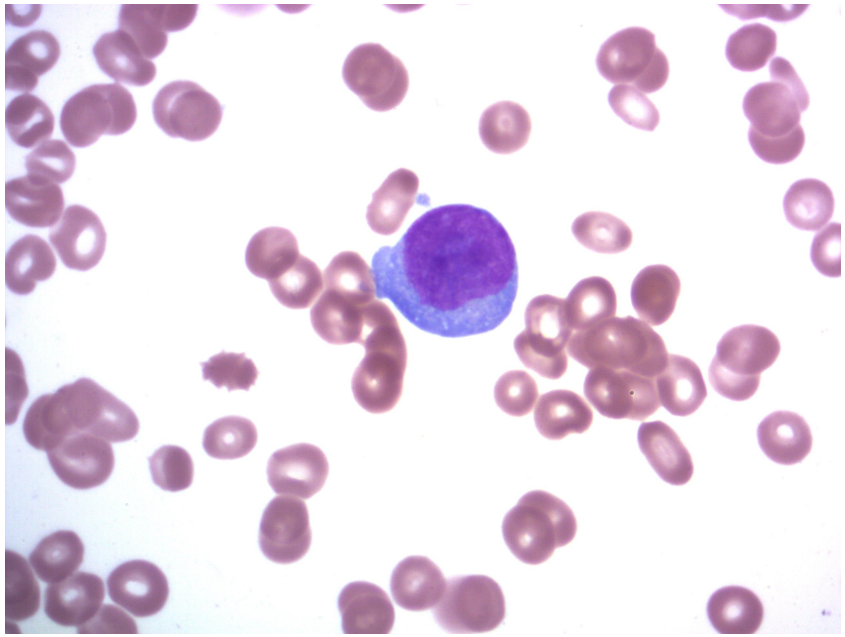
Patient #3

Patient #3

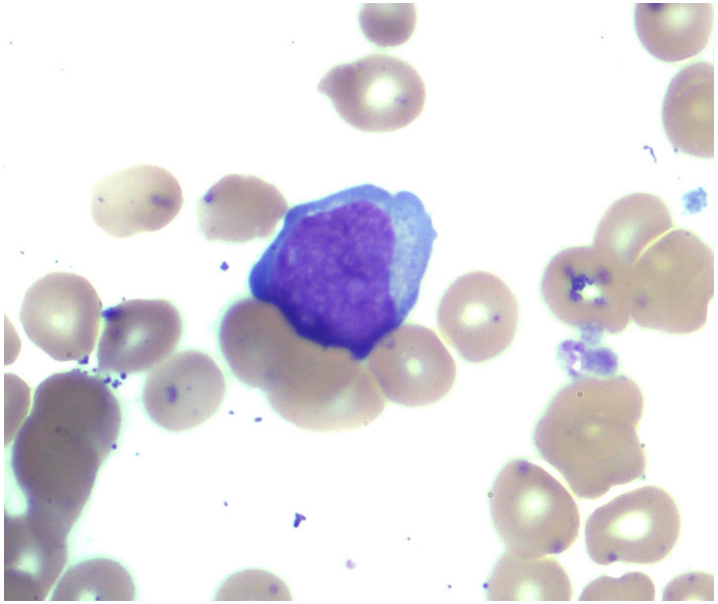
- 68-year-old man admitted to GI service with concern for GI bleed after being found to have a hemoglobin of 5.7 g/dL at an urgent care clinic
- Had presented with a history of fatigue, worsening over the past month
- PMH of diabetes, depression, and chronic kidney disease
- Colonoscopy a few months before was normal, EGD in the hospital was normal

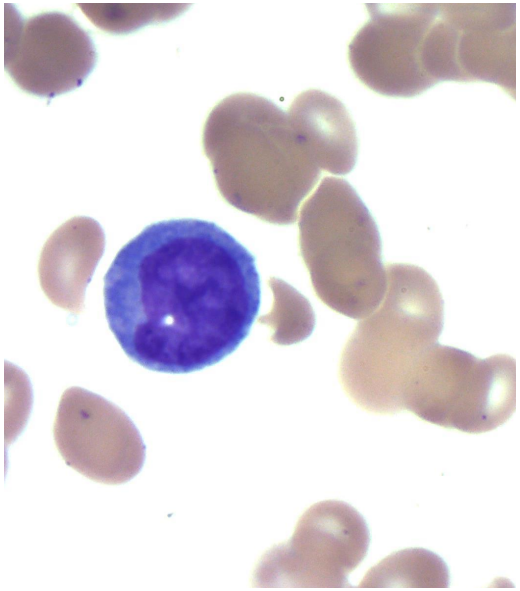
Patient #3: Peripheral Blood

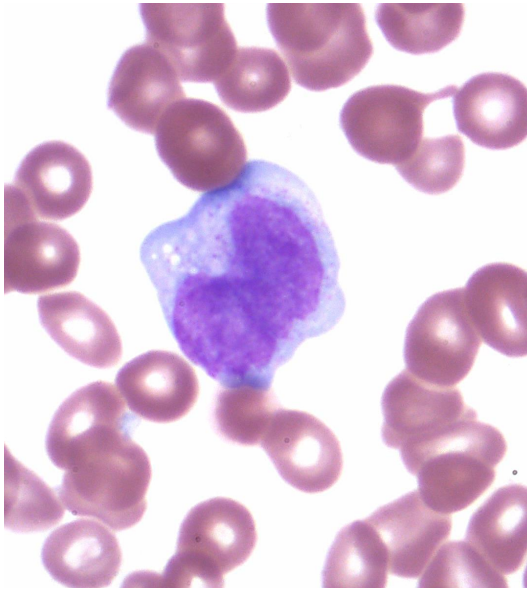
WBC	4.0 - 11.5 TH/mm3	2.3 ↓
RBC	4.50 - 5.90 Mil/mm3	2.66 ↓
Hemoglobin	13.5 - 17.5 g/dL	7.4 ↓
Hematocrit	41.0 - 53.0 %	21.1 ↓
MCV	80.0 - 100.0 fL	79.1 ↓
MCH	26.0 - 34.0 pg	27.8
MCHC	31.0 - 37.0 g/dL	35.2
RDW	12.0 - 16.0 %	19.3 ↑
Platelets	125 - 415 Th/mm3	84 ↓
MPV	6.9 - 10.6 fL	8.6
Neutrophils, %	39 - 69 %	7 ↓
Lymphocytes, %	24 - 44 %	79 ↑
Monocytes, %	2 - 10 %	12 ↑
Blasts, %	<=0 %	2 ↑



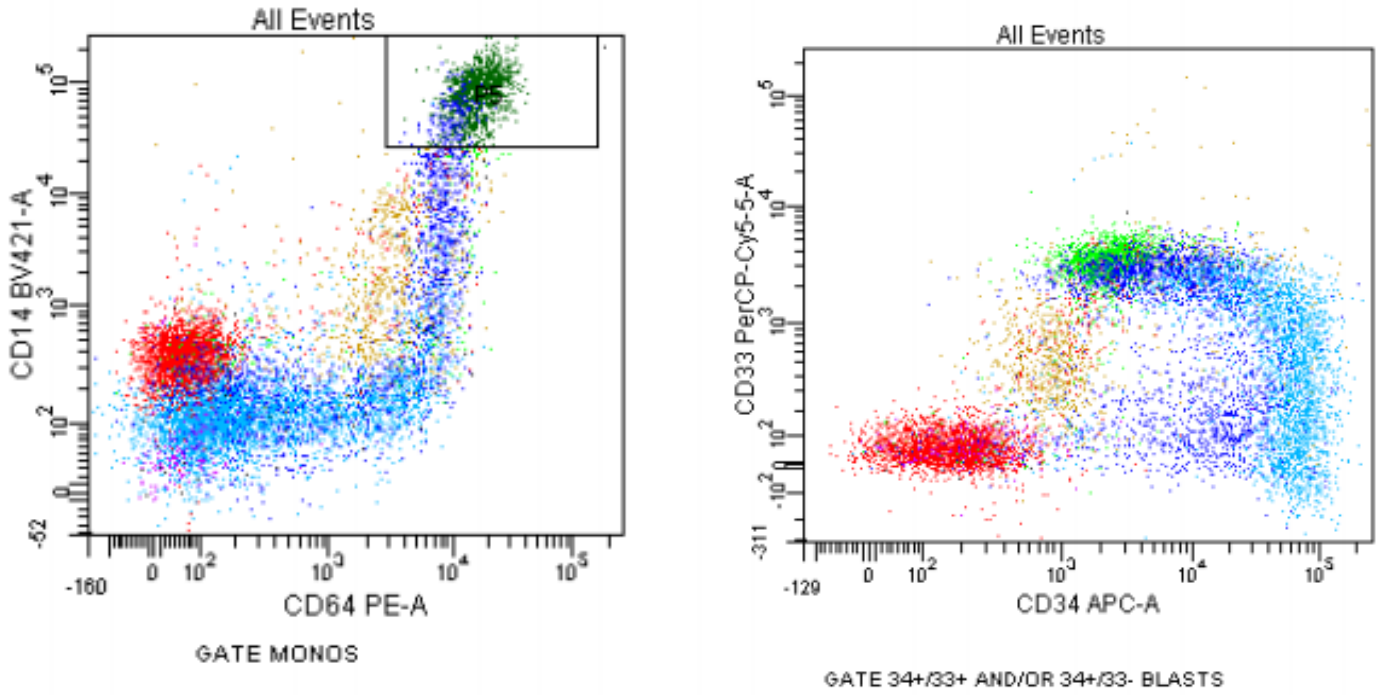
Patient #3: Bone Marrow: Acute Myeloid Leukemia With Monocytic Differentiation







Patient #3: Bone Marrow



Light blue and dark blue is normal maturation pattern of monocytes. Green is mature monocytes. Gold is granulocytes. Red is lymphocytes.

WHO Classification of AML

AML is a complex, dynamic disease, characterized by multiple somatically acquired driver mutations, coexisting competing clones, and disease evolution over time.

- **AML with recurrent genetic abnormalities**
  - 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
  - APL with PML-RARA
  - AML with t(9;11)(p21.3;q23.3); MLLT3-KMT2A
  - 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;q13.3); RBM15-MKL1
  - Provisional entity: AML with *BCR-ABL1*
  - AML with mutated *NPM1*
  - AML with biallelic mutations of *CEBPA*
  - Provisional entity: AML with mutated *RUNX1*
- **AML with myelodysplasia-related changes**
- **Therapy-related myeloid neoplasms**
- **AML, not otherwise specified**
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation
  - Acute myelomonocytic leukemia
  - Acute monoblastic/monocytic leukemia
  - Pure erythroid leukemia
  - Acute megakaryoblastic leukemia
  - Acute basophilic leukemia
  - Acute panmyelosis with myelofibrosis

WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue; 2017.

NCCN Guidelines Version 2.2018
Acute Myeloid Leukemia

EVALUATION FOR ACUTE LEUKEMIA

- History and physical (H&P)
- Complete blood cell (CBC) count, platelets, differential, comprehensive metabolic panel, uric acid, lactate dehydrogenase (LDH)
- Prothrombin time (PT), partial thromboplastin time (PTT), fibrinogen
- Bone marrow core biopsy and aspirate analyses, including immunophenotyping and cytochemistry
- Cytogenetic analyses (karyotype ± FISH)
- Molecular analyses (KIT, FLT3 [ITD and TKD], NPM1, CEBPA, IDH1, IDH2, TP53, and other mutations)[a]
- Human leukocyte antigen (HLA) typing for patient with potential hematopoietic cell transplantation (HCT) in the future (except for patients with a major contraindication to HCT)
- CT of brain without contrast, if CNS hemorrhage suspected[b]
- Brain MRI with contrast, if leukemic meningitis suspected[b]
- PET/CT, if clinical suspicion for extramedullary disease
- Lumbar puncture (LP), if symptomatic[b] (category 2B for asymptomatic)
- Evaluate myocardial function (echocardiogram or MUGA scan) in patients with a history or symptoms of cardiac disease or prior exposure to cardiotoxic drugs or radiation to thorax
- Central venous access device of choice

FOOTNOTES FOR EVALUATION FOR ACUTE LEUKEMIA

[a]A variety of gene mutations are associated with specific prognoses (category 2A) and may guide medical decision making (category 2B) (See AML-A). Currently, c-KIT, FLT3-ITD, FLT3-TKD, NPM1, CEBPA, IDH1/IDH2, and TP53 are included in this group; however, this field is evolving rapidly. While the above mutations should be tested in all patients, multiplex gene panels and next-generation sequencing analysis may be used to obtain a more comprehensive prognostic assessment (Papaemmanuil E, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med 2016;374:2209-2221). The information obtained may have prognostic impact in AML, may influence medical decision making regarding consolidation with chemotherapy versus an allogeneic hematopoietic stem cell transplant, or determination for eligibility for clinical trial participation (see Discussion). If a test is not available at your institution, consult pathology prior to performing the marrow evaluation about preserving material from the original diagnostic sample for future testing at an outside reference lab. Circulating blasts from peripheral blood may alternatively be used to detect molecular abnormalities in patients with a minimum of 10% involvement by the myeloid neoplasm to prevent false-negative results.

NGS Considerations

- What are diagnostically significant genetic mutations?
- What are prognostically significant genetic mutations?
- What are therapeutically significant genetic mutations?
- Can the detected mutations be used to track MRD?

- What is the turnaround time?
- What specimen can I send?
- Will the patient's insurance pay for the testing?

NGS, next generation sequencing; MRD, minimal residual disease.

Test name (Lab)	Genes tested	TAT (specimen)
NeoTYPE AML Prognostic Profile (NeoGenomics)	28 genes - ASXL1, BCOR, BRAF, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, FLT3, HRAS, IDH1, IDH2, JAK2 including V617F and Exons 12+14, KIT, KMT2A (MLL), KRAS, NPM1, NRAS, PDGFRA, PHF6, PTPN11, RUNX1, SETBP1, STAG2, TET2, TP53 and WT1	14 days (PB, BM, FFPE)
IntelliGEN Myeloid (Lab Corp)	50 genes - ABL1, ASXL1, BCOR, BCORL1, BRAF, CALR, CBL, CDKN2A, CEBPA, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, IDH1, IDH2, IKZF1, JAK2, JAK3, KDM6A, KIT, MKT2A, KRAS, MPL, NF1, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PML, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TAT2, TP53, U2AF1, WT1, ZRSR2	Not provided (PB, BM)
Myeloid Molecular Profile (Genoptix)	44 genes - ASXL1, BCOR, BRAF, CALR, CBL, CEBPA, CSF3R, DDX41, DNMT3A, ETNK1, ETV6, EZH2, GATA2, GNAS, GNB1, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NF1, NPM1, NRAS, PDGFRA, PHF6, PPM1D, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SH2B3, SMC1A, SMC3, SRSF2, STAG2, STAT3, STAT5B, TET2, TP53, U2AF1, WT1, ZRSR2	10-12 days (PB, BM)
FoundationOneHeme (Foundation Medicine)	>400 genes	2 weeks (PB, BM, FFPE)

NGS Considerations

- What are diagnostically significant genetic mutations?
  - Some mutations define subtypes of AML, e.g., t(15;17)
  - Some mutations are associated with clonal hematopoiesis of indeterminate potential (CHIP), e.g., DNMT3A, TET2, ASXL1

NGS Considerations

- What are prognostically significant genetic mutations?
  - How does karyotype affect prognostic significance?
  - What do combinations of different mutations mean?
  - What is significant allele frequency?

NGS Considerations

- What are therapeutically significant genetic mutations?
  - Is there a specific drug available for a specific mutation?
  - Does turnaround time affect ability to act on mutations?
  - Will this make the patient eligible for clinical trial?
  - What is significant allele frequency?

NGS Considerations

- Can the detected mutations be used to track MRD?
  - Some patients don't have a detectable mutation
  - Some mutations can (NPM1) and some mutations can't (FLT3)
    - Look at whole panel
  - What is significant allele frequency?
    - Established limit of detection of 5-10% allele frequency
  - When is the right time to test?
    - Well defined in APL to look for t(15;17) after consolidation
    - ~1/3 of D14 with MRD will be negative later with no additional treatment
  - Some patients with MRD don't relapse, some patients relapse who don't have detectable MRD
  - Does change in treatment based on finding MRD change disease outcome?
  - Lab variance

NEJM 2018;378:1189-99; Curr Hematol Malig Rep 2017;12:547-56.

Patient #3: Genetic Results

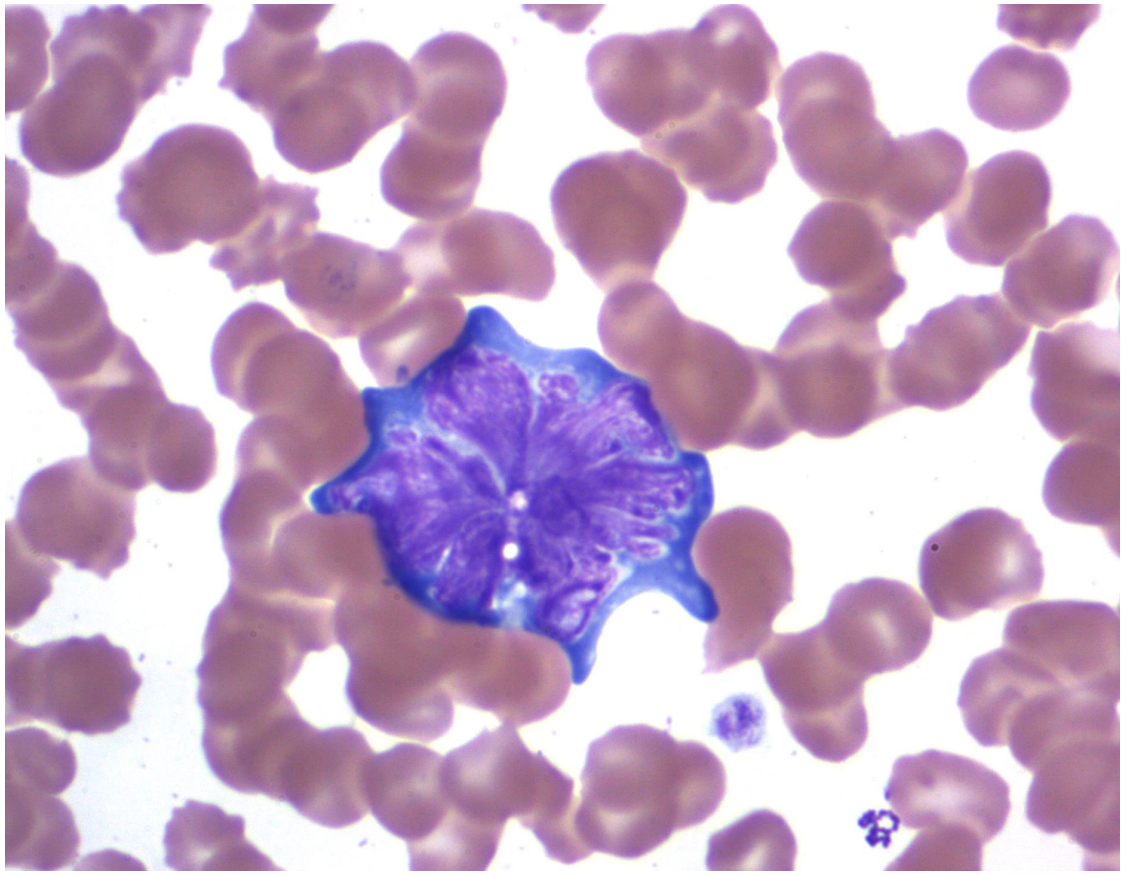
- NGS panel: No mutations detected
  - Tested for FLT3, CEBPA, IDH1, IDH2, NPM1, TP53, KRAS, NRAS
  - **Note:** This finding does not exclude the presence of genetic alteration occurring at allele frequency below our established detection limit of 5-10%, or other genetic alterations present in untested gene regions.

Patient #3: Genetic Results

- FLT3 mutation analysis: Suspicious for low level FLT3-ITD mutation (mutated to un-mutated allelic ratio <0.05).
  - Note: This FLT3-ITD result is detected below the established reproducible limit of assay sensitivity.
- Karyotype: 45,XY,-7[3] 45,X,-Y[16] 46,XY[1]
  - Monosomy 7: poor prognostic finding
  - Loss of Y: typically considered age related

Ancillary Testing in Hematopoietic Neoplasms

- What information will affect diagnosis, prognosis, treatment for the patient?
- What specimen is needed in order to get that information?
- This field is evolving rapidly.
- Keep an open conversation with pathology.



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