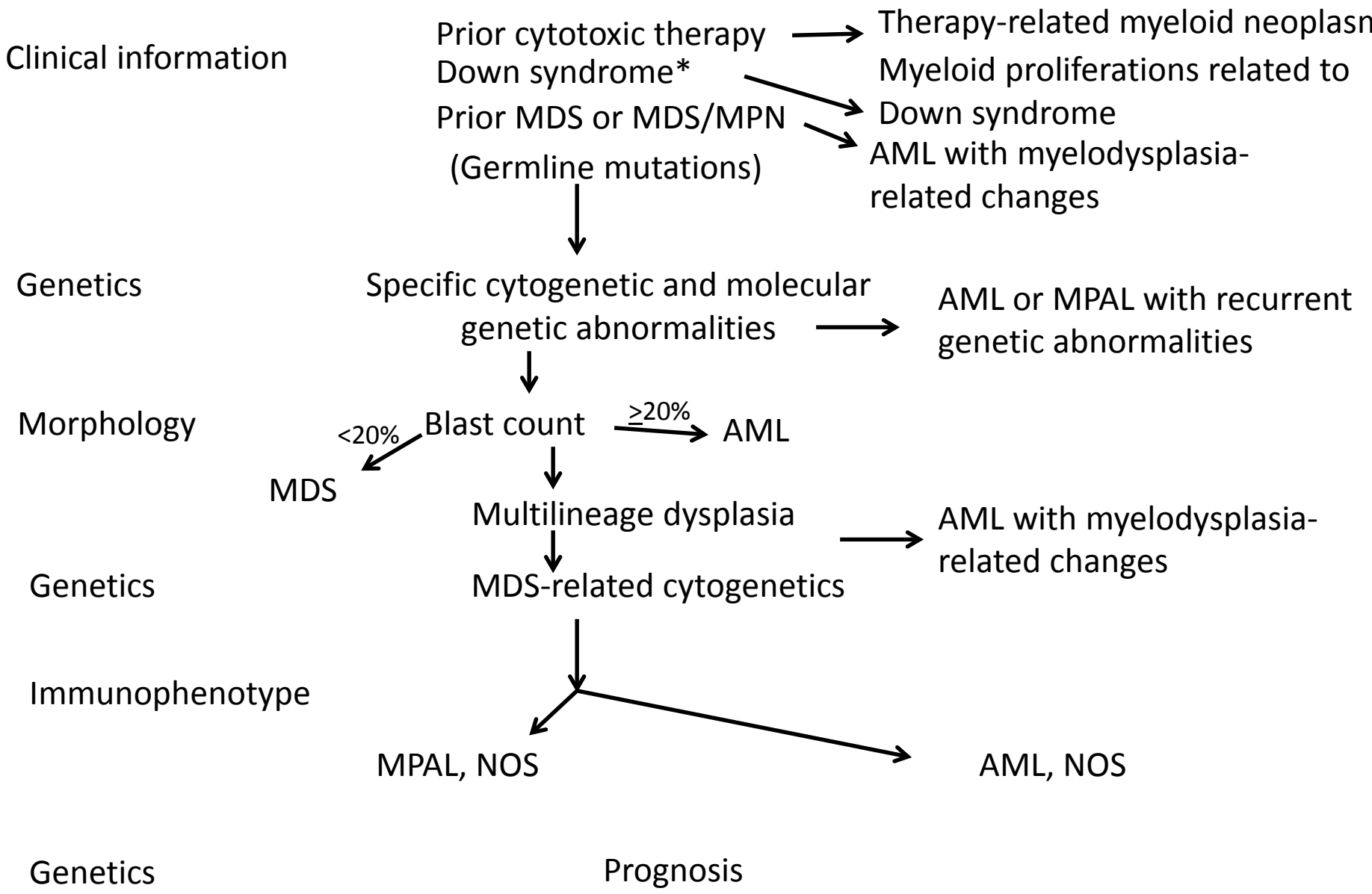
Genetic Testing in Diagnosis of Acute Leukemias: Summary

Daniel A. Arber, MD
University of Chicago
Marian H. Harris, MD, PhD
Boston Children's Hospital

The Diagnosis of Acute Leukemia

- Genetics play an increasingly important role in the diagnosis of acute leukemia
- But,
- An accurate diagnosis using the WHO classification requires more than just genetics:
  - Clinical information
  - Morphology
  - Immunophenotype
  - Cytogenetics
  - Molecular genetics

Diagnostic Hierarchy of Acute Myeloid (and Mixed Phenotype) Leukemias

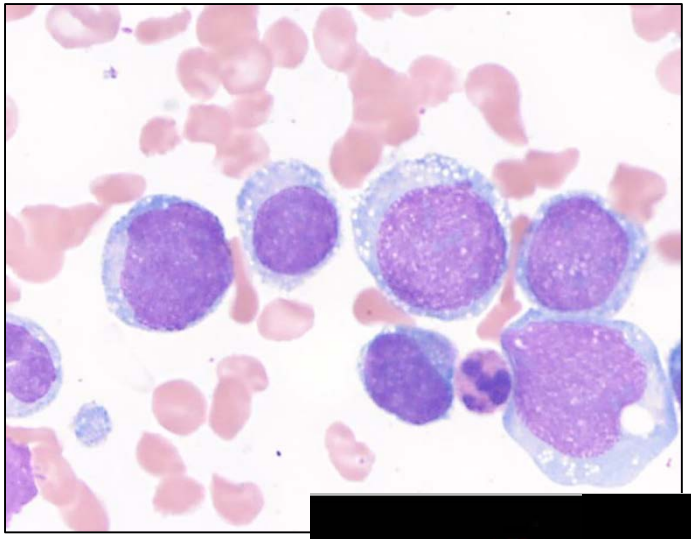


AML with Recurrent Genetic Abnormalities

Case #	Contributor	Panel Diagnosis	
SH2017-0150	M. Vasef University of New Mexico	APL with *PML-RARA*	Cytogenetic and FiSH negative, but PCR positive
SH2017-0180	**C.C. Yin** **MD Anderson Cancer Center**	**APL with variant *RARA* rearrangement (*IRF2BP2-RARA*)**	
SH2017-0118	R. Juskevicius Vanderbilt University Medical Center	APL with variant *RARA* rearrangement (*ZBTB16-RARA*)	
SH2017-0262	V. Reddy University of Alabama at Birmingham	1. AML with t(8;21) 2. CLL	
SH2017-0290	H. Yu VA Boston Healthcare System	AML with inv(3)	Myeloid and T markers
SH2017-0291	M. Menon Henry Ford Health System	AML with *BCR-ABL1*	
SH2017-0299	**A. Vogel** **Thomas Jefferson University**	**AML with *BCR-ABL1***	
SH2017-0335	S. Garces MD Anderson Cancer Center	AML with *BCR-ABL1*	Acquired
SH2017-0053	H. Kurt MD Anderson Cancer Center	AML with mutated *RUNX1*	Aberrant CD79A and TdT (subset)
SH2017-0164	G. Mikita Weill Cornell Medicine	AML with mutated *RUNX1*	Ambiguous immunophenotype and myeloid genetic mutations.
SH2017-0281	**D. Yang** **University of Wisconsin**	**AML with mutated *RUNX1***	**Other co-mutations**
SH2017-0313	A. Quesada MD Anderson Cancer Center	AML with mutated *RUNX1*	Salmon pink granules
SH2017-0370	S. Jain University of Pittsburgh	AML with mutated *RUNX1*	

AML with *BCR-ABL1*

- Difficult to distinguish from myeloid blast crisis of chronic myeloid leukemia
- Deletion of antigen receptors (IGH, TCR), *IKZF1* and/or *CDKN2A* may support a diagnosis of de novo disease
- Patients may benefit from targeted (TKI) therapy
- New provisional entity

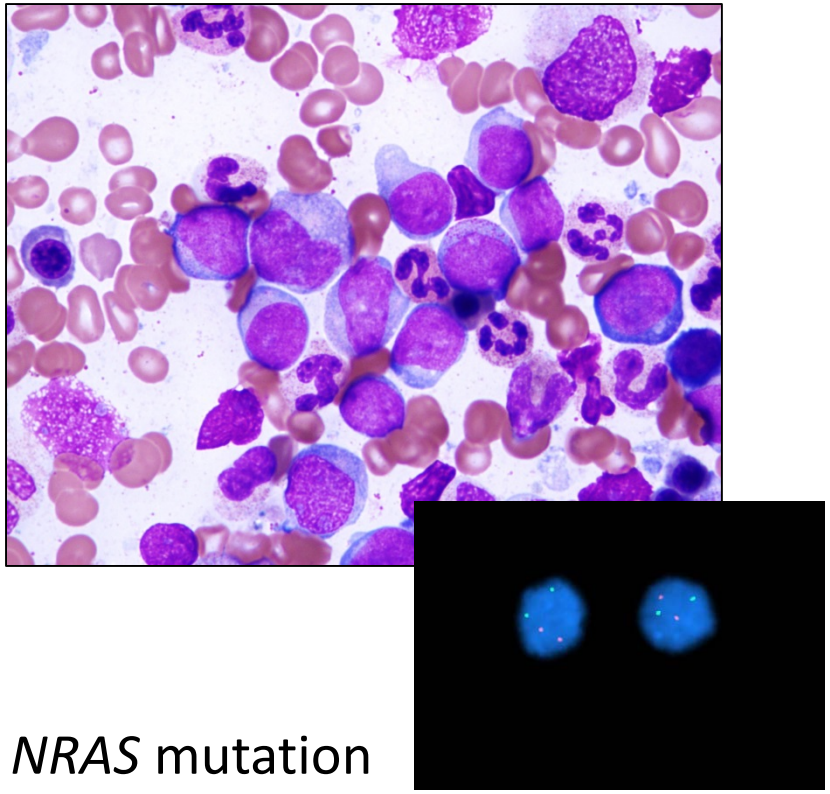


Case 299
A. Vogel

Soupir CP, et al. Am J Clin Pathol 127:642, 2007
Konoplev S, et al. Leuk Lymphoma 54:138, 2013
Nacheva EP, et al. Br J Haematol 161:541, 2013

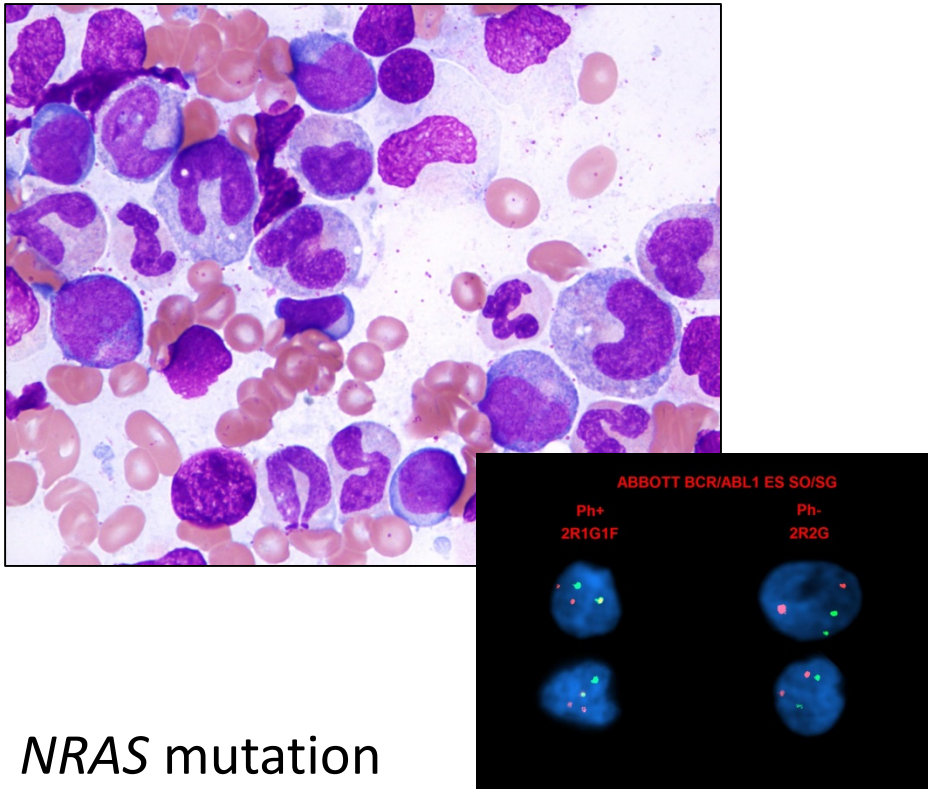
AML with Acquired *BCR-ABL1*

Initial Marrow



NRAS mutation
BCR-ABL1 negative

Refractory AML



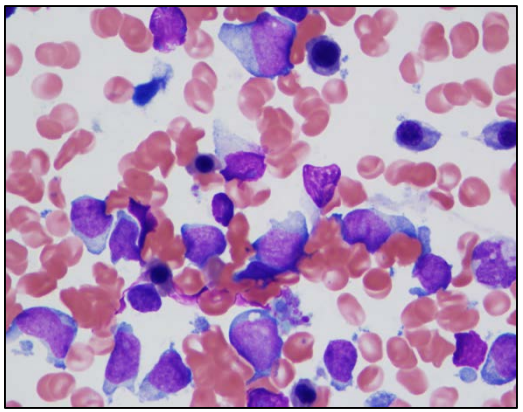
NRAS mutation
BCR-ABL1 positive

Case 335
S. Garces

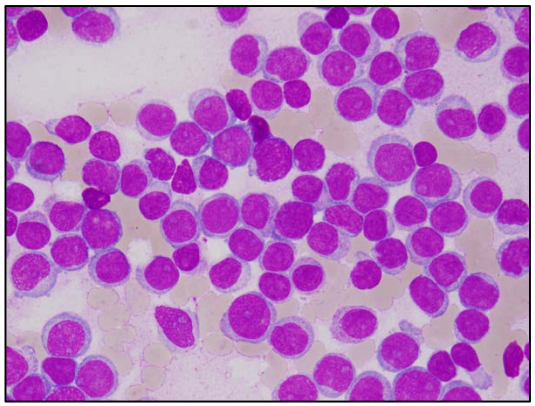
AML with mutated *RUNX1*

- Gene located at 21q22
- Encodes the alpha subunit of the core binding factor
- Mutation in 12.5-13.2% of AML
- More frequent in older male patients
- Frequent prior history of MDS, or prior exposure to radiation
- Frequent among FAB M0 cases, but wide morphologic spectrum
- Frequently associated *KMT2A*-PTD, *IDH1, IDH2* or *ASXL1* mutations
- Rare *CEBPA* or *NPM1* mutations
- Poor response to therapy with shortened survival
- Germline mutations should be evaluated

Case 53
H. Kurt
CD79A/TdT



Case 281
D. Yang
Mutations of
U2AF1, *WT1*,
PHF6, and *FLT3*



Tang et al. Blood 114:5352, 2009
Mendler et al. J Clin Oncol 30:3109, 201

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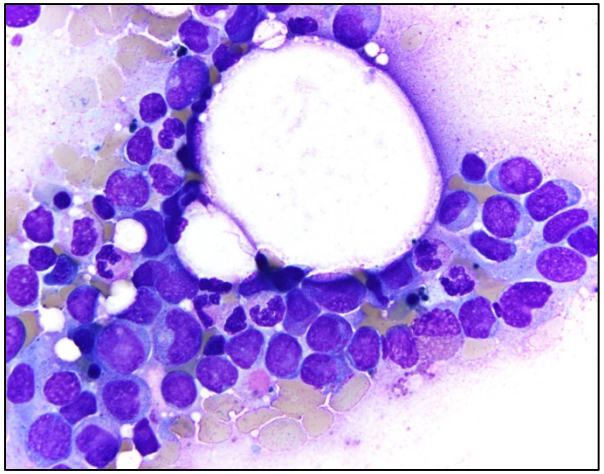
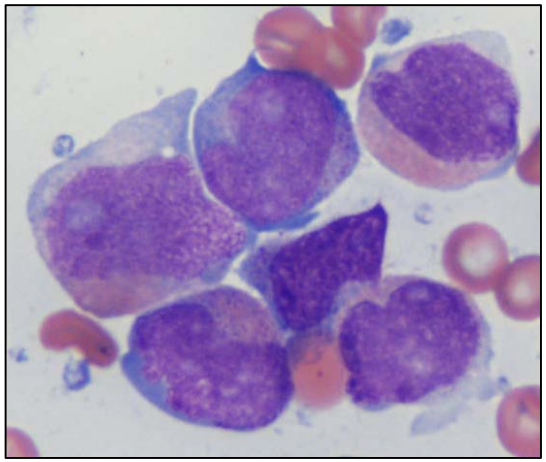
Case 370
S. Jain
IDH1 and *ASXL1* mutations

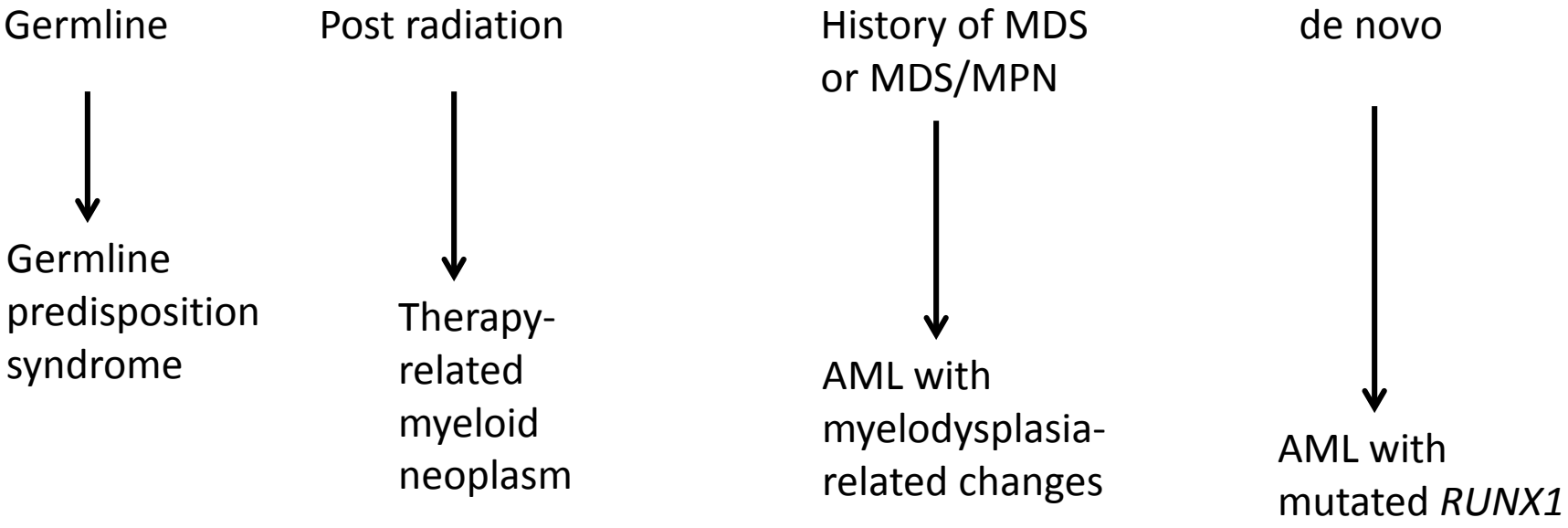


Case 313
A. Quesada



Tang et al. Blood 114:5352, 2009
Mendler et al. J Clin Oncol 30:3109, 2012

Mutations in *RUNX1*



Other AML Types

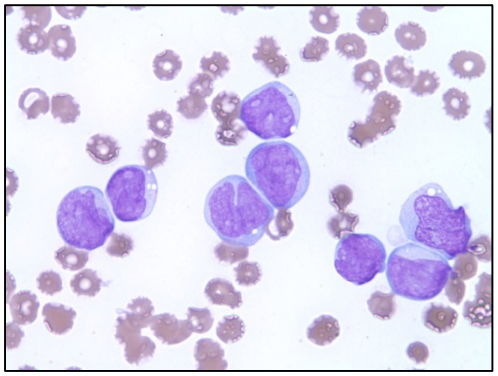
Case #	Contributor	Panel Diagnosis	
SH2017-0024	N. Baste University of Oklahoma Health Sciences Center	AML-MRC	Normal karyotype; *ASXL1* mutation; history of aCML
SH2017-0054	B. Chen UMass Memorial Medical Center	AML-MRC	i(17q); *FLT3*-ITD, *IDH2*, *NRAS* and *BCOR* mutations
SH2017-0107	C. Liu New York University	AML-MRC	Diagnosis limited by biopsy only; CD34 negative; complex karyotype
SH2017-0201	M. Jan Massachusetts General Hospital	AML-MRC	Complex karyotype that included t(4;17); *RARA* translocation excluded
SH2017-0325	M. Foshat University of Texas Health San Antonio	AML-MRC	High WBC, MLD, splenomegaly, marrow fibrosis, *CALR* mutation. AML vs BC of MPN
SH2017-0128	M. Yabe Memorial Sloan Kettering Cancer Center	1. Therapy-related myeloid neoplasm 2. Recurrent B-ALL	10% total blasts reported; *KMT2A* translocation detected in myeloid component only
SH2017-0288	R. Crotty Massachusetts General Hospital	Therapy-related myeloid neoplasm (AML)	PEL with *ZYMA-REL* rearrangement
SH2017-0323	C. Cotta Cleveland Clinic	AML with t(8;21); favor therapy-related	3 years post lenalidomide therapy for myeloma
SH2017-0328	**K. Holder** **University of Texas Health San Antonio**	**Therapy-related myeloid neoplasm (AML) with t(9;11)**	**Post therapy for APL**
SH2017-0276	N. Patel Columbia University	AML-NOS	Morphologic features of megakaryocytic lineage

Other AML Types

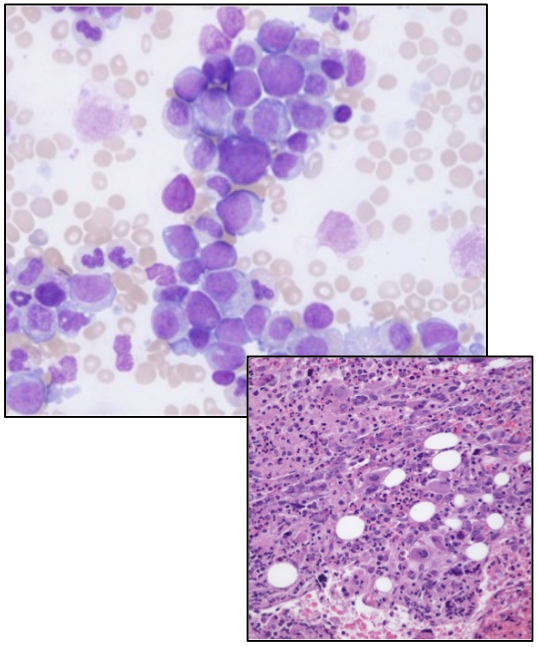
AML with myelodysplasia-related changes

- May arise from MDS or MDS/MPN
- Recurring cytogenetic abnormalities not included in AML RGA are rare, but do occur
- Revised 2016 WHO criteria have de novo cases with *NPM1* or biallelic *CEBPA* mutations trumping this category

Case 54
B. Chen
i(17q)



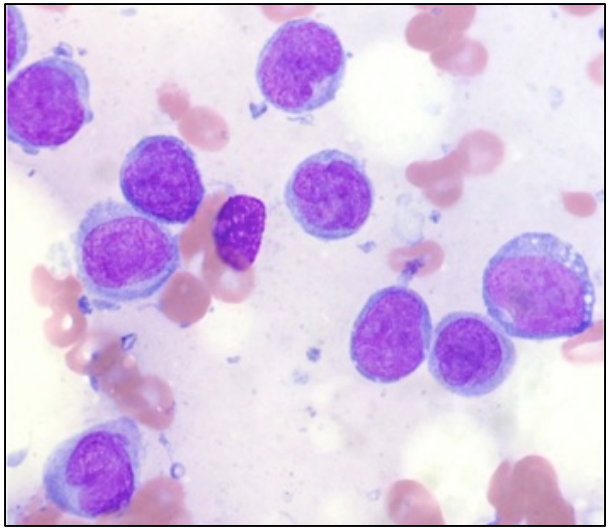
Case 325
M. Foshat
CALR mutation



Other AML Types

Therapy-related myeloid neoplasms

- May have recurring cytogenetic abnormalities that impact prognosis and should be noted (cases 128, 323 and 328)
- May occur after therapy for another AML type (case 328)

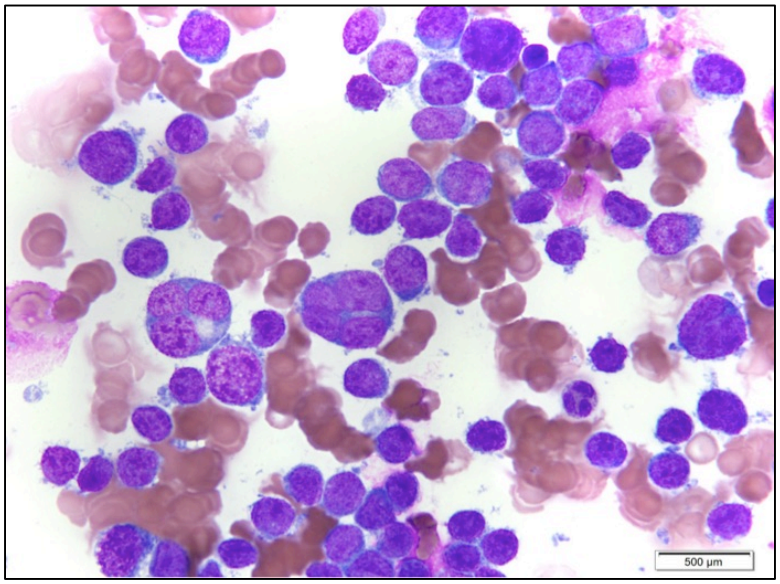


Case 328
K. Holder
t-MN (AML) with t(9;11)

Other AML Types

AML, not otherwise specified

- Morphology is often enough to subclassify
- Megakaryocyte lineage, however, must be confirmed by immunophenotyping



Case 276

N. Patel

AML Genetic Testing Recommendations

- Karyotype
- Molecular testing
  - For all or most cases
    - *FLT3*-ITD, *NPM1*, *CEBPA*, *RUNX1*
    - Others: *IDH1*, *IDH2*, *TET2*, *WT1*, *DNMT3A*, *TP53*, (*FLT3*-TKD), (*ASXL1*)
  - For select cases
    - *KIT* for core binding factor leukemias
    - *PML-RARA* if APL suspected

Based on CAP/ASH Guidelines Arch Pathol Lab Med. 2017 Feb 22. [Epub ahead of print].

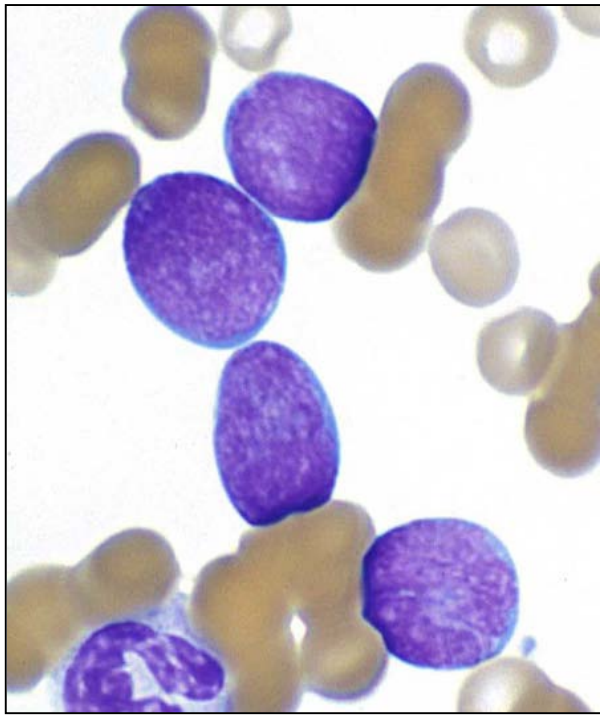
Genes in parentheses are not part of the guideline, but are included due to newly available drug targeting the abnormality (*FLT3*-TKD) or required for full evaluation of ELN risk group (Blood. 2017 Jan 26;129(4):424-447).

Immature Ambiguous and T-cell Neoplasms

Case #	Contributor	Panel Diagnosis	
SH2017-0113	E. Castro-Echeverry University of Pittsburgh	MPAL, B/myeloid, not otherwise specified	cryptic *NUP98/NSD1* t(5;11)(q35;p15) by SNP microArray analysis
SH2017-0163	G. Griffin Boston Children's Hospital	MPAL, B/T	
SH2017-0188	J. Aster Brigham & Women's Hospital	MPAL, T/myeloid, not otherwise specified favored	*NOTCH1*, *DNMT3A*, *ETV6*, and *IKZF1* mutations ETP-ALL vs. MPAL
SH2017-0221	Y. Xie University of California San Francisco	MPAL, T/myeloid, not otherwise specified	
SH2017-0319	Z. Hu MD Anderson Cancer Center	MPAL, T/myeloid, not otherwise specified	
SH2017-0026	M. Xu Yale University	AUL	
SH2017-0103	S. Williams University of Minnesota	Acute leukemia, not definitively classifiable	
SH2017-0189	J. Cortazar Brigham & Women's Hospital	T-ALL	*IL7R* exon 6 insertion mutation
SH2017-0259	G. Wertheim Children's Hospital of Philadelphia	T-ALL favored	NGS support for ETP lymphoma?

Early T-Precursor Acute Lymphoblastic Leukemia (ETP-ALL)

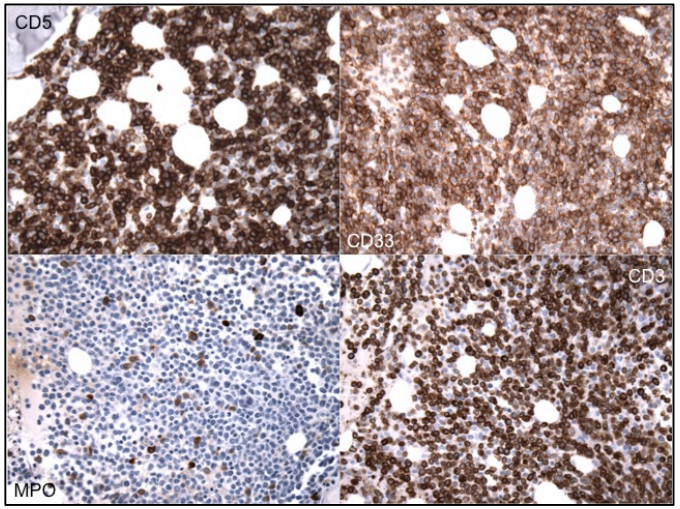
- Early T-Precursor (ETP) ALL comprises 10-15% of T-ALL
- Defined immunophenotypically by expression of CD7, CD3 (surface or rarely cytoplasmic) but not CD1a or CD8
  - Express one or more of the following CD34, CD117, HLA-DR, CD11b, CD65, CD33, or CD13, but not MPO
  - Usually express CD2; CD5 negative or absent in 25% or more of cells
- Molecular genetics
  - Increase in AML-associated mutations (*FLT3*, *NRAS/KRAS*, *DNMT3A*, *IDH1*, *IDH2*)
  - Infrequent NOTCH pathway (T-ALL-associated) mutations
- Initially considered high risk due to higher rate of induction failure
- Recent COG study suggests no outcome difference with current T-ALL therapy



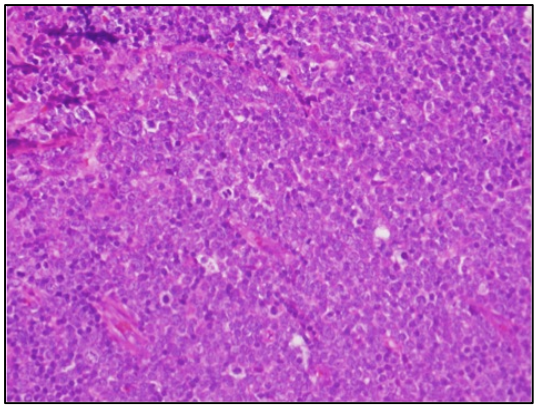
- Coustan-Smith E, et al. Lancet Oncol 10:147, 2009
- Haydu JE and Ferrando AA. Curr Opin Hematol 20:369, 2013
- Wood BL, et al. Blood 124:1, 2014

Immature Ambiguous and T-cell Neoplasms

- Both ETP-ALL and MPAL are diagnosed by immunophenotype
  - MPAL is subclassified based on detection of *KMT2A* translocations or *BCR-ABL1*
- Other more specific disease categories trump MPAL
- As currently defined, ETP-ALL must be MPO negative



Case 188 J. Aster



Case 259 G. Wertheim

MPAL and T-ALL Genetic Testing Recommendations

- MPAL
  - Karyotype
  - *BCR-ABL1*
  - *KMT2A* translocations
- T-ALL
  - Karyotype
  - *NOTCH1*, *FBXW7* mutations may be performed

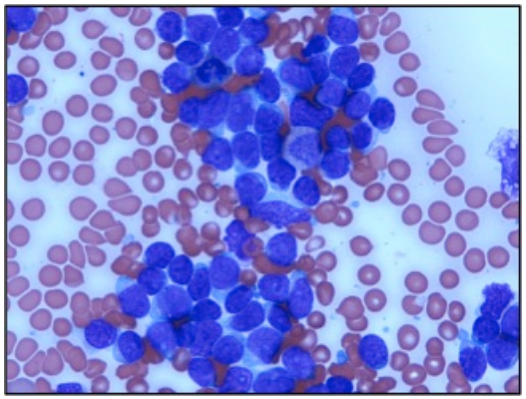
CAP/ASH Guidelines Arch Pathol Lab Med. 2017 Feb 22. [Epub ahead of print].

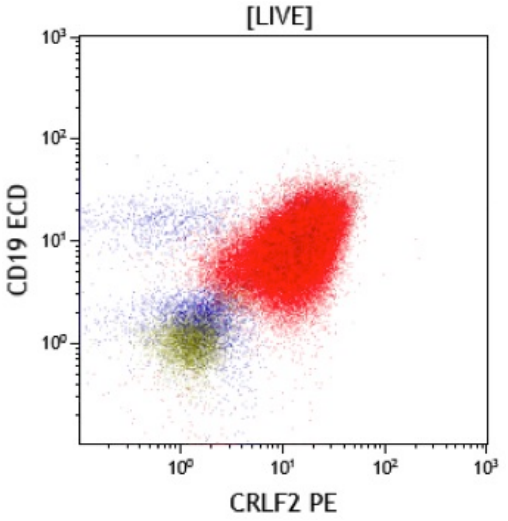
B-ALL, *BCR-ABL1*-like

Case #	Contributor	Panel Diagnosis	
SH2017-0078	K. D. Li University of Utah	B-ALL, *BCR-ABL1*-like	*IGH-CRLF2* rearrangement
SH2017-0110	C. S. Wilson University of New Mexico	B-ALL, *BCR-ABL1*-like	Ph-like gene expression signature and *JAK2* mutation
SH2017-0120	A. Wu UCLA	B-ALL, *BCR-ABL1*-like	Xp22 translocation, presumably involving *CRLF2*
SH2017-0123	Y. Hui University of Pennsylvania	B-ALL, *BCR-ABL1*-like	novel *GOLGA5-JAK2* fusion and sensitivity Ruxolitinib
SH2017-0131	K. Ganapathi University of California San Francisco	B-ALL, *BCR-ABL1*-like	
SH2017-0142	S. Li MD Anderson Cancer Center	B-ALL, *BCR-ABL1*-like	with *CRLF2* rearrangement and *JAK2* Mutation
SH2017-0151	V. Leventaki St. Jude Children's Research Hospital	B-ALL, *BCR-ABL1*-like	with *IGH-CRLF2* rearrangement in a children with Down syndrome.
SH2017-0233	V. Leventaki St. Jude Children's Research Hospital	B-ALL, *BCR-ABL1*-like	with *IGH-CRLF2* rearrangement
SH2017-0242	M.-L. Zhu Cleveland Medical Center	B-ALL, *BCR-ABL1*-like	with CRLF2 positivity by flow cytometry, cryptic *CRLF2* translocation t(Yp11.32;?), as well as t(X;20)(p22;q13.3) and deletion of *IKZF1*
SH2017-0282	C. Ryder Cleveland Medical Center	B-ALL, *BCR-ABL1*-like	*P2YR8-CRLF2* translocation, *JAK2* and *IL7R* Mutations, and somatic trisomy 21
SH2017-0311	T. Zhou Baylor College of Medicine	B-ALL, *BCR-ABL1*-like	with *CRLF2* expression and rearrangement
SH2017-0322	M. Alikhan University of Chicago	B-ALL, *BCR-ABL1*-like	*JAK2* mutation with *CRLF2* alteration in two siblings: a possible inherited cancer predisposition
SH2017-0343	**S. Ondrejka** **Cleveland Clinic**	**B-ALL, *BCR-ABL1*-like**	***PDGFRB* rearrangement**
SH2017-0362	R. Pillai City of Hope	B-ALL, *BCR-ABL1*-like	with *P2RY8-CRLF2* fusion, *JAK2* and *JAK1* mutations

BCR-ABL1-like B-ALL

- *BCR-ABL1*-like B-ALL is a high risk ALL with a gene expression profile similar to that of *BCR-ABL1*+ ALL
- Accounts for 10% of pediatric and 25% of adult ALL; associated with Hispanic ethnicity; poor clinical outcomes; some cases respond to TKI therapy
- Need to establish clear diagnostic criteria
  - *CRLF2* translocations
    - Usually show increased expression of CRLF2 by flow cytometry analysis
  - Some have activating mutations or translocations of genes, such as *ABL1*, *ABL2*, *JAK2*, *PDGFRB*, *NTRK3*, *TYK2*, *CSF1R*, and/or *EPOR*
  - The full spectrum of genetic changes is still being investigated



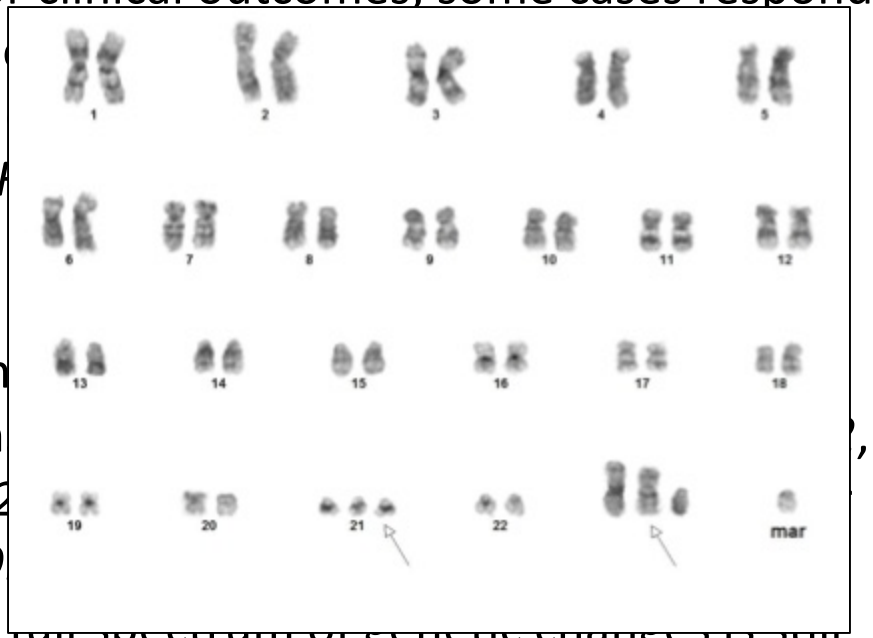


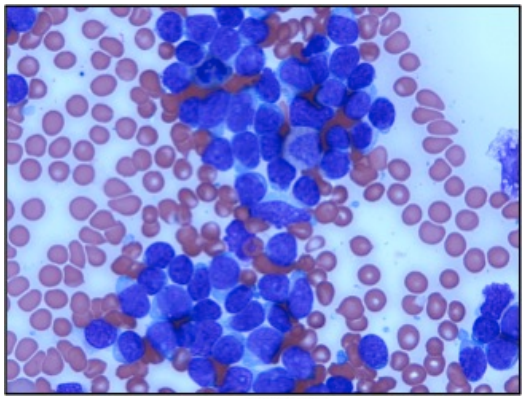
Case 322 M. Alikhan

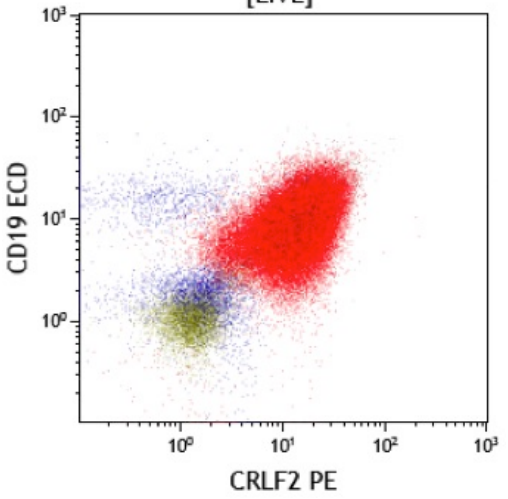
van der Veer et al. Blood 122:2622, 2013
Izraeli. Curr Opin Hematol 21:289, 2014

BCR-ABL1-like B-ALL

- *BCR-ABL1*-like B-ALL is a high risk ALL with a gene expression profile similar to that of *BCR-ABL1*+ ALL
- Accounts for 10% of pediatric and 25% of adult ALL; poor clinical outcomes; some cases respond to TKI th
- Need to
  - *CRL*
    -
  - Som
    tran
    JAK2
    EPO
  - The
    being investigated







Case 322 M. Alikhan

van der Veer et al. Blood 122:2622, 2013
Izraeli. Curr Opin Hematol 21:289, 2014

B-ALL, *BCR-ABL1*-like

- 12 of 14 cases had some evidence of *CRLF2* abnormalities (expression, FISH, karyotype, sequencing)
- 6 of 6 cases with clear data of testing were *JAK2* mutated or translocated
- 7 of 14 cases had a *BCR-ABL1*-like signature on a send out panel
- 1 case (343) had a *PDGFRB* translocation
- We are probably still missing a significant percentage of cases with current testing approaches

Other ALL Types

Case Number	Last Name	Final panel diagnosis	
SH2017-0235	M. Koo UCLA	B-ALL with iAMP 21	
SH2017-0366	**M. Harris** **Boston Children's Hospital**	**B-ALL with iAMP 21**	
SH2017-0045	C. Roth Baylor College of Medicine	B-ALL hypodiploid with *TP53* mutation	
SH2017-0108	**E. Mason** **Vanderbilt University Medical Center**	**LPL in setting of B-ALL**	***KMT2A* rearrangement**
SH2017-0183	J. Cheng University of Chicago	B-ALL, possibly therapy-related	
SH2017-0347	J. Gomez-Gelvez Henry Ford Health System	B-ALL, *BCR-ABL1* positive	vs MPAL

B-ALL with iAMP21

- Intrachromosomal amplification of chromosome 21 (iAMP21) accounts for about 2% of pediatric B-ALL
- Uncommon in adults
- Adverse outcomes when treated with standard risk therapy; but improved when treated as high risk ALL
- Presence of 5 or more copies of *RUNX1* on a single cell or 3 or more extra copies on a single abnormal chromosome 21 in metaphase FISH
- Reliably detected by FISH for *RUNX1* used to evaluate for B-ALL with *ETV6-RUNX1*

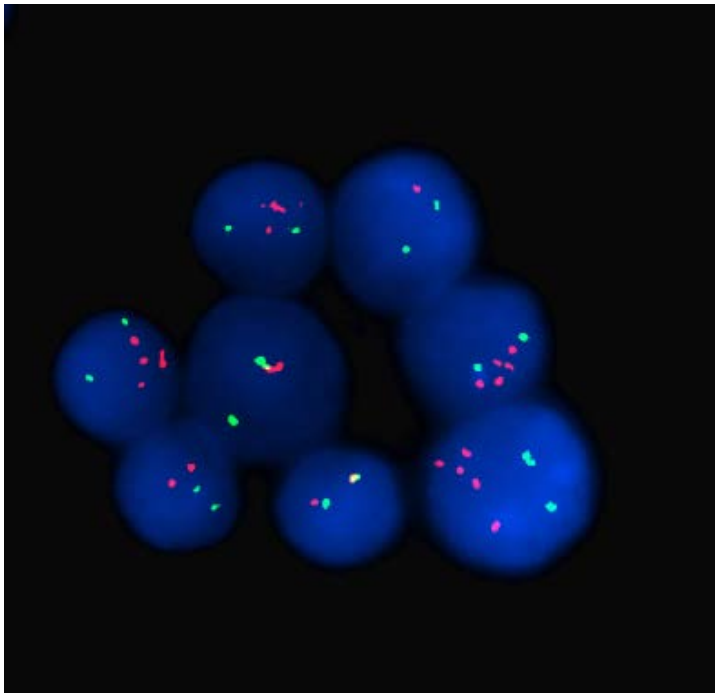


Image provided by Dana Bangs

Harrison et al. Leukemia 28:1015, 2014

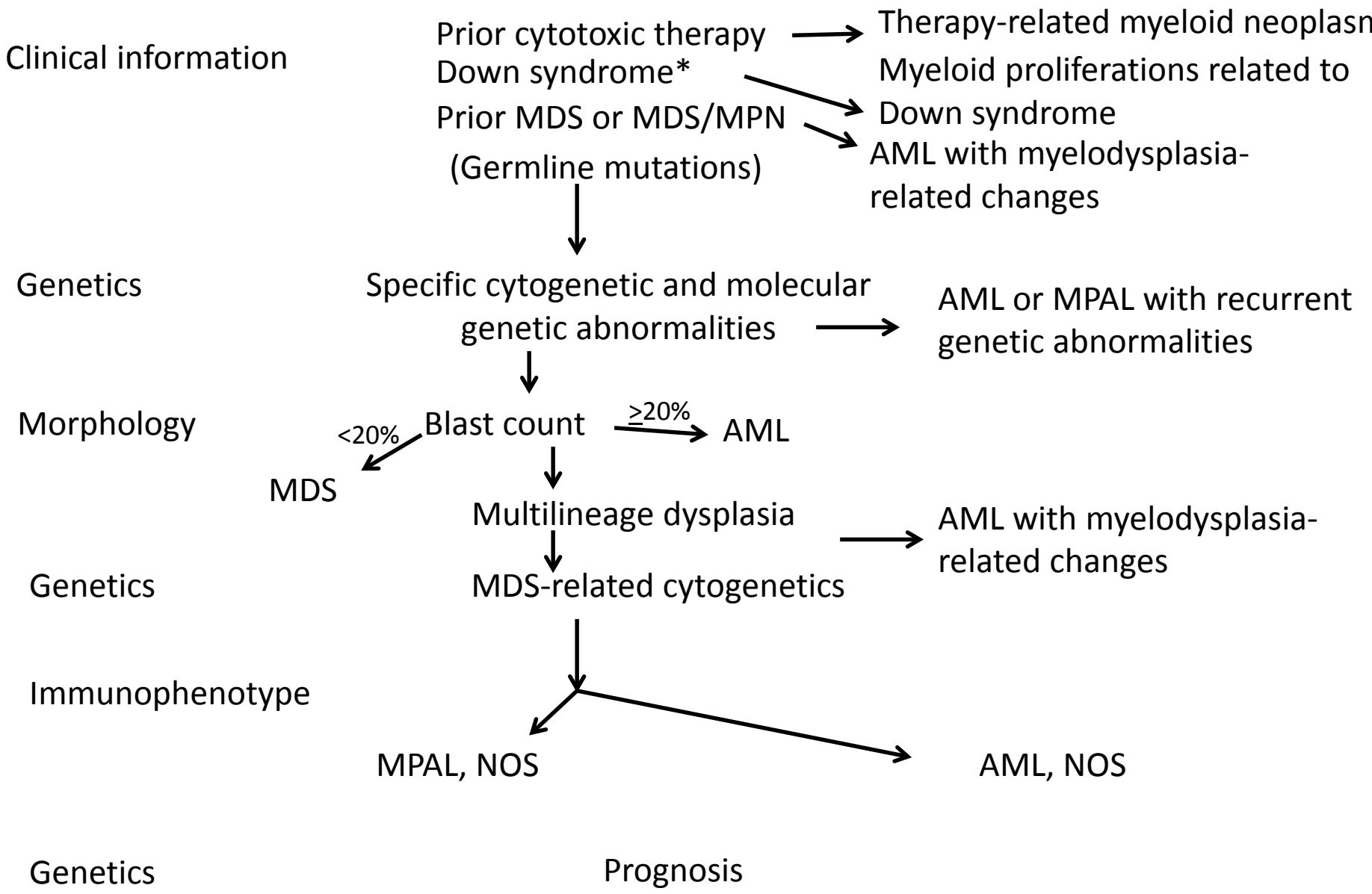
B-ALL Genetic Testing Recommendations

- Karyotype
- *BCR-ABL1*
- *KMT2A* translocations
- *PAX5*, *JAK1*, *JAK2*, *IKZF1* mutations may be performed*
- Pediatrics
  - t(12;21)(p13.2;q22.1); *ETV6-RUNX1*
  - iAMP21
  - Trisomy 4 and 10

CAP/ASH Guidelines Arch Pathol Lab Med. 2017 Feb 22. [Epub ahead of print].

*commercial assays for *BCR-ABL1*-like ALL not readily available

Diagnostic Hierarchy of Acute Myeloid (and Mixed Phenotype) Leukemias



Thank you for your attention