Work up of Acute Leukemia

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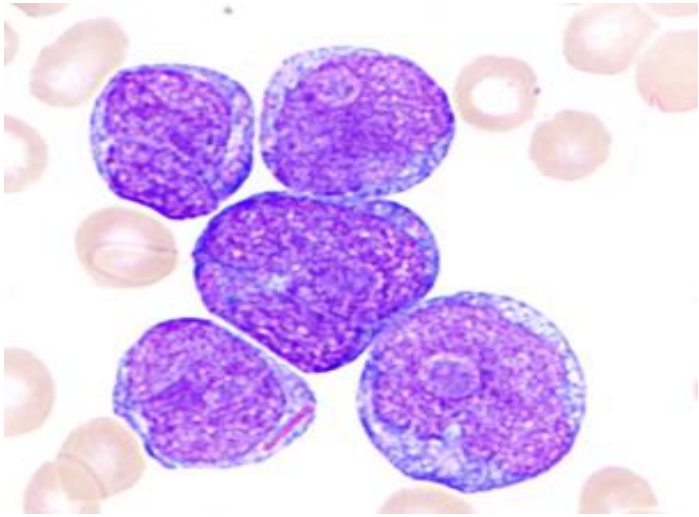
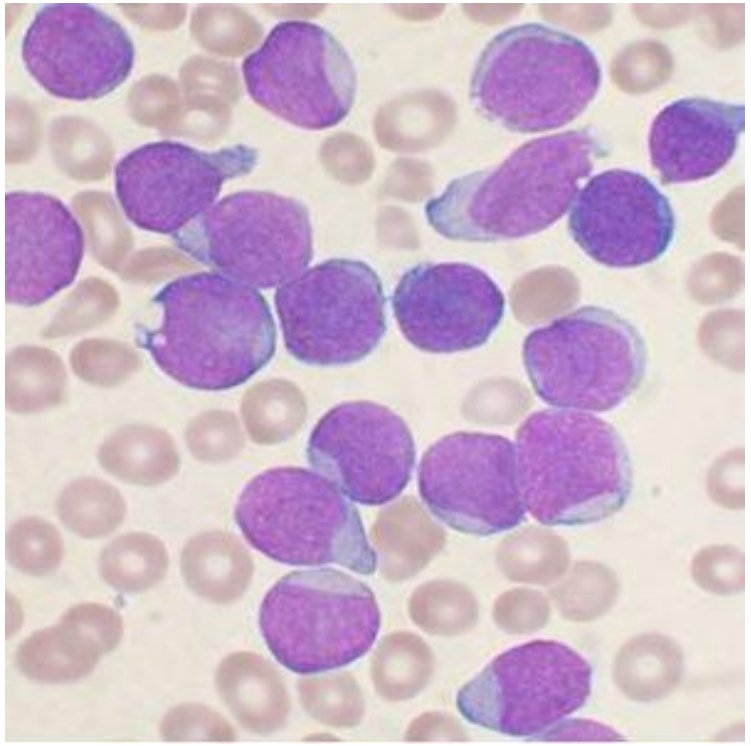




Learning objectives

- Discuss the updated testing guidelines for acute leukemia from College of American Pathologist (CAP)/American Society of Hematology (ASH)
- To know about the samples and tests needed at the time of initial evaluation on all patients
- Discuss the tests needed on a subset of acute leukemia patients
- Understand the prognostic/therapeutic implications of newer molecular tests in acute leukemia
- To be familiar with the newly approved targeted therapies

Agenda

- Introduction and CAP/ASH guidelines for specimen requirement and testing guidelines
- Discuss the broader classification of acute leukemia
- Discuss the specific subtypes of Acute lymphoblastic leukemia (ALL)
- Discuss the specific subtypes of Acute myeloid leukemia (AML)
- Elaborate the molecular genetics gene mutations with prognostic/therapeutic implications in Acute myeloid leukemia (AML)

Introduction

- Definition
    - ≥ 20% blasts (blood or marrow)
    - Select recurrent genetic abnormalities (with or without 20% blasts)
- Two broad categories: Lymphoid and Myeloid

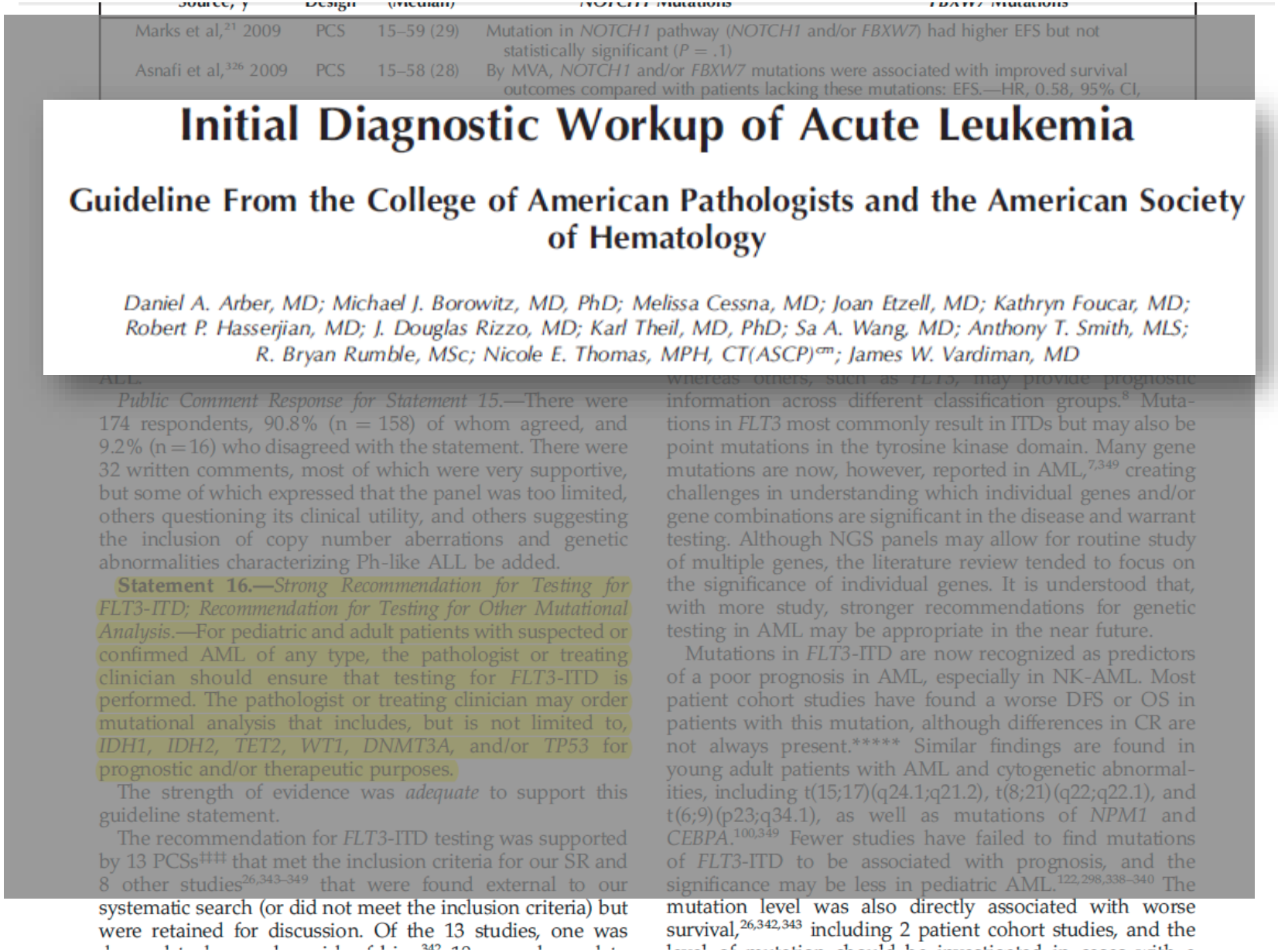
- Complete diagnosis requires knowledge of clinical information, peripheral smear and bone marrow evaluation, immunophenotyping and karyotype analysis

- Molecular studies are often required

Introduction: Statistics

- Acute lymphocytic leukemia (ALL): 5,960 new cases/year
  - 75% cases seen in <6 years
  - 80-85% are precursor B-cell phenotype
  - 5 year survival rare $\approx$ 85%

- Acute myeloid leukemia 19,520 new cases/year
  - Commonly seen in adults
  - 5 year survival rare $\approx$ 27%



Source, y	Design	(Median)	NOTCH1 Mutations	FBXW7 Mutations
Marks et al,[21] 2009	PCS	15–59 (29)	Mutation in *NOTCH1* pathway (*NOTCH1* and/or *FBXW7*) had higher EFS but not statistically significant ($P = .1$)	
Asnafi et al,[326] 2009	PCS	15–58 (28)	By MVA, *NOTCH1* and/or *FBXW7* mutations were associated with improved survival outcomes compared with patients lacking these mutations: EFS.—HR, 0.58, 95% CI,	

Initial Diagnostic Workup of Acute Leukemia

Guideline From the College of American Pathologists and the American Society of Hematology

Daniel A. Arber, MD; Michael J. Borowitz, MD, PhD; Melissa Cessna, MD; Joan Etzell, MD; Kathryn Foucar, MD; Robert P. Hasserjian, MD; J. Douglas Rizzo, MD; Karl Theil, MD, PhD; Sa A. Wang, MD; Anthony T. Smith, MLS; R. Bryan Rumble, MSc; Nicole E. Thomas, MPH, CT(ASCP)^{cm}; James W. Vardiman, MD

Public Comment Response for Statement 15.—There were 174 respondents, 90.8% (n = 158) of whom agreed, and 9.2% (n = 16) who disagreed with the statement. There were 32 written comments, most of which were very supportive, but some of which expressed that the panel was too limited, others questioning its clinical utility, and others suggesting the inclusion of copy number aberrations and genetic abnormalities characterizing Ph-like ALL be added.

Statement 16.—*Strong Recommendation for Testing for FLT3-ITD; Recommendation for Testing for Other Mutational Analysis.*—For pediatric and adult patients with suspected or confirmed AML of any type, the pathologist or treating clinician should ensure that testing for *FLT3*-ITD is performed. The pathologist or treating clinician may order mutational analysis that includes, but is not limited to, *IDH1*, *IDH2*, *TET2*, *WT1*, *DNMT3A*, and/or *TP53* for prognostic and/or therapeutic purposes.

The strength of evidence was *adequate* to support this guideline statement.

The recommendation for *FLT3*-ITD testing was supported by 13 PCSs‡‡‡ that met the inclusion criteria for our SR and 8 other studies[26,343–349] that were found external to our systematic search (or did not meet the inclusion criteria) but were retained for discussion. Of the 13 studies, one was

whereas others, such as *FLT3*, may provide prognostic information across different classification groups.[8] Mutations in *FLT3* most commonly result in ITDs but may also be point mutations in the tyrosine kinase domain. Many gene mutations are now, however, reported in AML,[7,349] creating challenges in understanding which individual genes and/or gene combinations are significant in the disease and warrant testing. Although NGS panels may allow for routine study of multiple genes, the literature review tended to focus on the significance of individual genes. It is understood that, with more study, stronger recommendations for genetic testing in AML may be appropriate in the near future.

Mutations in *FLT3*-ITD are now recognized as predictors of a poor prognosis in AML, especially in NK-AML. Most patient cohort studies have found a worse DFS or OS in patients with this mutation, although differences in CR are not always present.***** Similar findings are found in young adult patients with AML and cytogenetic abnormalities, including t(15;17)(q24.1;q21.2), t(8;21)(q22;q22.1), and t(6;9)(p23;q34.1), as well as mutations of *NPM1* and *CEBPA*.[100,349] Fewer studies have failed to find mutations of *FLT3*-ITD to be associated with prognosis, and the significance may be less in pediatric AML.[122,298,338–340] The mutation level was also directly associated with worse survival,[26,342,343] including 2 patient cohort studies, and the

*Reprinted from Arber DA, Borowitz, MJ, Cessna M, Etzell J, Foucar K, Hasserjian RP, Rizzo JD, Theil K, Wang SA, Smith AT, Rumble RB, Thomas NE, and Vardiman JW. Initial Diagnostic Workup of Acute Leukemia: Guideline From the College of American Pathologists and the American Society of Hematology. *Arch Pathol Lab Med.* doi: 10.5858/arpa.2016-0504-CP with permission from *Archives of Pathology & Laboratory Medicine*.

Key questions asked during initial work up

- What clinical and lab information should be available?
- What specimens and sample types should be evaluated?
- What tests are required for all patients?
- Which tests should be performed on only a subset of patients?
- Where should laboratory testing be performed?
- How should test results and diagnosis be correlated and reported?

1. What clinical and lab information should be available?

- Why do we need clinical information?

  - Down syndrome
  - Myeloid neoplasm with germline predisposition
  - Prior therapy
  - Use of recombinant granulocytic growth factors
  - Vitamin B12 or folic acid deficiency

2. What specimens and sample types should be evaluated on all cases?

- Peripheral blood, bone marrow (BM) aspirate and/or touch imprints
- BM core biopsy and/or marrow clot*
- Peripheral blood (PB) may be used for ancillary studies
  - If there are adequate blasts
  - BM is inadequate
  - There is compelling reason to avoid BM
- Tissue biopsy for extramedullary disease without apparent BM or PB involvement
- Flow cytometry - should be comprehensive enough to distinguish between AML, B-ALL,T-ALL, and acute leukemia of ambiguous lineage
  - **Essential for lineage assignment**
- Conventional cytogenetics

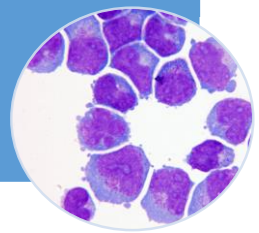
2.What specimens and sample types should be evaluated? -continued

- If sufficient BM aspirate is not available for flow, a second core biopsy can be used for flow and genetic studies
- Should be unfixed (culture media)
- Non-decalcified paraffin-embedded (FFPE) or unstained BM aspirate can be used for nucleic acid extraction
  - Usually the clot sections
  - Depends on the lab and the validation

Acute Leukemia: broader classification

- MPO (by flow cytometry, immunohistochemistry, or cytochemistry
- Monocytic differentiation (≥2 of the following: non-specific esterase, CD11c, CD14, CD64, lysozyme)

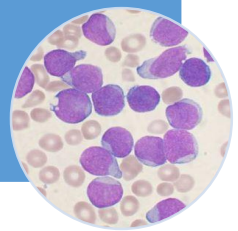
Acute myeloid leukemia



•B-cell lineage: strong CD19 with ≥ 1 of the following strongly expressed: CD79a,cytoplasmic CD22, CD10 or weak CD19 with ≥2 of the above

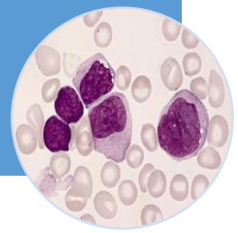
•T-cell lineage: Cytoplasmic CD3 (by flow or immuno *) or surface CD3

Acute lymphoid leukemia



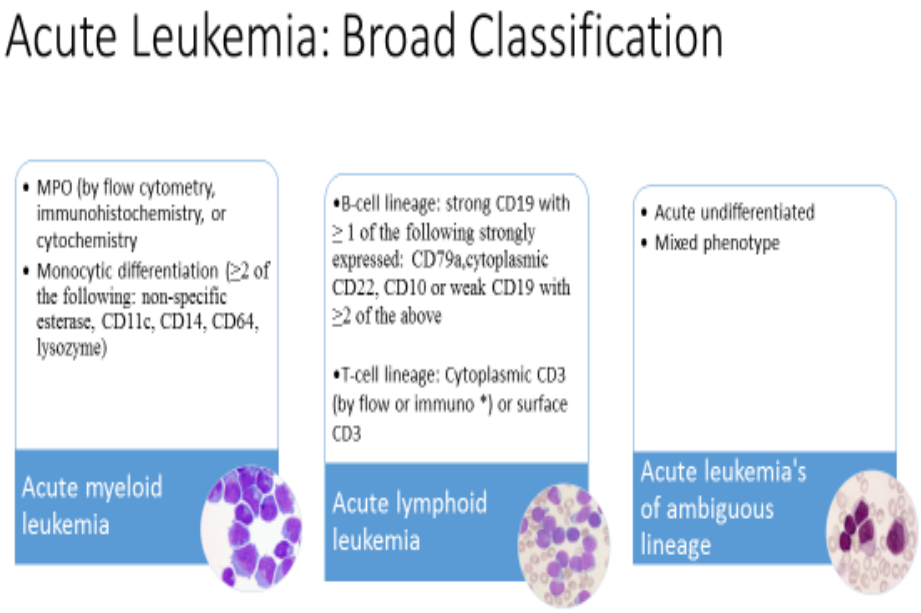
- Acute undifferentiated
- Mixed phenotype

Acute leukemia's of ambiguous lineage



Utility of cytochemical stains

- Not useful for ALL
- Can be useful sometime for AML
  - MPO and non-specific esterase
  - Speed and low cost



Cytogenetics and FISH studies

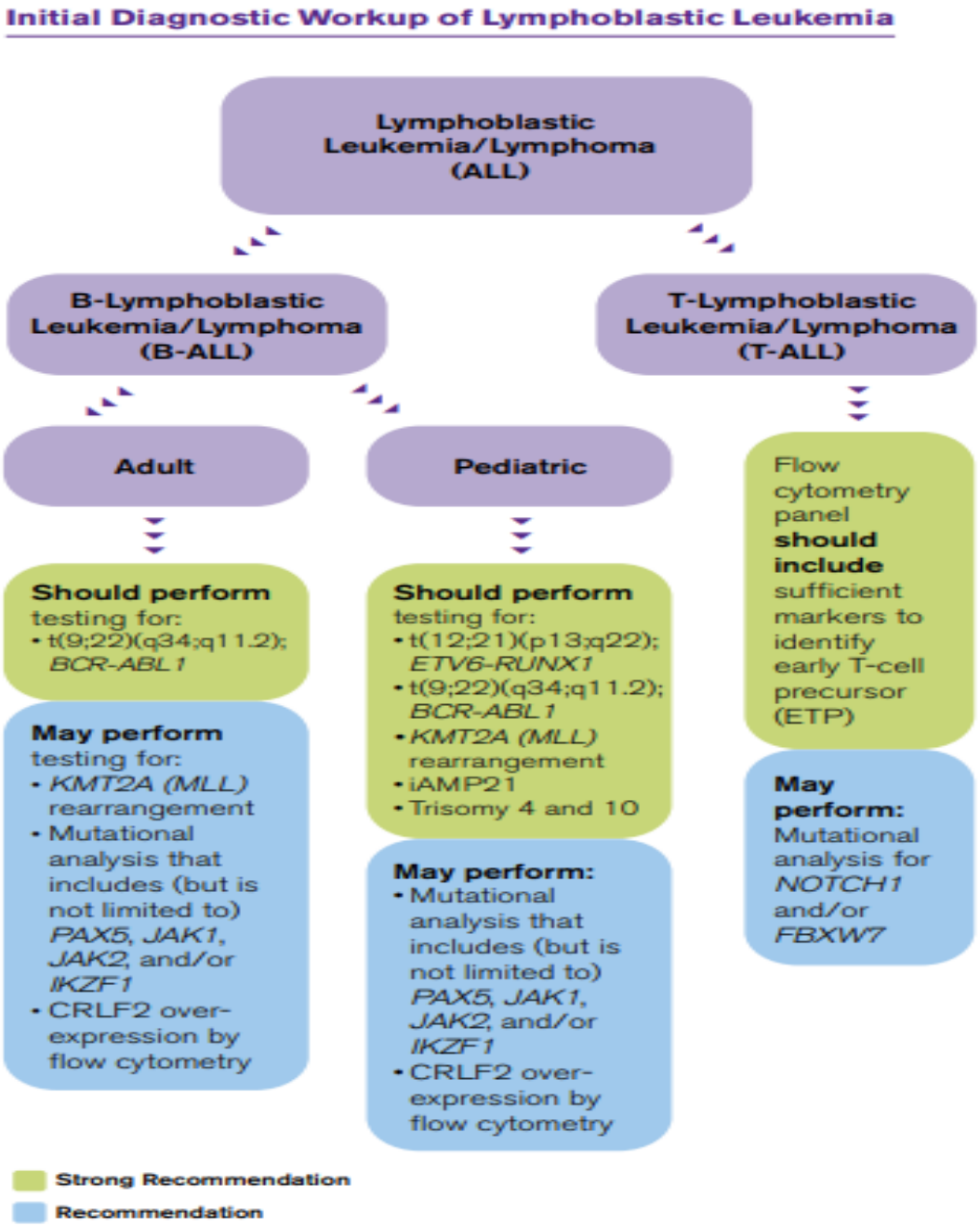
- Role of cytogenetics is critical for prognostic implications
- Provides a basis for classification and choice of initial and post remission therapy
- FISH -complimentary to an adequate cytogenetics
  - Many of the abnormalities of ALL are cryptic t(12;21) *ETV6-RUNX1* or intrachromosmal amplification of chromosome 21
  - STAT FISH can be very helpful in acute promyelocytic leukemia (APL)
  - In other AMLs ?

Molecular studies

- Most of the molecular studies can be performed on EDTA PB (if enough blasts) or bone marrow
- DNA and RNA extract and hold should be done on all the sample
- Molecular studies can be added later
- DNA or RNA extraction can also be performed on cryopreserved cells

Further work up of ALL

Initial Diagnostic Workup of Acute Leukemia
Presented by ASH and the College of American Pathology (CAP) in 2017, adapted from Initial diagnostic workup of acute leukemia: guideline from the College of American Pathologists and the American Society of Hematology. *Archives of Pathology & Laboratory Medicine.* 2017.



T-acute lymphoblastic leukemia (T-ALL)

- Early T-cell precursor should be identified
  - 10-13% of T-ALL
  - Limited T-cell differentiation
  - Express cytoplasmic CD3
  - CD7+, lacks CD8 and CD1a and is positive for one or more myeloid associated markers (CD11b, CD13, CD33)
  - usually negative for CD5 and may express CD2 and/or CD4
  - Mutation profile by NGS similar to AML
- *NOTCH1* and *FBXW7* mutations frequently seen
  - Lack prognostic significance

B-acute lymphoblastic leukemia (B-ALL):FISH

Pediatric

- ETV6/RUNX1 (TEL/AML1) t(12;21) – good prognosis
- Trisomy 4 and 10 - good prognosis
- BCR/ABL1 t(9;22)
- MLL (11q23)
- iAMP21

Adult

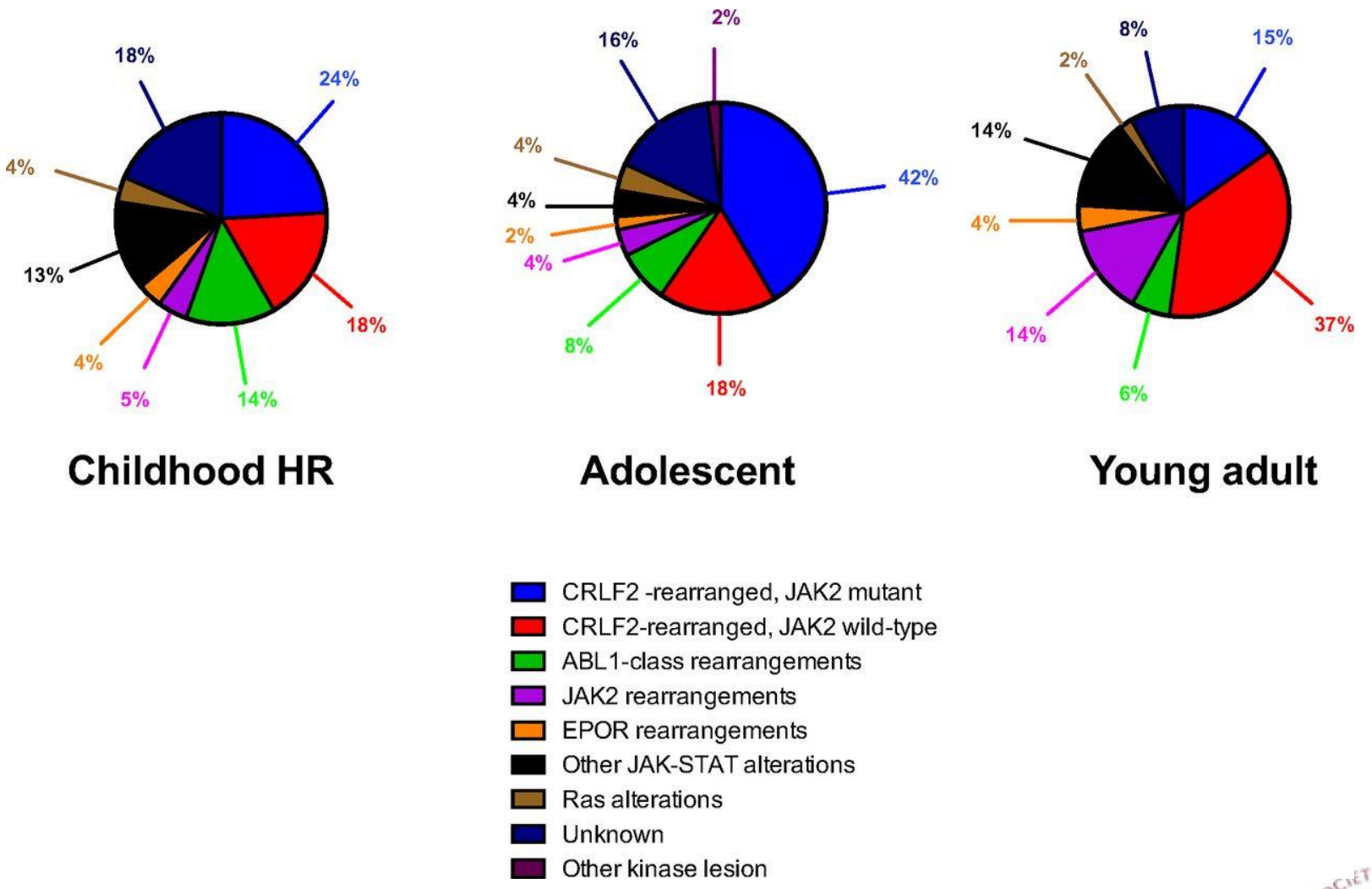
- BCR/ABL1 t(9;22)

BCR-ABL1 or Ph-like B-ALL: Why is it important to identify these patients?

- 10-15% in children and 25% in adults

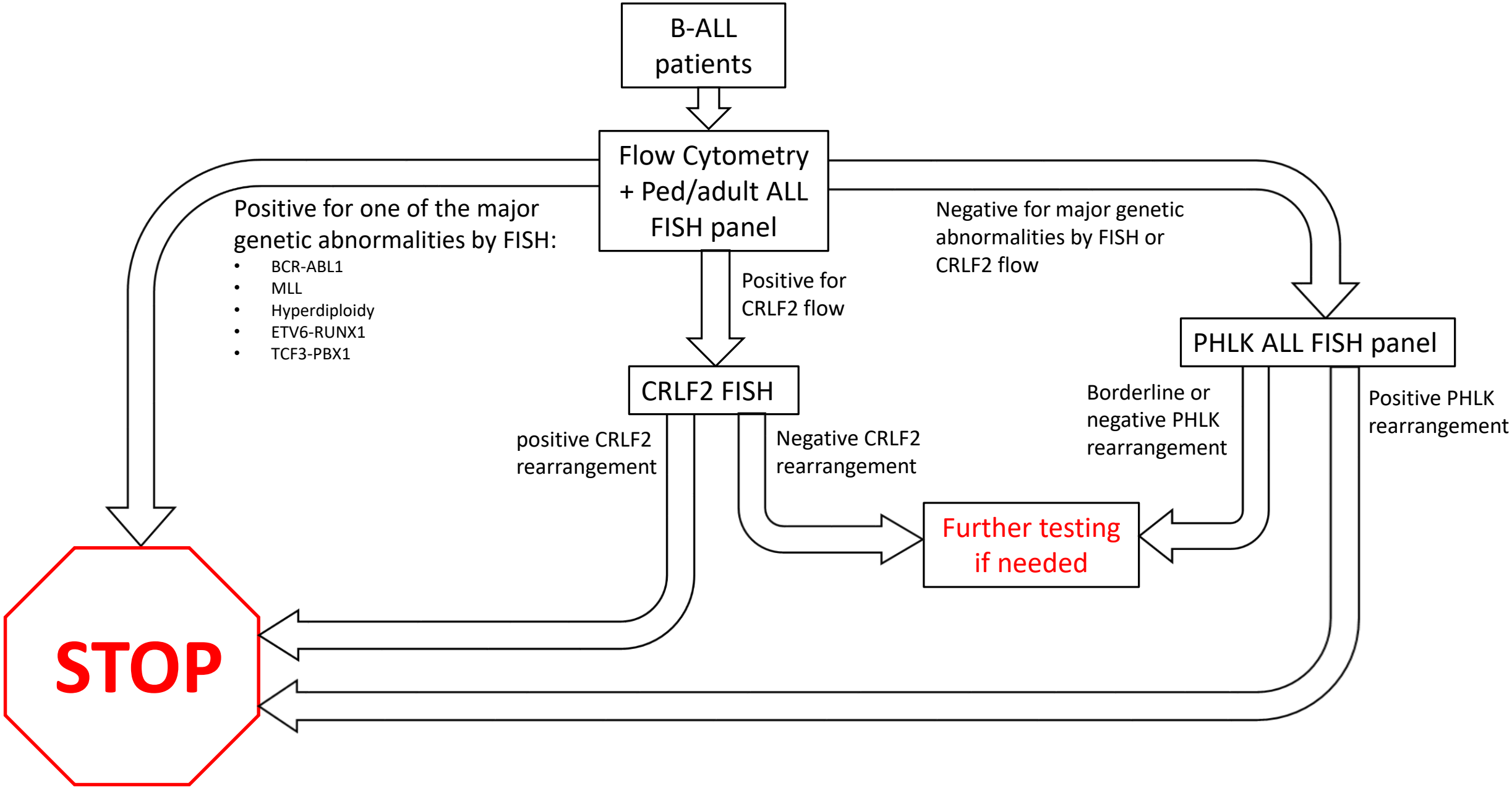
- The most common abnormalities include CRLF2 rearrangements, JAK mutations, and erythropoietin receptor (EPOR) rearrangements
  - All three of these categories lead to activation of the JAK/STAT pathway
- Mutations involving ABL-class genes include ABL1, ABL2, CSF1R, PDGFRA, and PDGFRB. Other mutations and fusions include IKZF1, FGFR1, and RAS
  - Can be treated with tyrosine kinase inhibitors

Distribution of Ph-like ALL subgroups among children, adolescents, and young adults.



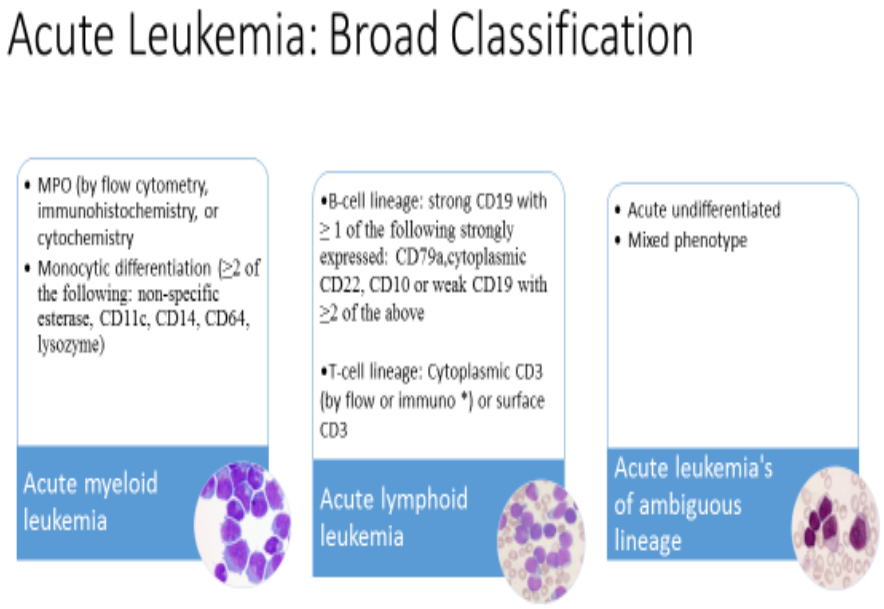
Thai Hoa Tran, and Mignon L. Loh Hematology 2016;2016:561-566

Ph-like ALL test algorithm



Acute leukemia of ambiguous lineage

Broader classification



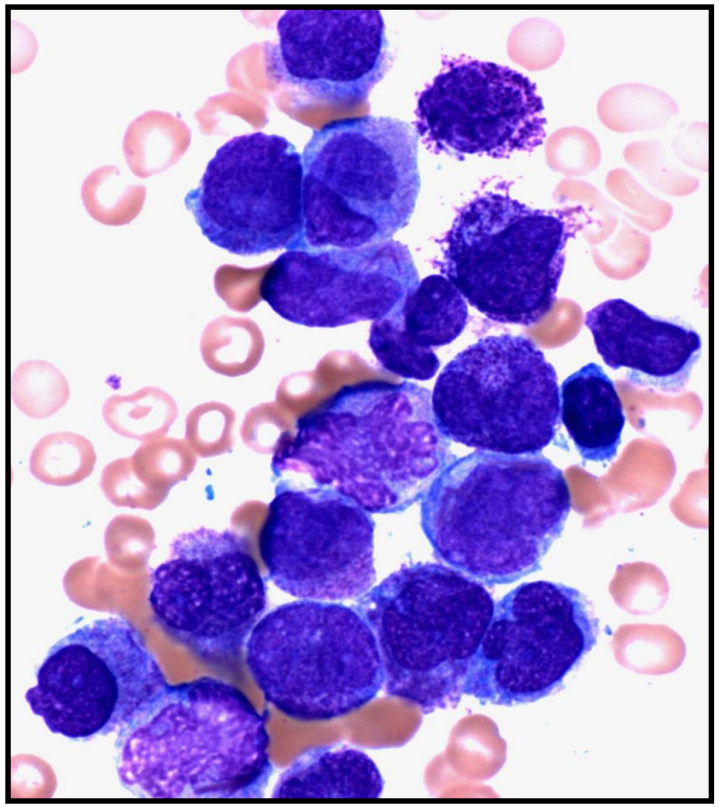
Acute leukemia of ambiguous lineage

- Acute undifferentiated
- Mixed phenotype
  - Mixed phenotype acute leukemia with t(9;22) *BCR-ABL1*
  - Mixed phenotype acute leukemia with t(v;11q23.3) *KMT2A*-rearranged
  - Mixed phenotype acute leukemia, B/myeloid not otherwise specified
  - Mixed phenotype acute leukemia, T/myeloid not otherwise specified

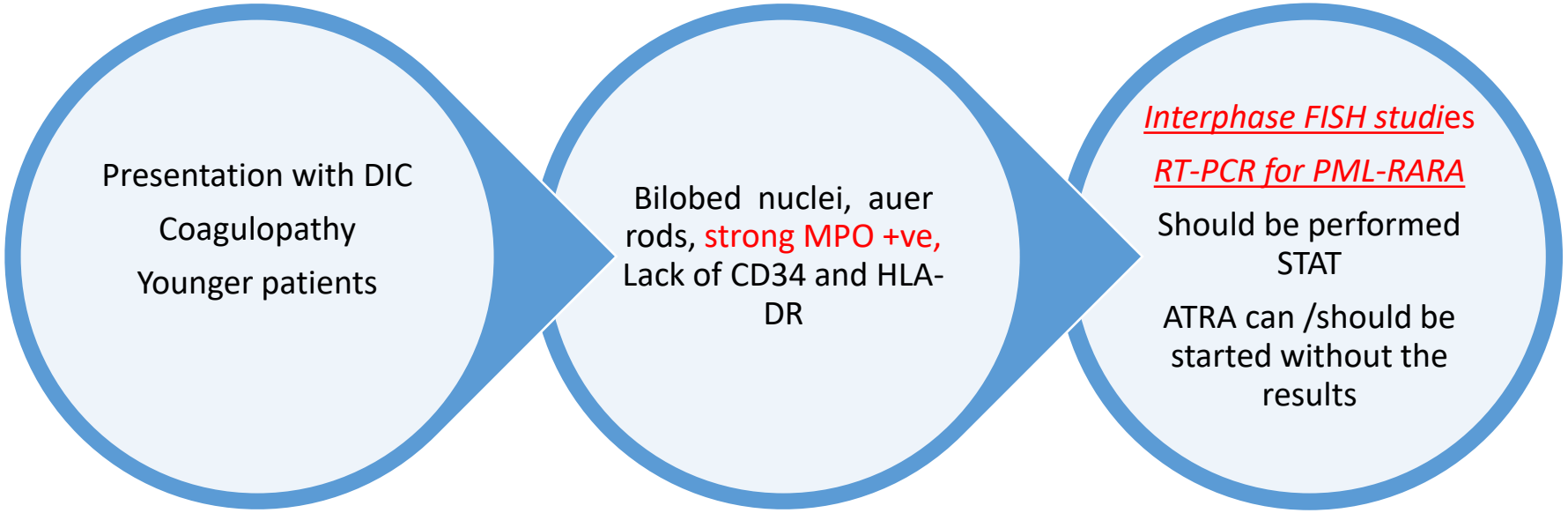
Acute myeloid leukemia (AML)

Acute promyelocytic leukemia (APL)

- Bone marrow packed with highly granular abnormal promyelocytes (no maturation)
  - Hypergranular or microgranular
- Unique risk of fatal hemorrhage due to activation of both coagulation and fibrinolytic pathway on top of production defect
- Medical emergency
- Highly curable: Vitamin A (ATRA) / arsenic trioxide and chemotherapy



APL: rapid diagnosis



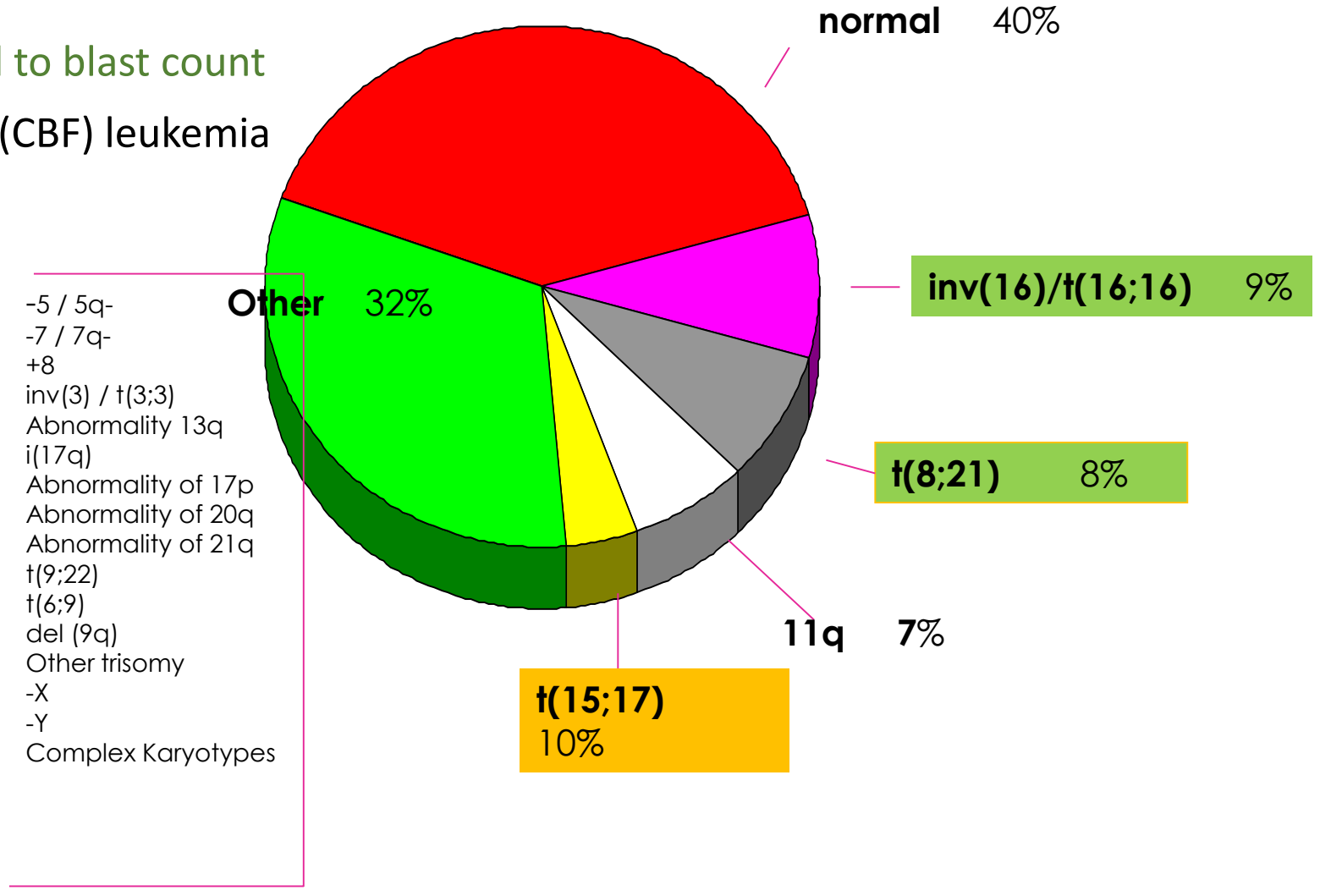
AML: further testing

- For pediatric or adult patients with suspected or confirmed AML of any type
  - FLT3-ITD should be performed on all AML cases

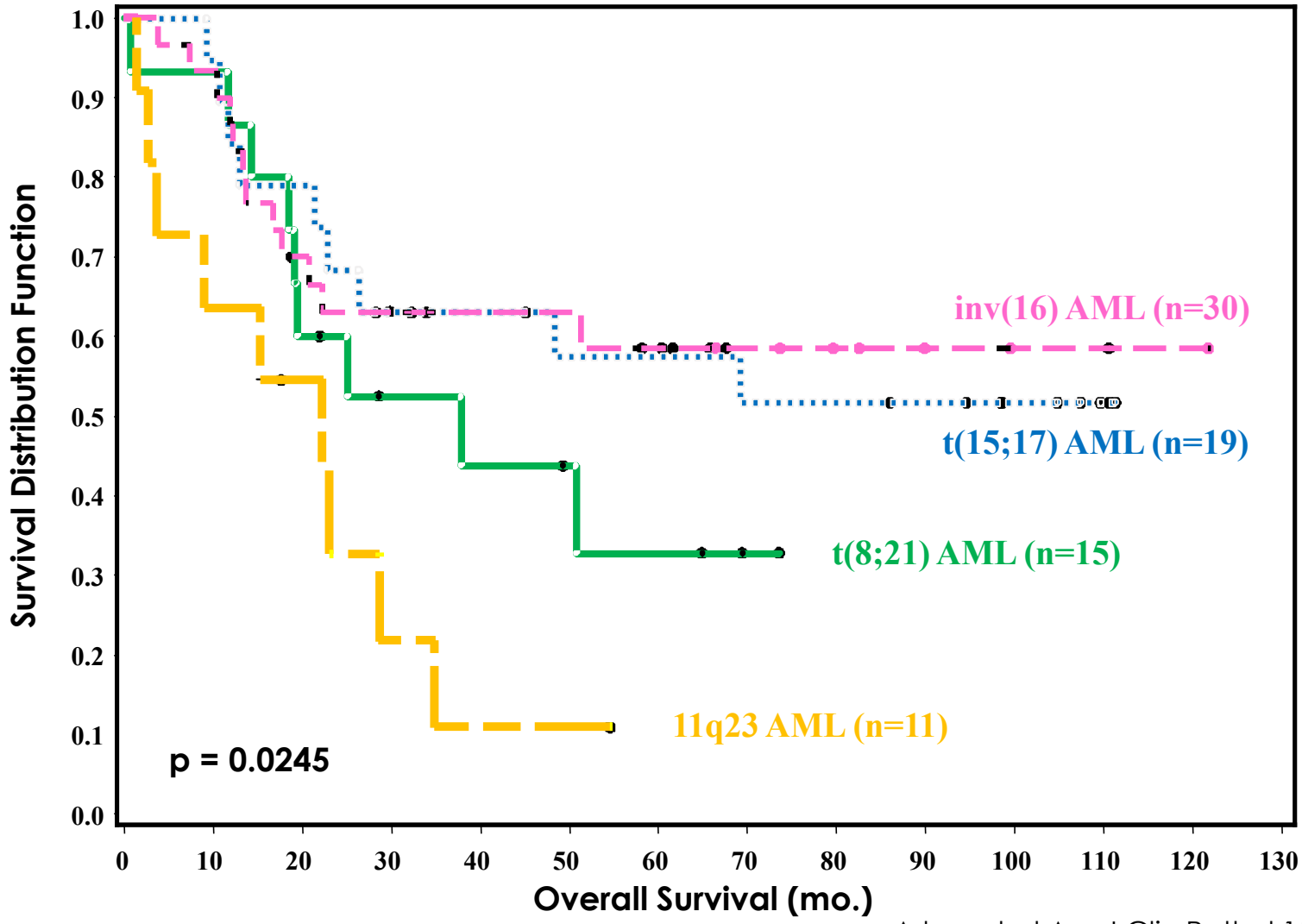
- Other mutational testing including *IDH1, IDH2, TET2, WT1, DNMT3A* and or *TP53* is recommended

AML: Cytogenetics

- AML without regard to blast count
- Core binding factor (CBF) leukemia and Kit mutation



Recurring Cytogenetic Abnormalities in Adult AML



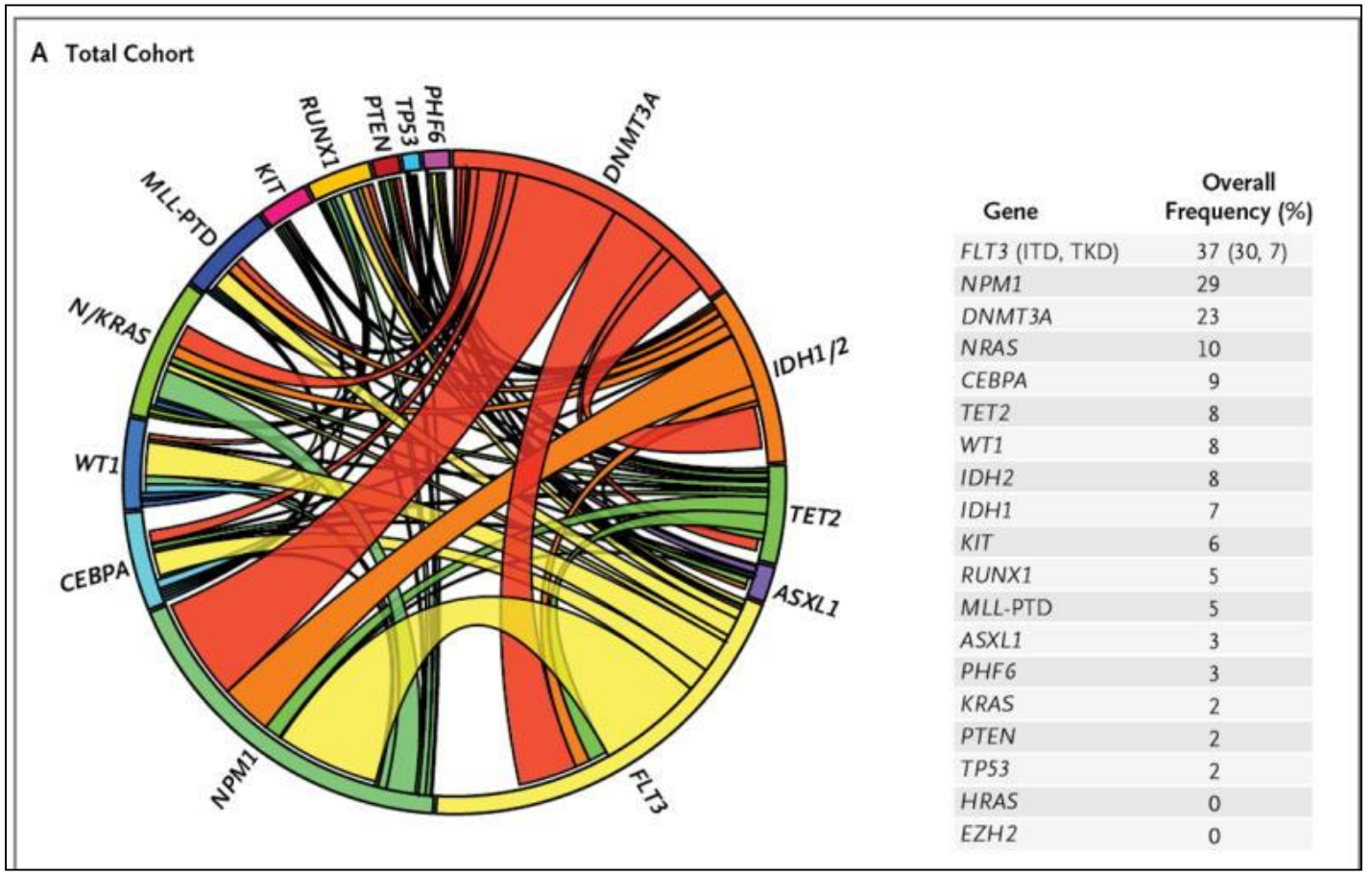
Arber et al Am J Clin Pathol 119:672, 2003

Cytogenetic Risk Groups

Low	t(8;21) inv(16)/t(16;16) t(15;17)	High	Complex (≥3) abnormalities -7 inv(3q) del(9q) without t(8;21) 11q23, 17p, 20q or 21q abnormalities t(9;22) t(6;9) +13 dmin/hsrs
Intermediate	Normal karyotype Single abnormalities +8 +11 -Y 12p abnormalities		

Molecular studies in AML

Mutational complexity of AML



Gene	Overall Frequency (%)
FLT3 (ITD, TKD)	37 (30, 7)
NPM1	29
DNMT3A	23
NRAS	10
CEBPA	9
TET2	8
WT1	8
IDH2	8
IDH1	7
KIT	6
RUNX1	5
MLL-PTD	5
ASXL1	3
PHF6	3
KRAS	2
PTEN	2
TP53	2
HRAS	0
EZH2	0

Majority of AML patients have multiple mutations.

JP Patel et al. N Engl J Med 2012;366(12):1079-89.

FLT3 and *IDH1/2*

FLT3 (FMS-like tyrosine kinase 3)

- **Receptor tyrosine kinase involved in hematopoiesis**
- **Frequency in AML**

  ITD ~23% TKD ~7%
- **Prognostic significance**

  ITD – negative TKD – unclear
- **Therapeutic significance**

Midostaurin and other drugs approved for *FLT3* mutated AML

- **How to measure?**
  - Fragment analysis/RT PCR
  - Next generation sequencing

IDH1 and *IDH2* isocitrate dehydrogenase 1, 2

- **Cellular metabolism and epigenetic regulation, DNA methylation**
- **Frequency in AML**

IDH1 – 6-10% *IDH2* – 8-19%

- **Prognostic significance**

  unclear
- **Therapeutic significance**

Enasidenib and other drugs approved to treat relapsed/ refractory *IDH1*/*IDH2* mutated AML

- **How to measure?**
  - RT PCR, Sanger sequencing, NGS

Nat Rev Clin Oncol 2016;13:305, Levis M. Blood 2017;129:3403.

NPM1 and CEBPA

NPM1 (nucleophosmin)

- **Phosphoprotein involved in ribosome biogenesis, cell proliferation, and apoptosis**
- **Frequency in AML:**
  - 27-35%
- **Prognostic significance**

NPM1^{mut} and *FLT3*-ITD^{wt} favorable

NPM1^{mut} better prognosis than normal karyotype AML and NPM1^{wt}

- **Therapeutic significance** May not need alloHCT in first remission

CEBPA (CCAAT/enhancer- binding protein alpha

- **Transcription factor involved in neutrophil differentiation**
- **Frequency in AML**
  - Monoallelic 3-4%
  - Biallelic 4-6%
- **Prognostic significance**

Monoallelic similar to wild type

Biallelic and normal karyotype has favorable prognosis

- **Therapeutic significance** May not need alloHCT in first remission

Falini B et al. N Engl J Med 20005;352:254, Yohe S. J Clin Med 2015;4:460, Dohner H et al. N Engl J Med 2005;106:3740, Thiede C et al. Blood 2006;107:4011, Coombs CC et al. Nat Rev Clin Oncol 2016;13:305.

KIT (v-KIT Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog)

Deletions or insertions in exons 8 and 17

- **Receptor tyrosine kinase involved in proliferation, differentiation, survival**
- **Frequency in AML** <5%
- **Prognostic significance**

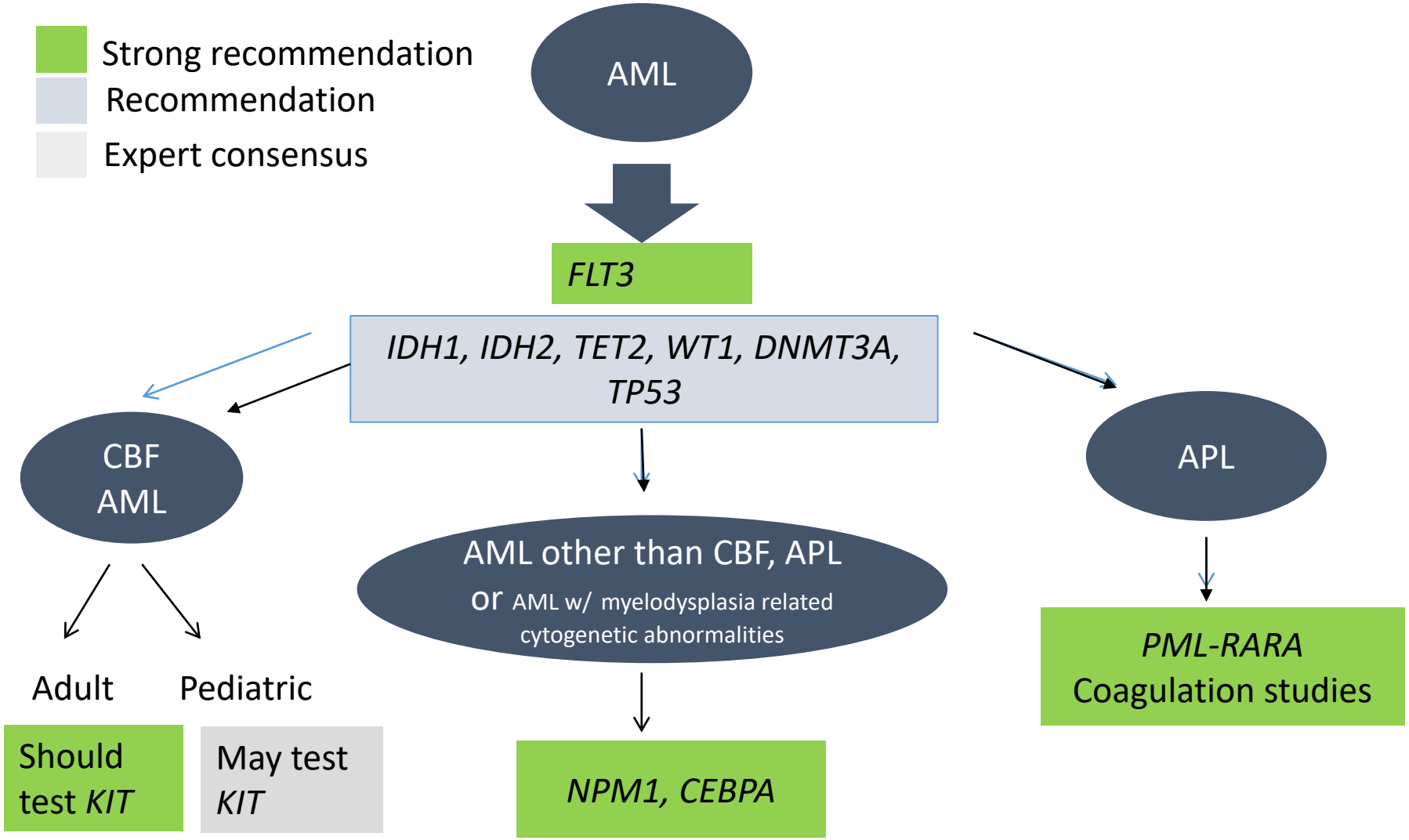
Adults: KIT (high >25% VAF)+ CBF AML worse prognosis

Pediatric: unclear

- **Therapeutic significance**
  alloHCT in relapsed/refractory KIT+ CBF AML
- **How to measure?**
  - PCR, NGS

Yohe S. J Clin Med 2015;4:460, Dohner H et al. N Engl J Med 2015;373:1136, Papaemmanuil E et al N Engl J Med 2016;374:2209, Ustun C et al. Cancer Medicine 2018 [epub ahead of print]

Molecular Testing Algorithm



PCR and NGS methodologies used for molecular testing in routine practice

	AML Testing Method Characteristics
PCR-based methods: • Real-time PCR • Allele specific PCR	• Potential to be cheaper than NGS on a single biomarker[1] • High sensitivity, potential to be <0.01%[1] • Well established methods with minimal laboratory requirements[2] • Limited degree of multiplexing[3] (restricted range of information per experiment)[3] • Able to test one gene/region at a time[2] • Faster TAT
Traditional Sequencing • Sanger sequencing • Fragment analysis	• Long read lengths (500-750 bases)[4] • High degree of raw accuracy[5] • Well established methods with minimal laboratory requirements[2] • Low sensitivity (~10-20%)[2] • Low throughput when analyzing large genes[2]
NGS	• Minimal DNA input[2] • High sensitivity[2] • Reduced costs for labs when multiple genes being tested (ex: *IDH1/IDH2*, *FLT3*, *NPM1*, ...)[6] • May be more expensive than PCR-based[2] • Complex and long data analysis, requiring expertise on bioinformatics and dedicated softwares[2] • Long TAT[2]

1. Shires K et al. *Med Technol SA*. 2011;25:39. 2. Black JS et al. *Pathogenesis*. 2015;2:9. 3. ten Bosch JR et al. *J Mol Diagn*. 2008;10(6):484-492. 4. Chaisson M et al. *Bioinformatics*. 2004;20:2067. 5. Shendure J et al. *Nat Biotechnol.* 2008;26:1135. 6. Wertheim G, Bagg A*J Mol Diagn*. 2011;13:605.

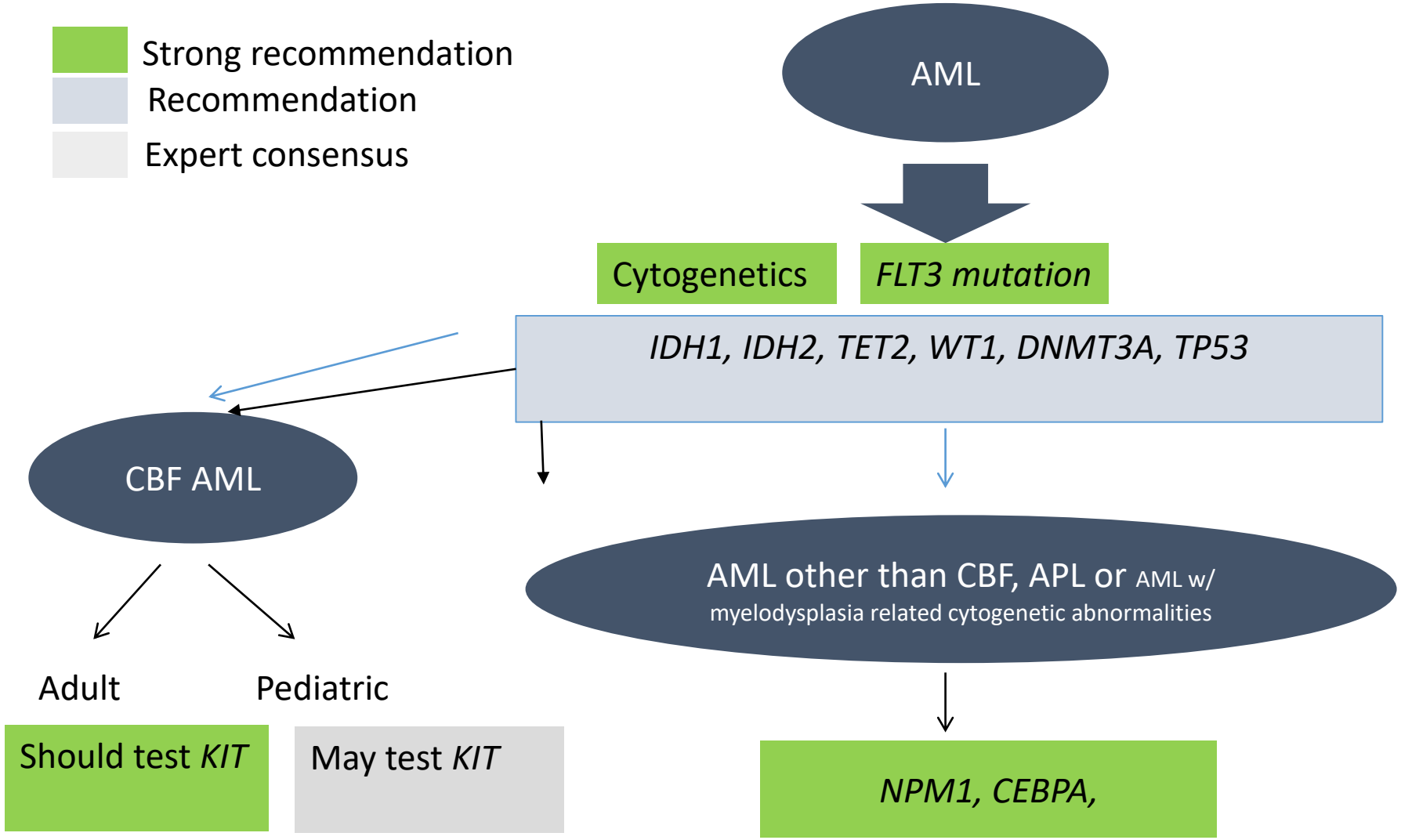
Case #1

- A 37-year-old man presents to the emergency department complaining of fatigue and shortness of breath with two-week history of worsening exercise tolerance and a rather abrupt onset of shortness of breath over the past several hours. The patient has no major past medical history and works as an architect. Her laboratory results reveal the following:
- White blood cells -74.1 × 10^9/L
- Hemoglobin-7.3 g/dL
- Platelet count- 24 × 10^9/L
- White blood cell (WBC) differential is notable for 39% blasts (don’t look like promyelocytes

Next Step

- Flow cytometry was performed
- Showed CD34, CD13, CD33, HLADR, CD117 and MPO
  - AML
- What should be our next step?

Testing algorithm



Testing algorithm

- Two options
- Targeted PCR/RT or Sanger Sequencing- NPM1, CEBPA, FLT3
- NGS sequencing- will have all the genes
  - Turn around time is longer
  - Might not work for FLT3 testing

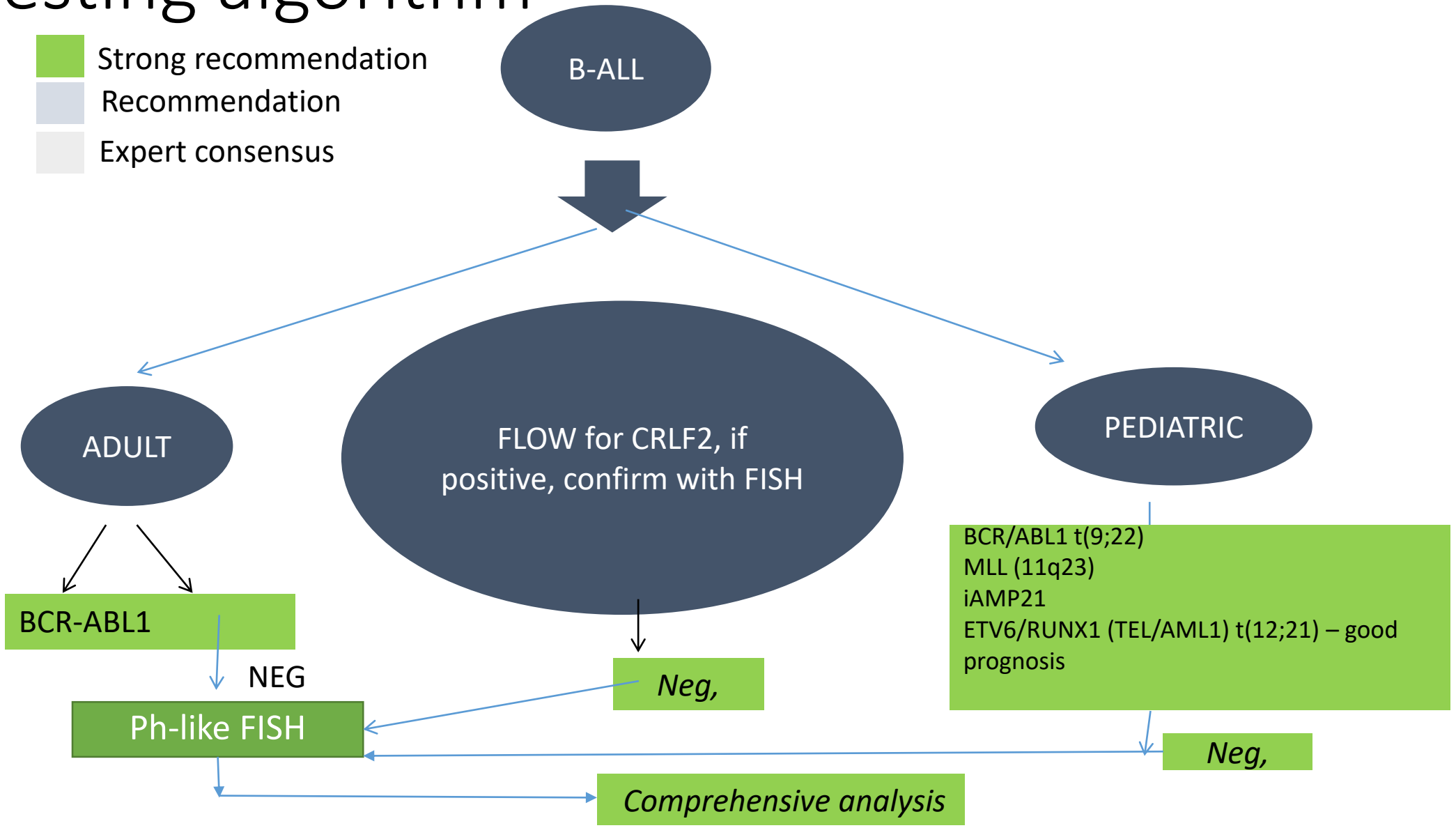
Case #2

- A 37-year-old man presents to the emergency department complaining of fatigue and shortness of breath with two-week history of worsening exercise tolerance and a rather abrupt onset of shortness of breath over the past several hours. The patient has no major past medical history and works as an architect. Her laboratory results reveal the following:
- White blood cells -74.1 × 10^9/L
- Hemoglobin-7.3 g/dL
- Platelet count- 24 × 10^9/L
- White blood cell (WBC) differential is notable for 39% blasts.

Next Step

- Flow cytometry was performed as the initial step
- Showed CD34, CD10, CD19, CD22 and TdT
  - Diagnosis - B-ALL
- What should be our next step?

Testing algorithm



Conclusion

- Laboratory evaluation is critical, though complex
- Morphologic evaluation, immunophenotyping, and karyotype analysis should be performed on all cases
- Molecular genetic testing is evolving with targeted therapies
- On going updates will be needed for the guideline to remain relevant

Acknowledgement

- Special thanks to Tracy George, MD, Jay Patel, MD,MBA and Xinje Xu, PhD

Thank you!