Classification of Leukemias and Lymphomas: Increasing Role of Molecular Testing

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Objectives

- Discuss how molecular and cytogenetic findings are used to:
  - More precisely classify leukemia and lymphoma.
  - Add prognostic information
    - May evolve into classification in the future.
- Discuss clonal hematopoiesis and “pre-MDS.”
- Include a mix of cases where molecular and/or cytogenetic data were used as above.

Genetically Defined Hematologic Malignancies

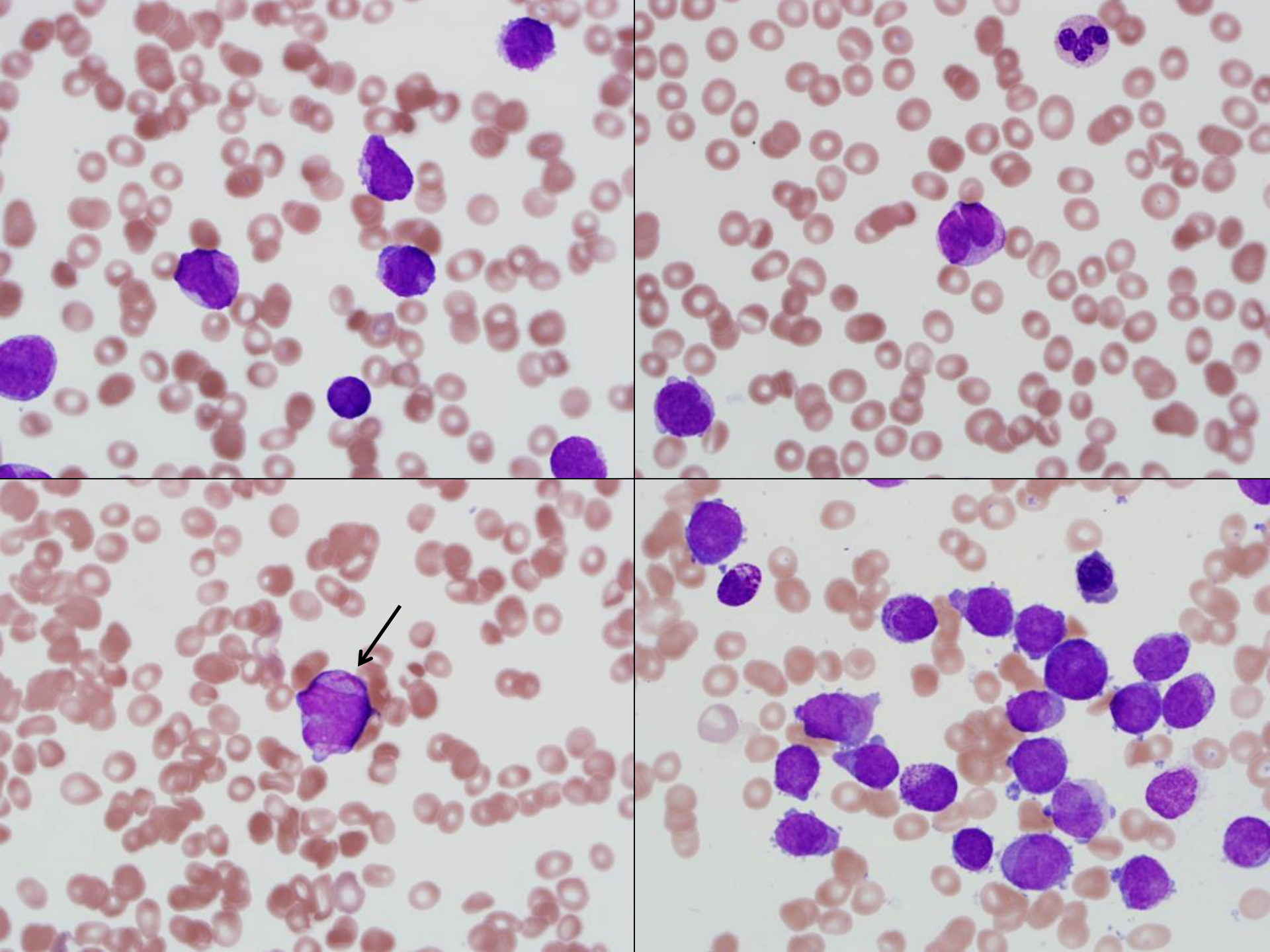
- CML was the first
  - Diagnosis requires t(9;22)

- Acute leukemia diagnosis: recurrent cytogenetic changes and mutations.
  - Diagnose AML with <20% blasts.

- Lymphoma: Limited role now, but expect it to increase.

Acute Myeloid Leukemia with Recurrent Genetic Abnormalities

- *AML with t(8;21)(q22;q22), *RUNX1-RUNX1T1 (CBFA/ETO)*
- *AML with inv(16)(p13q22) or t(16;16)(p13;q22), *CBFB-MYH11*
- *APL with t(15;17)(q22;q11-12), *PML-RARA*
- AML with t(9;11)(p22;q23), *MLLT3-MLL and other balanced translocations of 11q23 (MLL)*
- AML with t(6;9)(p23;q34), *DEK-NUP214*
- AML with inv(3)(q21;q26.2) or t(3;3)(p13;q13), *GATA2, MECOM*
- AML with t(1;22)(p13;q13), *RBM15-MKL1*

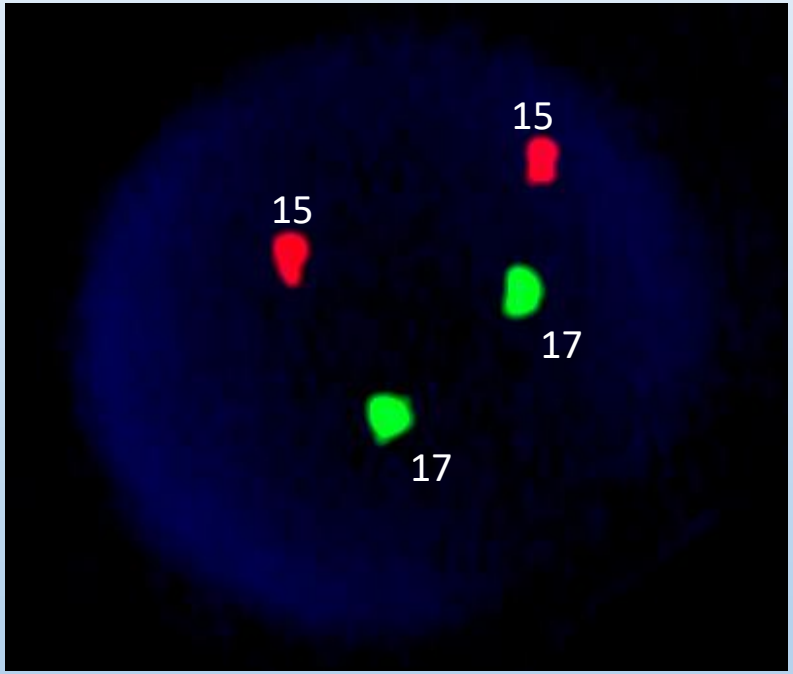


Morphology Highly Suggestive of Acute Promyelocytic Leukemia

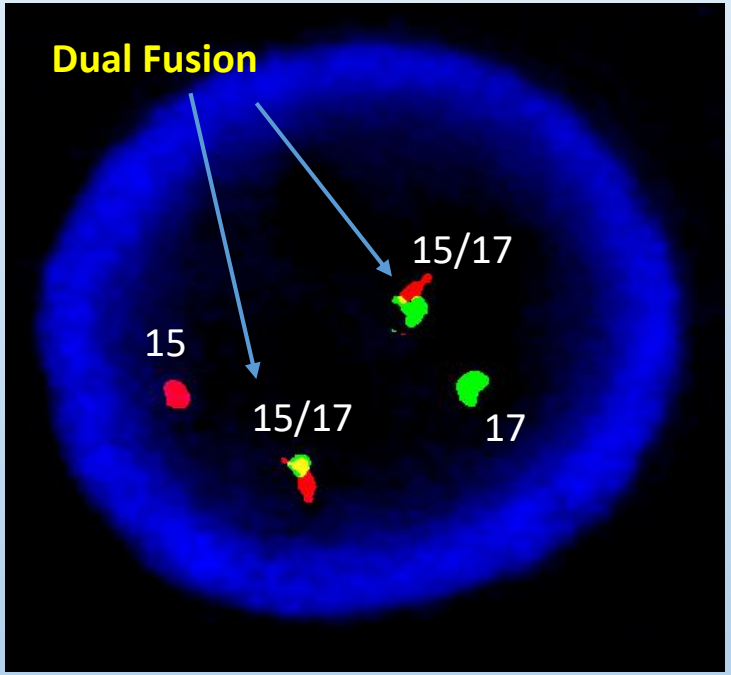
- Patients can present with DIC.
  - Thrombocytopenia, schistocytes.
- Emergent diagnosis required.
- Patients respond well to all-trans retinoic acid (ATRA).
- Treatment regimen distinct from other AMLs: ATRA, arsenic.
- Good prognosis.

t(15;17) *PML-RARA* fusion is diagnostic of APL

Normal



Abnormal



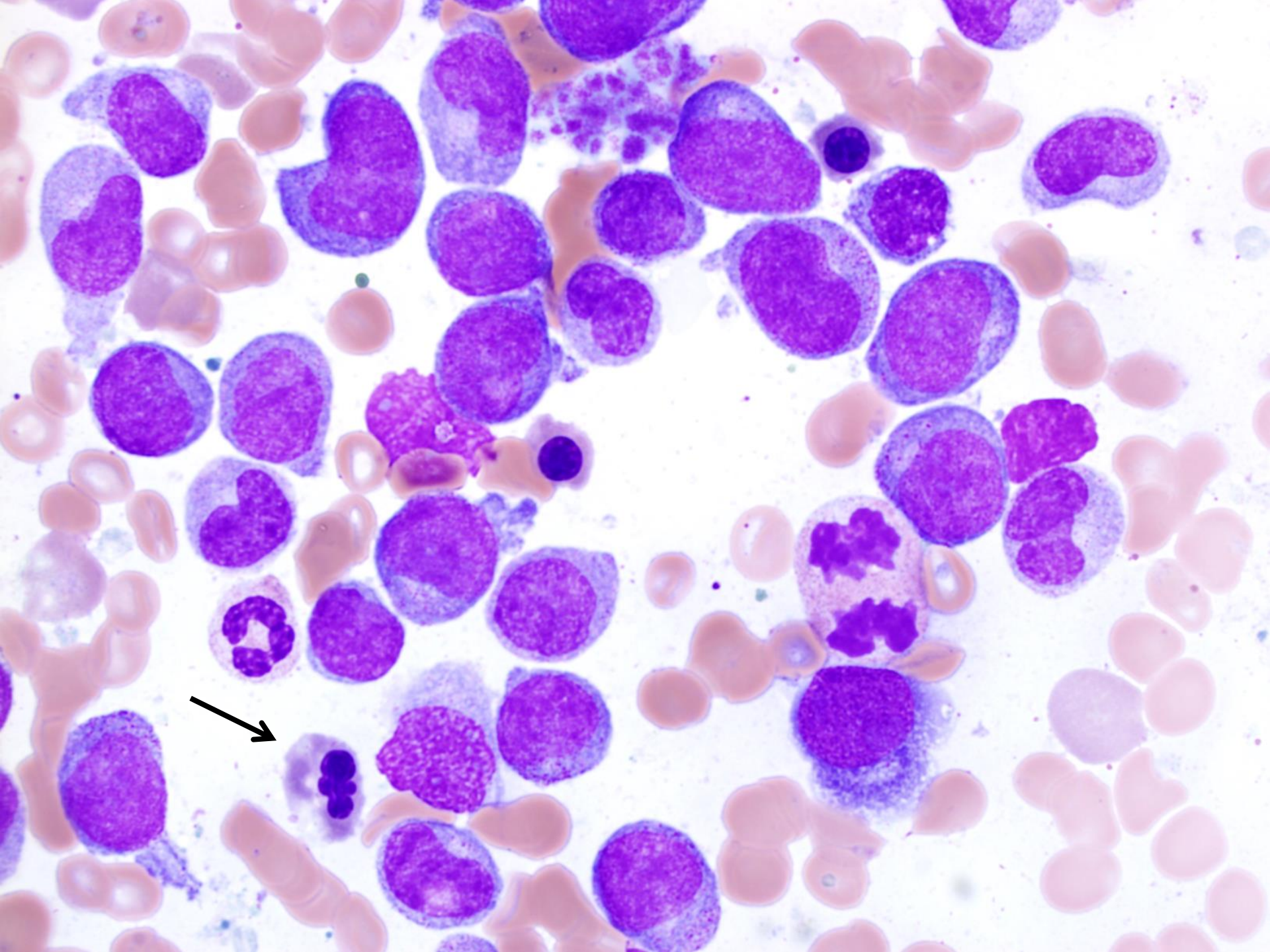
- FISH (or RT-PCR) is recommended at diagnosis for quick turn-around time.
- RT-PCR for *PML-RARA* fusion product for disease monitoring.

Cytogenetically Normal AMLs: Mutation Studies for Classification

- Mutations
  - *NPM1* (nucleophosmin)
  - *CEBPA* (CCAAT enhancer binding protein alpha)
- WHO 2017 Categories:
  - AML with mutated *NPM1*
  - AML with biallelic mutations in *CEBPA*
  - AML with mutated *RUNX1 (provisional)*

Case:

- 54-year-old woman noticed increased bleeding while brushing her teeth.
- CBC:
  - WBC: 7.2 k/µL with occasional circulating blasts
  - Hgb: 10.1 g/dL
  - HCT: 30.7%
  - PLT: 97 k/µL
- Bone marrow evaluation.



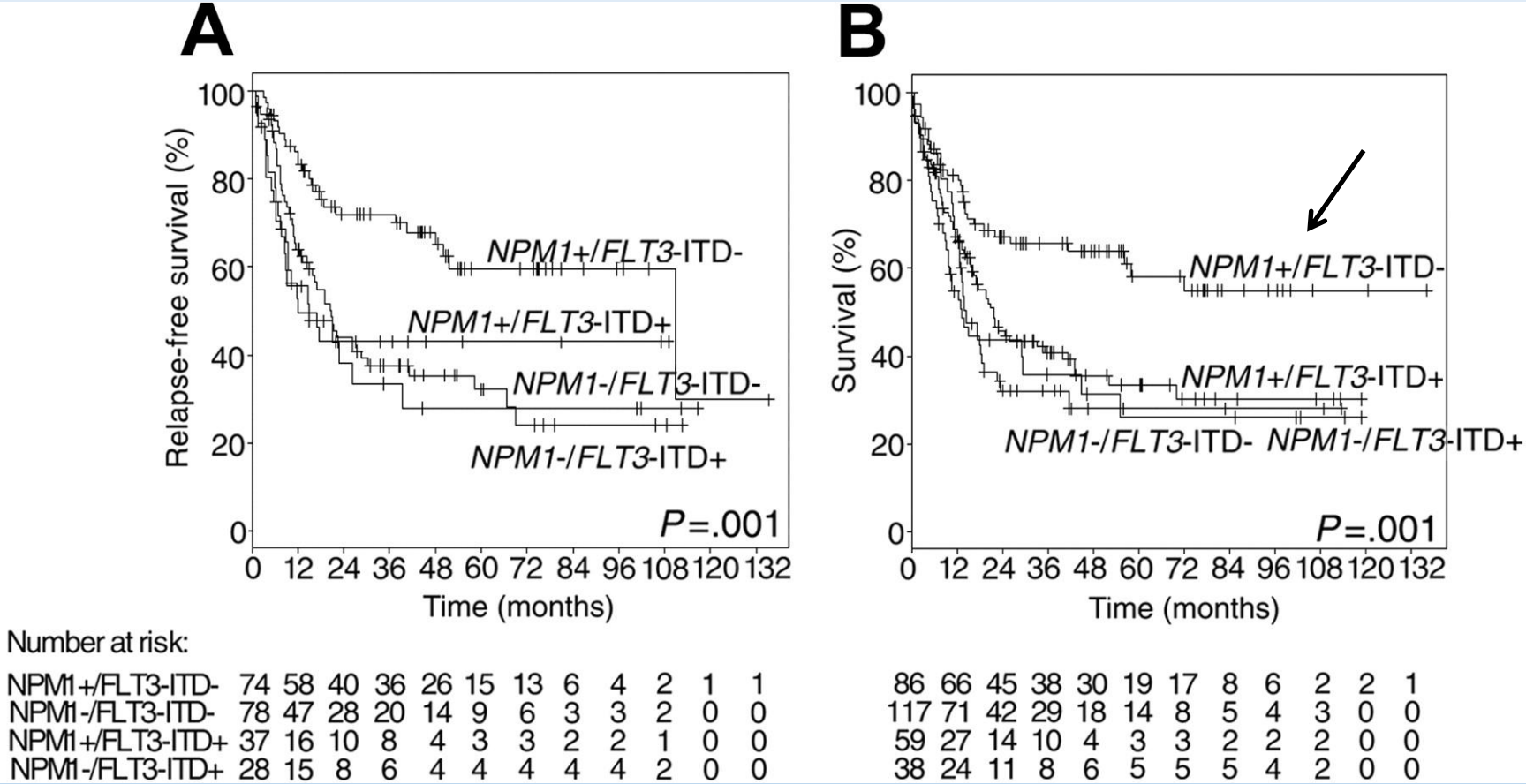
Hematopathology Work-up

- Blasts represented 57% of the bone marrow nucleated cells by differential count.
- Flow cytometry identified a prominent blast population which expressed CD13, CD33, CD117, CD34, CD7, myeloperoxidase and HLA-DR.
- Diagnosis: acute myeloid leukemia.
- 1-4 days later, normal AML FISH panel.
  - APL t(15;17) result first.
- 7 days later, normal karyotype: 46,XX [20].
- 14 days later, NGS panel results: *NPM1* mutation, no *FLT3* or *CEBPA* mutations.

Diagnosis: AML with mutated *NPM1*

- Original pathology report is amended to include precise classification.
- Significant dysplasia not associated with worse prognosis in this setting.
- Monitoring option: *NPM1* mutation by quantitative RT-PCR to detect minimal residual disease (MRD).
- Prognostic information.

NPM1 mutated, *FLT3* Wild Type Identifies a Subgroup with a Better Prognosis



Blood 106:3740, 2005.

AML Summary

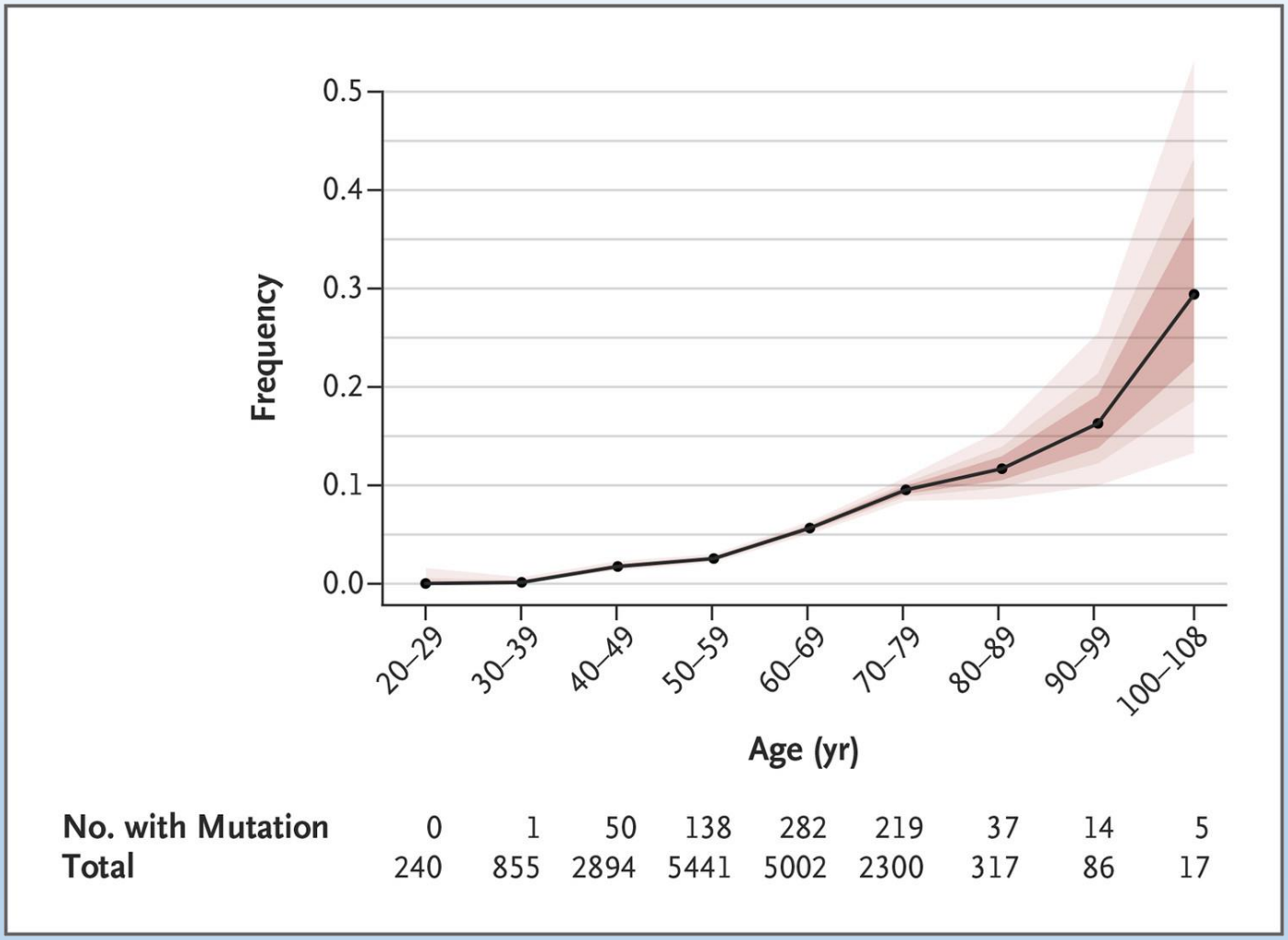
- Morphology still the first and most important step.
  - Most cases still diagnosed based on blast percentage.
- Cytogenetics are critical.
  - Define AML in absence of increased blasts.
  - Provide prognostic information.
- Molecular testing.
  - Specific classification and prognostic information.
- Amount of testing required depends on the clinical situation.

Clonal Hematopoiesis +/- Cytopenias

Clonal Hematopoiesis

- Mutations in leukemia-associated driver genes are commonly detected in apparently healthy older people with normal blood counts.
  - Predominantly *DNMT3A*, *TET2*, and *ASXL1*
- Mutations are present in a clone and confer a survival advantage.
  - Would not be detectable in a single cell.
- Associated with risk of developing myeloid neoplasms (sometimes lymphoid) or cardiovascular events.

Prevalence of Somatic Mutations Increases with Age



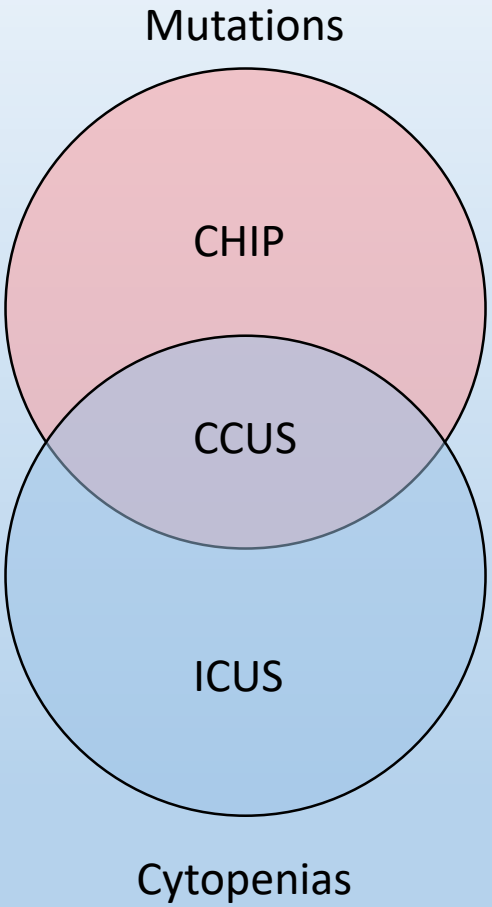
No. with Mutation	0	1	50	138	282	219	37	14	5
Total	240	855	2894	5441	5002	2300	317	86	17

Jaiswal S et al. N Engl J Med 2014;371:2488-2498.

The NEW ENGLAND JOURNAL of MEDICINE

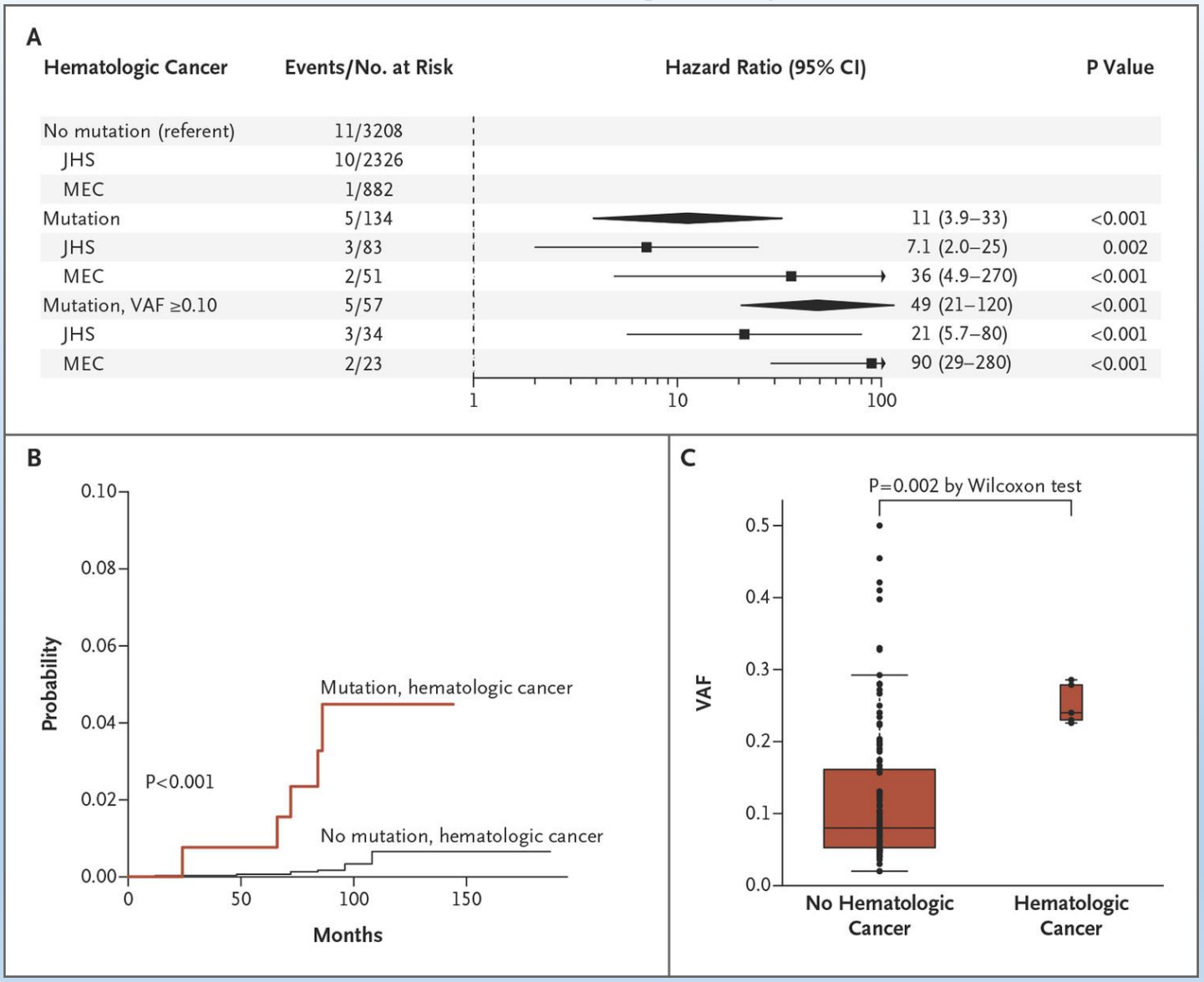
Terminology

- CHIP: Clonal Hematopoiesis of Indeterminate Potential.
  - By definition, patients lack cytopenias.
- CCUS: Clonal Cytopenias of Undetermined Significance.
- ICUS: Idiopathic Cytopenias of Undetermined Significance (no mutation detected).
- Clonality ≠ Malignancy
- MDS: Fulfills WHO 2017 criteria.



Steensma DP. Blood Advances 2018, v2.

Risk of Hematologic Cancers is Related to Mutant Variant Allele Frequency



Jaiswal S et al. N Engl J Med 2014;371:2488-2498.

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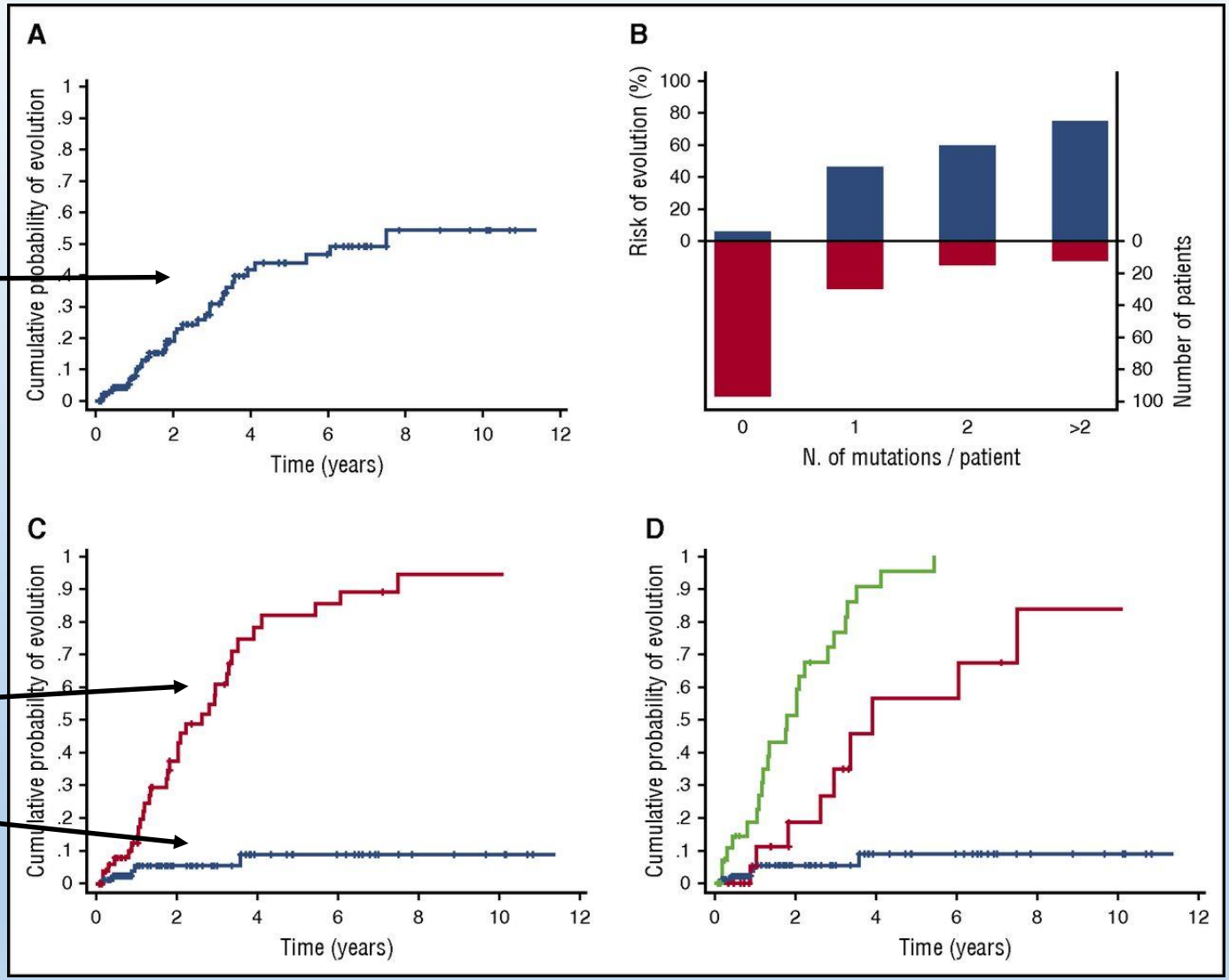
Probability of progression to a myeloid neoplasm in patients with cytopenias is higher when mutation(s) are detected

All patients with cytopenias

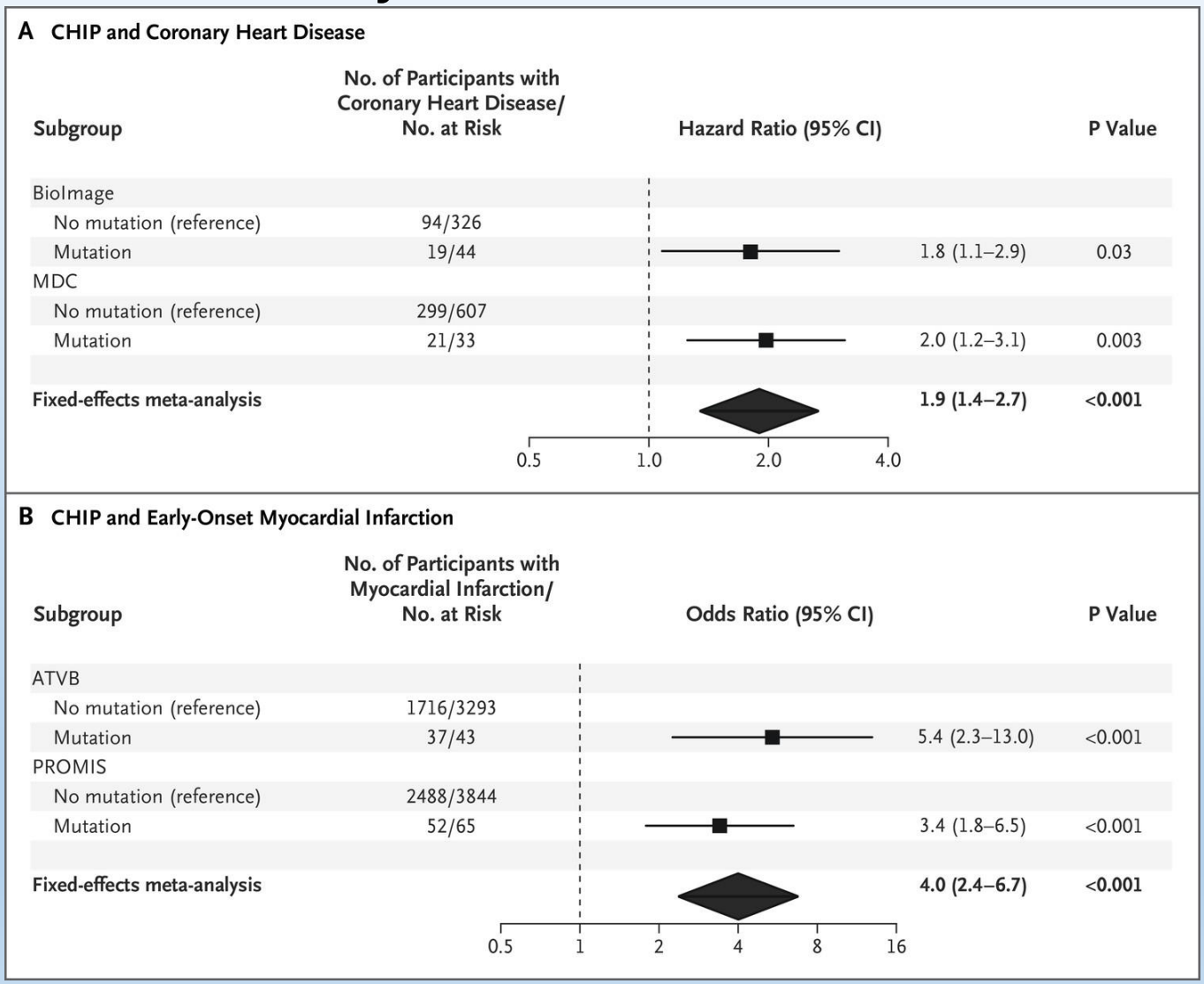
CCUS

ICUS



Luca Malcovati et al. Blood 2017;129:3371-3378

Association between Clonal Hematopoiesis of Indeterminate Potential (CHIP) and Coronary Heart Disease and Early-Onset Myocardial Infarction.



Jaiswal S et al. N Engl J Med 2017;377:111-121.

The NEW ENGLAND JOURNAL of MEDICINE

NGS Sequencing Panels

- Include many myeloid driver mutations including those most common in CHIP.

ARUP's Myeloid NGS Panel:

Genes – *ASXL1, ASXL2, BCOR, BCORL1, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT1, DNMT3A, EED, ELANE, ETNK1, ETV6, EZH2, FAM5C, FLT3, GATA1, GATA2, HNRNPK, IDH1, IDH2, JAK2, JAK3, KDM6A, KIT, KRAS, LUC7L2, MAP2K1, MLL, MPL, NOTCH1, NPM1, NRAS, NSD1, PHF6, PRPF40B, PRPF8, PTPN11, RAD21, RUNX1, SETBP1, SF1, SF3A1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, SUZ12, TET2, TP53, U2AF1, U2AF2, WT1, ZRSR2*

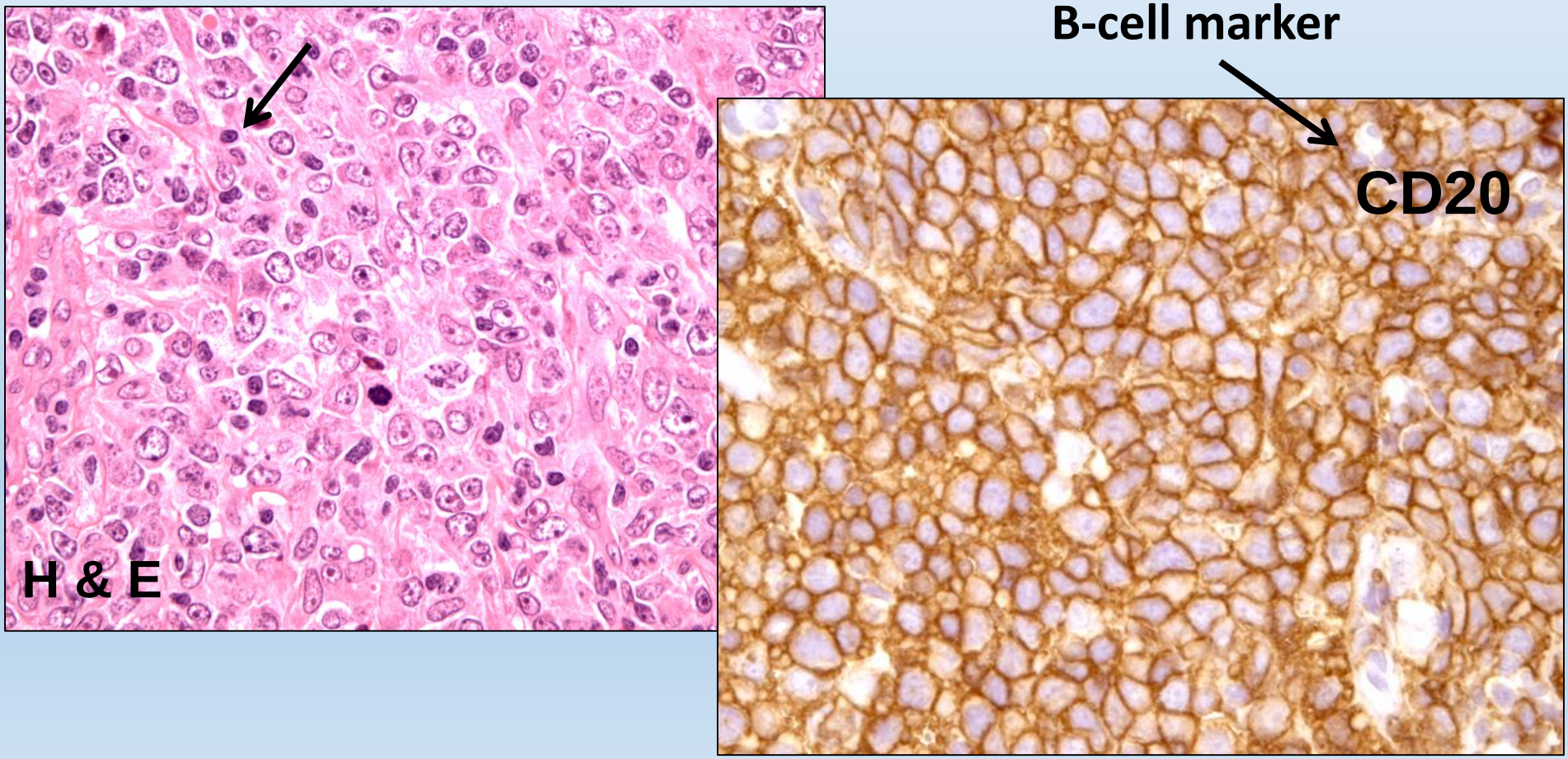
CHIP and Clonal Cytopenias: Key Points

- CHIP: when you run NGS on normal older patients.
- ICUS: Cytopenias without (detected) mutations
- CCUS: MDS without dysplasia.
  - Similar to cytogenetically-defined MDS.
  - Likely considered as such in future classification.
- Cardiovascular disease is much more common than MDS, so this is the bigger risk for patients with CHIP.
  - Likely involves clonal monocyte/macrophages and increased local inflammation in atherosclerotic plaques.
  - Anti-inflammatory anti-IL-1beta monoclonal antibody decreased recurrent events after MI.
  - Anti-inflammatory therapy for patients with CHIP?
  - Fasting lipid panel…and NGS myeloid panel?

Genetic Testing in Lymphoma

Diffuse Large B-cell Lymphoma

- Ancillary testing required for diagnosis: CD20.



Diffuse Large B-cell Lymphoma Ancillary Testing

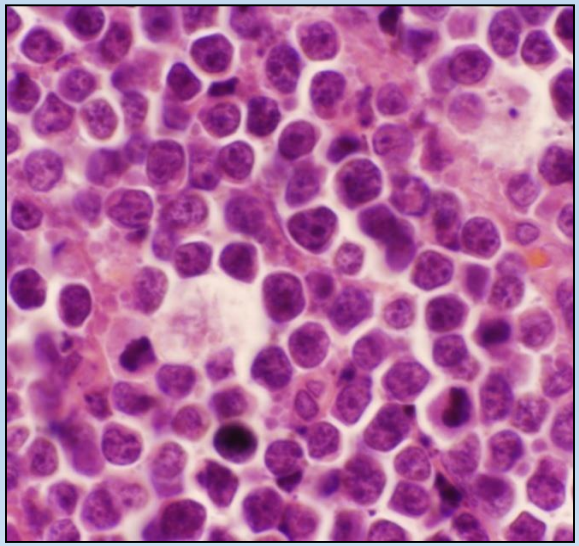
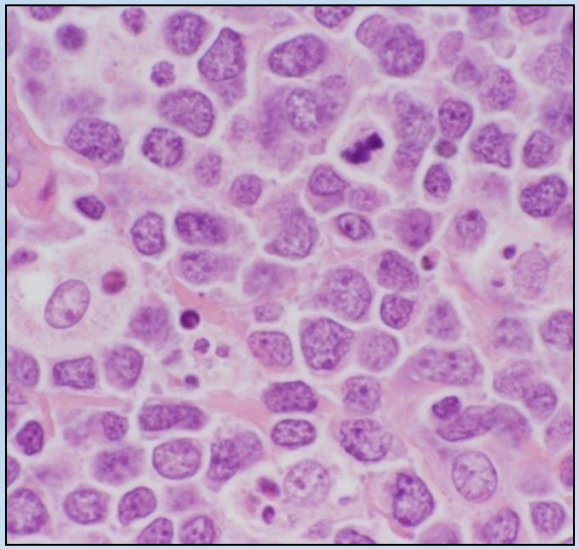
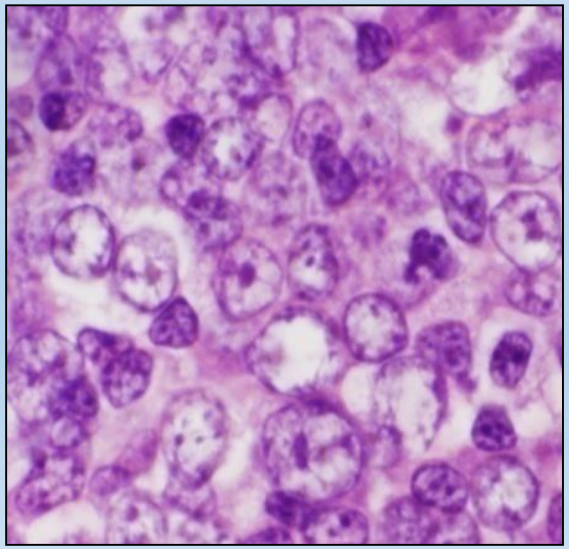
- Useful for for sub-classification and/or prognostic information
  - EBER
  - FISH for *MYC, BCL2, BCL6* translocations
  - MIB-1
  - GC vs. non-GC subtyping
  - MYC, BCL2 protein expression

MYC and *BCL2* Rearrangements and Protein Expression: Inform Prognosis and Guide Therapy

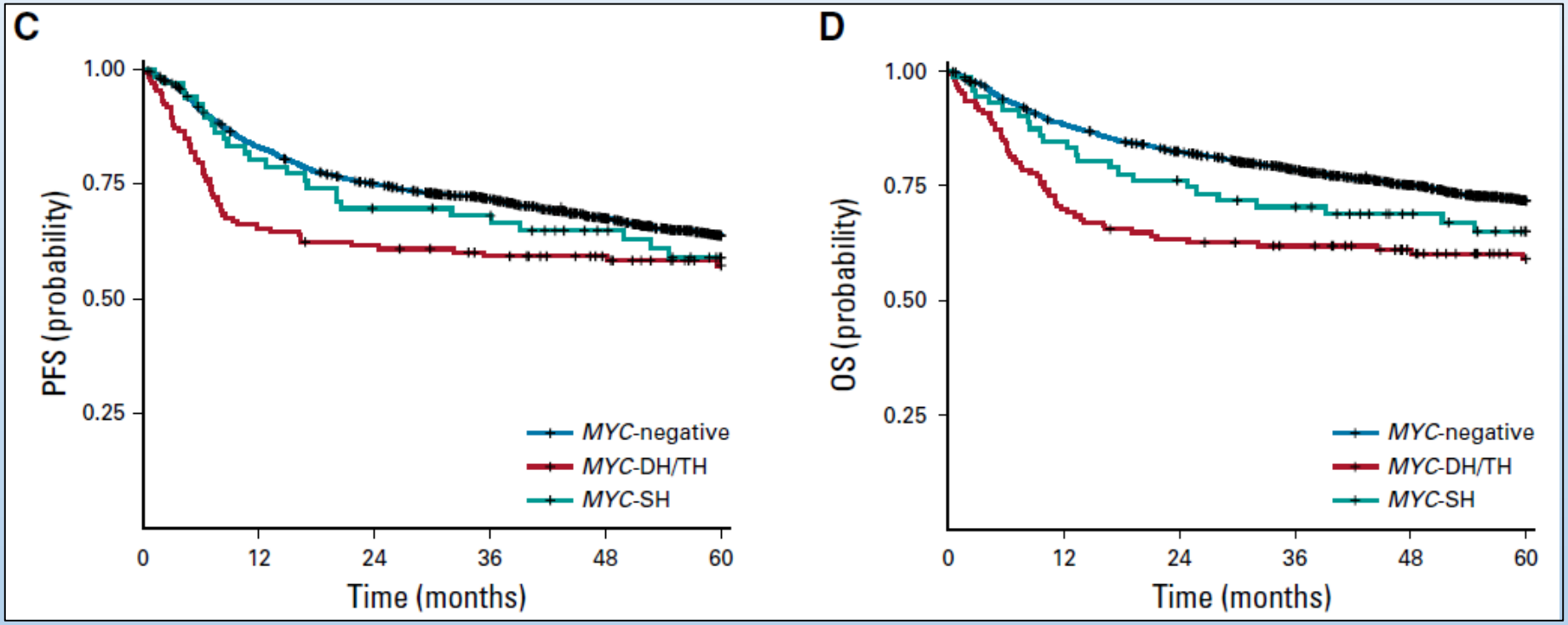
- Diffuse large B-cell lymphoma, NOS
- Double-expresser (DE) DLBCL, NOS
  - Expresses MYC (>40%) and BCL2 (>50%) protein
- High grade B-cell lymphoma double hit (HGBL-DH), 4-6% of DLBCL.
  - *MYC/BCL2*, 80% (includes 20% triple hit).
  - *MYC/BCL6*, 20%.

High-Grade B-cell Lymphoma with *MYC* and *BCL2* and/or *BCL6* Rearrangements (WHO 2017)

- Aggressive presentation, often disseminated (PB, BM, CSF).
- Can resemble BL with increased pleomorphism and/or atypical immunophenotype or genetic features.
- *MYC* complex karyotype is common.

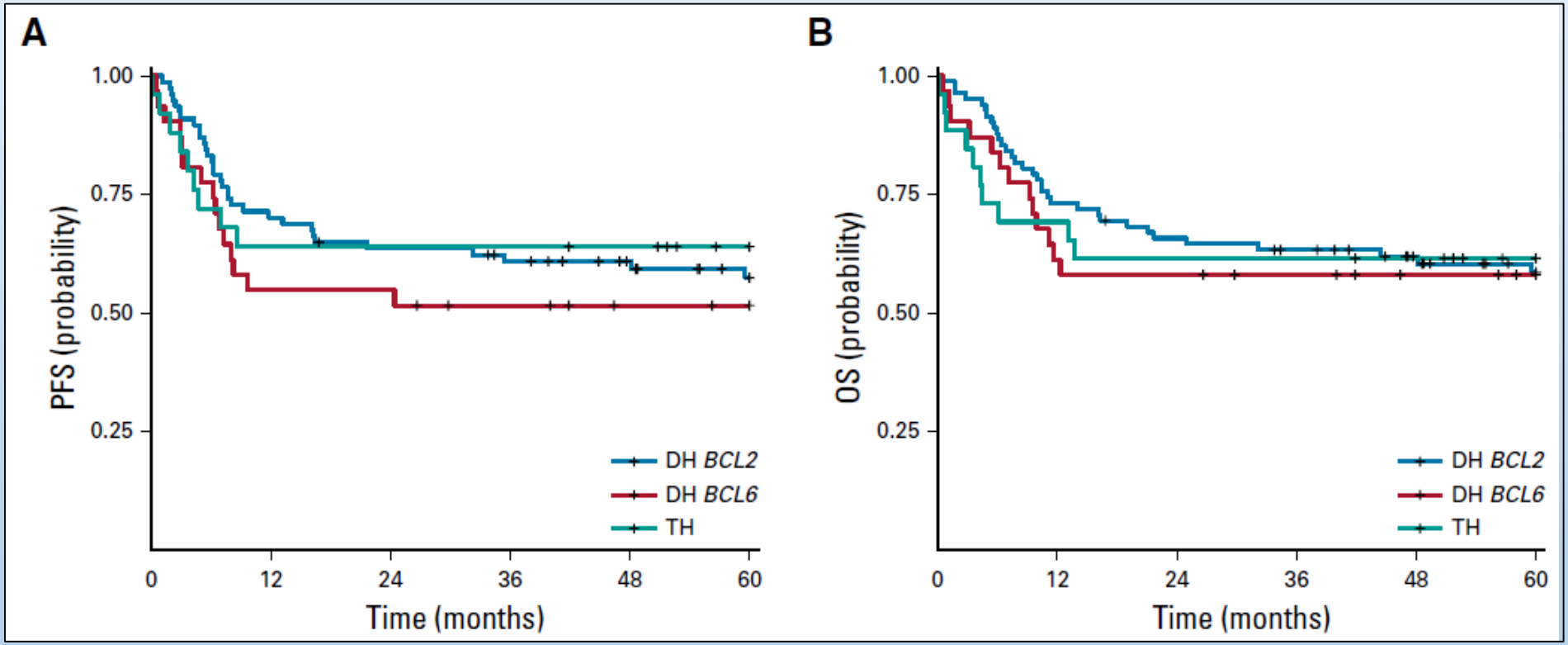




MYC DH/TH Have Worse Outcomes than MYC-N or MYC-SH



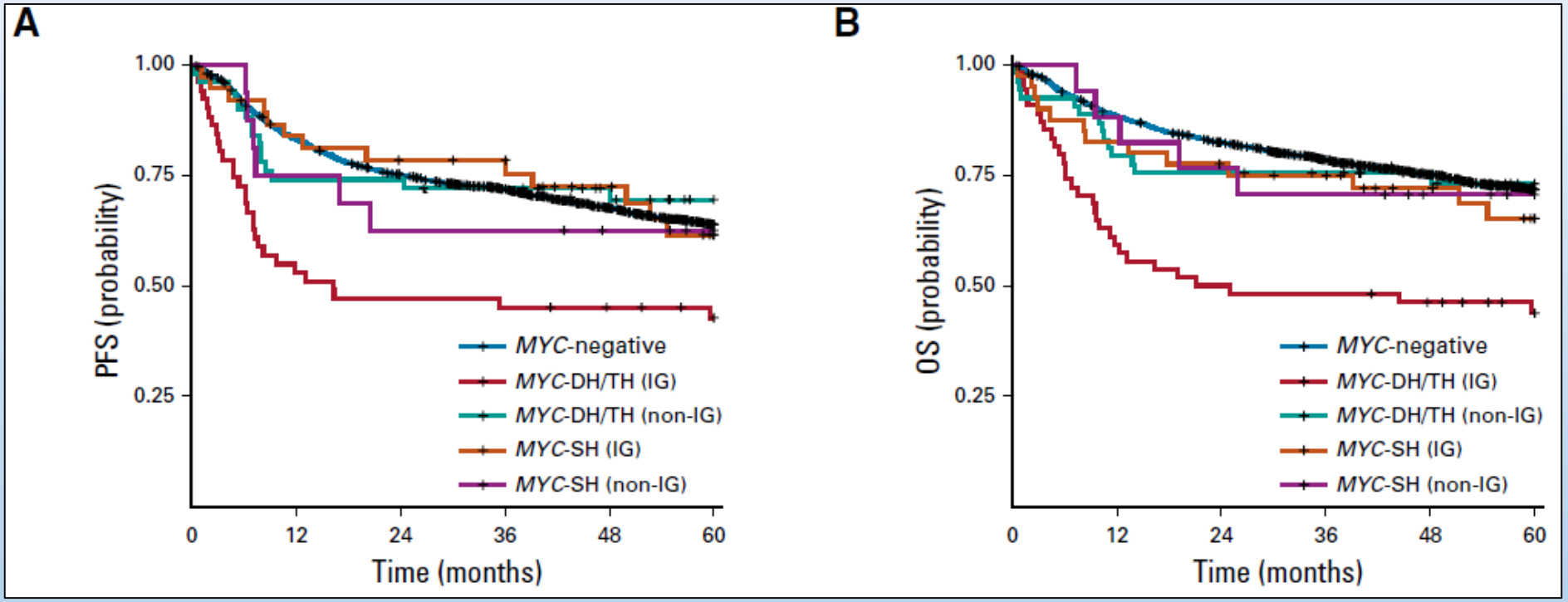
Rosenwald *et al.,* J Clin Oncol 37, 2019.

No Significant Difference Between MYC/BCL2, MYC/BCL6, or TH



Rosenwald *et al.,* J Clin Oncol 37, 2019.

Partner Matters: DH/TH with non-IG MYC Partners Don't Do Worse



- Suggests that MYC break-apart should be followed by FISH for MYC-IGH, IGK-MYC, MYC-IGL.
- IG promoters/enhancers drive the highest MYC Expression.

Rosenwald *et al.,* J Clin Oncol 37, 2019.

MYC/BCL2 Co-expression has adverse effect on survival. Neither MYC nor BLC2 expression alone impacts survival.

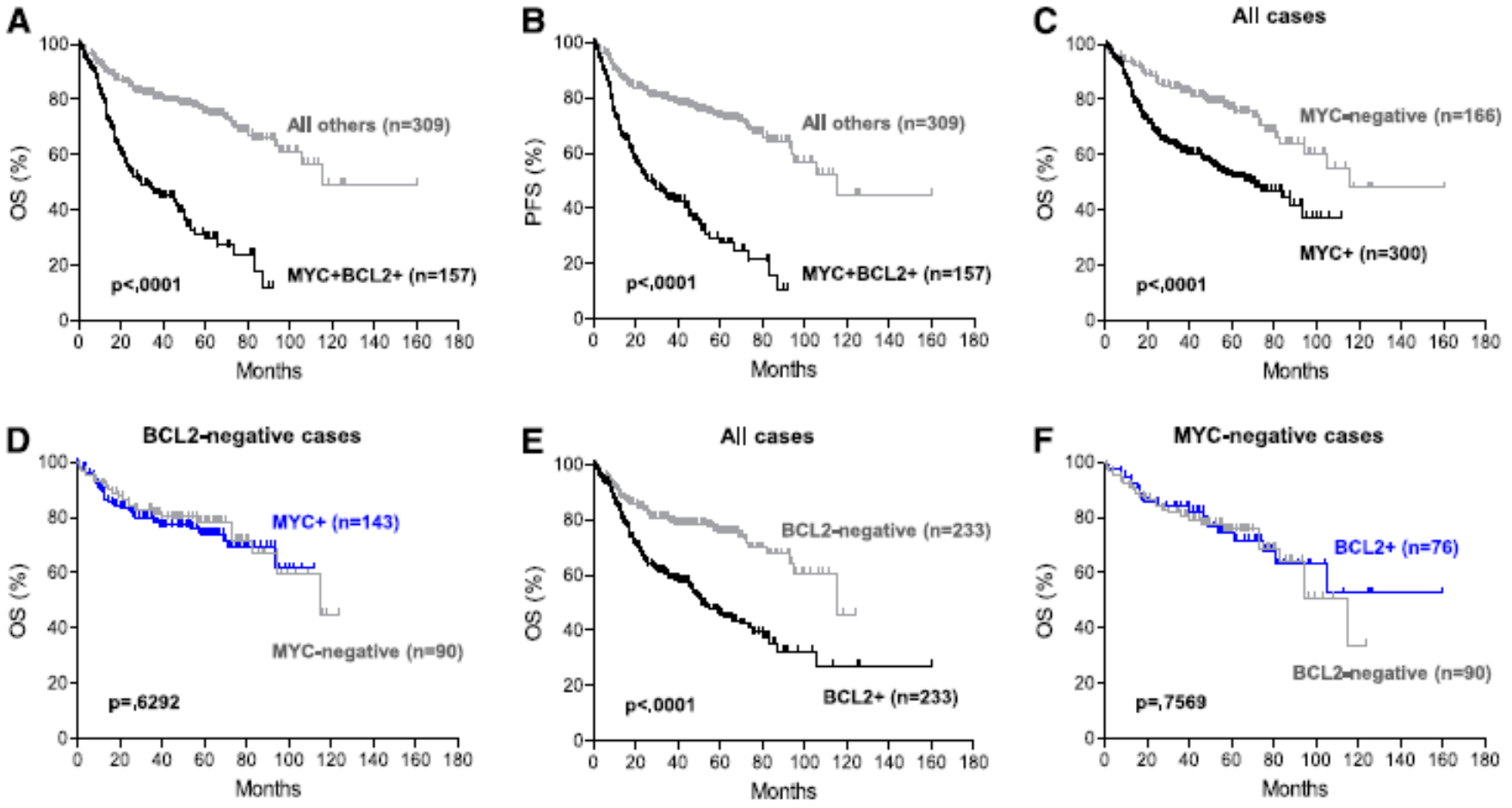
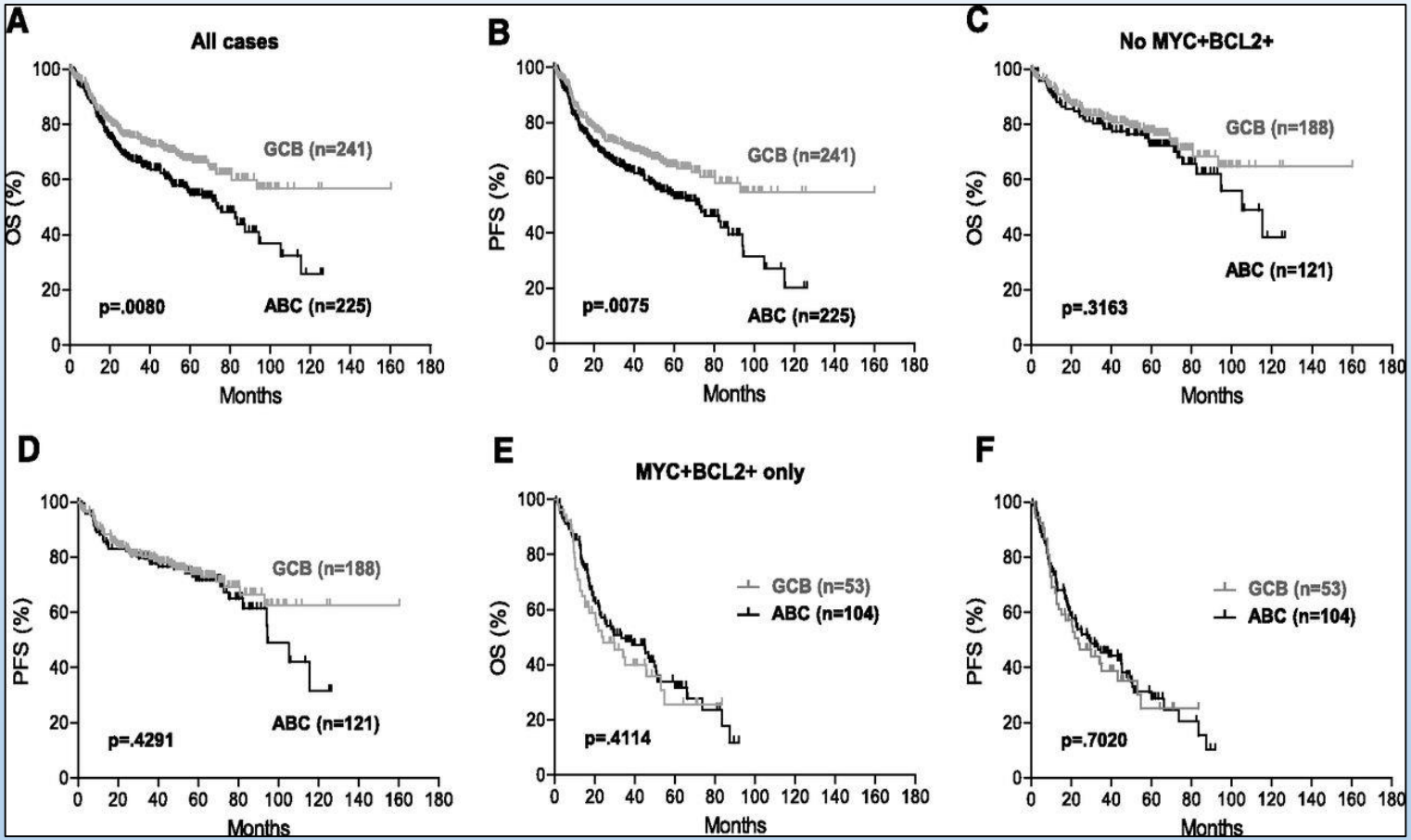


Figure 1. Prognostic impact of MYC/BCL2 coexpression in DLBCL. (A-B) OS (A) and PFS (B) of patients with DLBCL with MYC/BCL2 coexpression (MYC^+BCL2^+) in the training set. (C-D) OS of patients with MYC^+ DLBCL in the presence (C) or absence (D) of BCL2 coexpression in the training set. (E-F) OS of patients with $BCL2^+$ DLBCL in the presence (E) or absence (F) of MYC coexpression in the training set.

Hu *et al.*, Blood 121:4021-31, 2013.

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

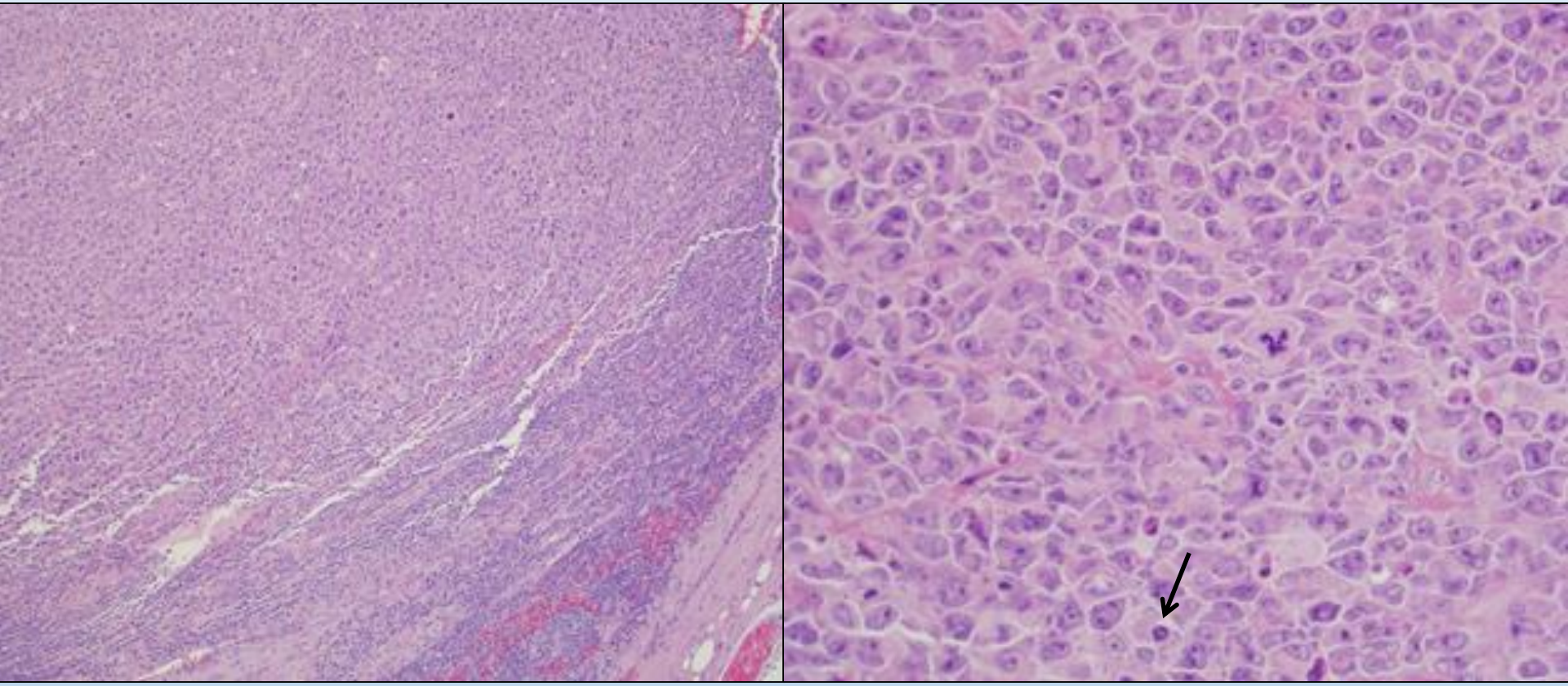
MYC/BCL2 coexpression contributes to the inferior prognosis of ABC-DLBCL.

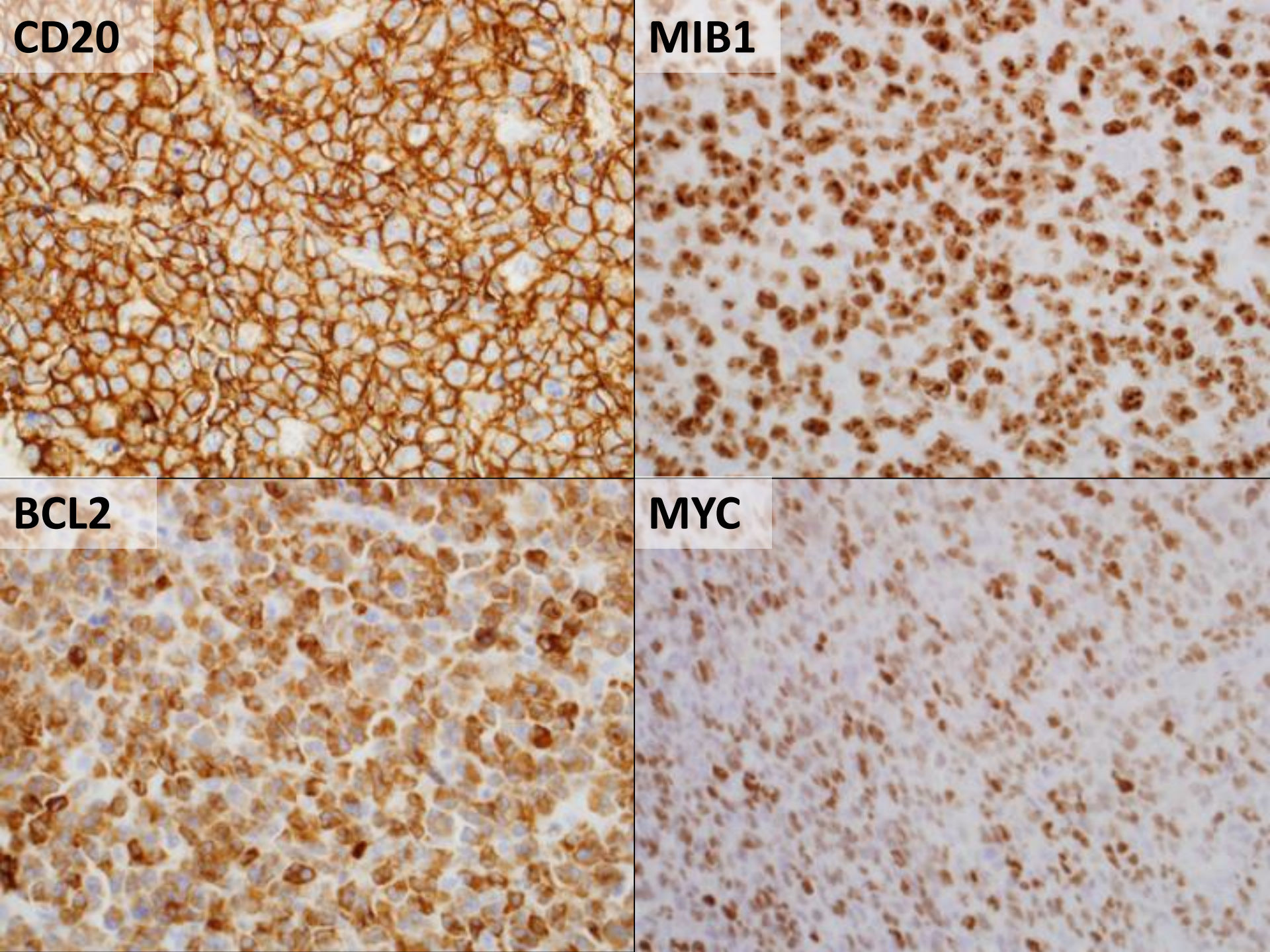


Hu S et al. Blood 2013;121:4021-4031

79-year-old woman with recent breast cancer diagnosis and enlarged lymph nodes.

- Right neck mass.



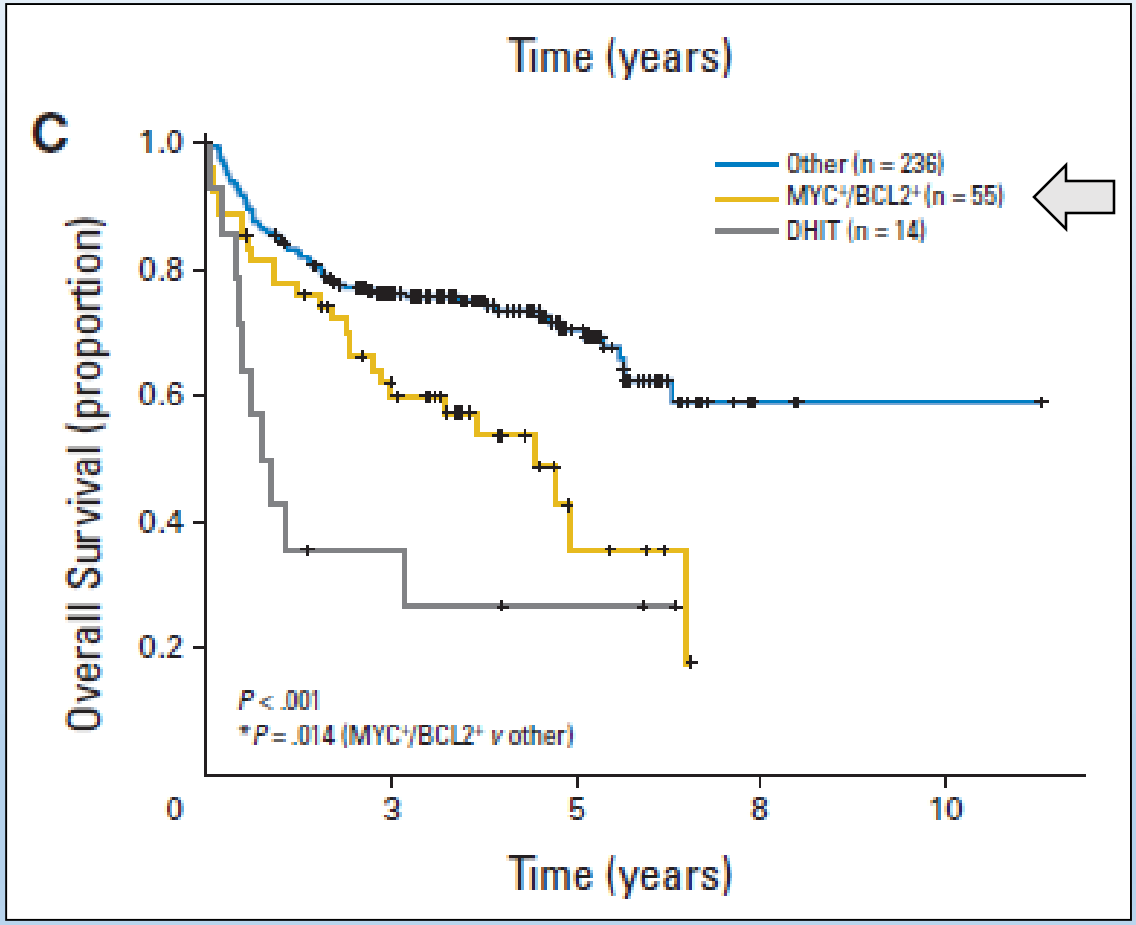

CD20
MIB1
BCL2
MYC

Results of FISH and Final Classification

- *MYC* breakapart probe assay negative.
  - *MYC* rearrangement excluded.
  - *MYC/BCL2* double hit lymphoma excluded.
- Classification:
  - Diffuse large B-cell lymphoma, activated B-cell subtype, with co-expression of MYC and BCL2.
- Prognosis: bad, but not as bad as *MYC/BCL2* double hit.

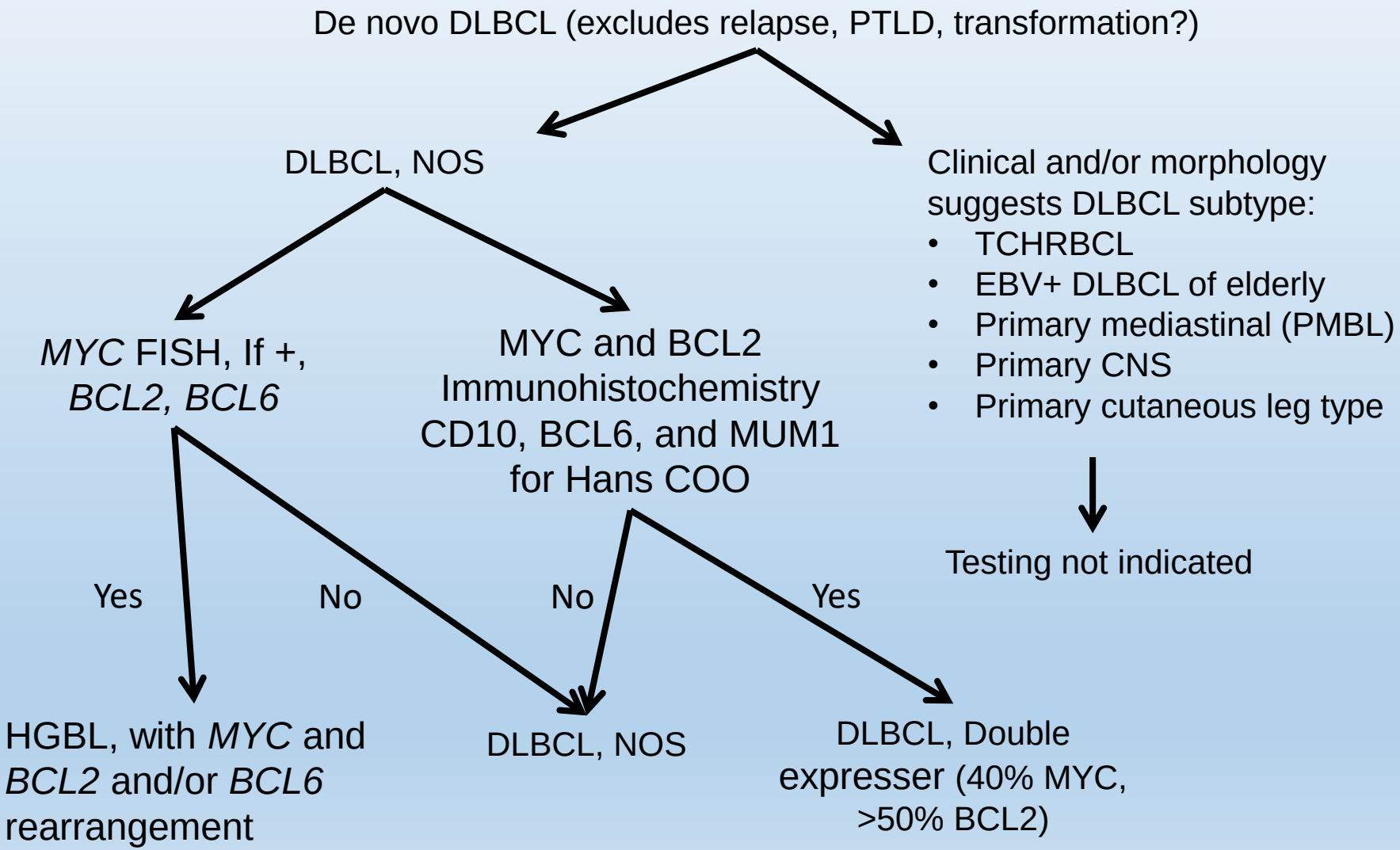
Prognosis and Treatment

- Consider DA-EPOCH-R rather than R-CHOP.



J Clin Oncol, 2012 30(28):3452-9.

DLBCL Prognostic Testing Strategy



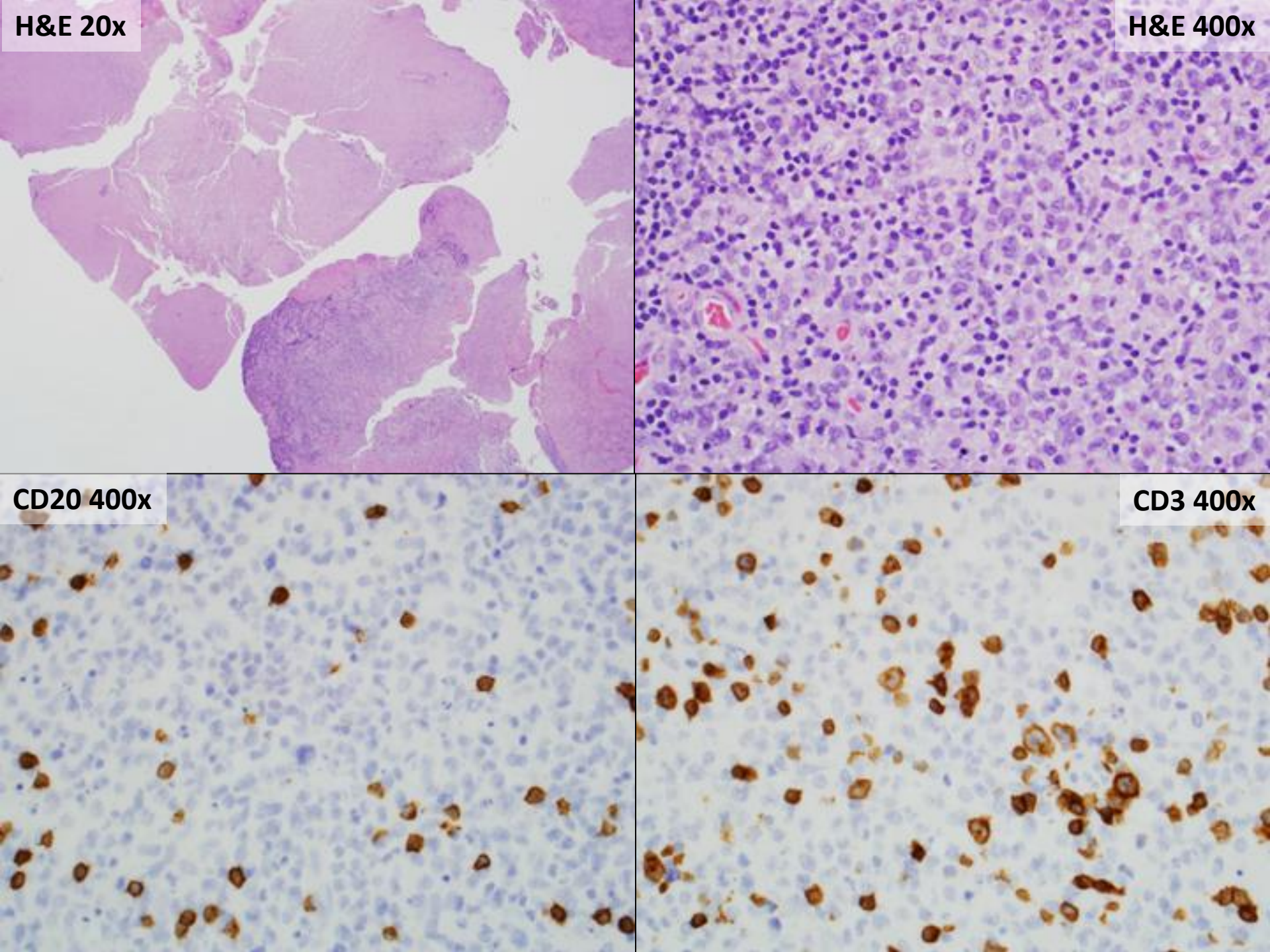
When should FISH be performed?

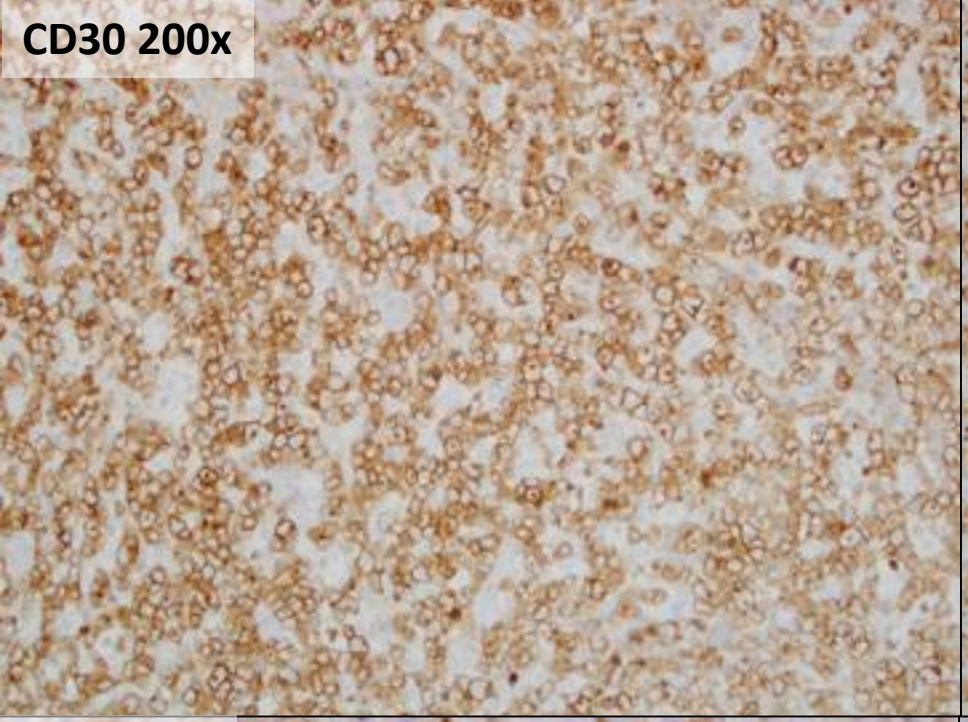
- FISH for *MYC* on most DLBCLs.
  - If positive, follow with *BCL2* and *BCL6* FISH.
- Clinical context should be considered.
  - Will it change clinical approach/therapy?

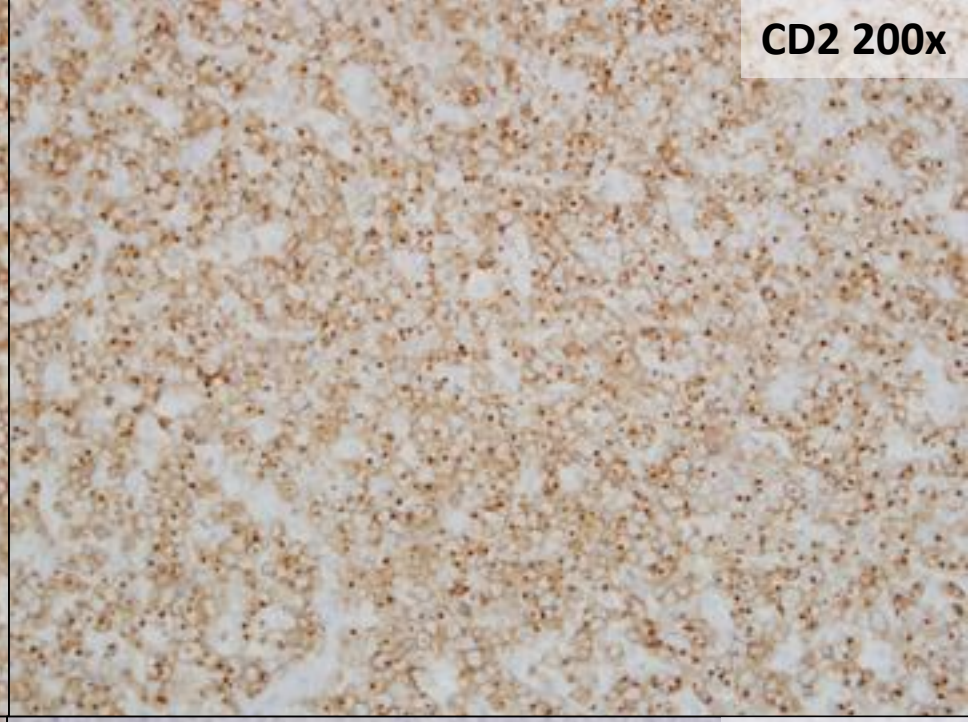
DLBCL Conclusions

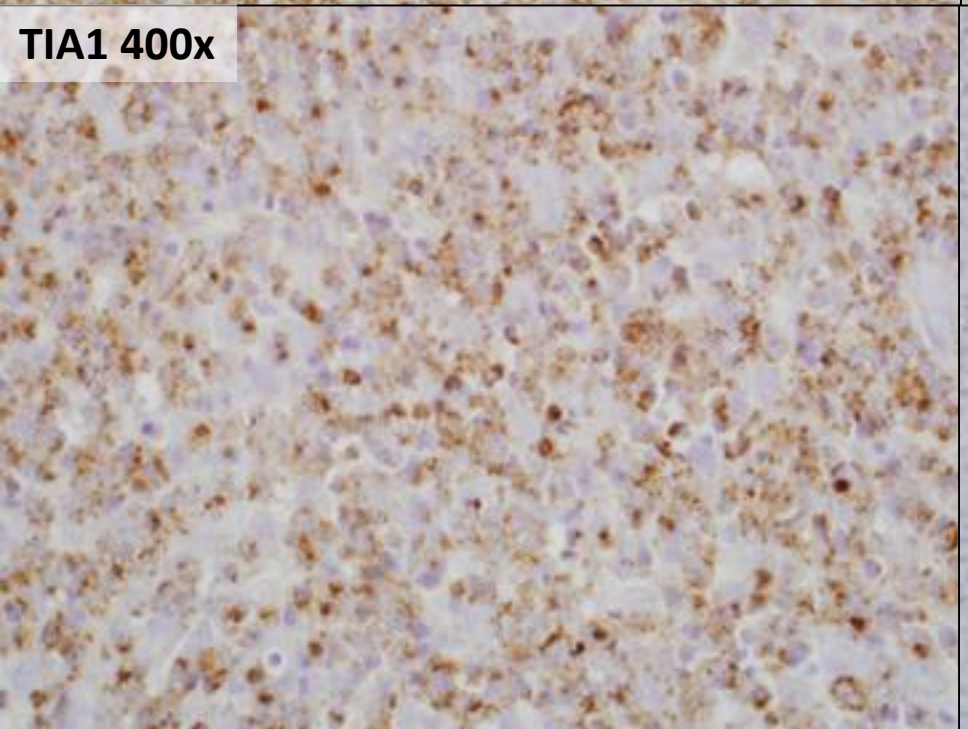
- Diagnosis of DLBCL requires only morphology and immunophenotype.
- Diagnosing or excluding the WHO 2017 category HGBL, with *MYC*+*BCL2* +/- *BCL6* rearrangement requires FISH.
  - A genetically-defined lymphoma.
- Testing should be performed when results will affect patient care.

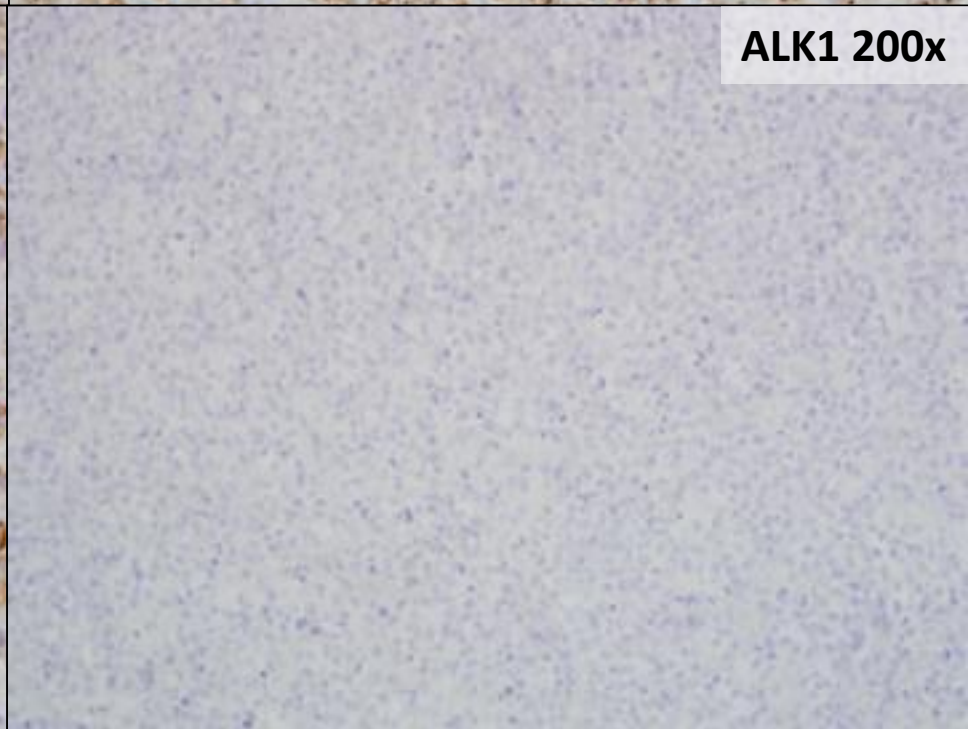
Case: 9-year-old boy with a mediastinal mass


H&E 20x
H&E 400x
CD20 400x
CD3 400x


CD30 200x


CD2 200x


TIA1 400x


ALK1 200x

Immunophenotype and Diagnosis

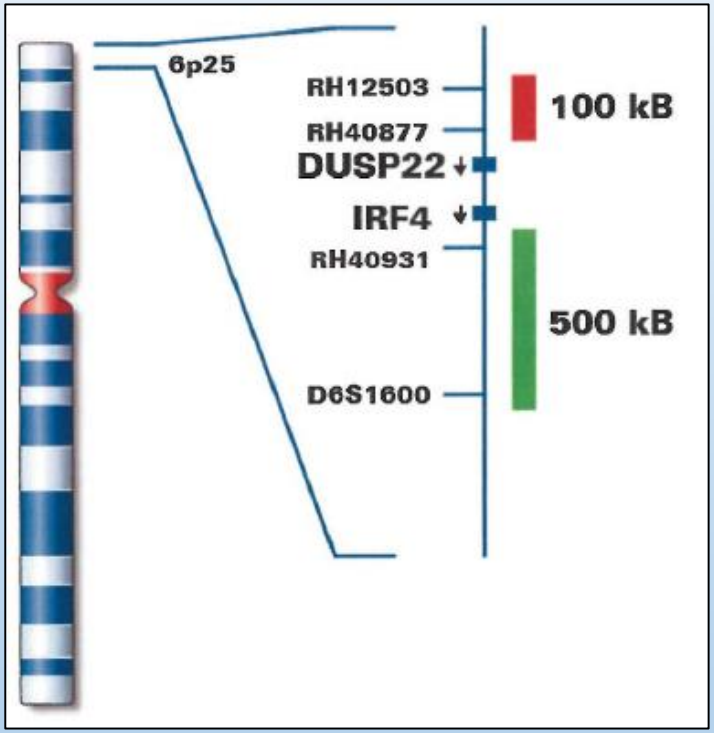
- Positive for CD2, CD30 (strong and diffuse), TIA1.
- Positive: CD45, CD4 (weak), CD7 (subset).
- Negative: CD20, PAX5, CD15, CD8, CD5.

- Diagnosis: Anaplastic Large Cell Lymphoma (ALCL), ALK1 negative.
- Other considerations:
  - Hodgkin: morphology; CD15-, PAX5-, CD3+
  - PTCL, NOS: CD30 too strong and diffuse.

Additional Studies (I thought we were done?)

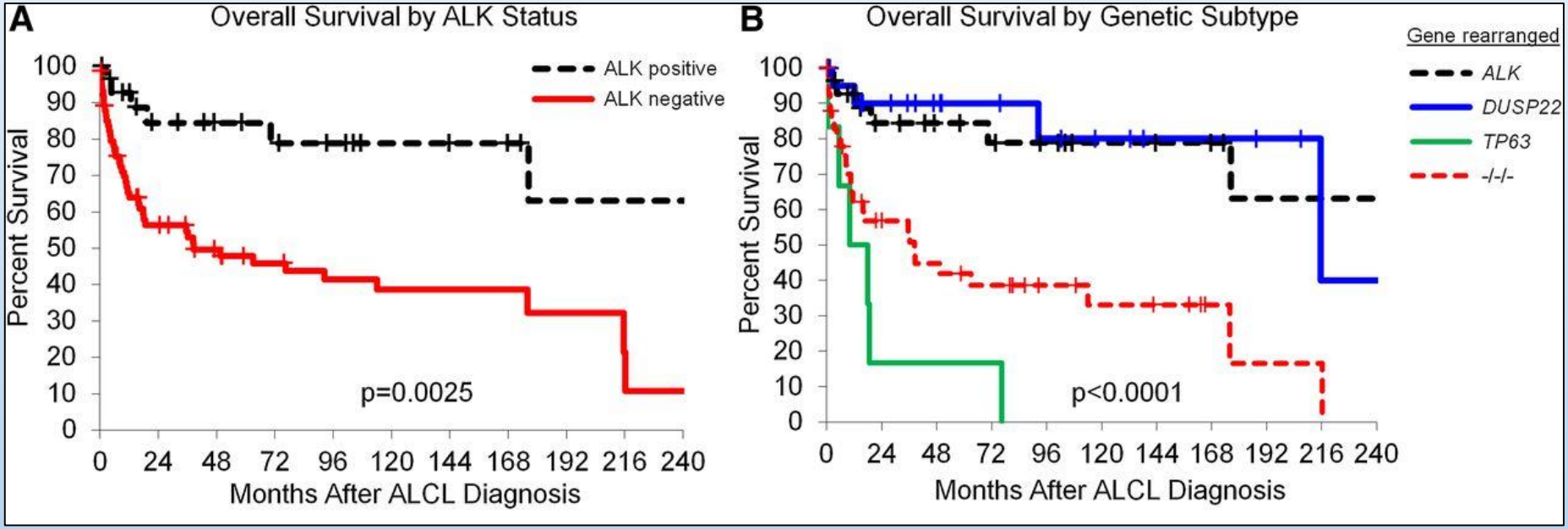
- FISH NEGATIVE for rearrangement of *DUSP22/IRF4*.

- Immunohistochemical stain for p63 is NEGATIVE in tumor cells.



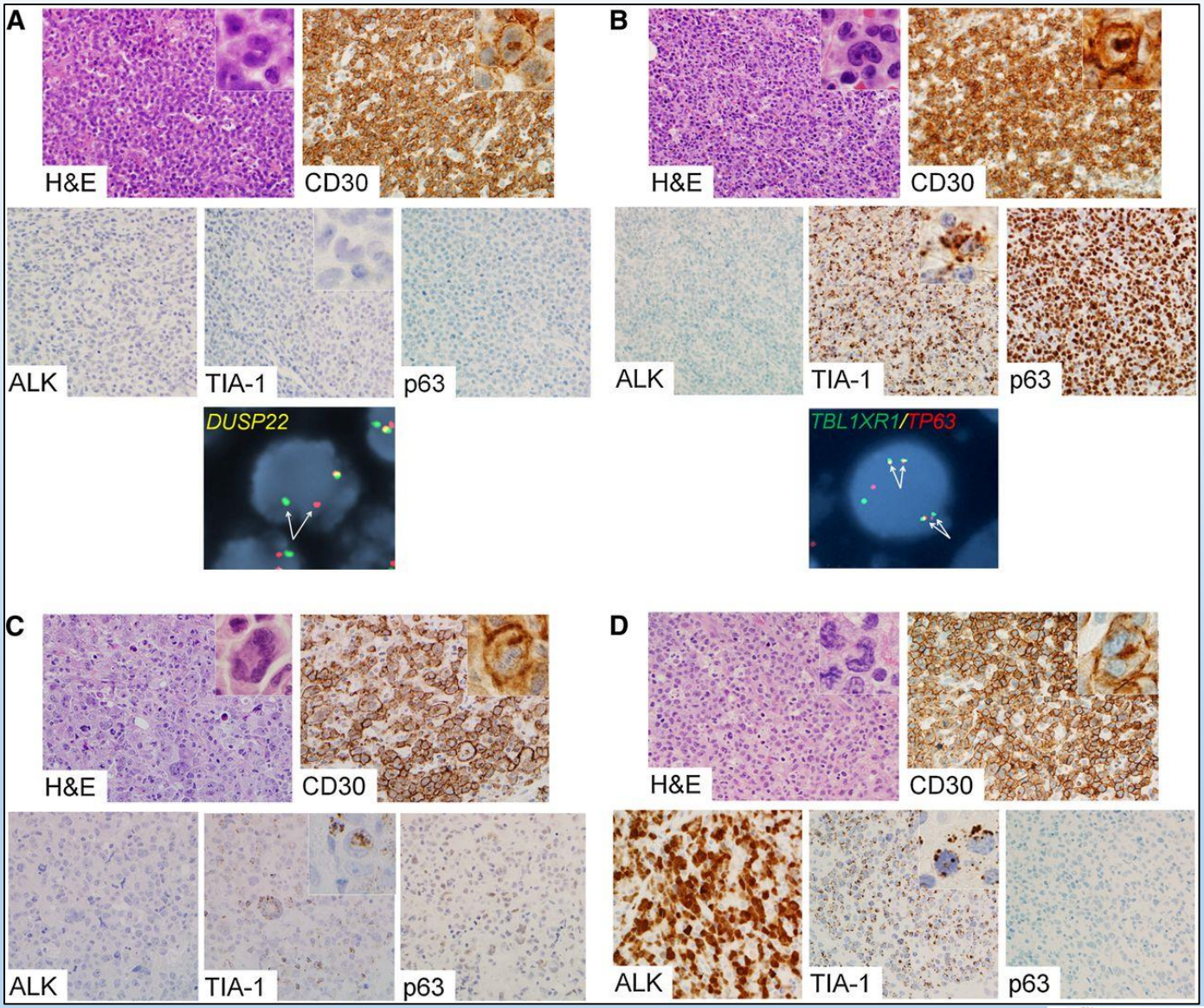
www.sh-eahp.org

IRF4/DUSP22 Rearranged ALK- ALCL Shows Outcomes Similar to ALK+ ALCL



Edgardo R. Parrilla Castellar et al. Blood 2014;124:1473-1480

Genetic subtypes of ALCL



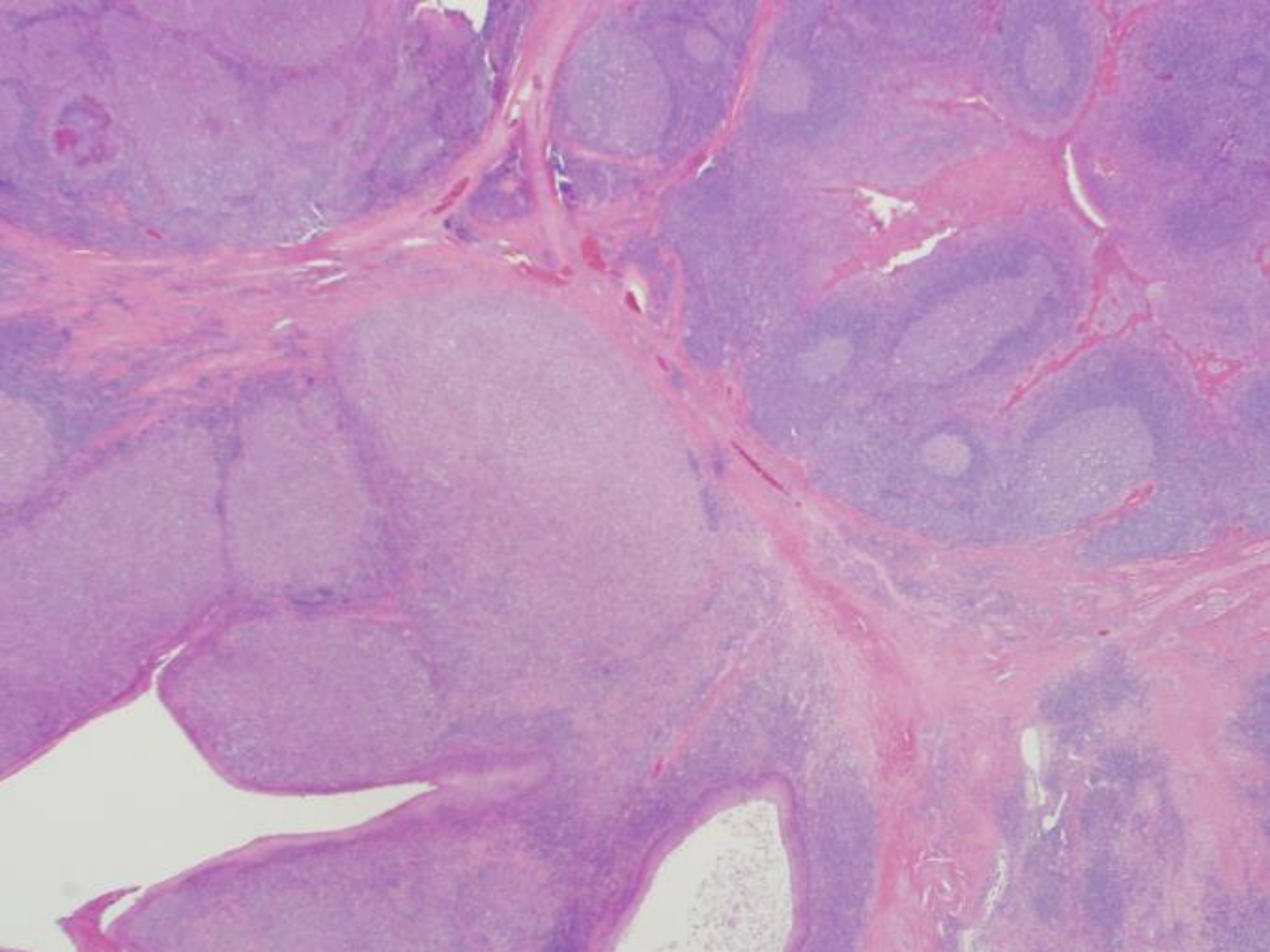
Edgardo R. Parrilla Castellar et al. Blood 2014;124:1473-1480

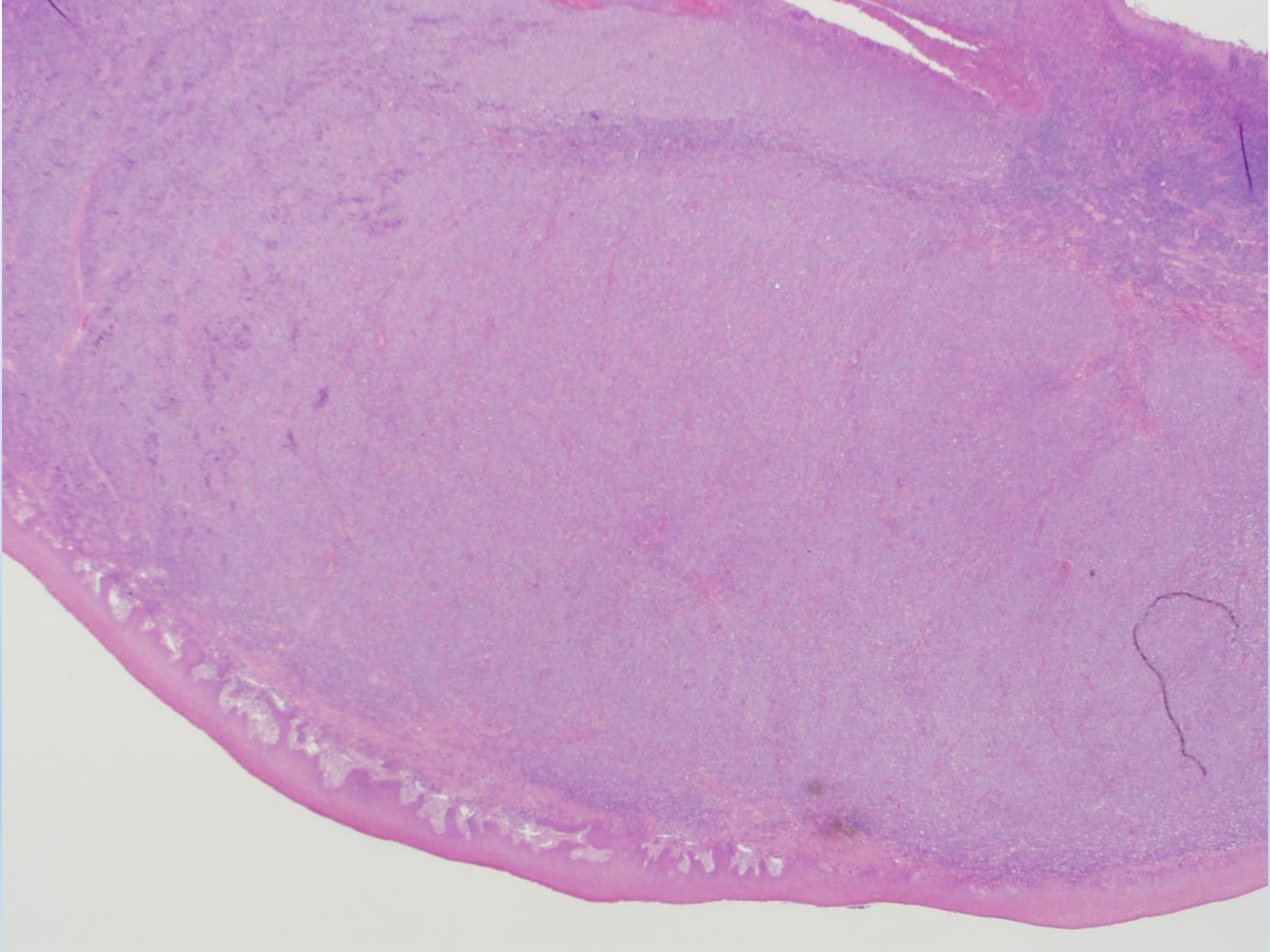
Approach to ALCL

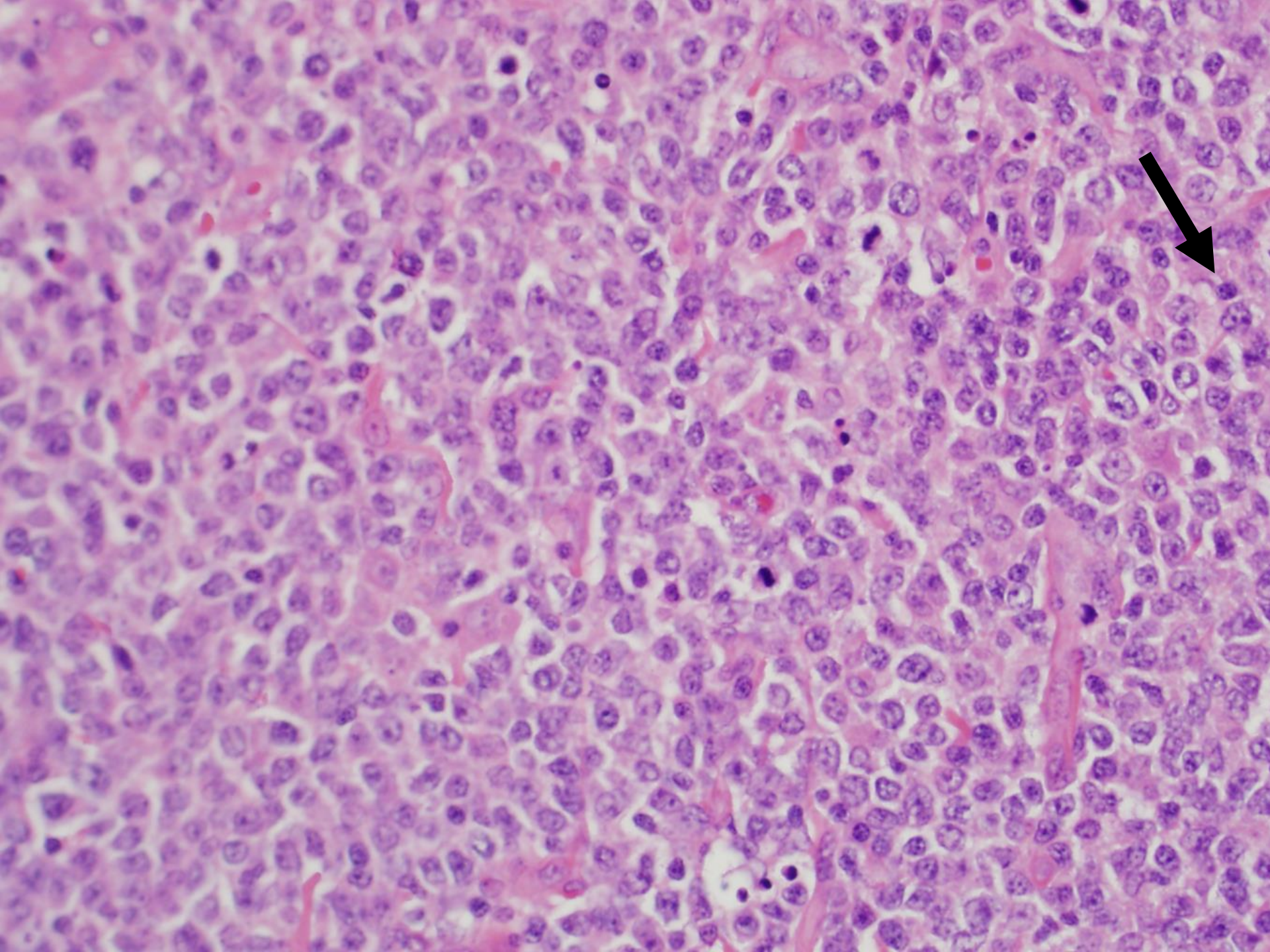
- ALK+ ALCL: Beware of morphologic variants, which can show varying amounts of CD30 expression and large, atypical cells.
  - Common pattern (60%): sheets of large, atypical cells.
  - Lymphohistiocytic pattern (10%): large, atypical cells can hide among histiocytes but cluster around vessels.
  - Small cell (5-10%): most cells are small to medium-sized, large cells cluster around vessels.
  - Hodgkin-like pattern (3%): Resembles NS-cHL.
- ALK negative ALCL
  - Diffuse or sinusoidal growth pattern.
  - Resembles common pattern of ALK+ ALCL with strong, diffuse CD30 (no variants recognized).
- p63 expressed in all cases with TP63 rearrangement, but also in some cases without rearrangement.
  - Expression did not have prognostic significance.
  - Negative p63 has good negative predictive value.

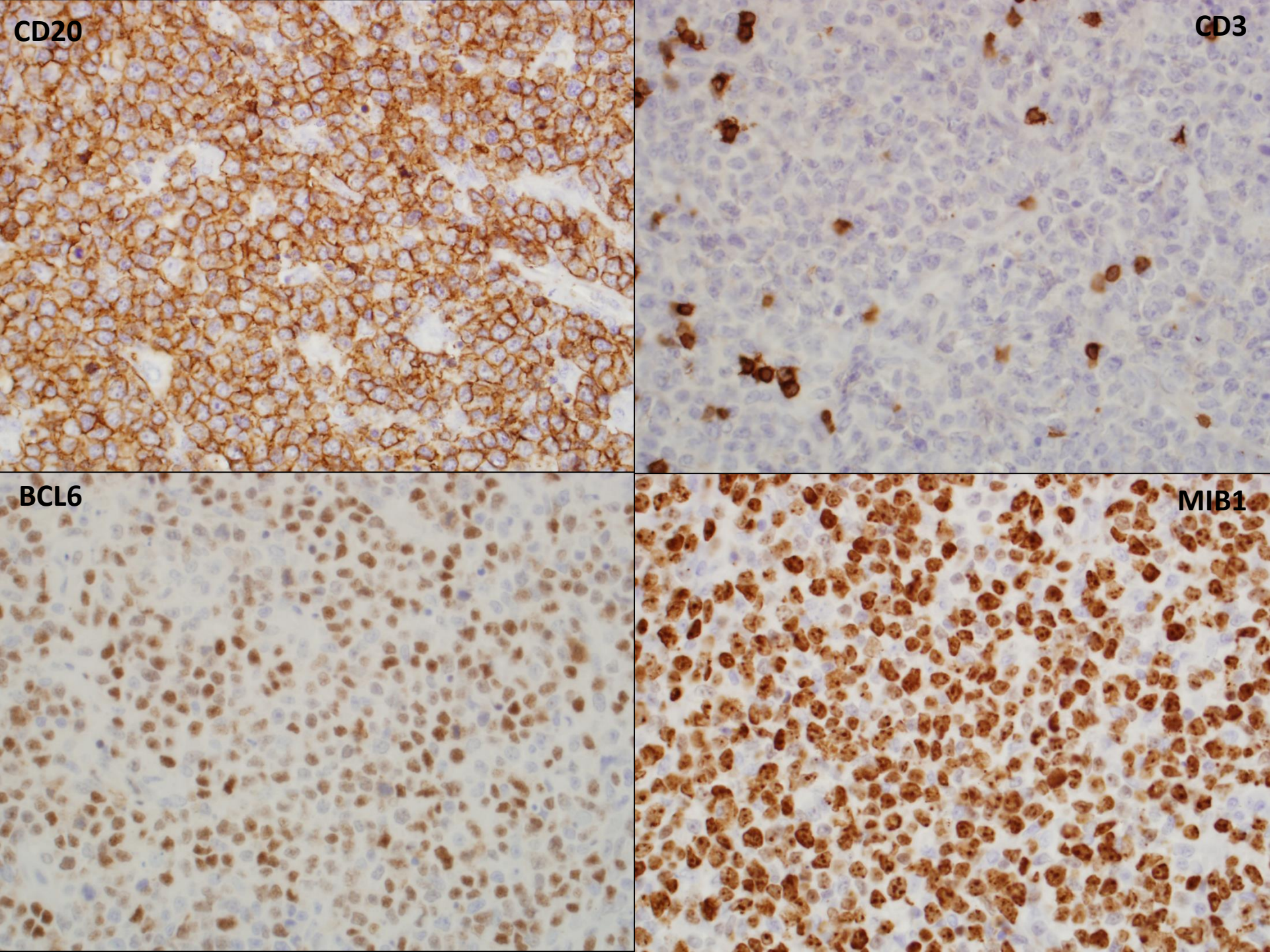
17-year-old girl with enlarged tonsils

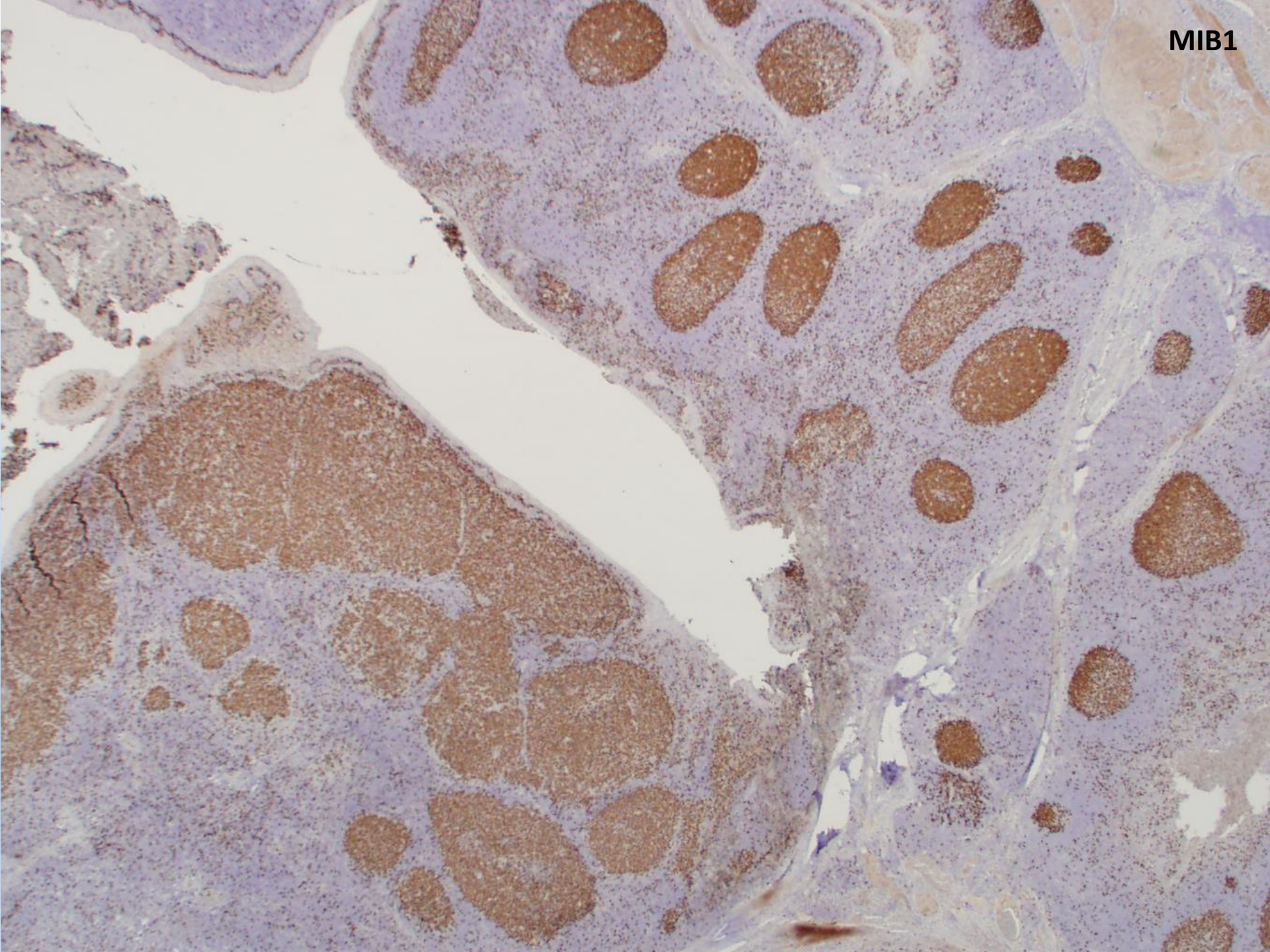
- Original pathology report:
- R tonsil: malignant lymphoma, favor high grade.
- L tonsil: follicular hyperplasia.
- “The overall features favor a high-grade lymphoma.”
- Implications: High-grade implies Burkitt lymphoma in this age group, could include double-hit lymphomas in older adults.
- Intensive chemotherapy regimen required.

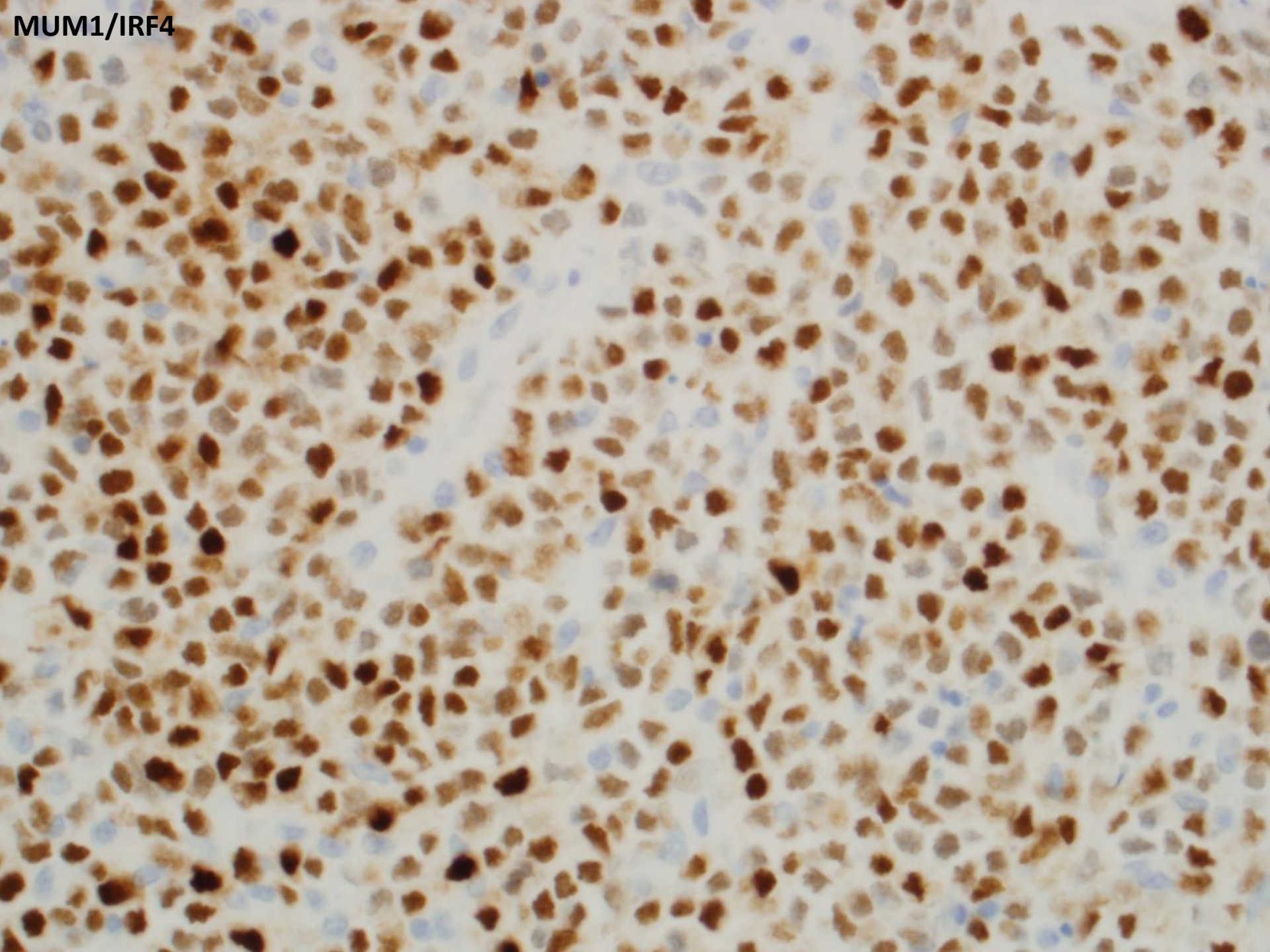







CD20
CD3
BCL6
MIB1


MIB1


MUM1/IRF4

Differential Diagnosis

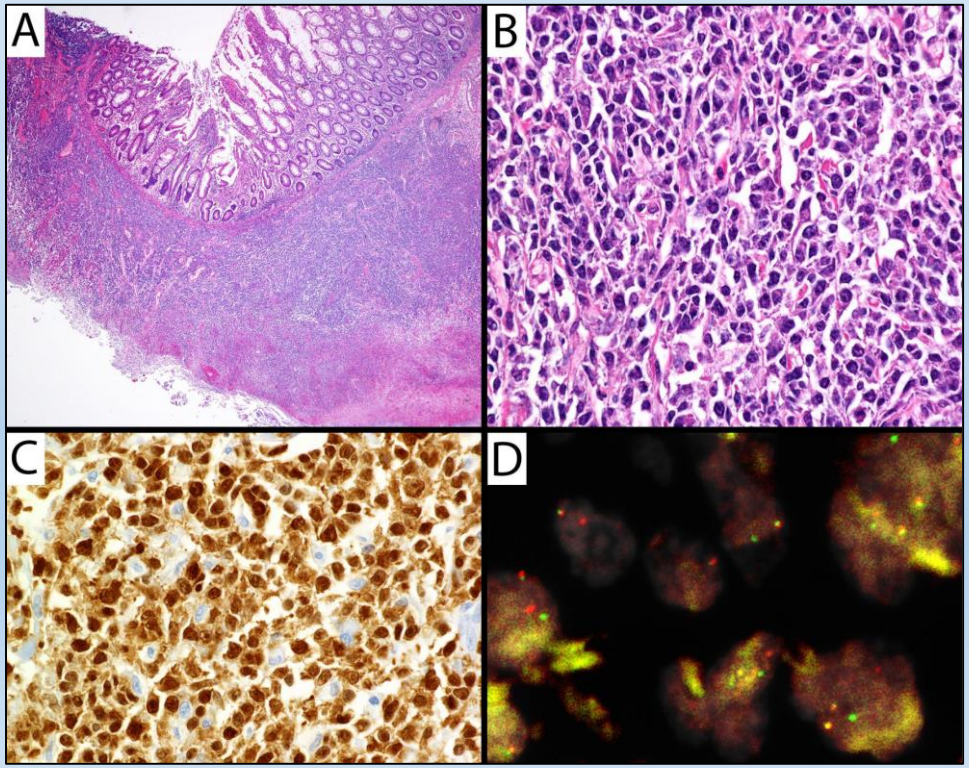
- Burkitt lymphoma: Excluded by morphology
  - Lacks tingible body macrophages, too pleomorphic.
- Follicular lymphoma, grade 3.
- Diffuse large B-cell lymphoma.
- Large B-cell lymphoma with *IRF4* rearrangement.
  - New entity in WHO 2017.
- FISH results:
  - FISH for *MYC* and *BCL6* and *BCL2* negative.
  - FISH for *IRF4/DUSP22* positive.

Large B-cell lymphoma with *IRF4* rearrangement.

- Localized in head and neck.
- Median age 12 (range 4-79).
- Morphologically fit into DLBCL, follicular lymphoma grade 3, pediatric type follicular lymphoma.
- Positive for BCL6 and IRF4/MUM1.
- Good outcome after chemotherapy.
  - Less intensive therapy than Burkitt lymphoma.
- In the appropriate clinical context, FISH for *IRF4/DUSP22* should be performed.

Overall uncommon (<1%), but more common in younger patients

- We studied 32 patients from Children's Oncology Group protocols.
- FISH for *IRF4/DUSP22* positive in 2/32 cases (6%).
  - One in tonsils.
  - One in ileum.



Genetically Defined Lymphomas

- ALK+ ALCL
- Mantle cell lymphoma
- HGBL, with *MYC*+*BCL2* +/- *BCL6* rearrangement
- Large B-cell lymphoma with *IRF4* rearrangement
- Lymphomas with highly characteristic genetic changes that do not define them:
  - Burkitt lymphoma: (*IG-MYC*)
  - Follicular lymphoma: t(14;18)
  - Lymphoplasmacytic lymphoma: *MYD88* mutation.
  - Hairy cell leukemia (BRAF V600E).

Summary

- Some entities are now defined in the WHO Classification by genetic (mutations, translocations) features.
  - More in AML, ALL
  - Emerging in lymphoma
- When clinically indicated, consider additional testing for precise classification.