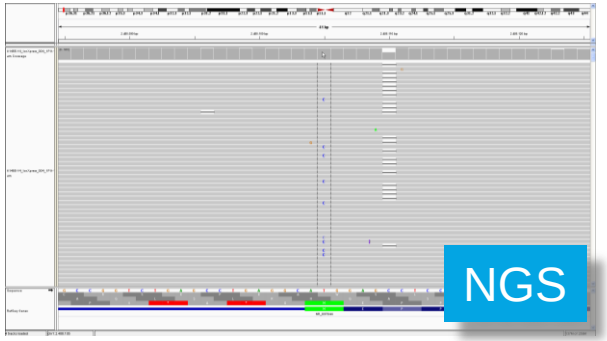
Eberhard-Karls-Universität
UKT
Universitätsklinikum Tübingen

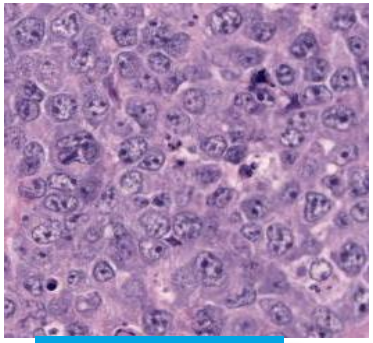
Institut für Pathologie

Methods used to diagnose lymphomas

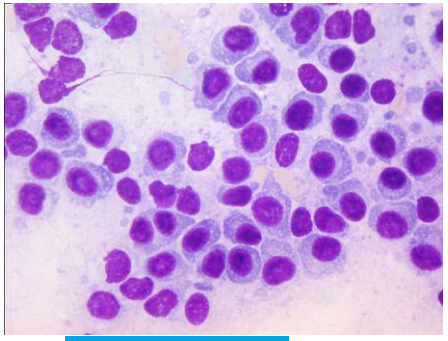
Prof. Dr.Med. Leticia Quintanilla-Fend



NGS

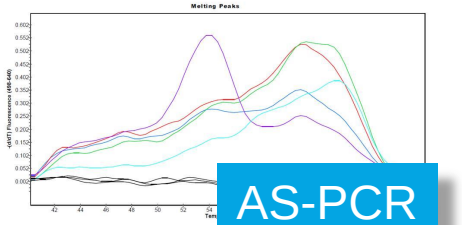


histology

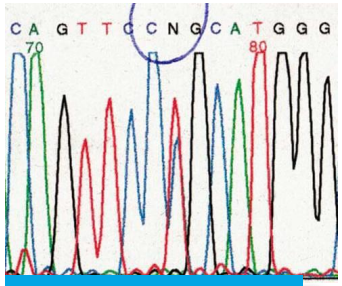


Cytology

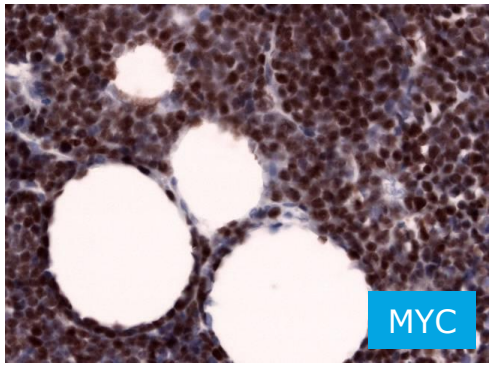
Molecular techniques



AS-PCR

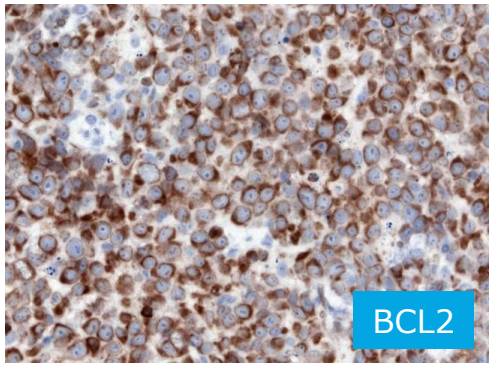


Sanger seq.

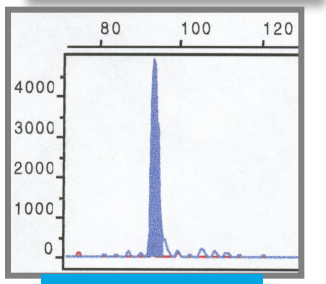


MYC

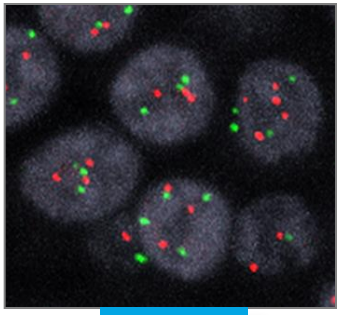
Immunohistochemistry



BCL2



Clonality



FISH

Methods use to diagnose lymphomas

- There is probably no other method that has so revolutionized the field of pathology during the past 50 years as the immunohistochemical technique

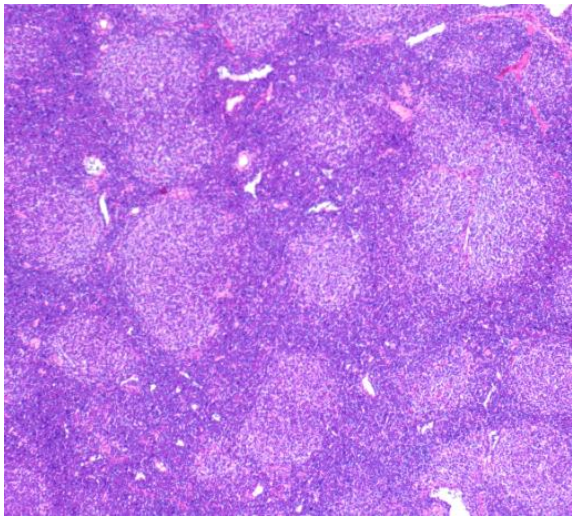
- Immunophenotyping has contributed importantly to the diagnosis and understanding of lymphomas

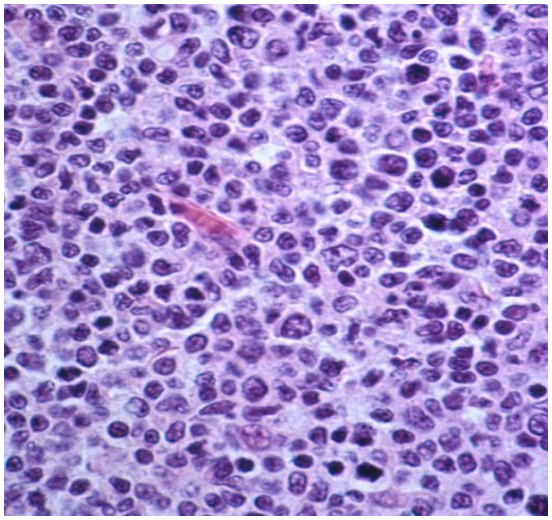
Immunohistochemical approach.

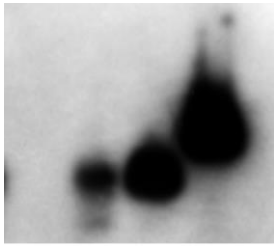
Immunophenotyping in Lymphomas :

- As a surrogate marker of molecular changes
- Increases diagnostic accuracy
  - ✓ Cell lineage determination
  - ✓ B-cell NHL 73% to 87%
  - ✓ Peripheral T-cell lymphomas from 41% to 86%
- Gives prognostic information

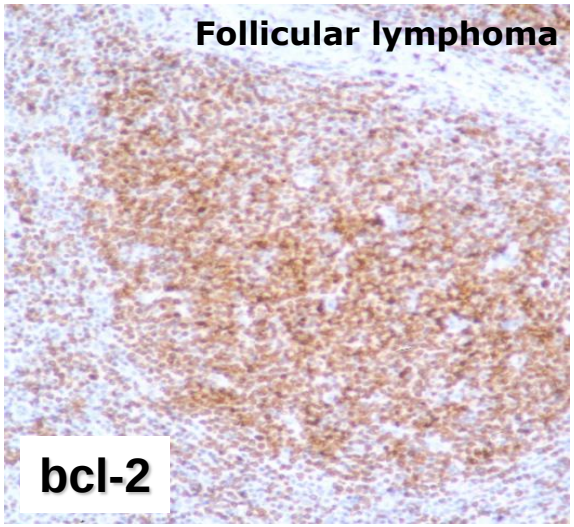
Surrogate marker of molecular changes

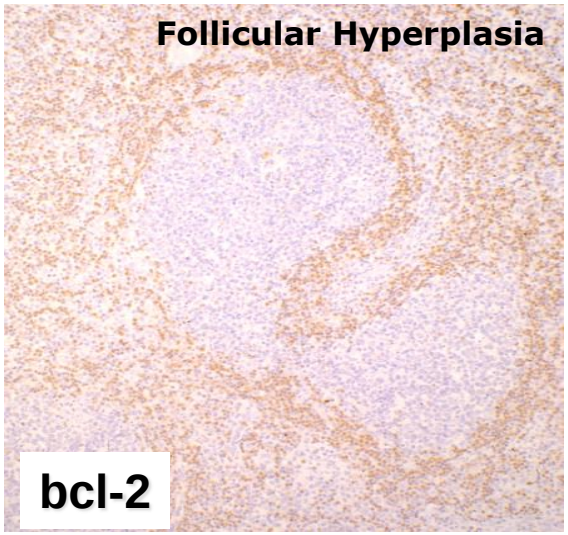


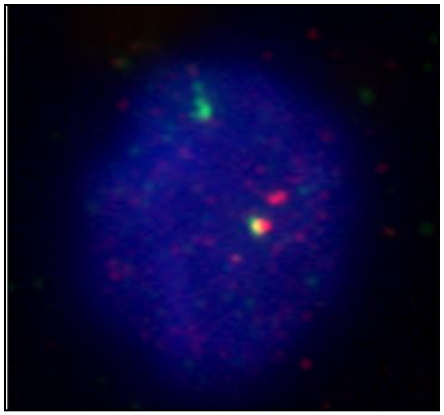




Rearrangement of BCL-2

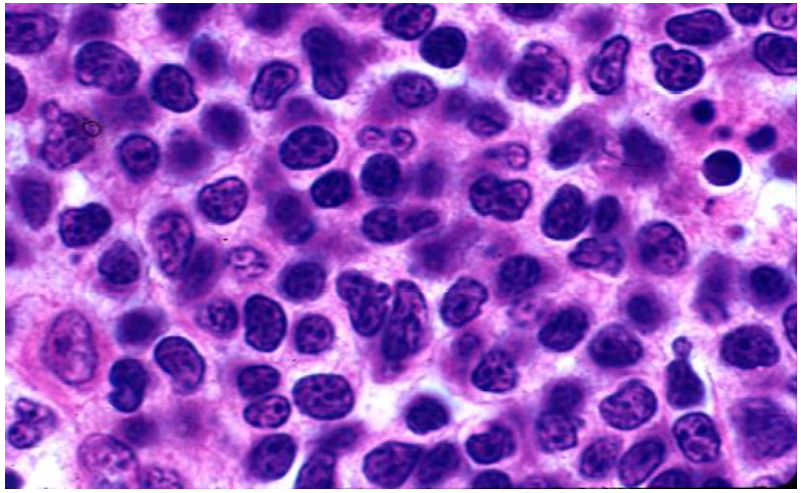




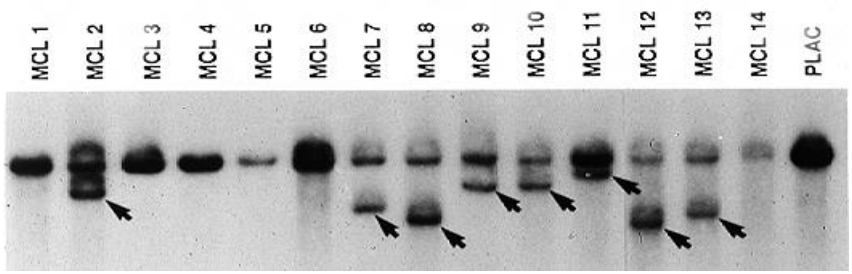


t (14;18)

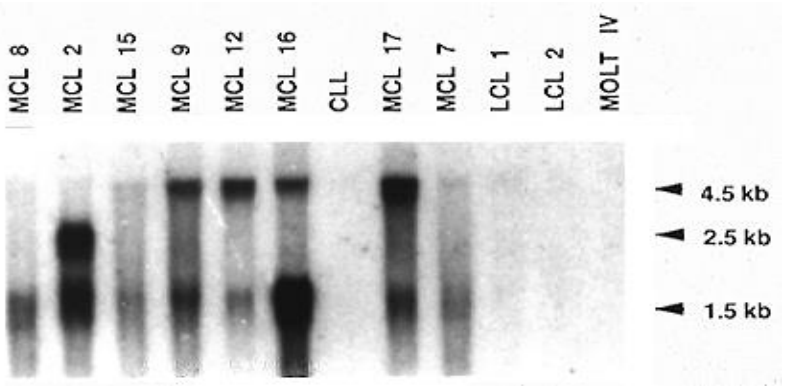
Surrogate marker of molecular changes



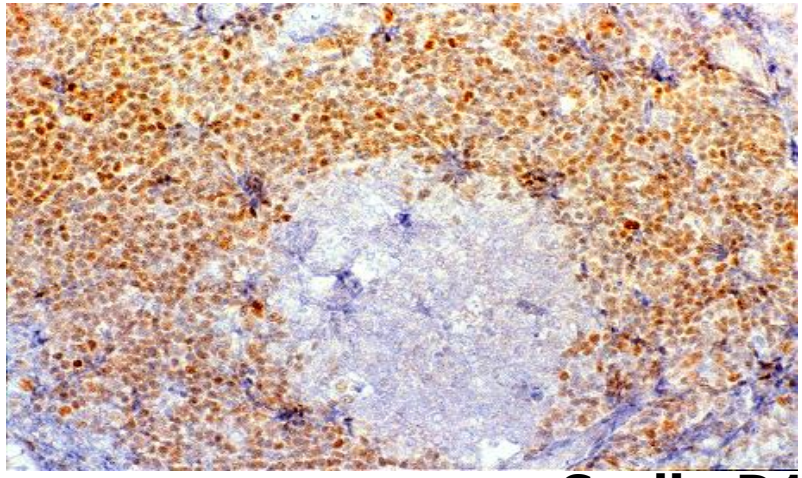
t (11;14)



Rearrangement of BCL-1

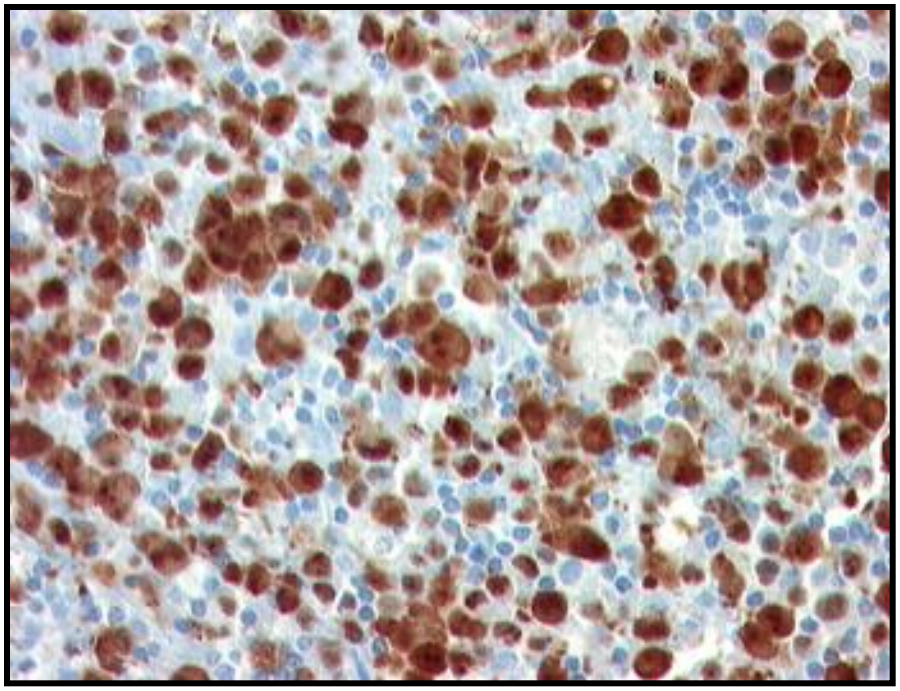


Northern blot cyclin D1

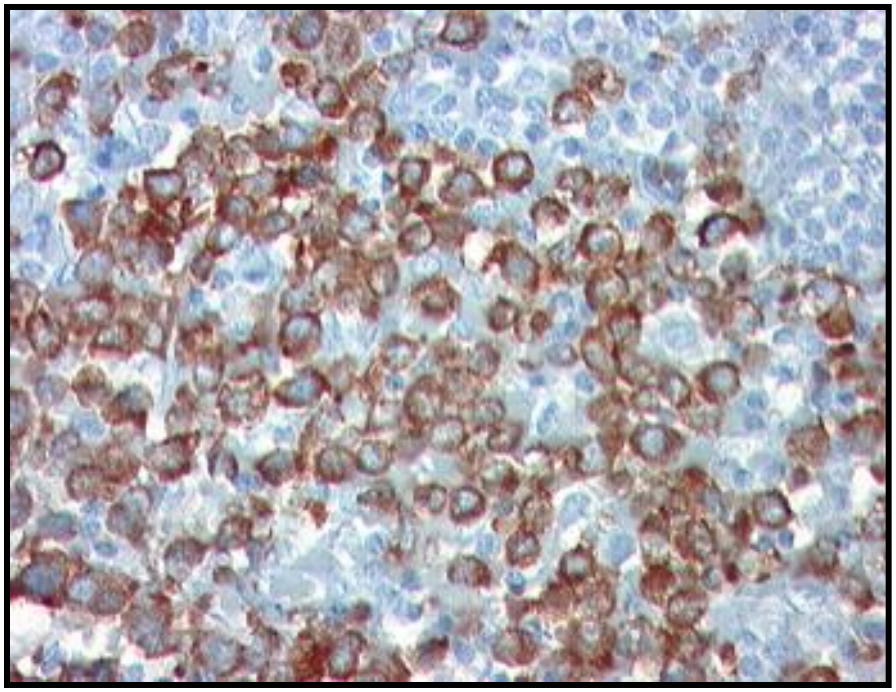


Cyclin D1

ALK+ Anaplastic large cell lymphoma.

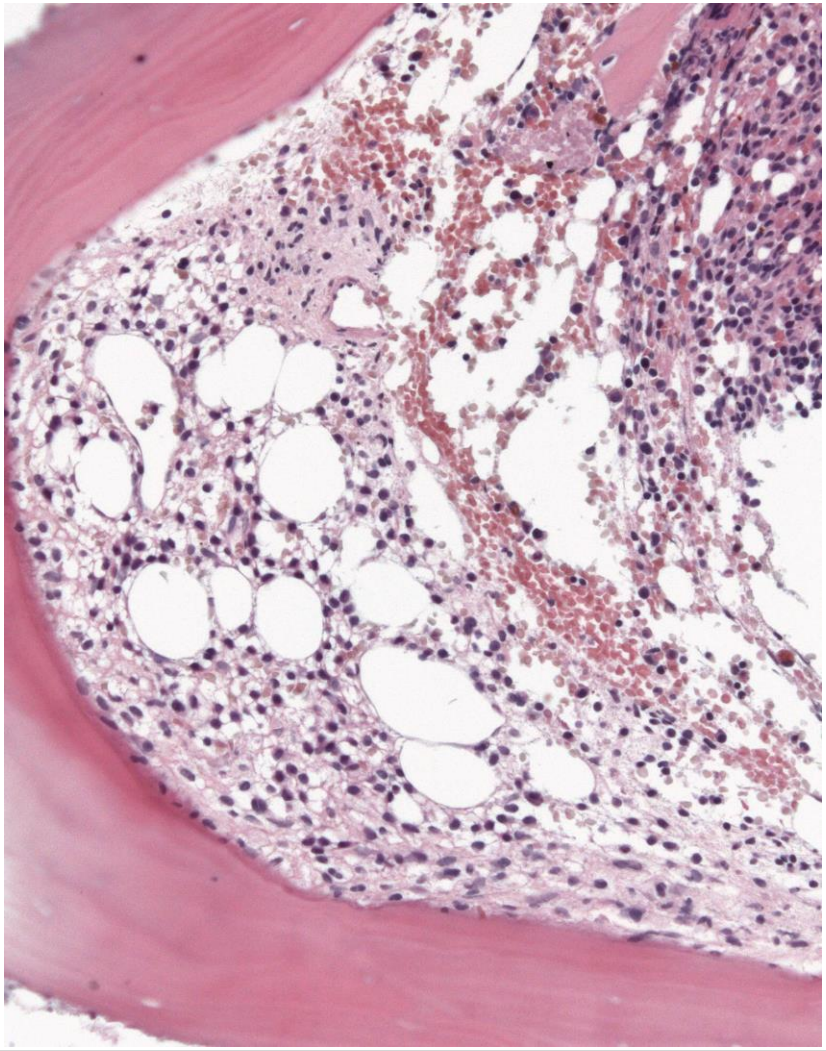


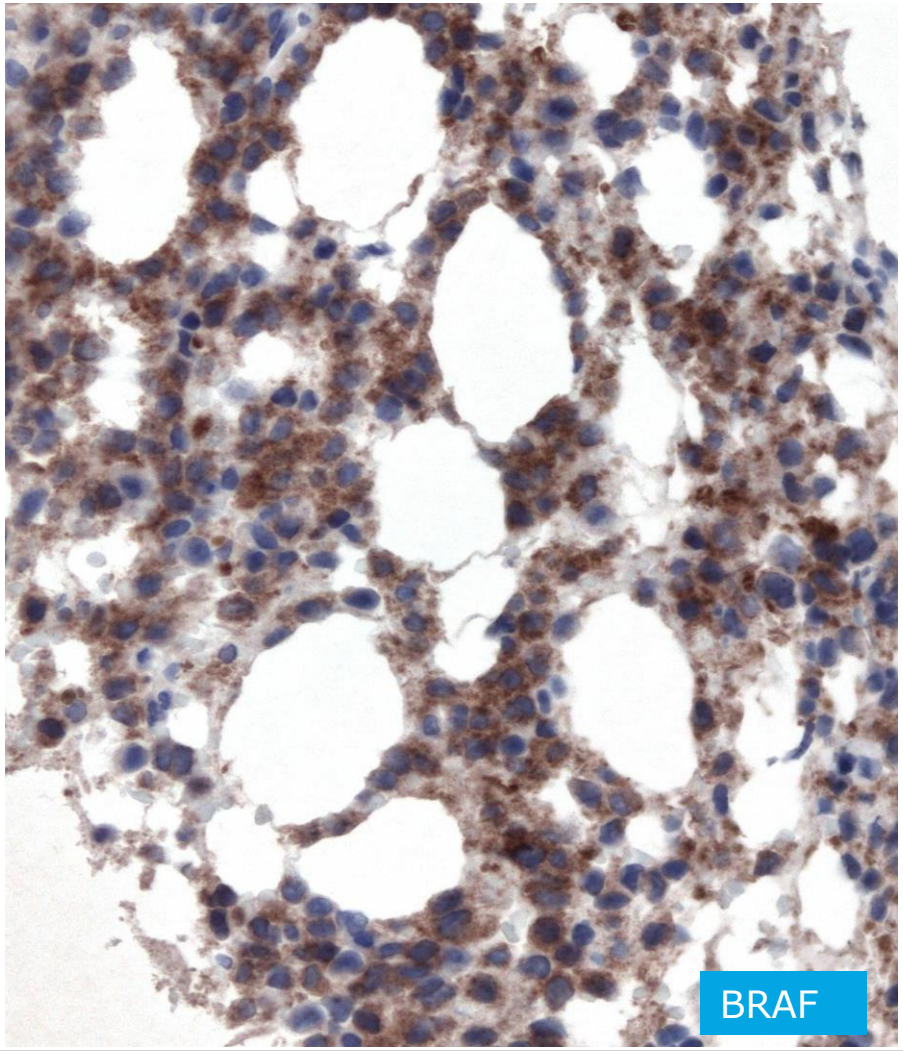
NPM-ALK - t(2;5)



Variant fusion protein

Hairy cell leukemia BRAF antibody





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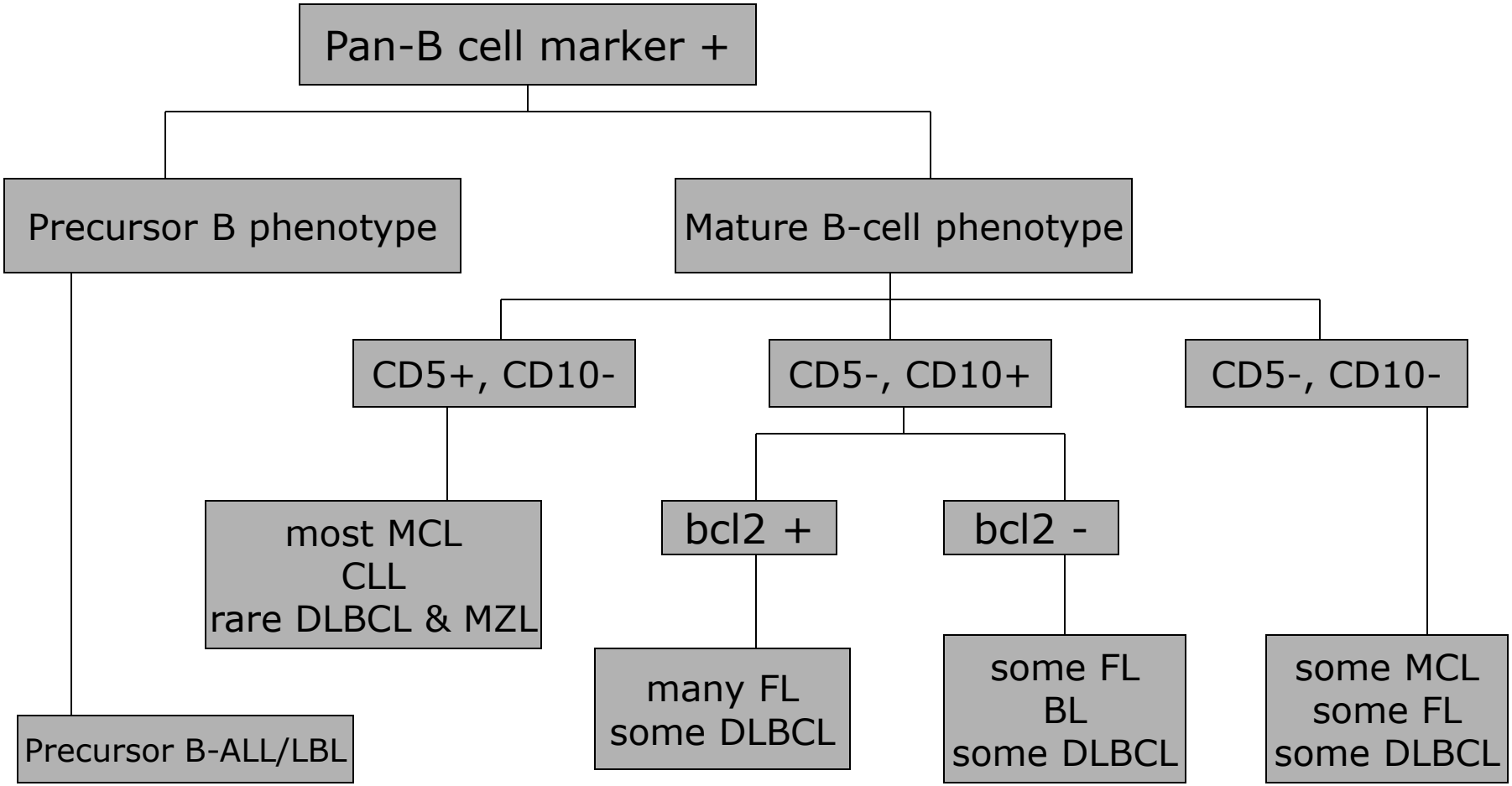
B-cell NHL 73% to 87%

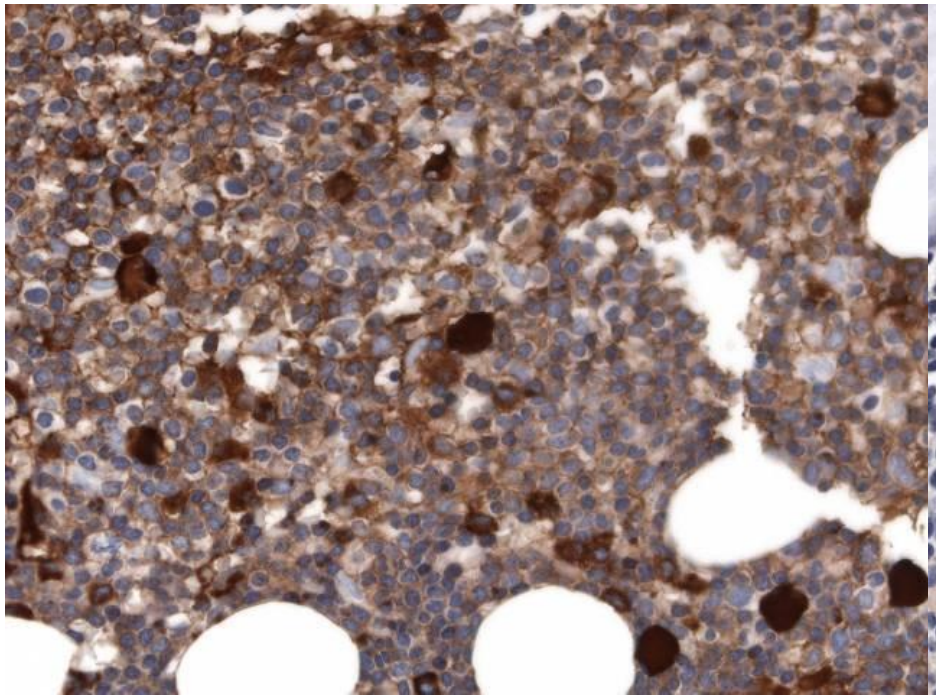
Peripheral T-cell lymphomas from 41% to 86%

- Gives prognostic information

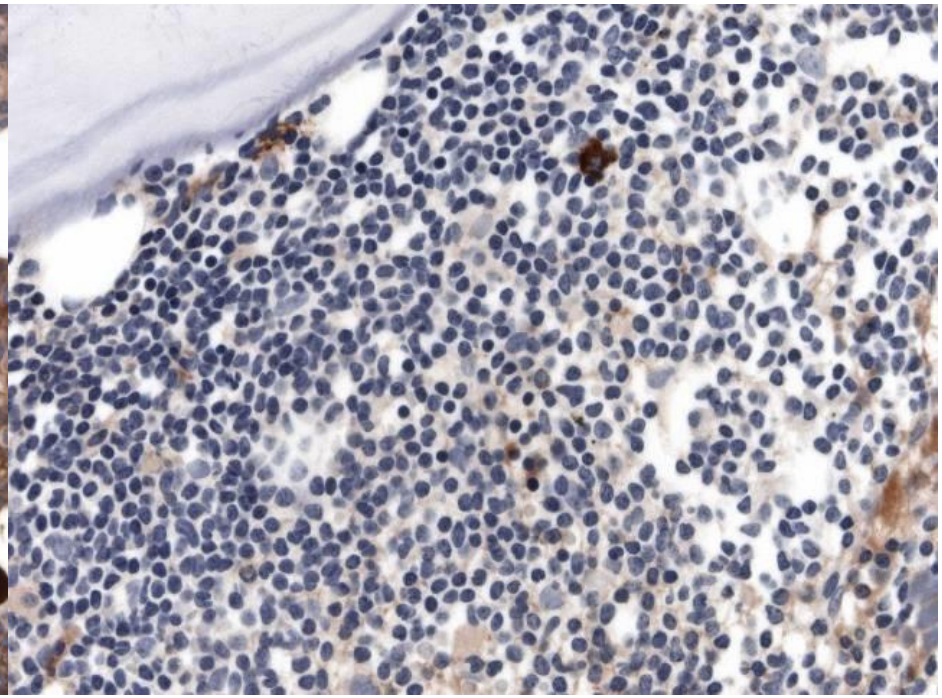
Diagnostic Accuracy in B-cell lymphomas



Light chain restriction for clonality

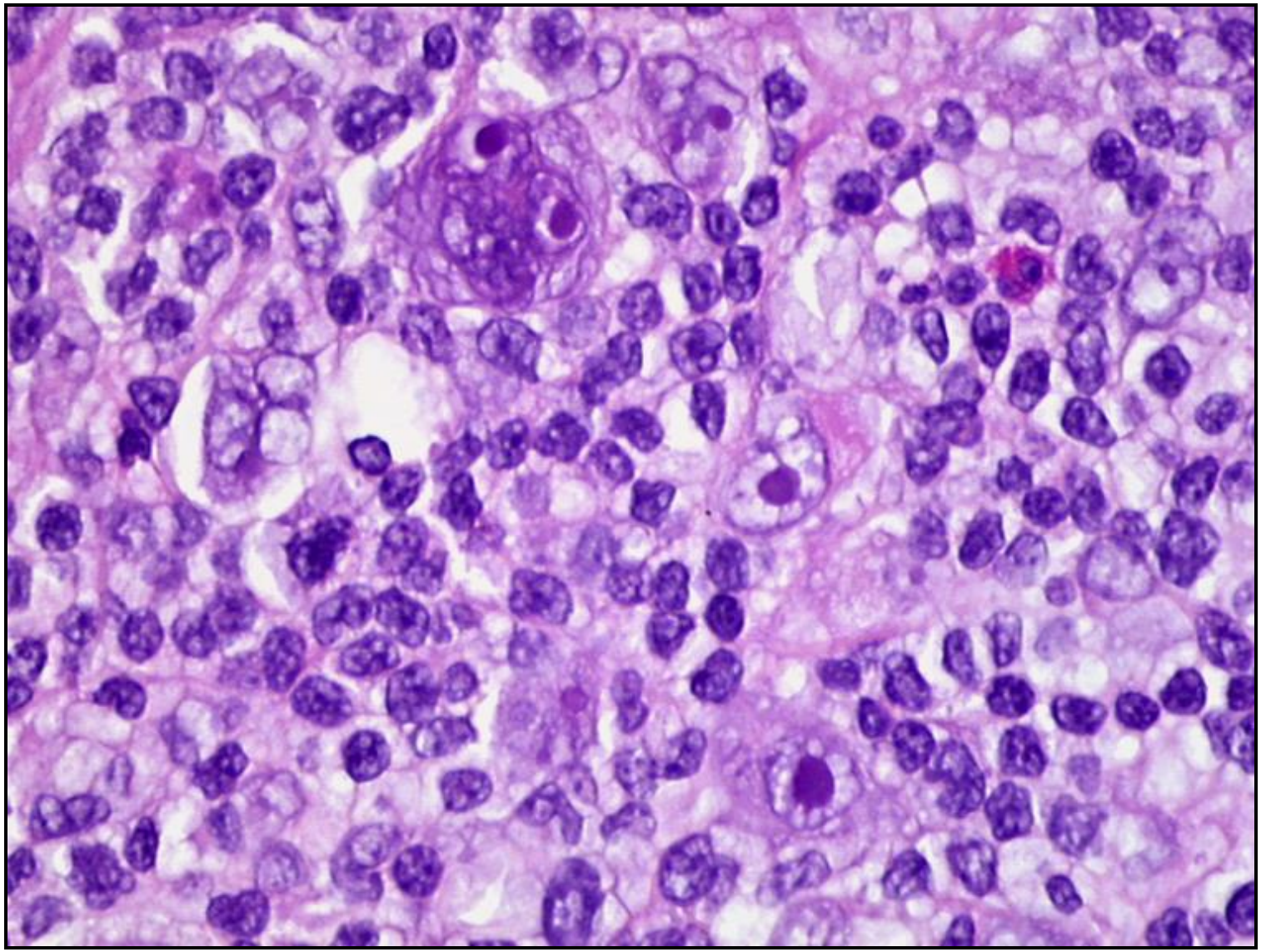


Kappa



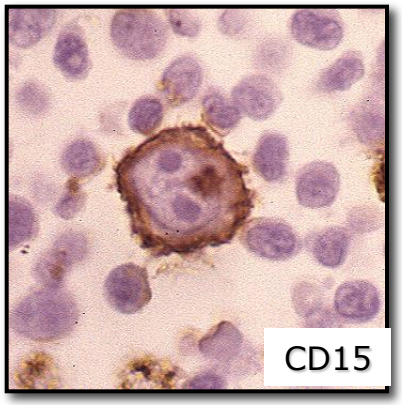
Lambda

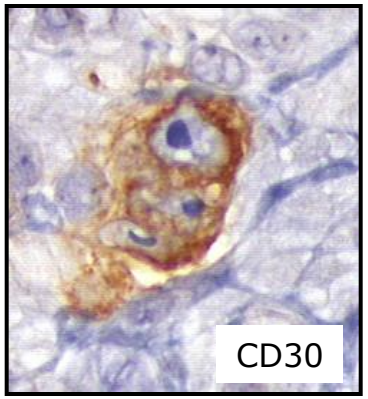
Accuracy in the diagnosis of CHL

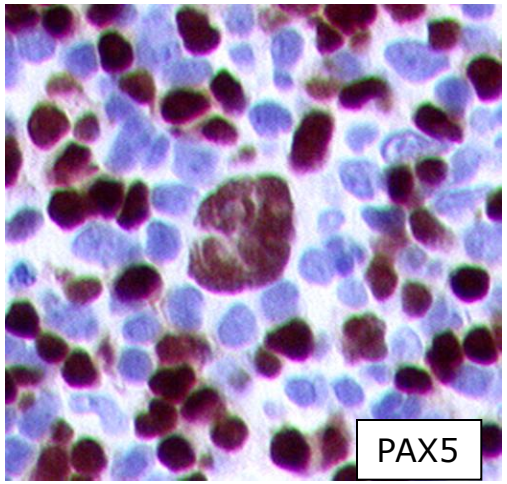


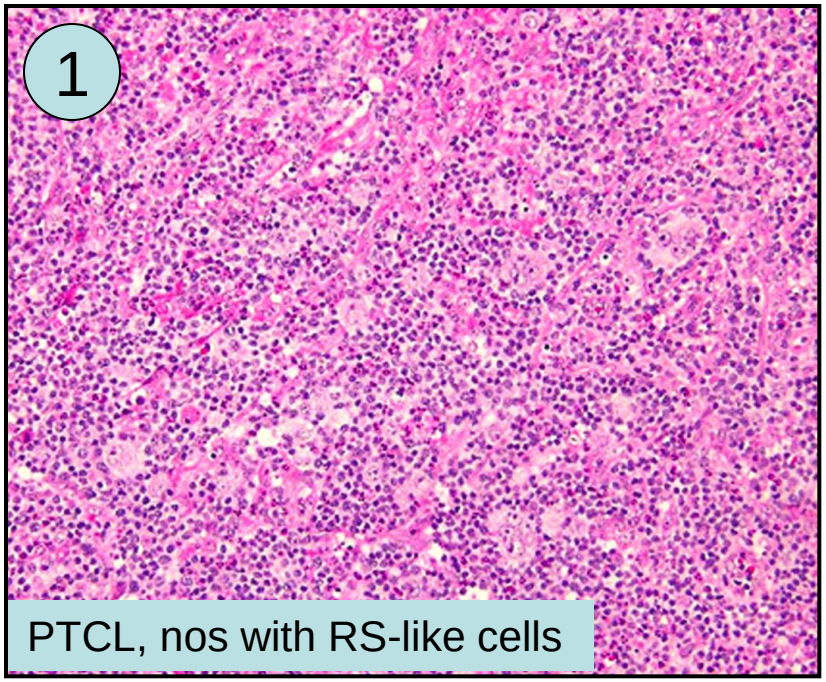
Accuracy in the diagnosis. Hodgkin lymphoma

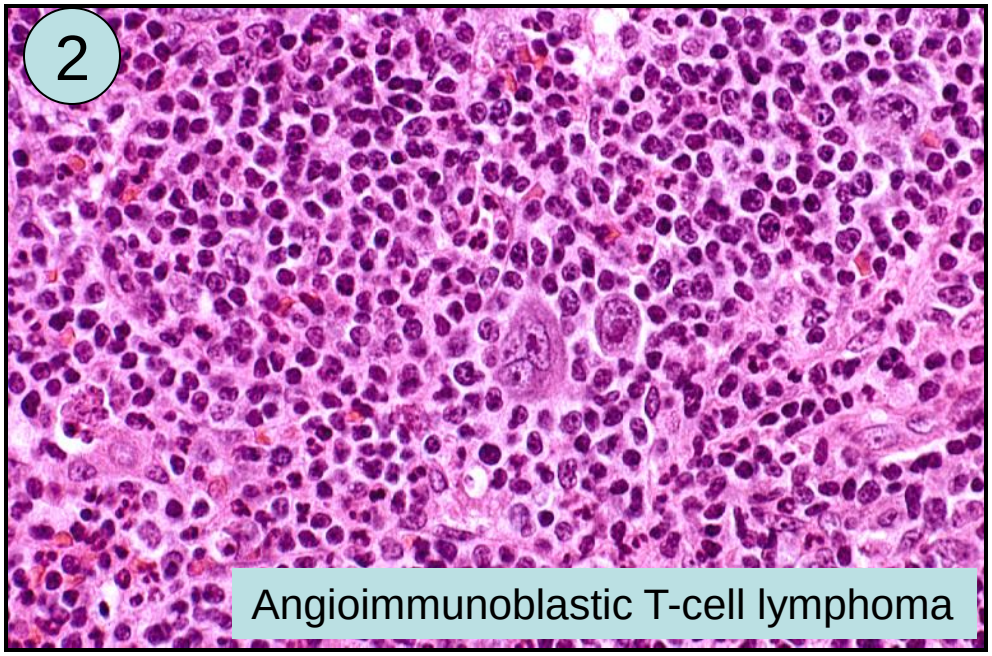
- Immunophenotype
- CD30 + (100%)
- CD15 + (75-85%)
- CD20 - (weak positivity in a minority of tumor cells)
- PAX5 (90%)
- MUM1
- LMP1 +/-
- Oct2 and BOB.1 -

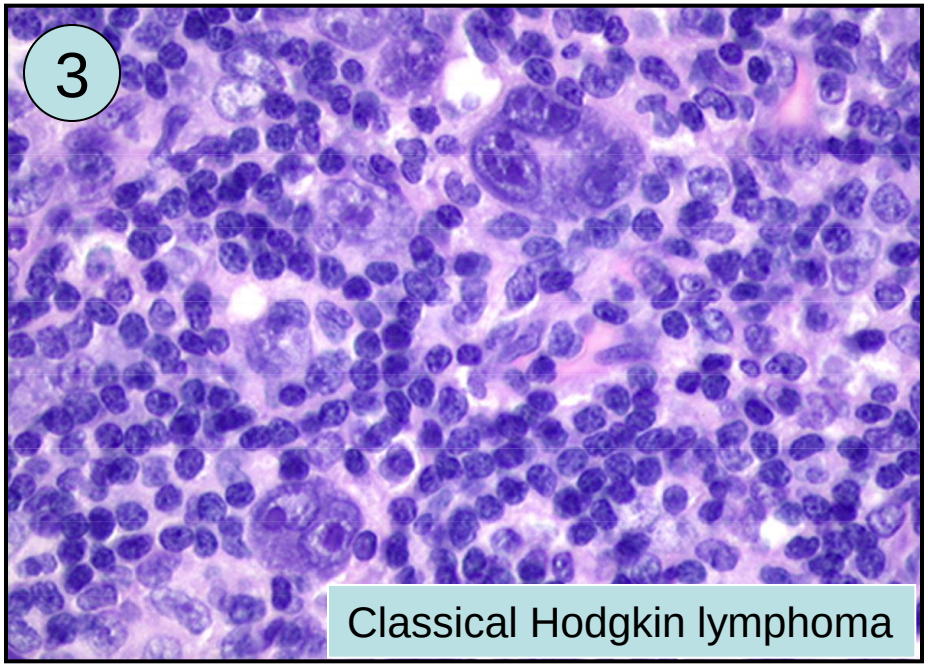


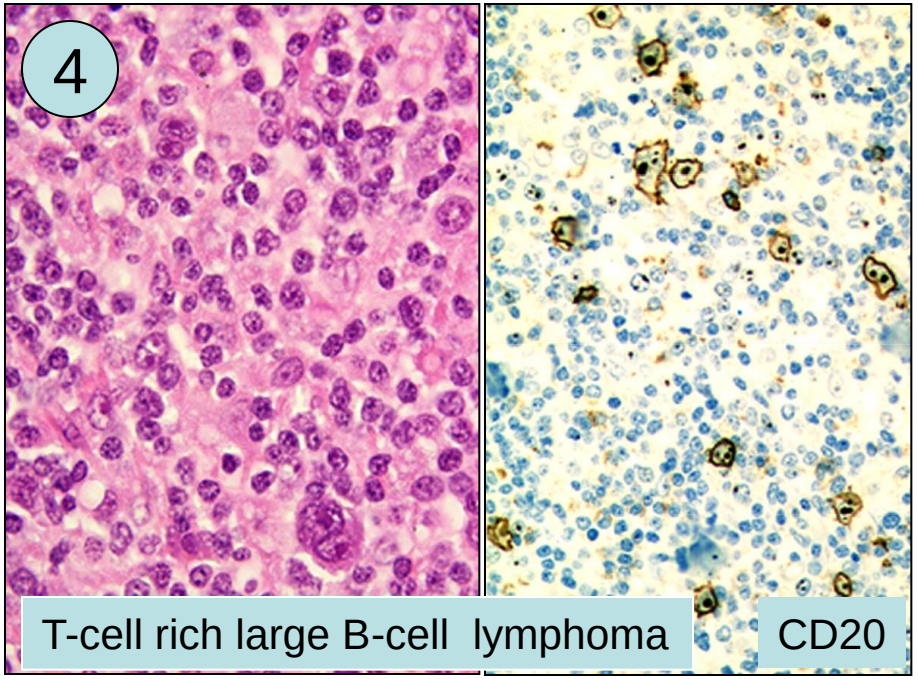




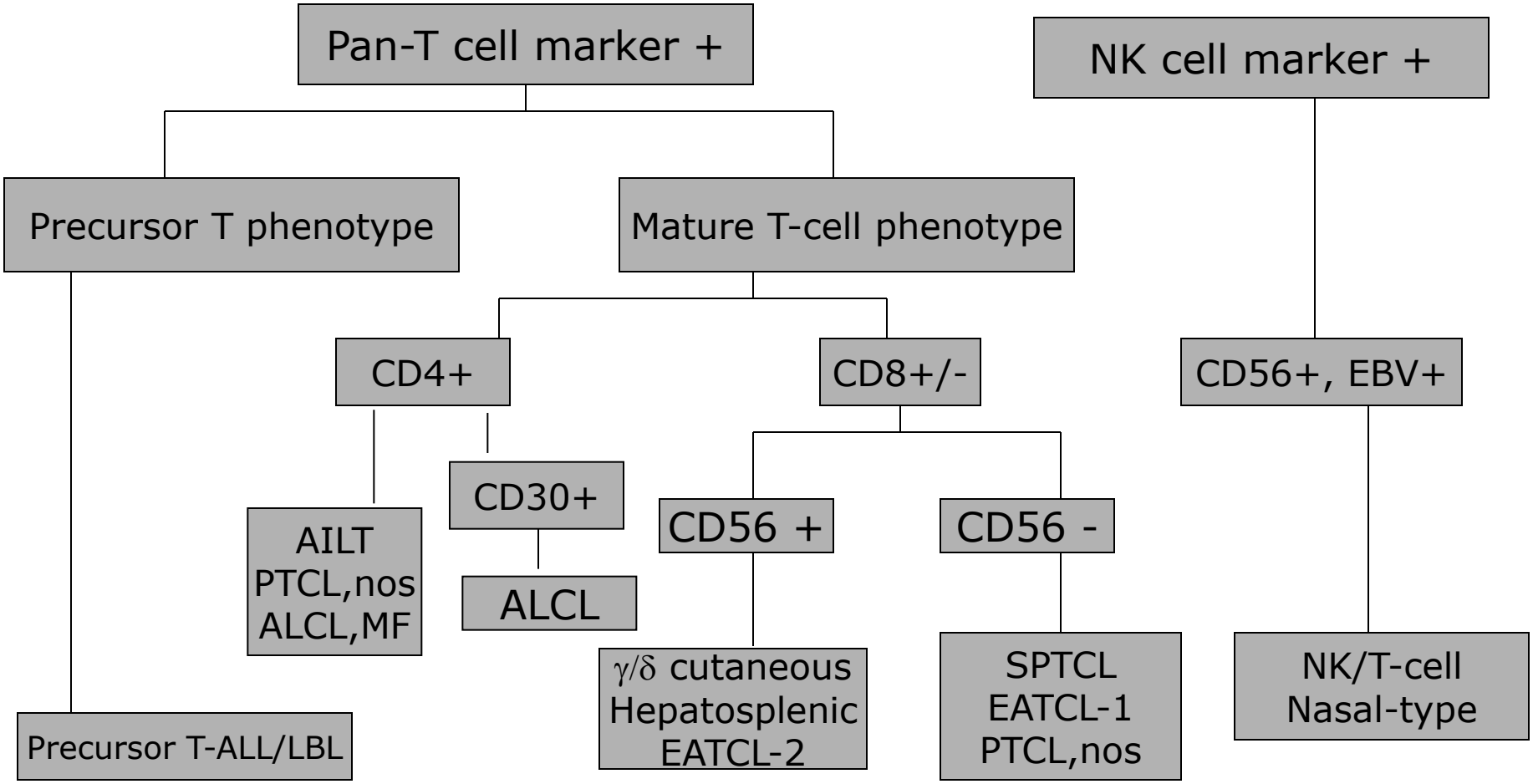

1
PTCL, nos with RS-like cells


2
Angioimmunoblastic T-cell lymphoma

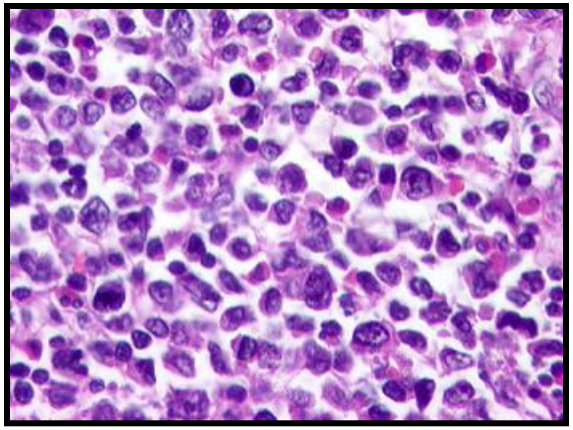

3
Classical Hodgkin lymphoma

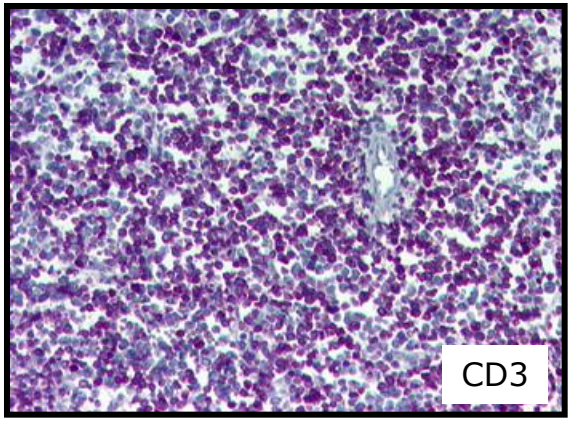

4
T-cell rich large B-cell lymphoma
CD20

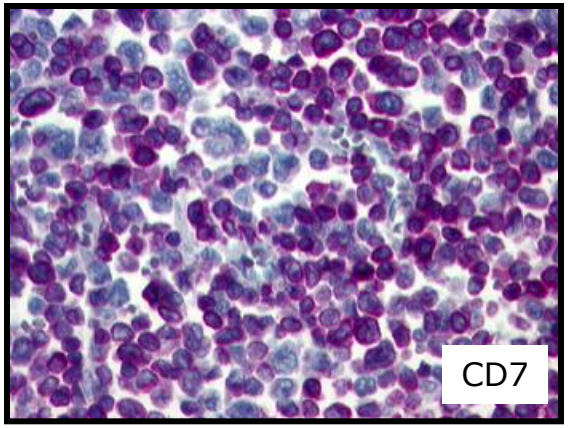
Diagnostic Accuracy in T/NK-cell lymphomas

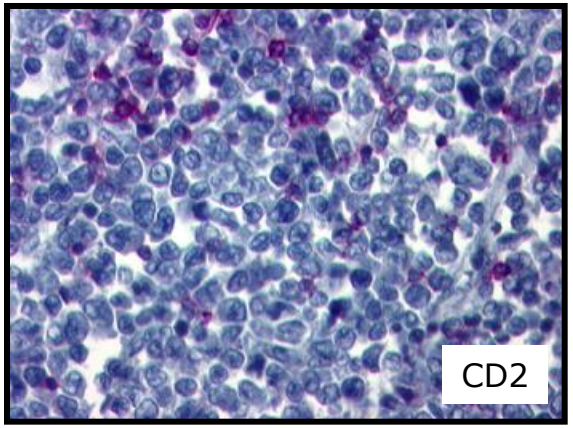


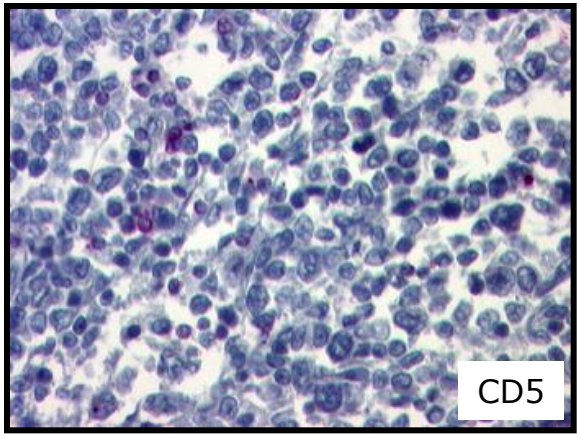
Diagnostic accuracy. T-cell NHL

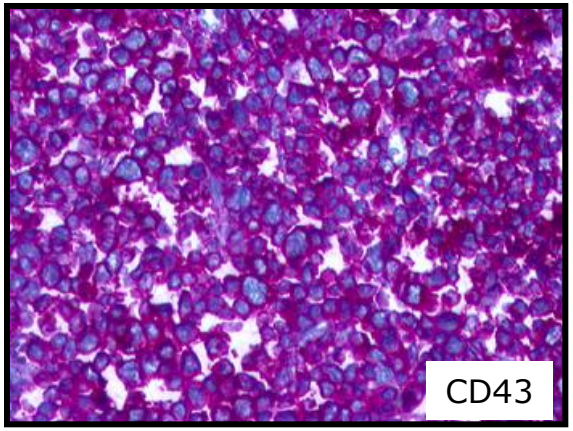






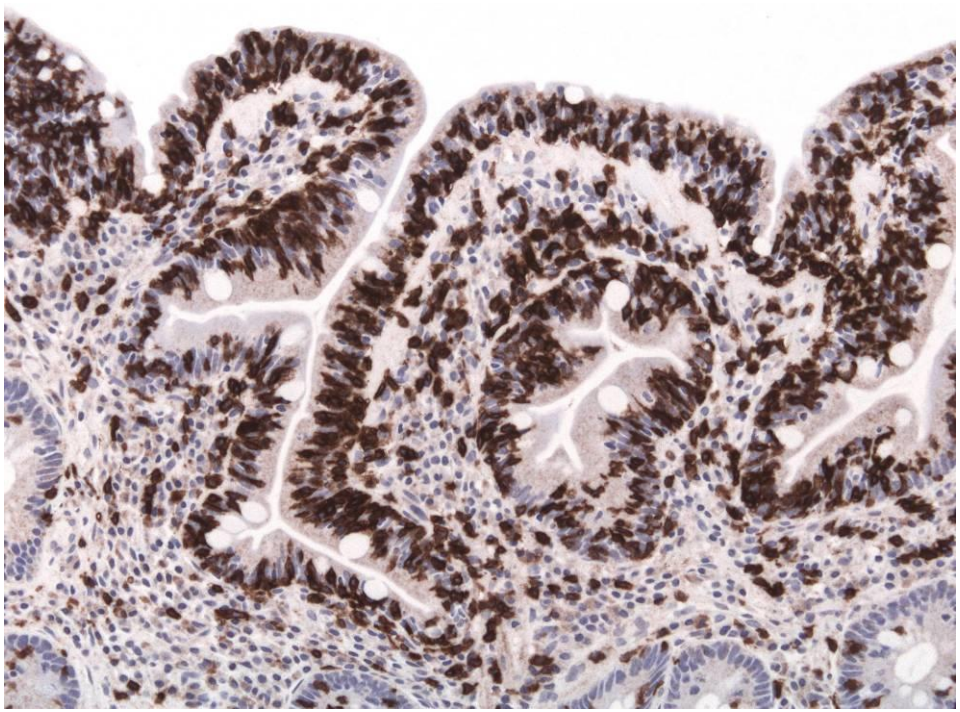




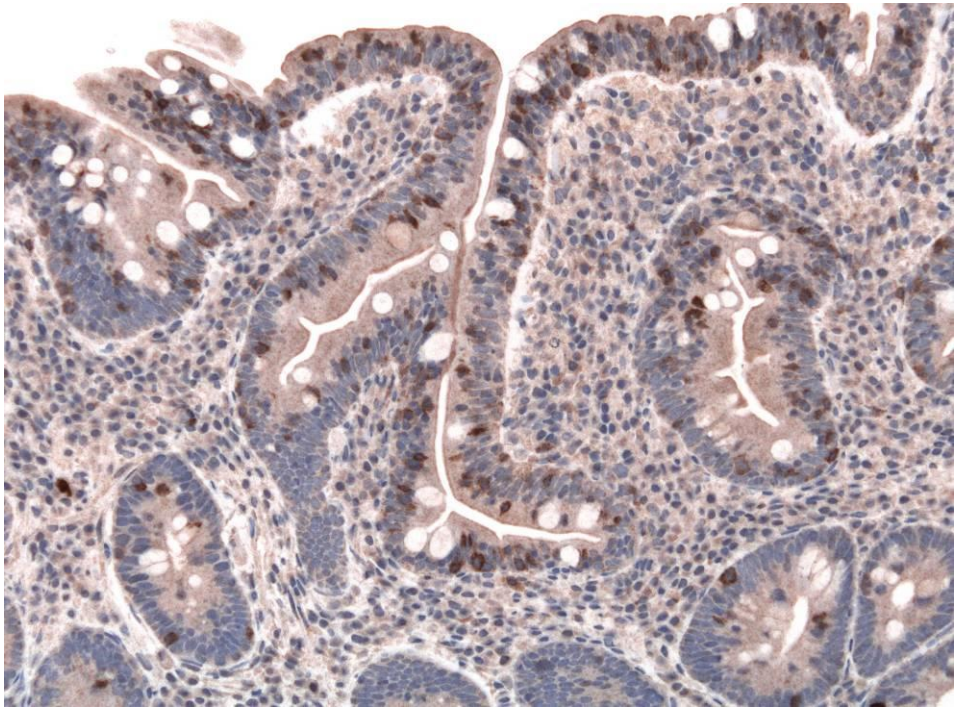


Aberrant phenotype: loss of one or more pan-T-cell markers

Biomarkers of cell lineage/differentiation



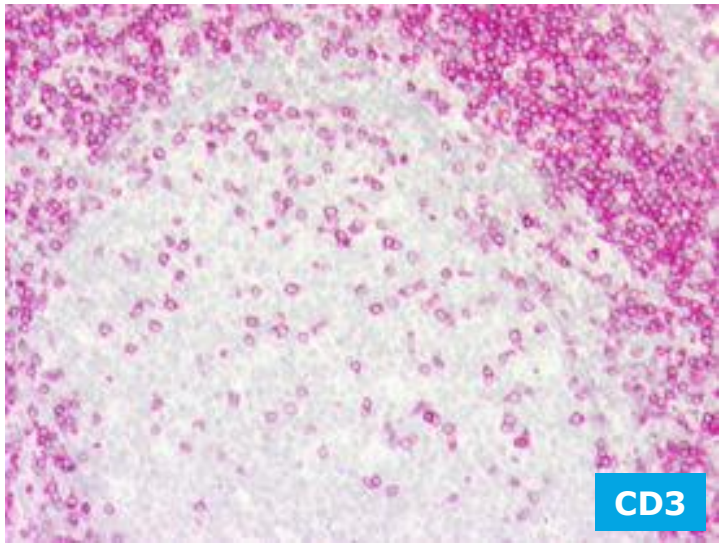
TCRαβ

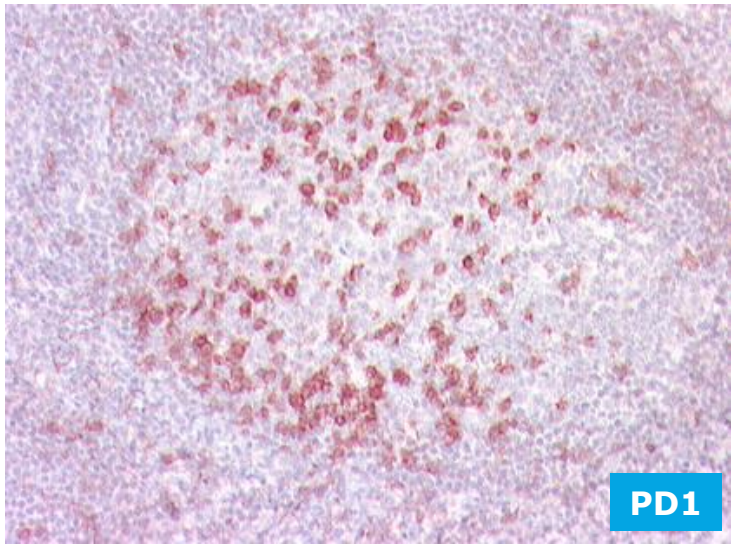


TCRγ

Follicular helper T-cells (T_{FH}) markers in AILT

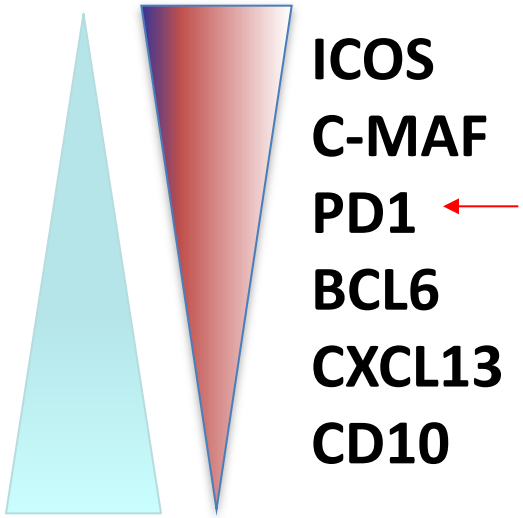
T_{FH} are a minor subset of T-cells normally residing in germinal centers and act as critical regulators of the B-cell immune response

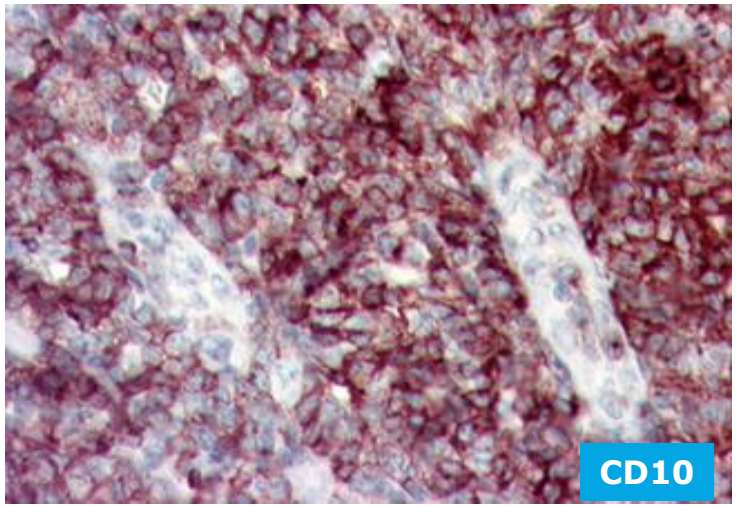


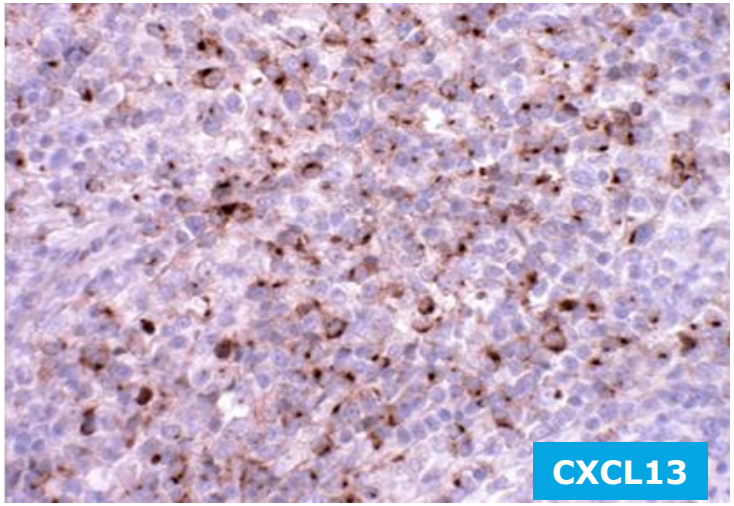


- Hypergammaglobulinemia and autoimmune manifestations
- T_{FH} express BCL6 and c-MAF

Follicular helper T-cells (T_{FH})

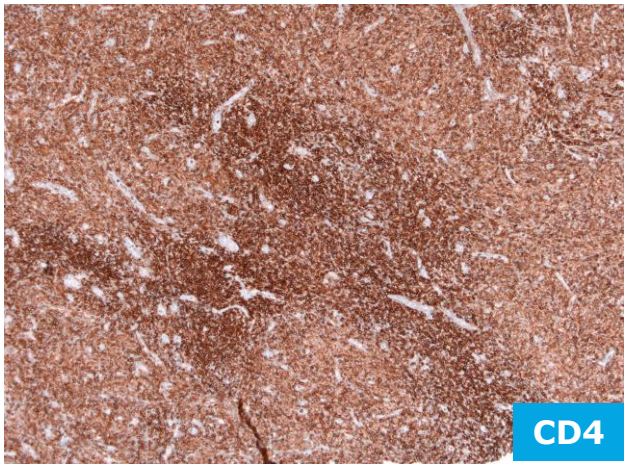


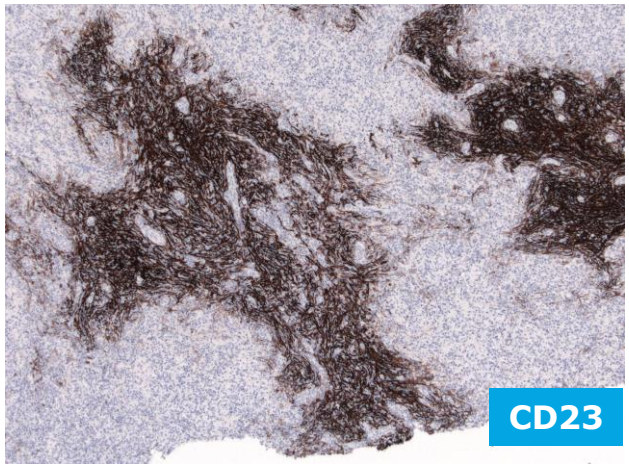


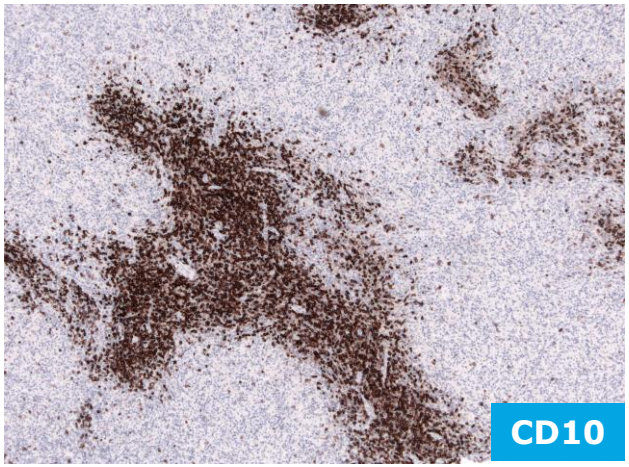


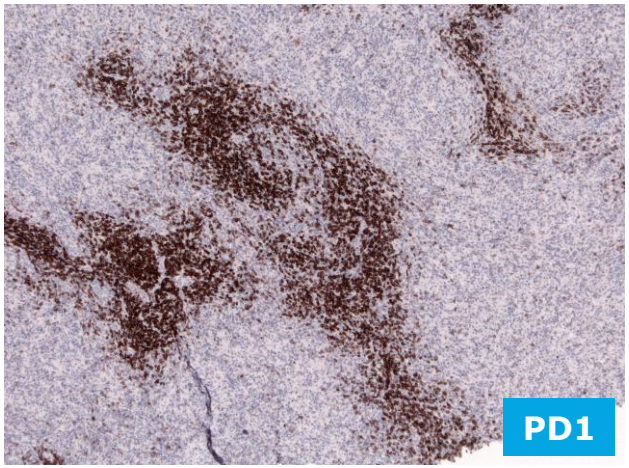
Am J Surg Pathol 2006, 30: 490
Modern Pathol 2006, 19: 337
Blood 2005, 106: 1501
Blood 2002, 99: 627

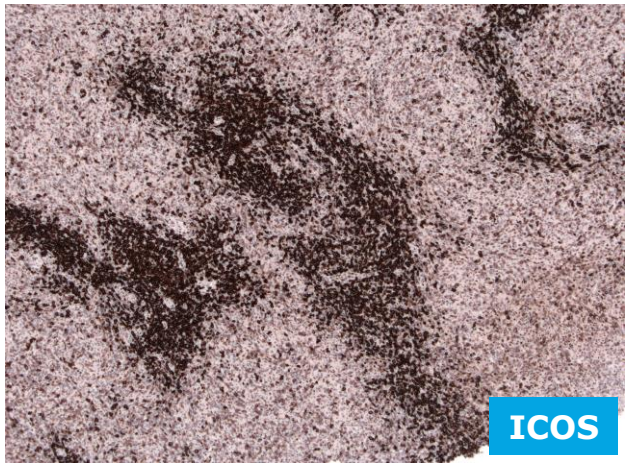
Microenvironmental imprint in AILT

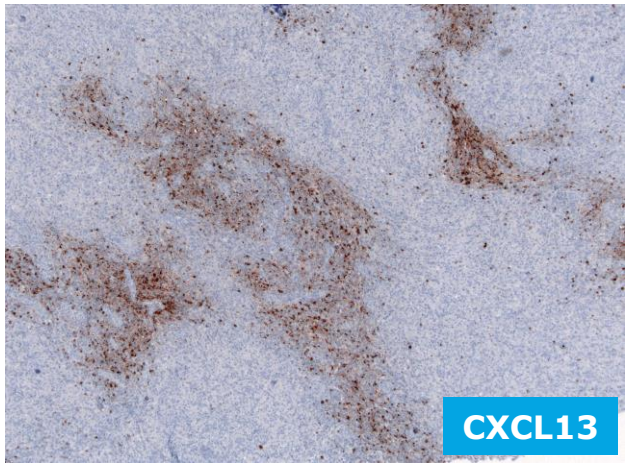








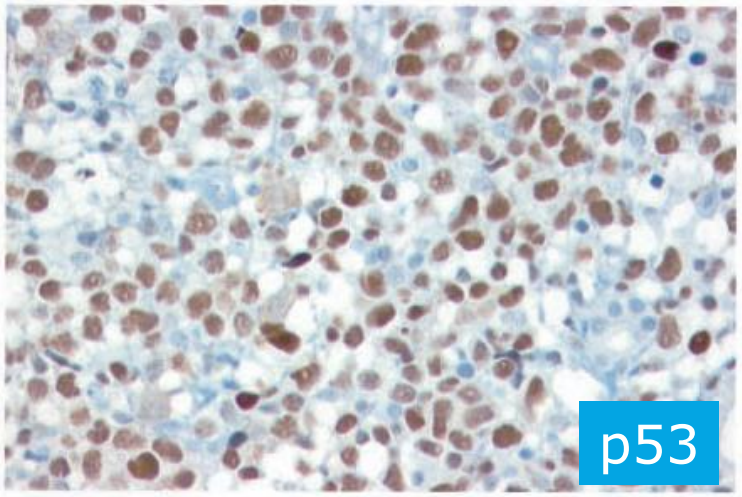


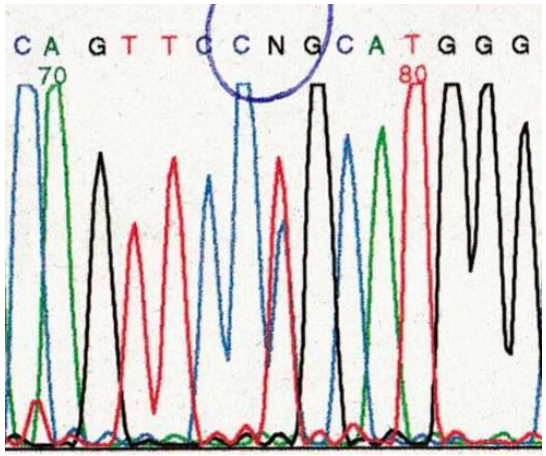


Biological Prognostic markers.

- Cell cycle
  - p53, p27, ki-67, cyclins
- Apoptosis related molecules
  - BCL-2, survivin
- Transcription factors
  - MYC, NOTCH1
- B-cell differentiation molecules
  - BCL-6, CD10, CD5, Zap-70, Foxp-1, CD21
- Other molecules
  - ICAM, sCD44, PD-L1

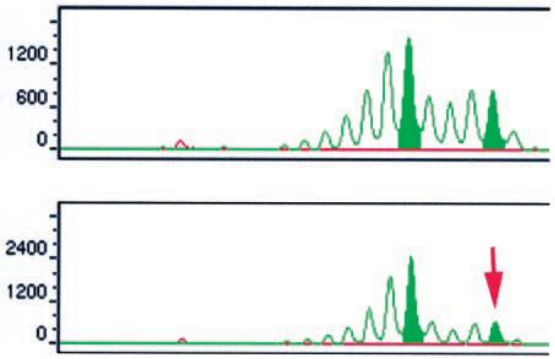
P53 mutational analysis in NK/T-cell lymphoma





20%

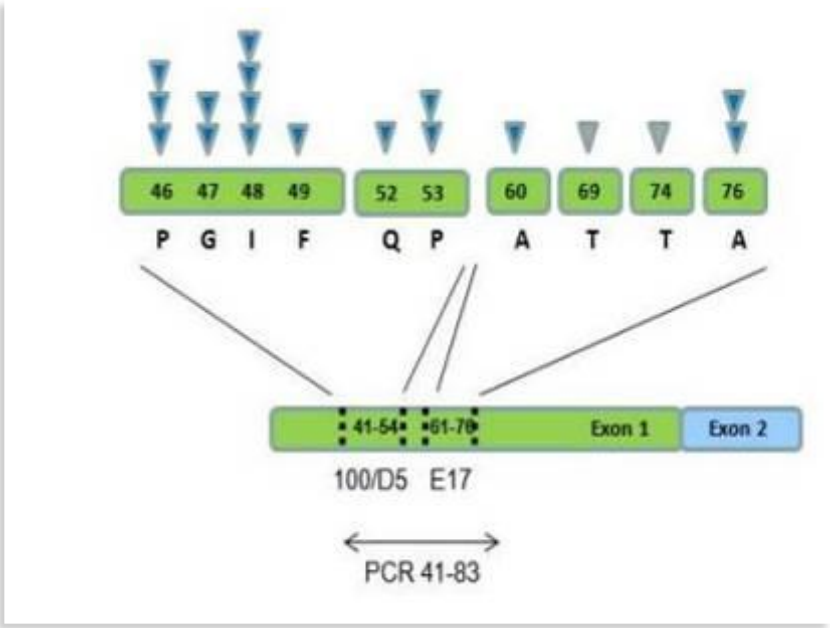
P53 exon 7, codon 242.
TGC → CGC, Cys→Arg

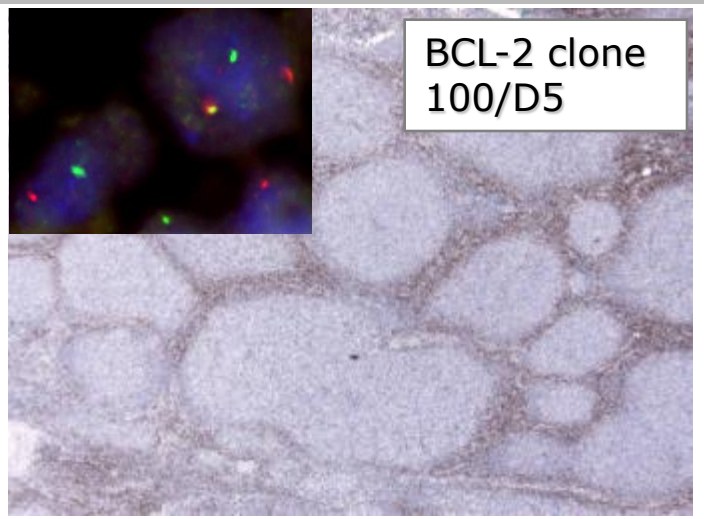


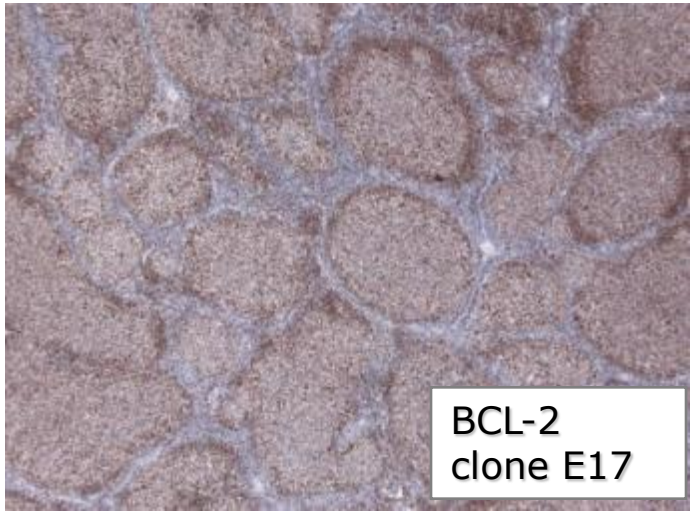
Quintanilla-Martinez et al, 2001 Am J Pathol:159;2095

BCL2 mutations in Follicular lymphoma

- **t(14;18)+ FL**
  - show Exon 1 missense mutations leading to conformational change of 100/D5 epitope
  - functional BH2 domains are spared

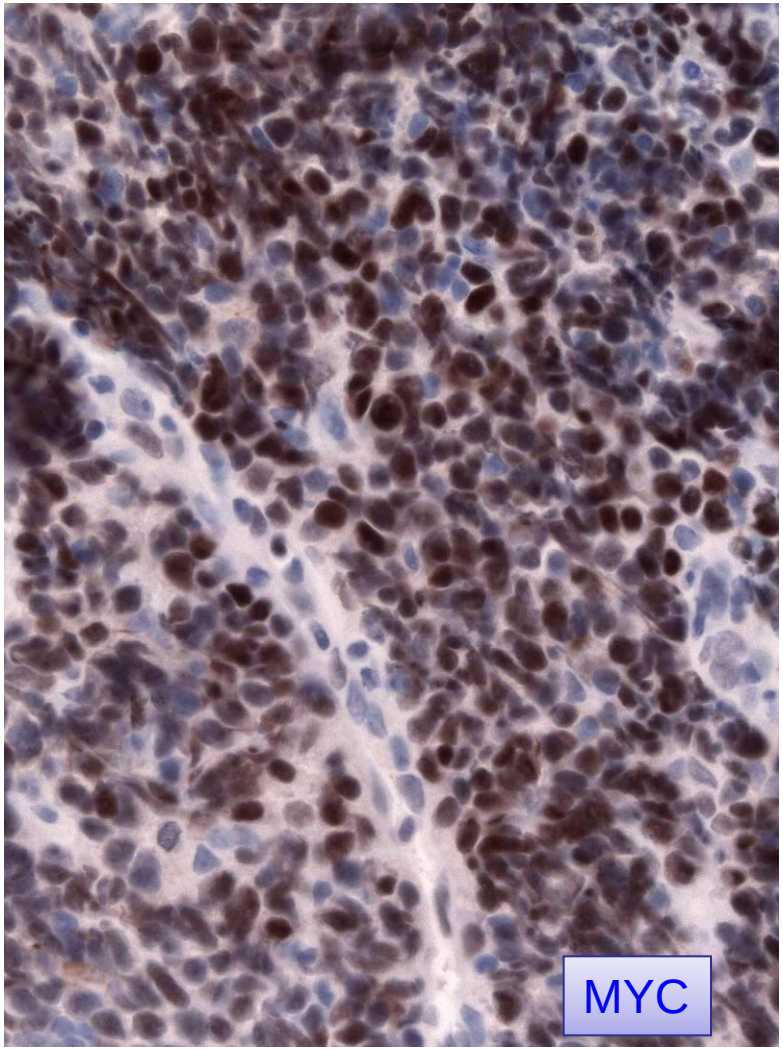




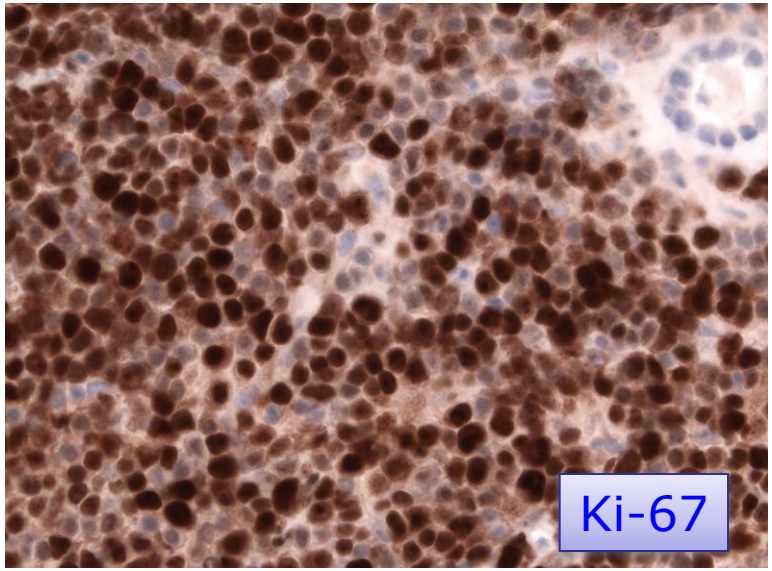


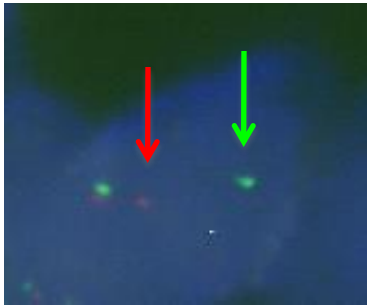
Adam et al, Hum Pathol 2013

DLBCL with *MYC* translocation



Monoclonal rabbit antibody



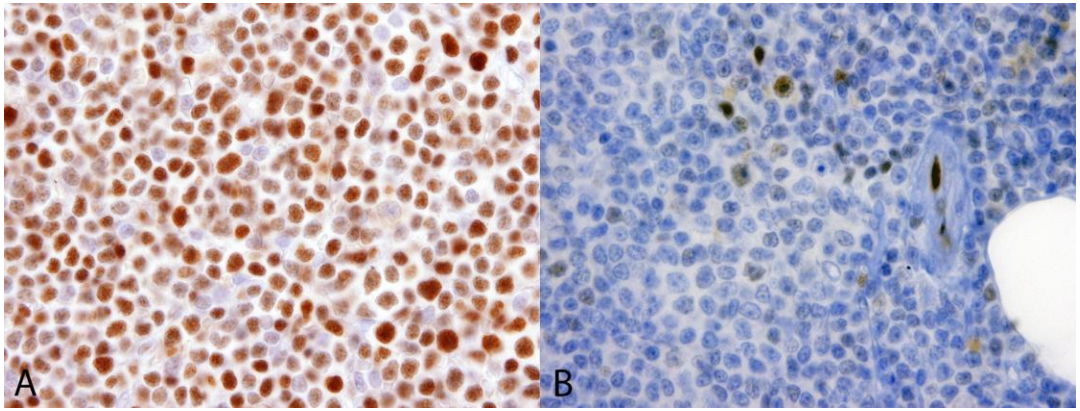


MYC break-apart

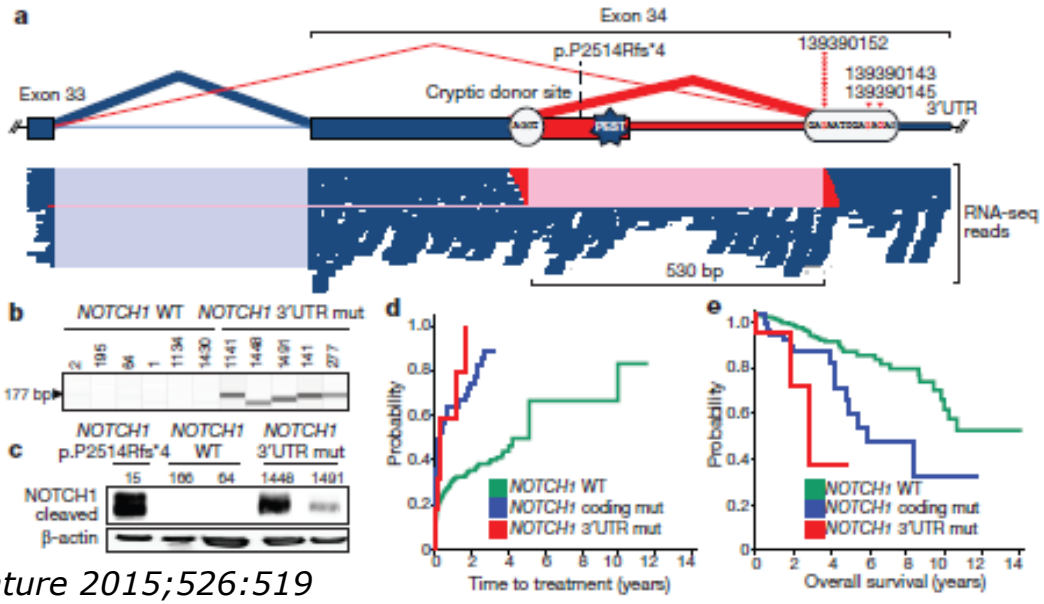
Ruzinova et al, AJSP 2010;34:882

NOTCH1 expression

- 50-60% of T-ALL have activating mutations
- 20% CLL
- 29% DLBCL
- 23% FL
- 5% MCL



Ho C and Rodig SJ, Sem Diag Pathol 2015;32:381



Puente et al Nature 2015;526:519

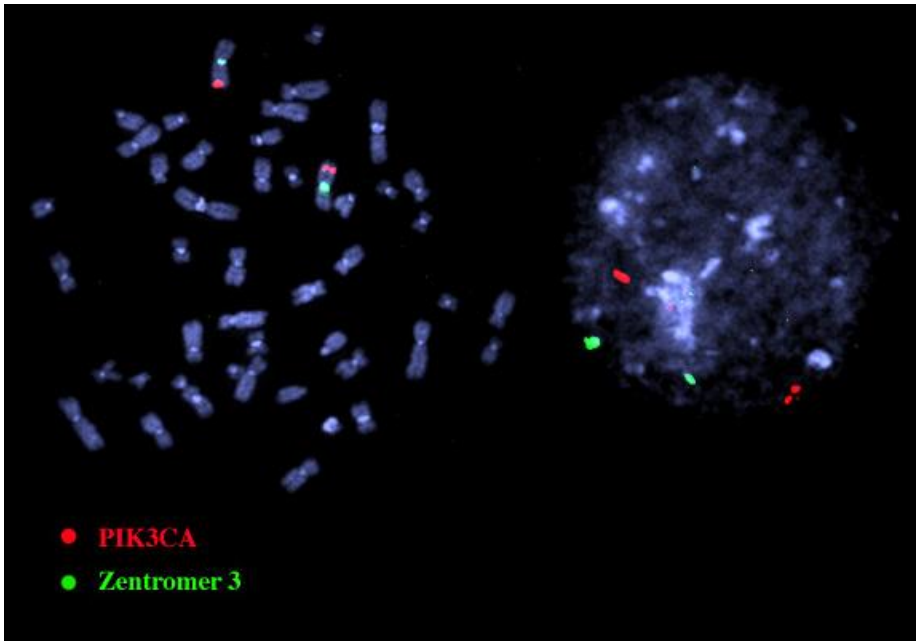
Techniques in molecular diagnosis

- Diagnostic ancillary methods
  - FISH (fluorescence *in situ* hybridization)
  - PCR based techniques
    - Quantitative (Real-time PCR)
      - Allele specific PCR (mutation detection)
    - Qualitative (clonality analysis)
    - Sequence analysis
  - Next generation sequencing

Fluorescence in situ hybridization (FISH)

- Examine individual cells
- Detect heterogeneity within tumor cells
- Interphase FISH can be apply to paraffin-tissue
- Best method to detect
  - translocations
  - amplifications
  - polisomy

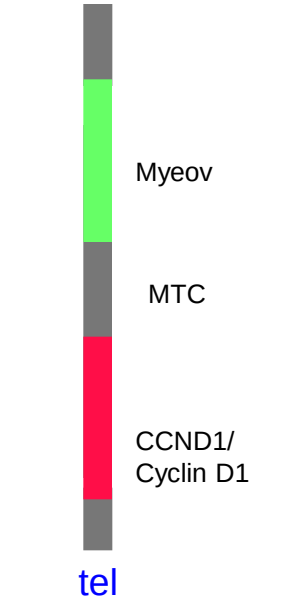
PIK3CA /Centromere 3



Metaphase Interphase

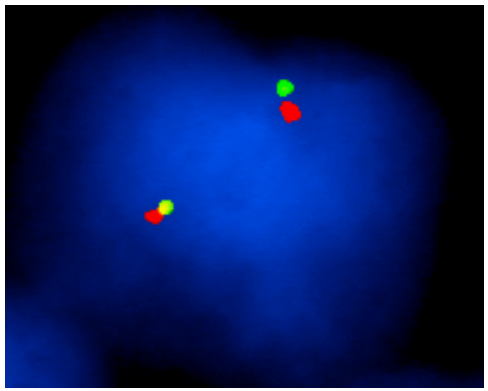
Haralambieva et al, J Pathol 2002, 198:163

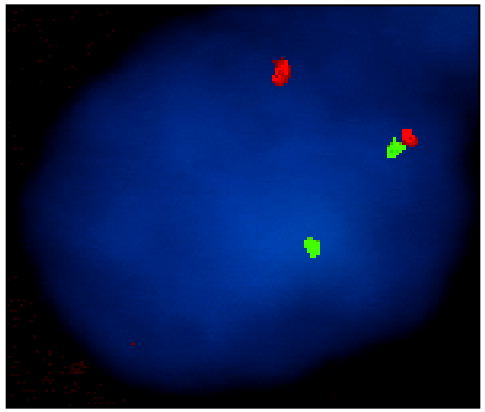
Fluorescence in situ hybridization (FISH)



Chromosome 11

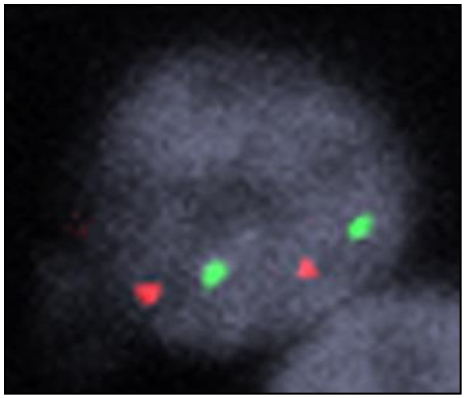
Segregation FISH

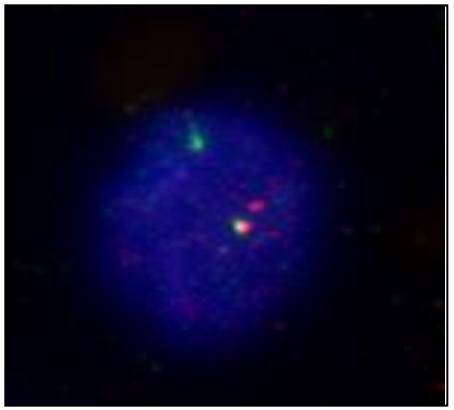




Break in chromosome 11

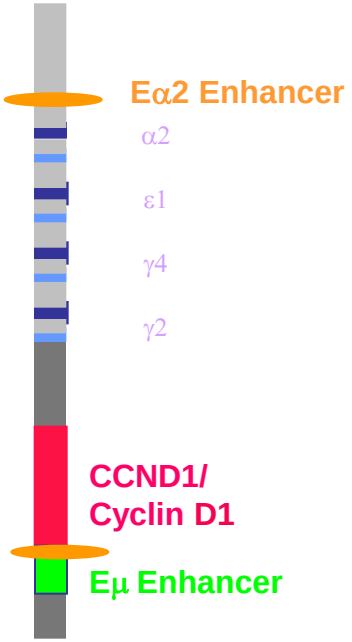
Colocalization FISH





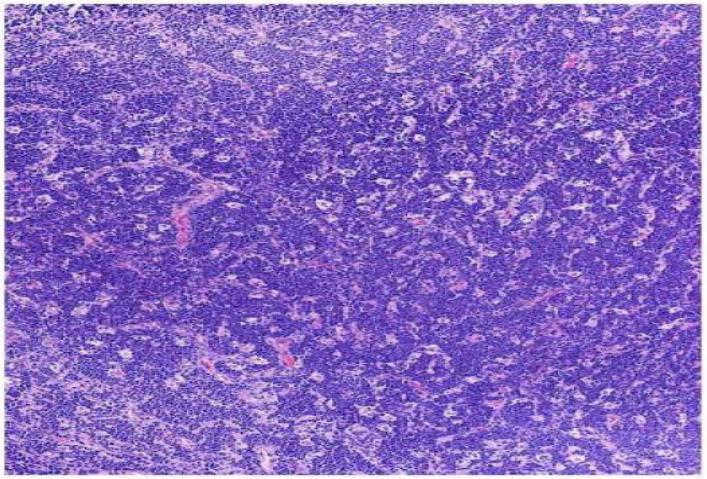
t(11;14)

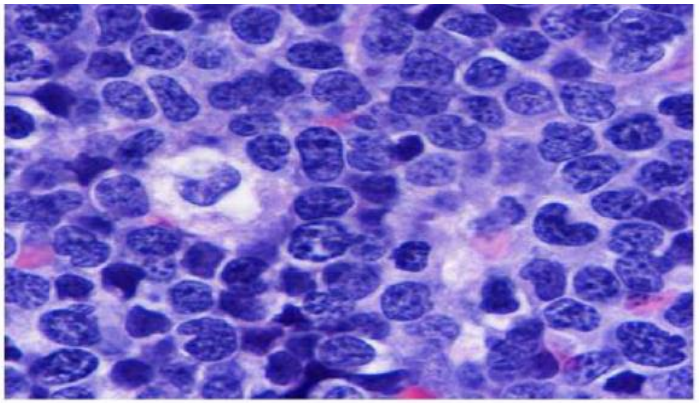
Chromosome 11
Chromosome 14



Chromosome 14

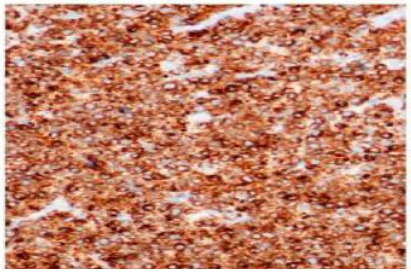
Transformed follicular lymphoma „double hit“

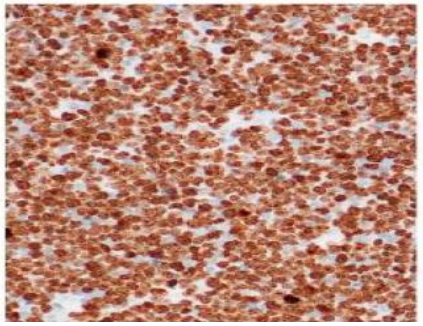


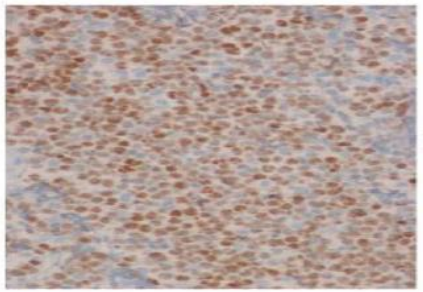


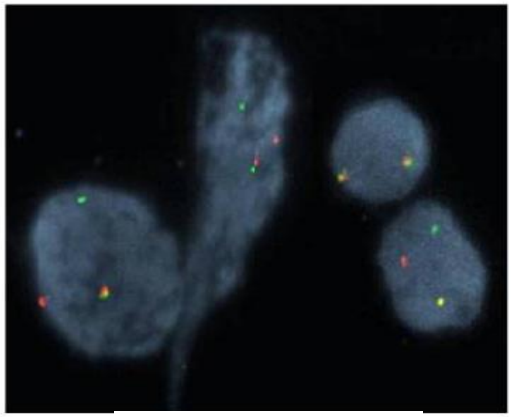
Low Power

High Power

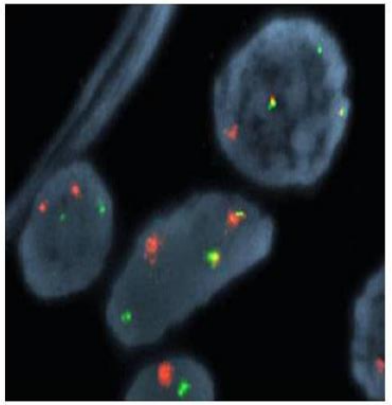








MYC break apart

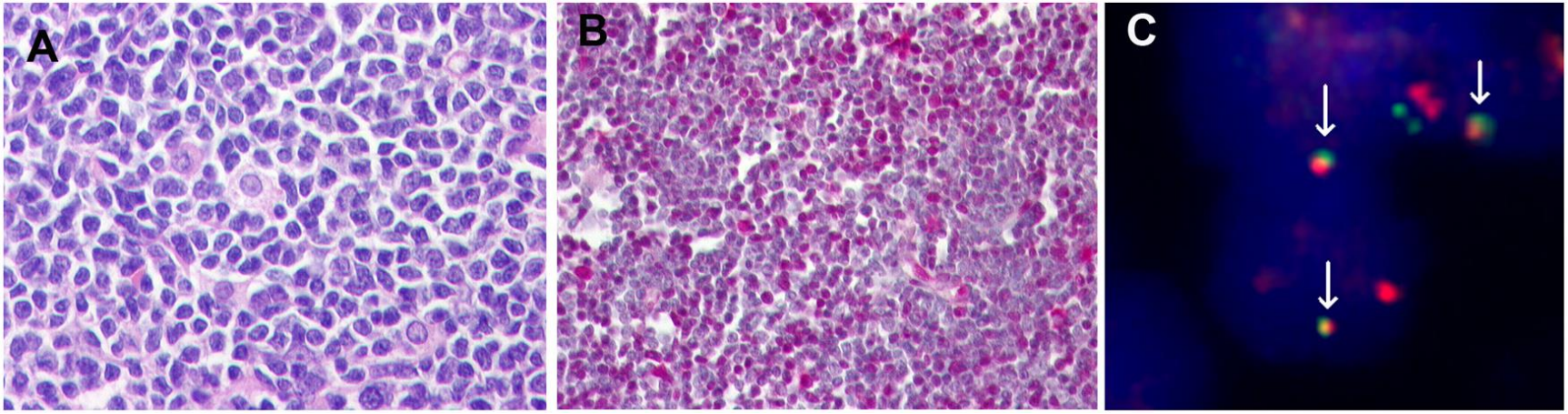


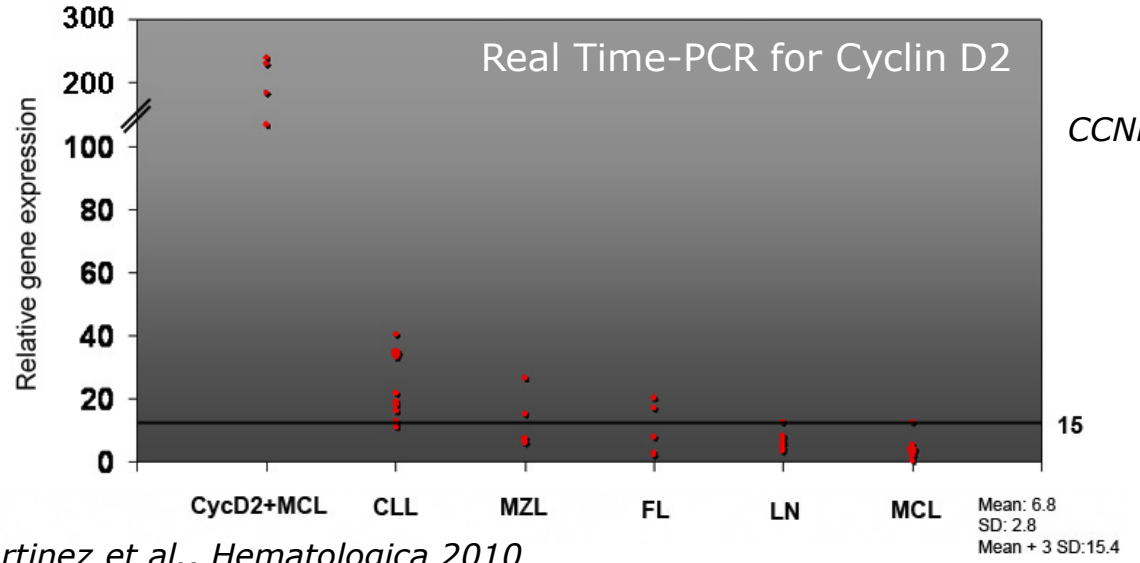
IGH-BCL2

Techniques in molecular diagnosis

- Diagnostic ancillary methods
  - FISH (fluorescence *in situ* hybridization)
  - PCR based techniques
    - Quantitative (Real-time PCR)
      - Allele specific PCR (mutation detection)
    - Qualitative (clonality analysis)
    - Sequence analysis
  - Next generation sequencing
    - Targeted amplification
    - clonality

Cyclin D2+ Mantle cell lymphomas





t(2;12)

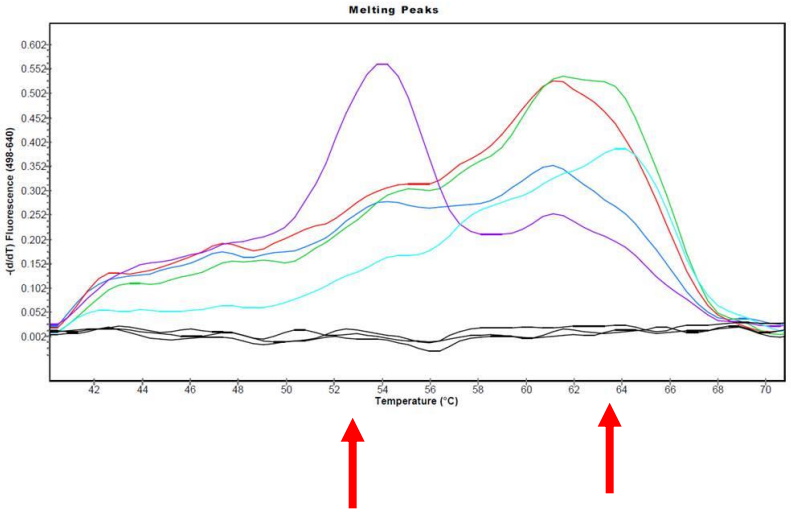
CCND2 (12p13) and *IGK* (2p12)

Quintanilla-Martinez et al., Hematologica 2010

Allelic specific PCR (mutation detection)

AS-PCR with melting curve for mutations with hot spots.

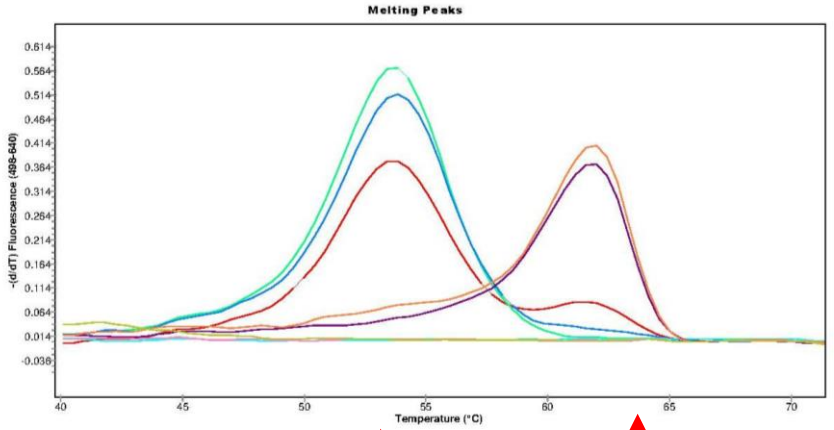
MYD88 mutation analysis (LPL)



Wild type

MYD88 **L265P** Mutation

RHOA mutation analysis (AITL)



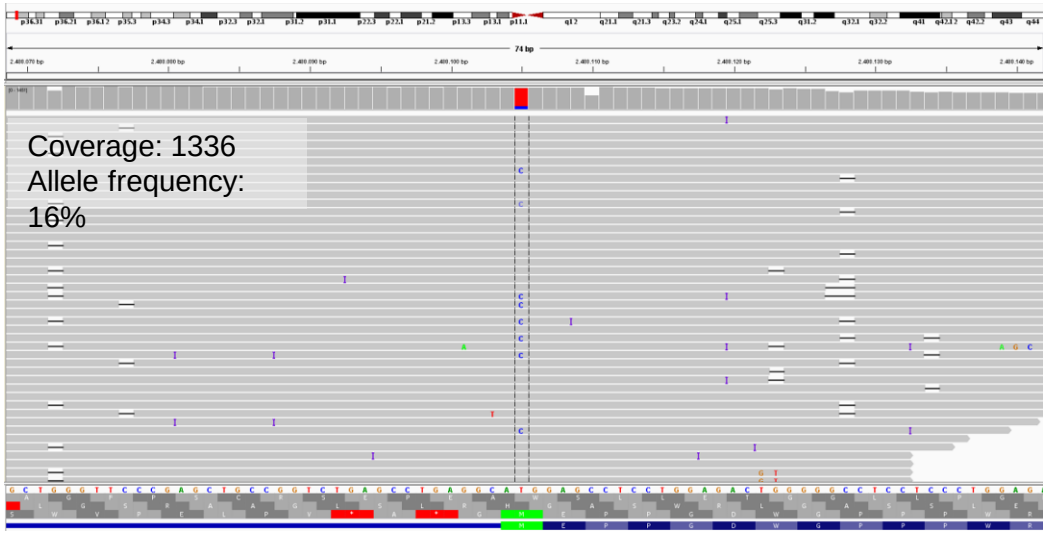
Wild type

RHOA **G17V** Mutation

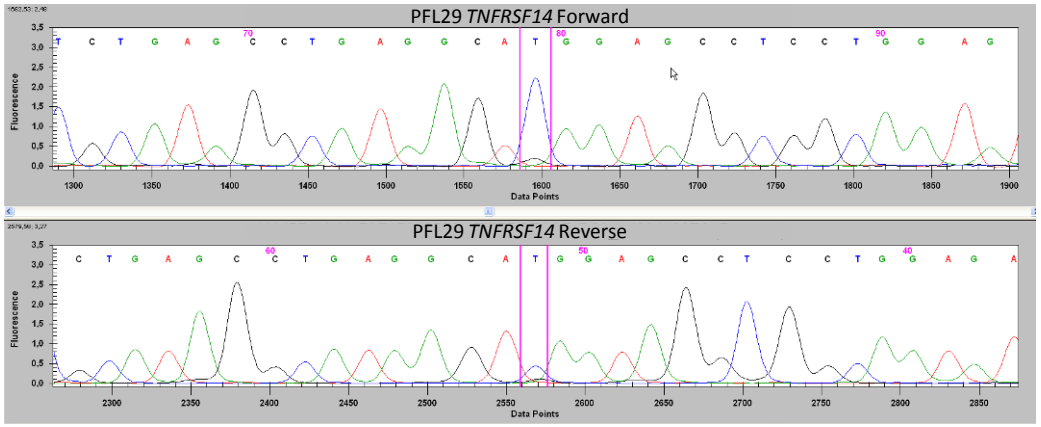
Schmidt J et al, BJH 2015, 169:795-803
Bonzheim I et al Blood 2015

TNFRSF14 mutations in PFL

A) Ion AmpliSeq Custom Panel



B) Validation Sanger sequencing

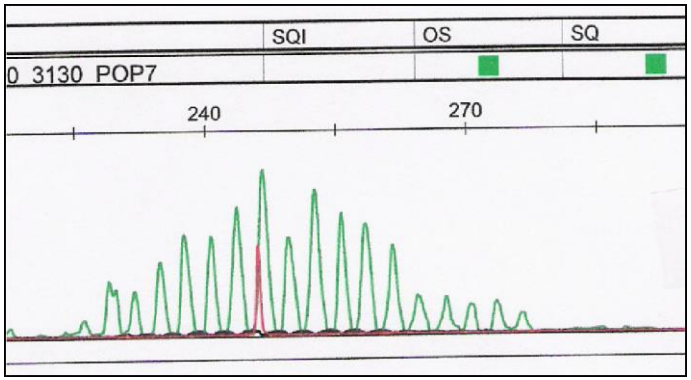


PFL29 *TNFRSF14*
p.Met1_Gln97del

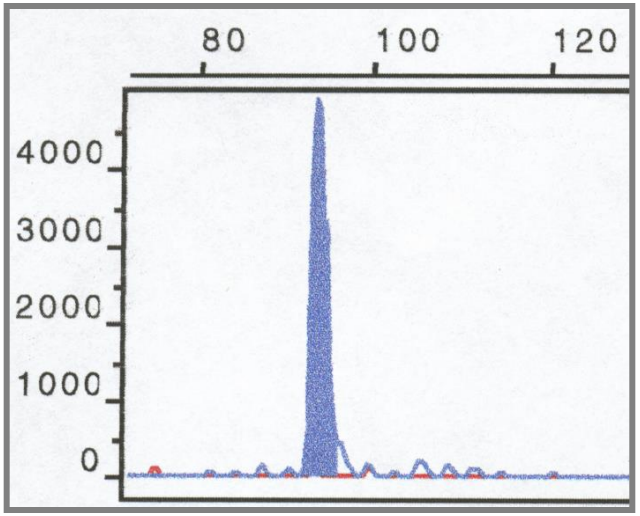
Clonality as marker of lymphoid neoplasia

- Immunoglobulin gene or T-cell receptor gene rearrangements
  - Malignant vs. bening
  - Cell lineage B vs T
- Diagnosis of residual disease
  - For MRD specialized approaches (e.g. patient specific probes) required
- In disease
  - Unique molecular fingerprint
  - Determination of clonal relationship
  - Transformation vs. second malignancy

IgH heavy chain clonality analysis



Polyclonal pattern FR2
3-bp spacing
Gaussian distribution



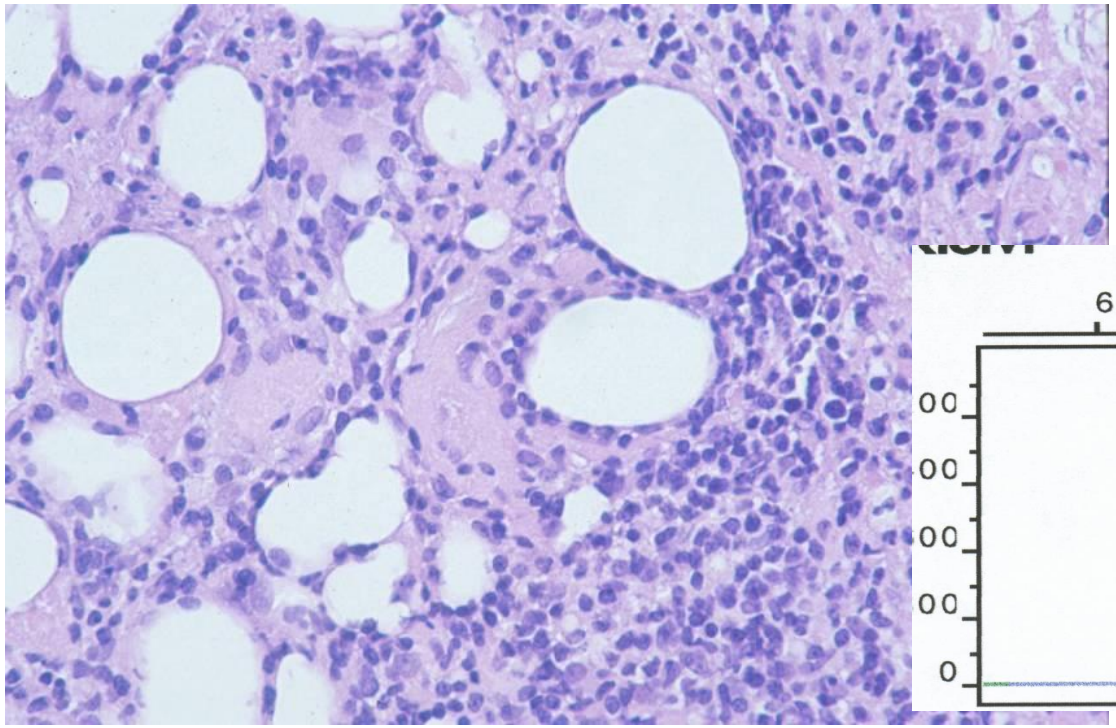
Automated fragment length analysis

Monoclonal peak FR3
Faint polyclonal background

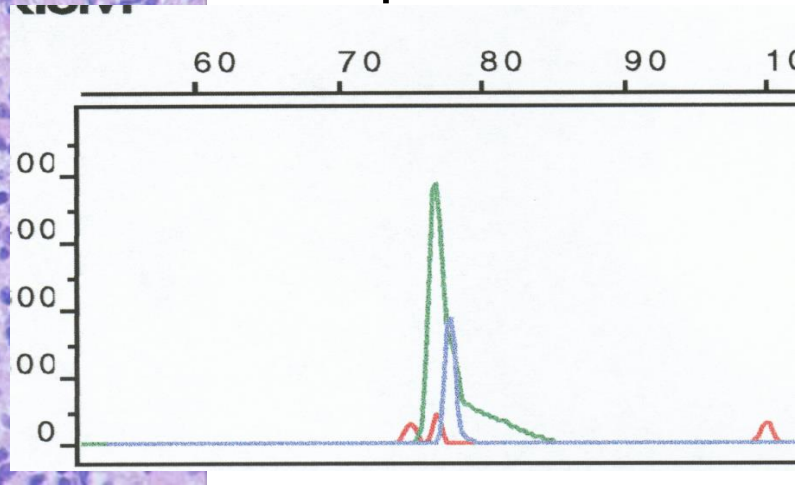
GeneScan

Benign vs. Malignant

- Polymorphous infiltrates in subcutaneous tissue
- Predominance of T-cells, rimming of fat cells

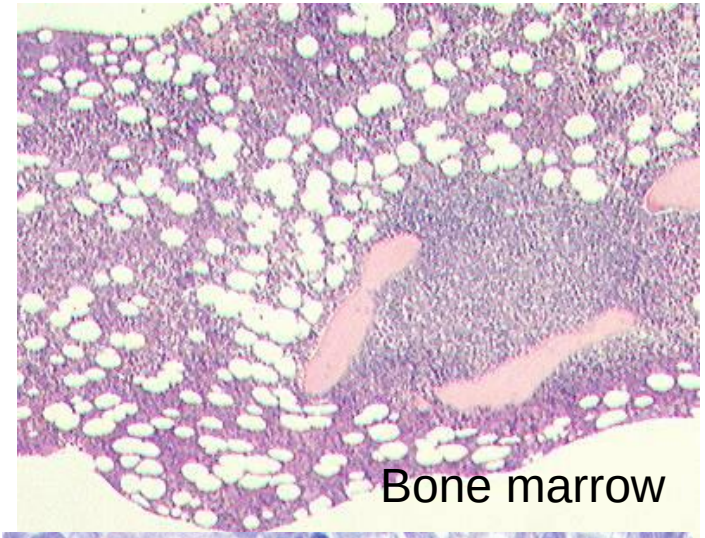


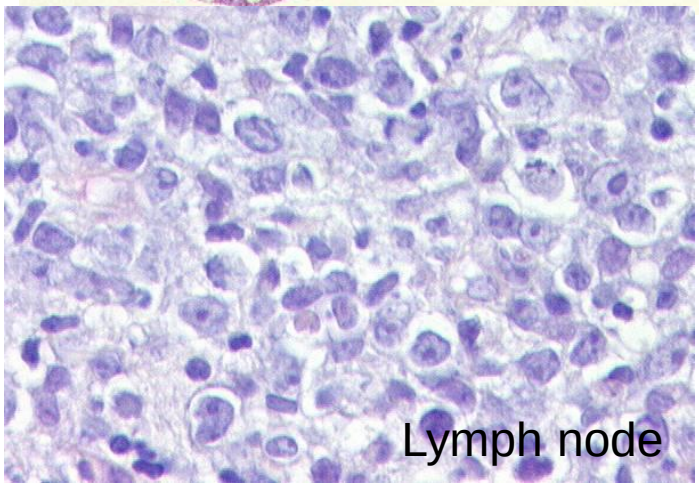
35-year-old woman,
Subcutaneous nodules
leukopenia

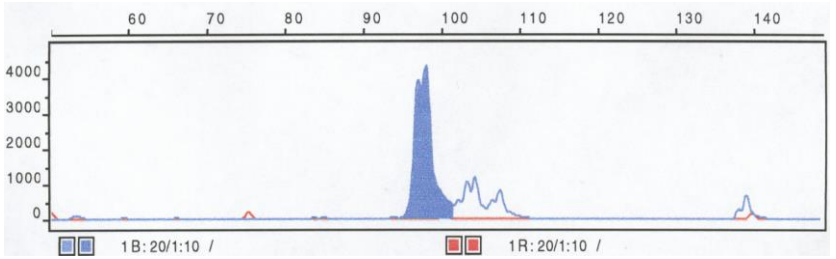


-Monoclonal TCRγ rearrangement

Discordant lymphomas

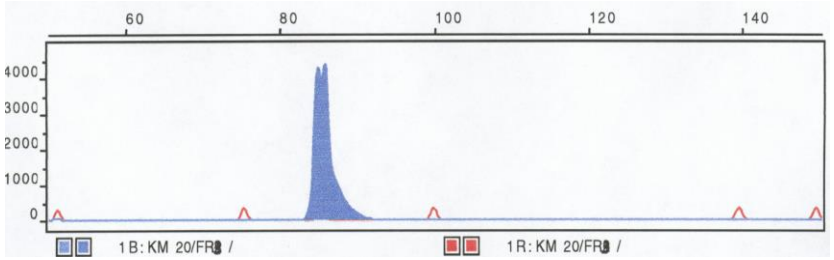






97 bp

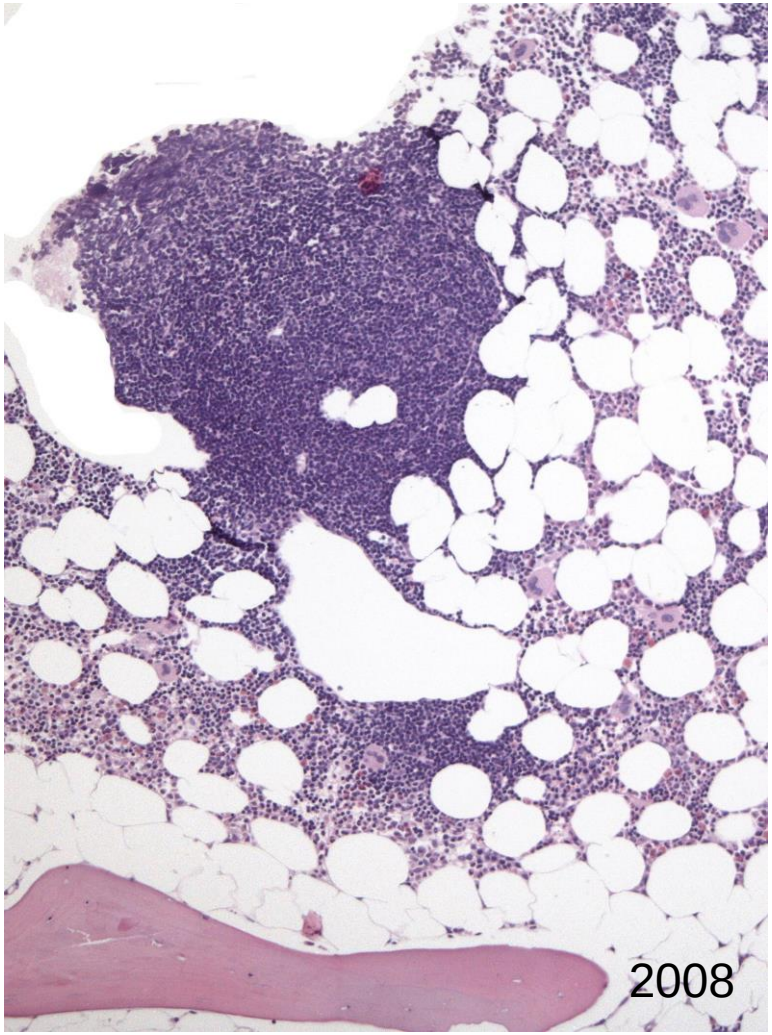
In 30% of the cases a second neoplasia is found!

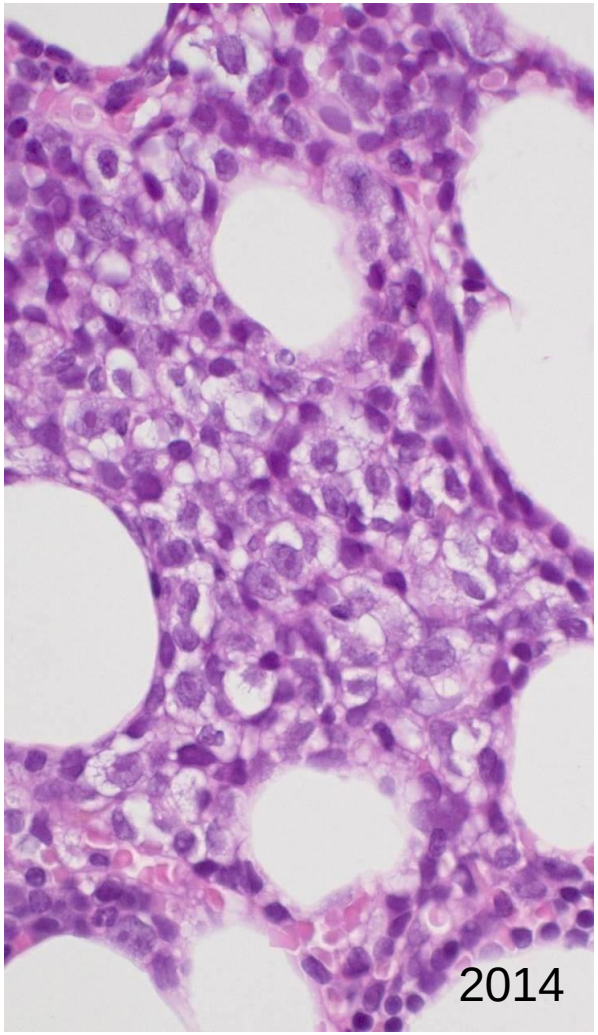


85 bp

Kremer et al, Lab Invest 2003

Lymphoma transformation



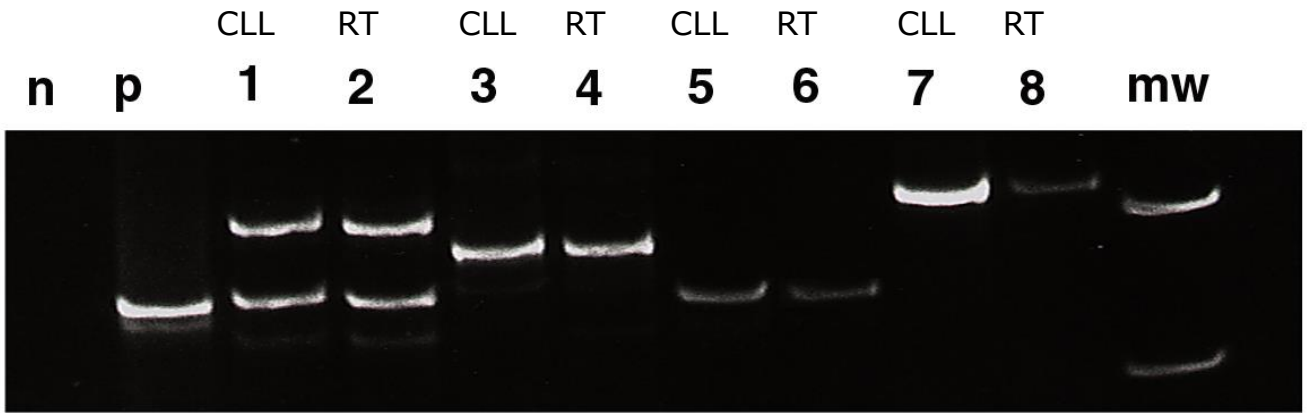


60-year-old male, with history of CLL now with retroperitoneal lymphadeno-pathy, B-symptoms, lymphocytosis of PB

Richter´s transformation

Transformation or secondary neoplasia?

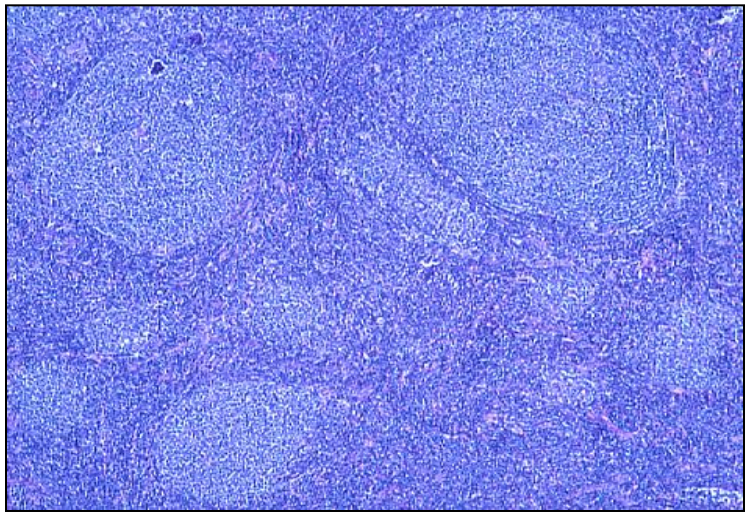
78% related
22% unrelated

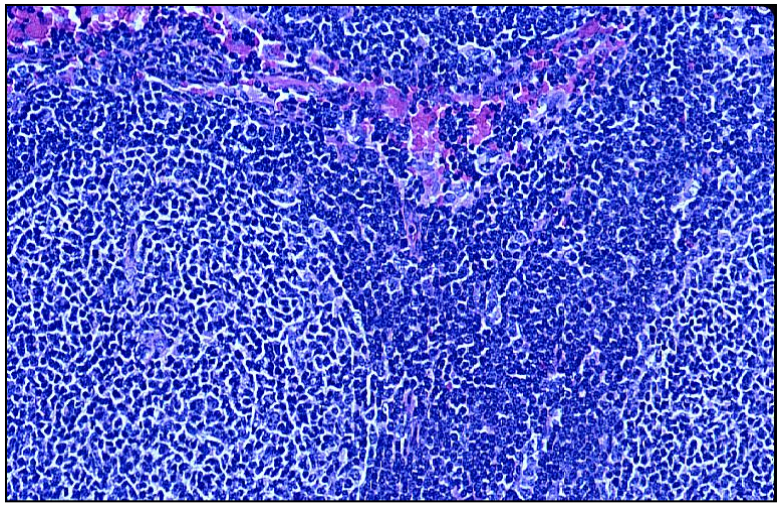


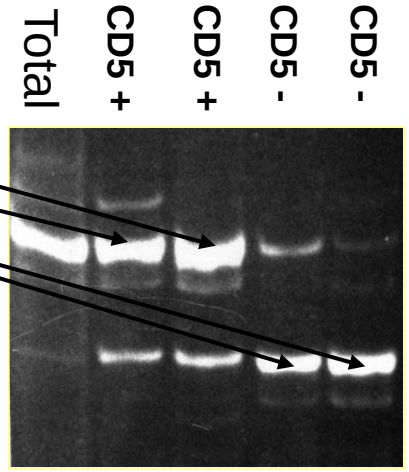
Mao, Quintanilla-Martinez et al, AJSP 2007

FR3 IgH-PCR

Composite lymphoma
Follicular lymphoma and CLL

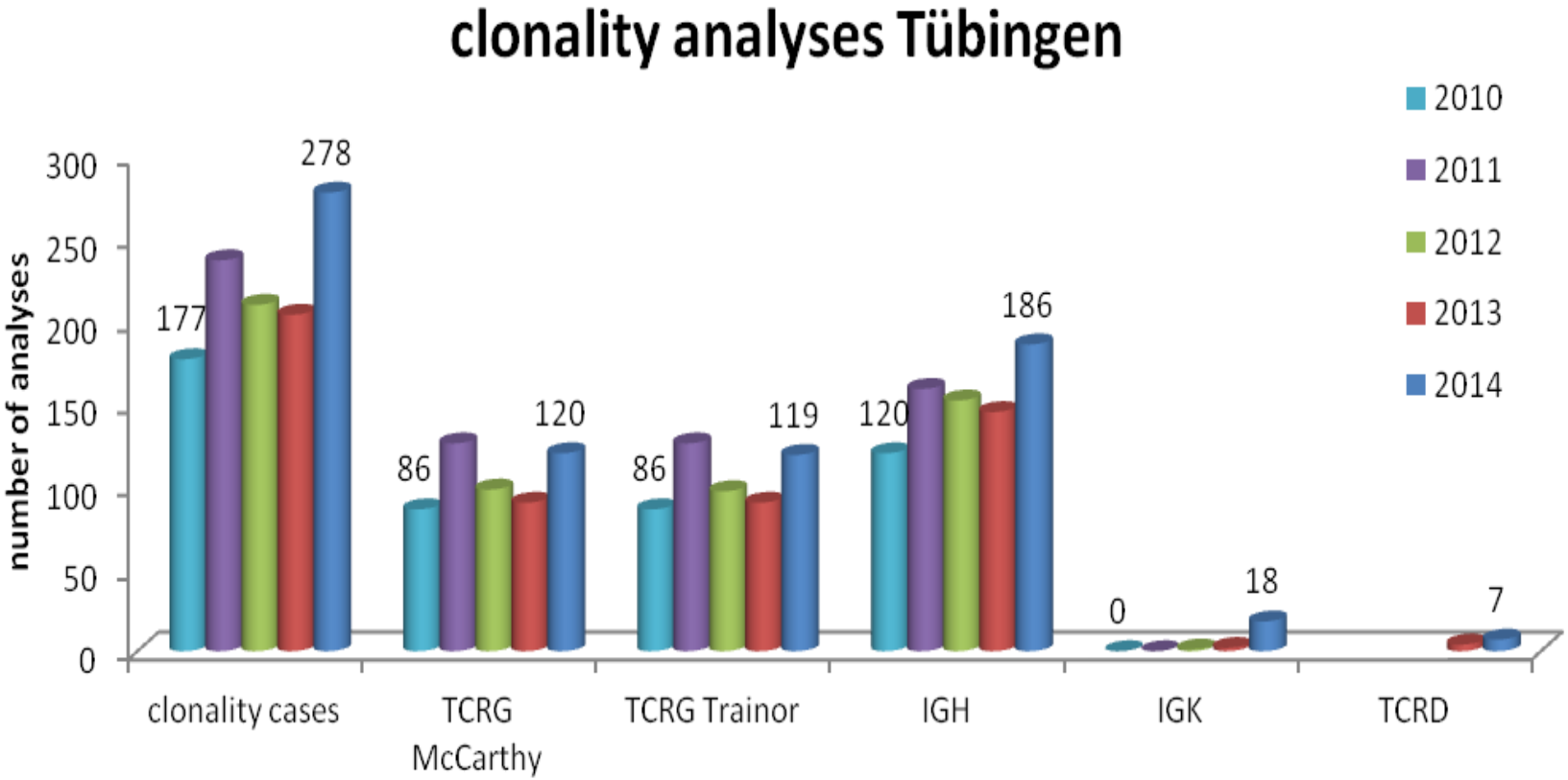


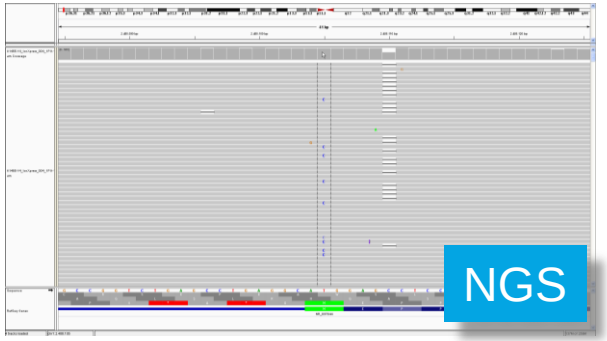


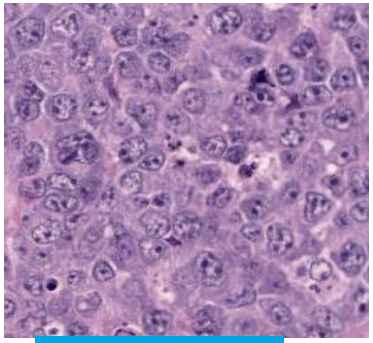


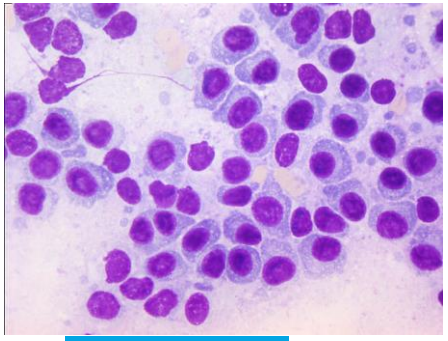
IgH-PCR
FR3

Molecular diagnosis in Hematopathology

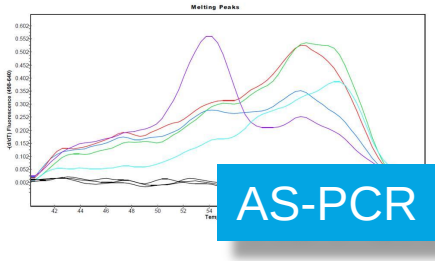


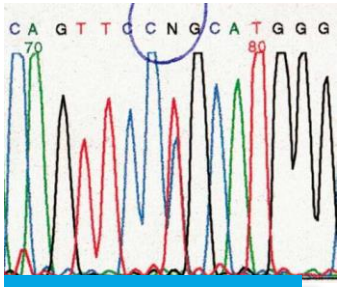


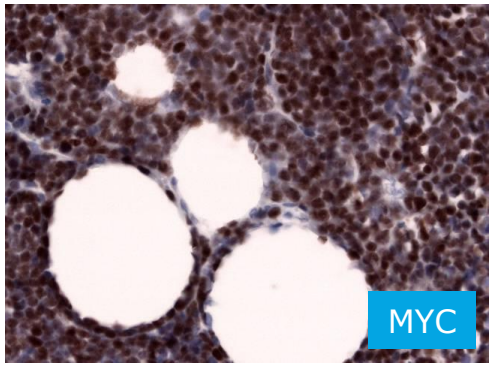




Molecular techniques







Immunohistochemistry