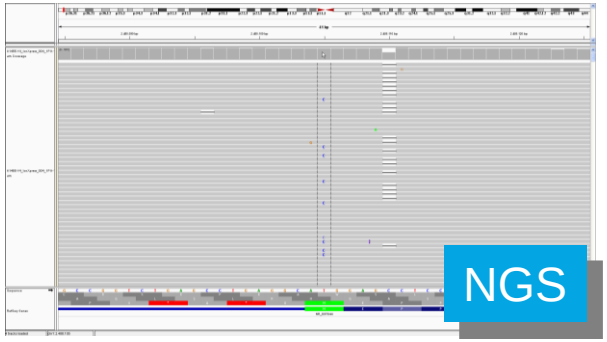
Eberhard-Karls-Universität
UKT
Universitätsklinikum Tübingen

Institut für Pathologie

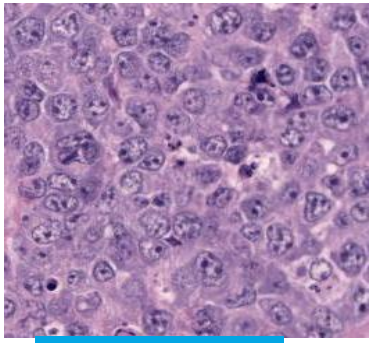
Methods used to diagnose lymphomas

Prof. Dr.Med. Leticia Quintanilla-Fend

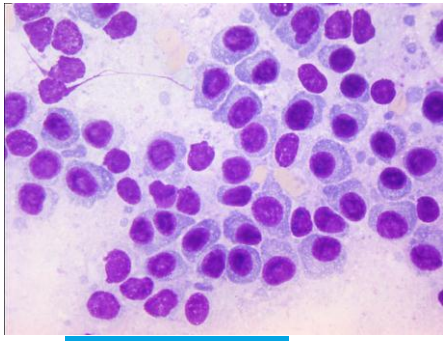
Comprehensive Cancer Center
Tübingen - Stuttgart



NGS

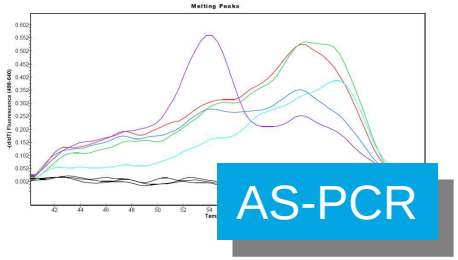


histology

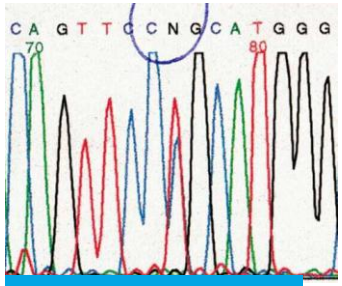


Cytology

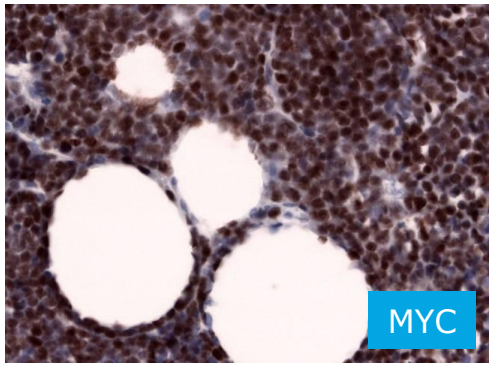
Molecular techniques



AS-PCR

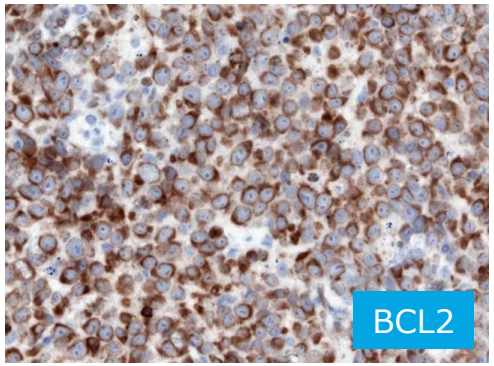


Sanger seq.

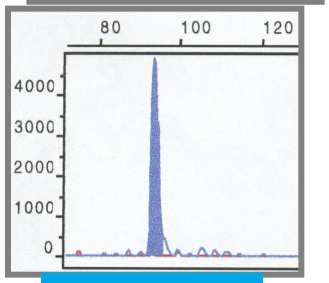


MYC

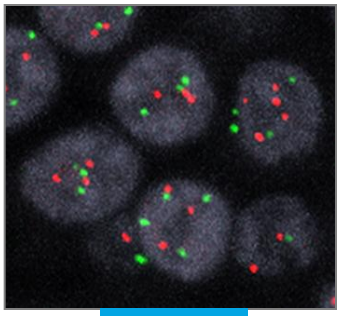
Immunohistochemistry



BCL2



Clonality



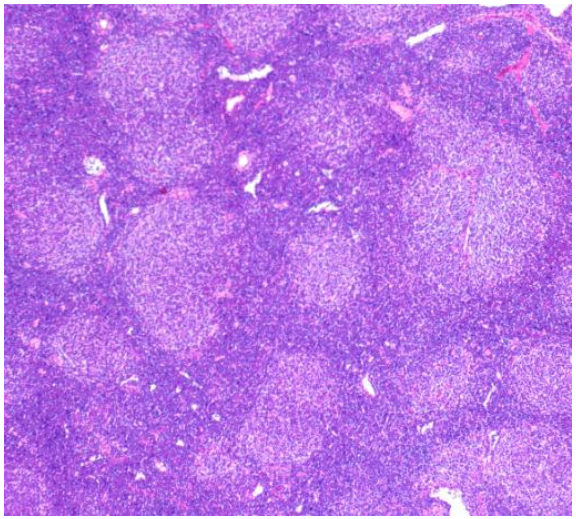
FISH

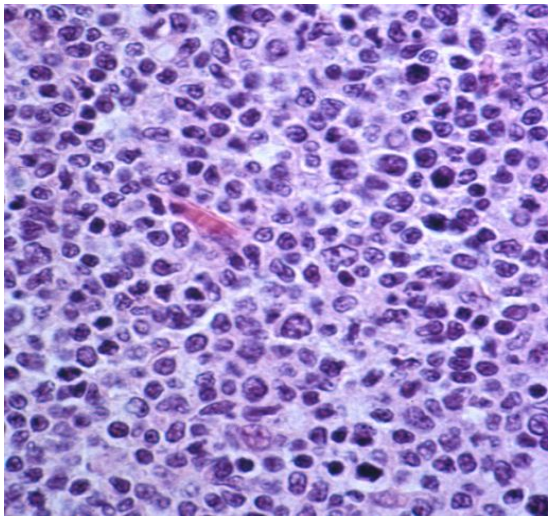
Immunohistochemical approach.

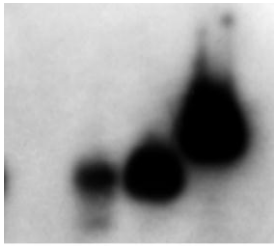
Immunophenotyping in Lymphomas :

- 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

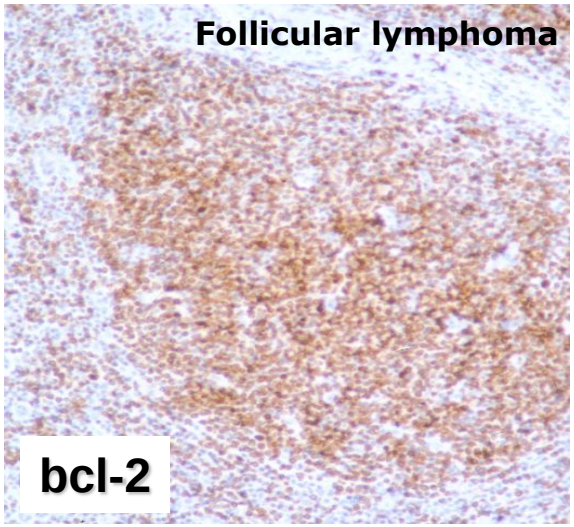
Surrogate marker of molecular changes

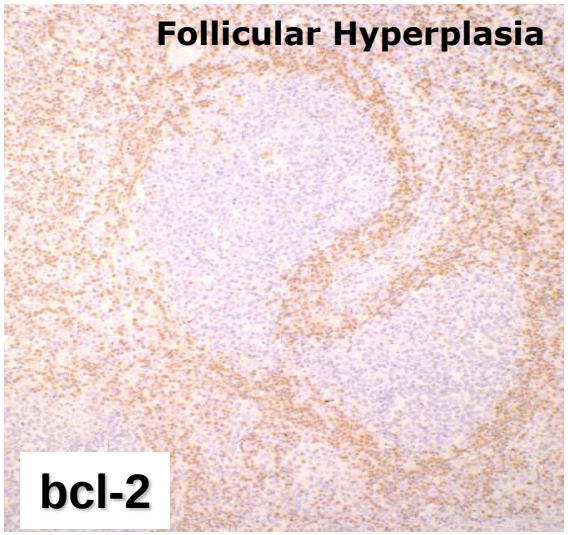


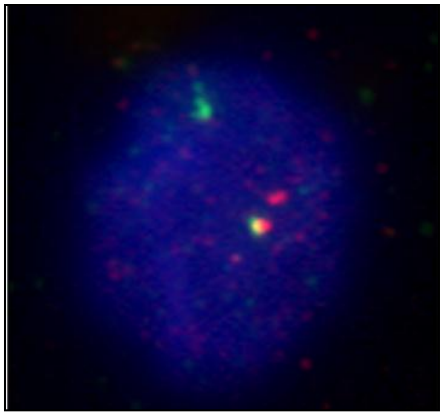




Rearrangement of BCL-2

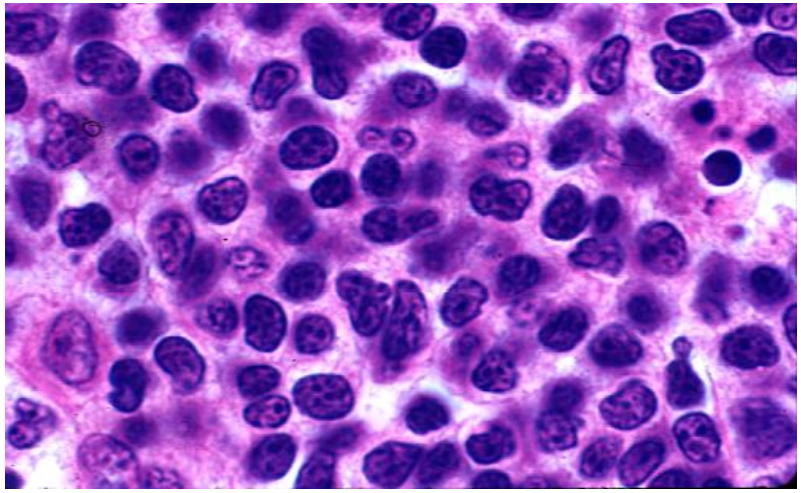




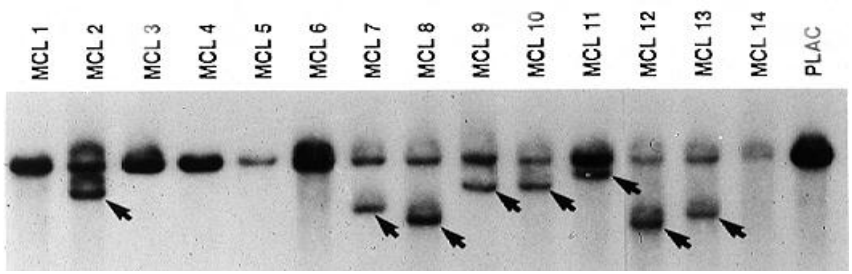


t (14;18)

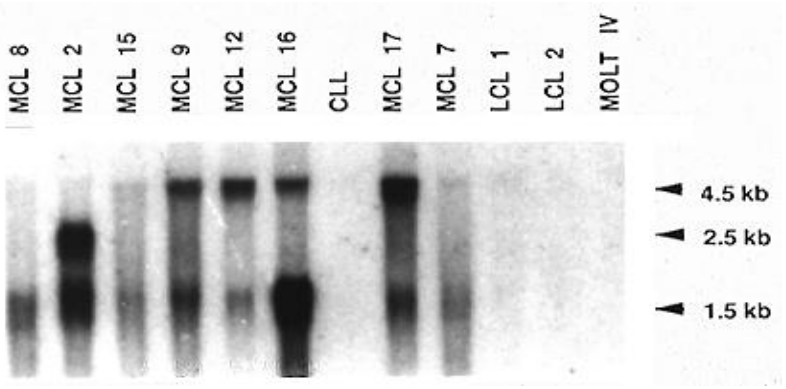
Surrogate marker of molecular changes



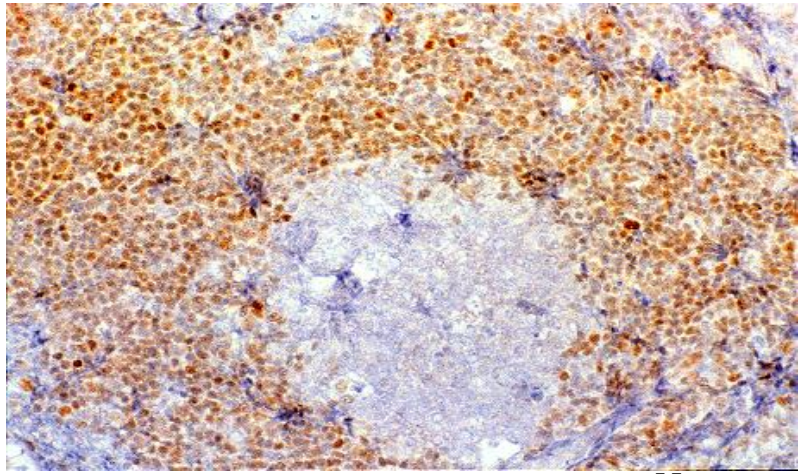
t (11;14)



Rearrangement of BCL-1

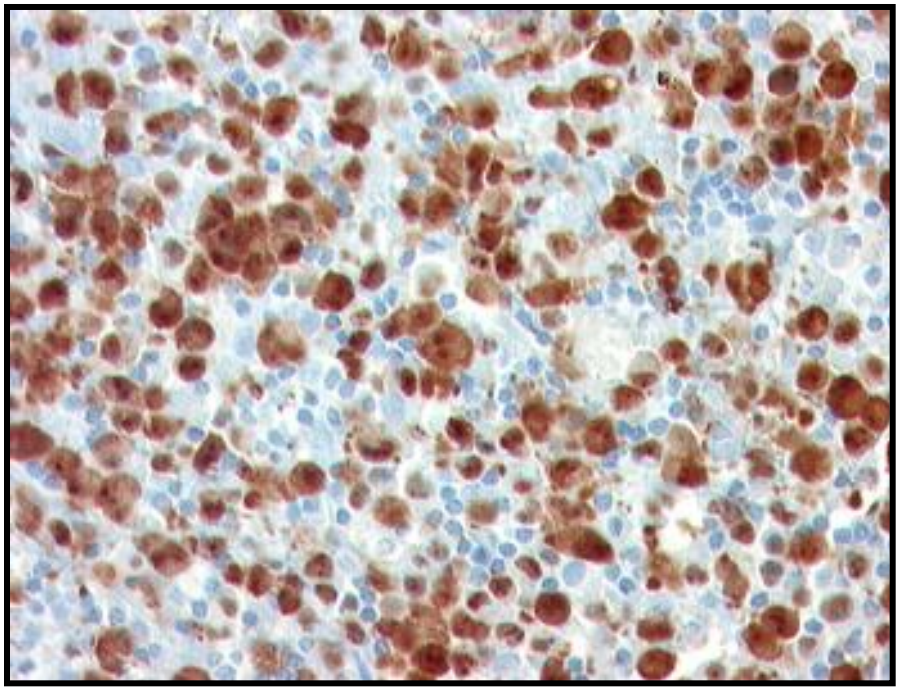


Northern blot cyclin D1

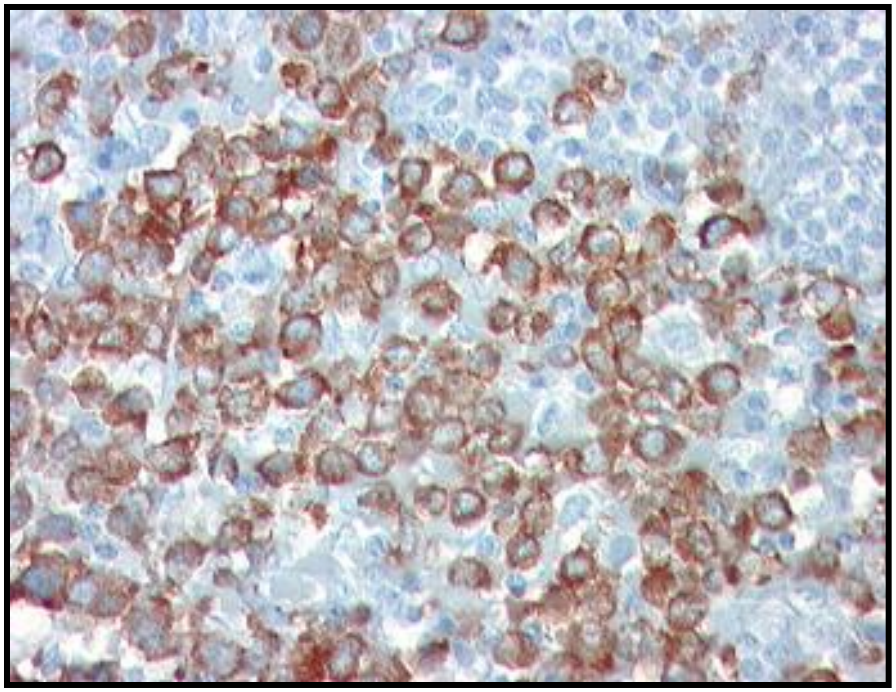


Cyclin D1

ALK+ Anaplastic large cell lymphoma.

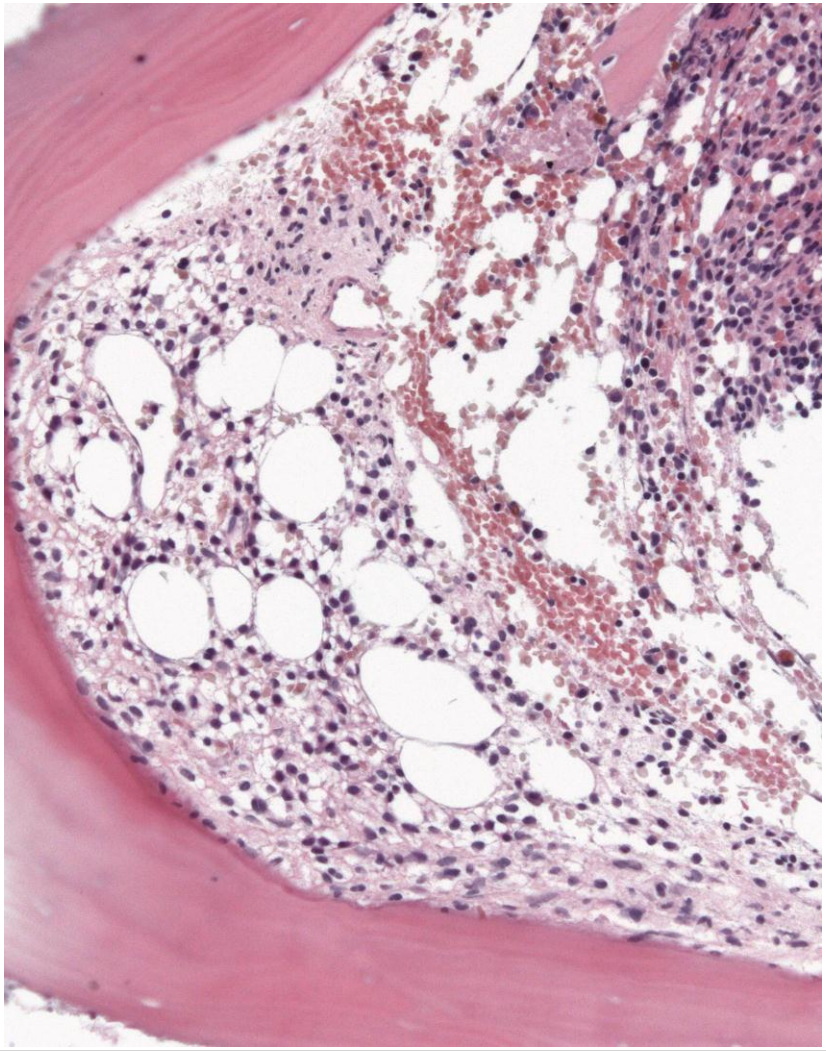


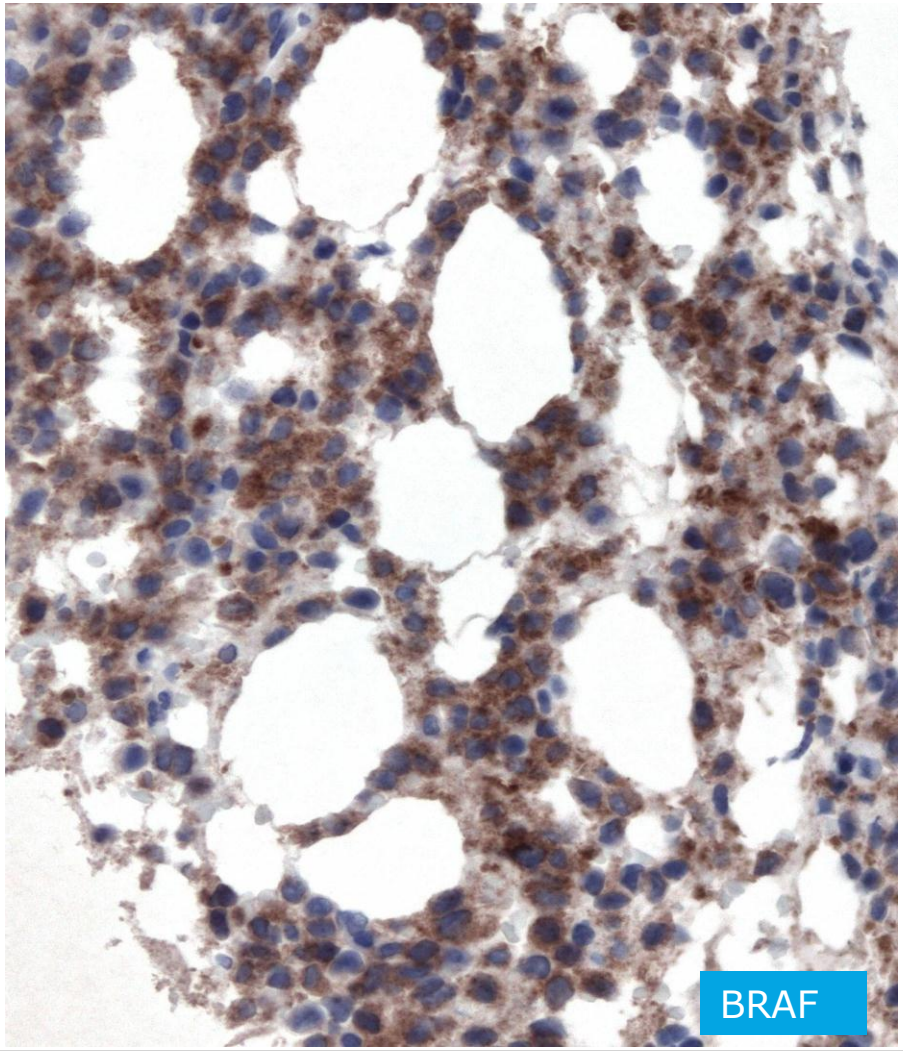
NPM-ALK - t(2;5)



Variant fusion protein

Hairy cell leukemia BRAF antibody





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Peripheral T-cell lymphomas from 41% to 86%

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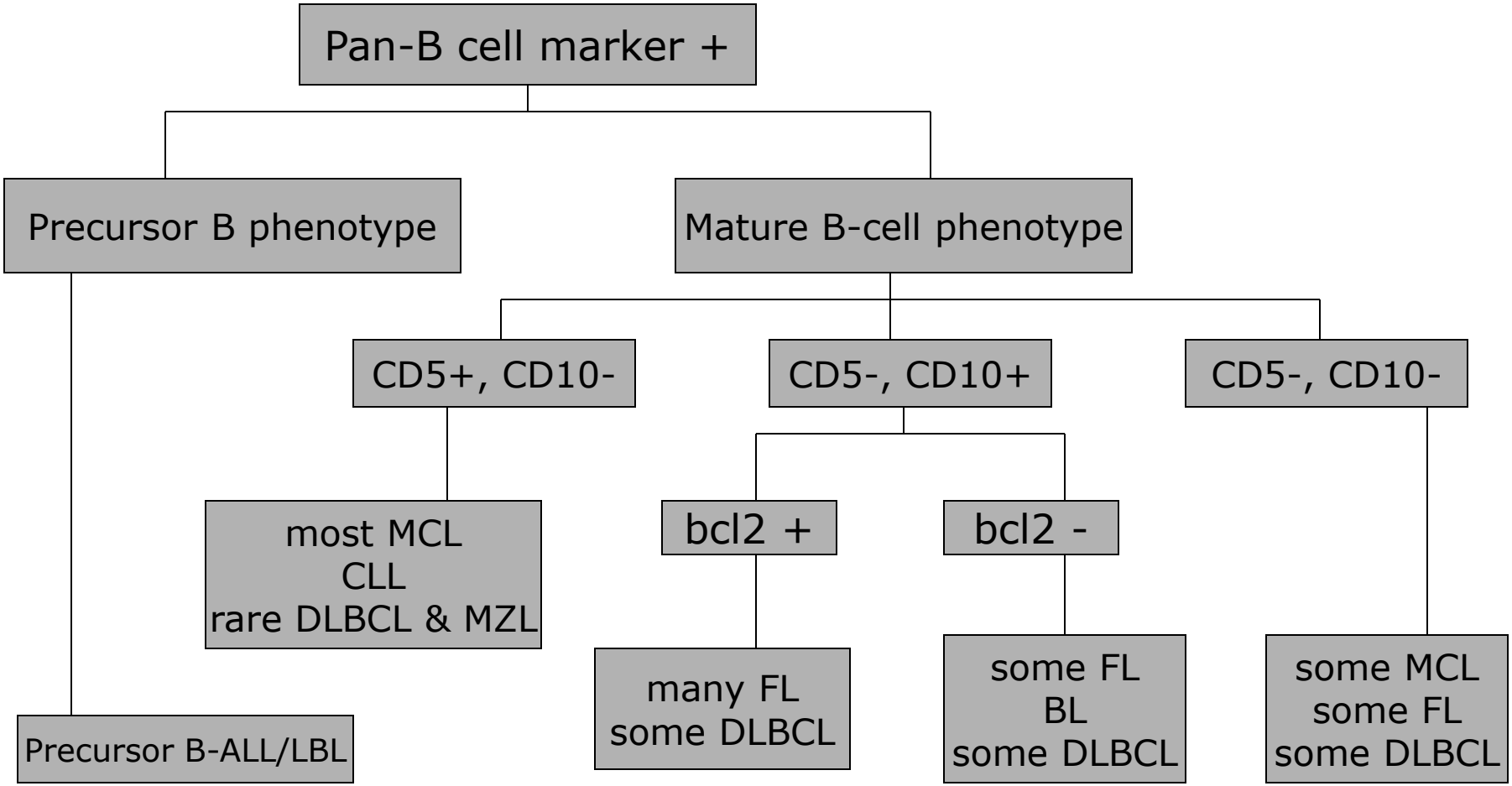
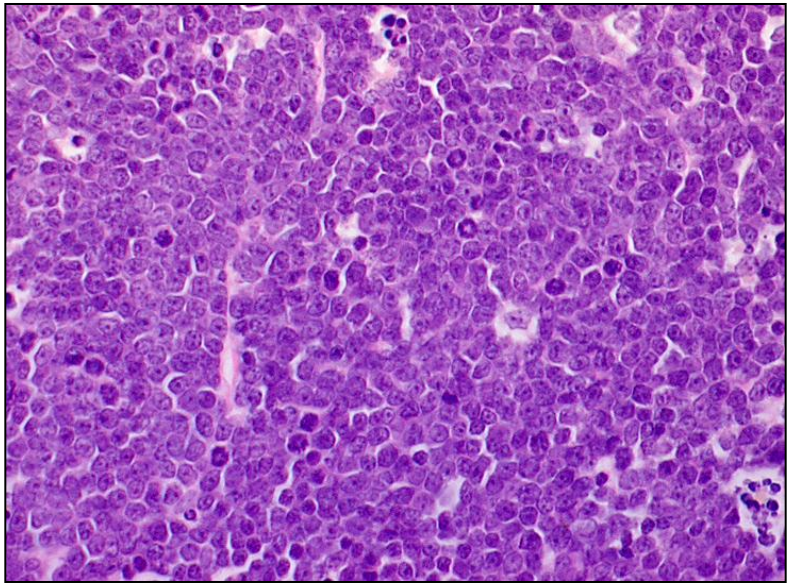
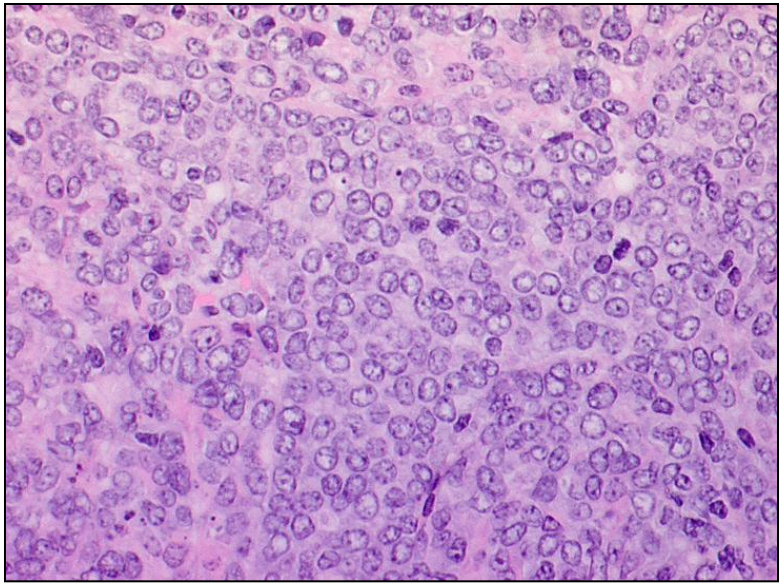
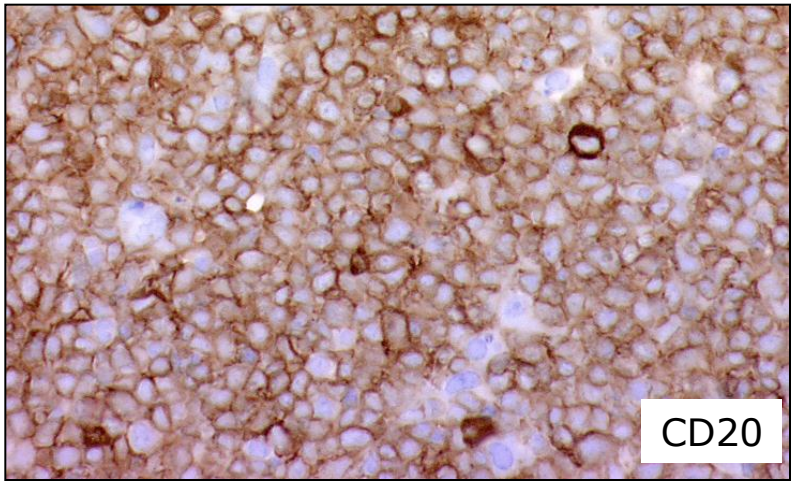
Diagnostic Accuracy in B-cell lymphomas

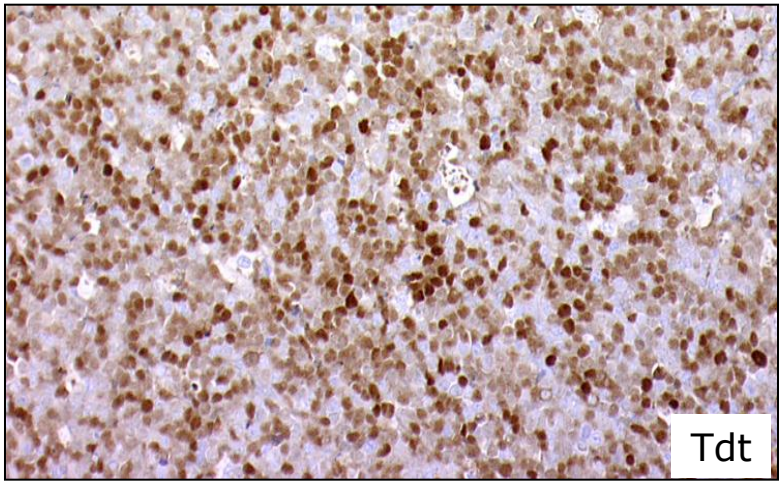


Precursor B-cell lymphoblastic/lymphoma



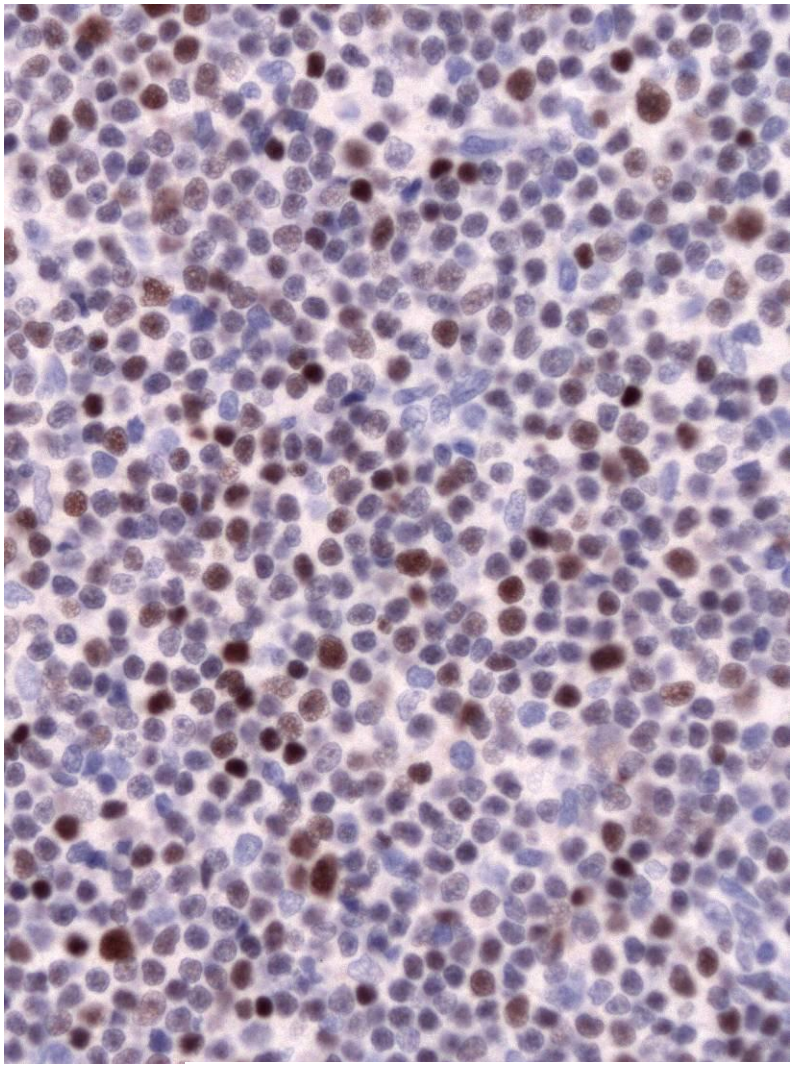






Lymphoid enhancer binding factor-1 (LEF-1)

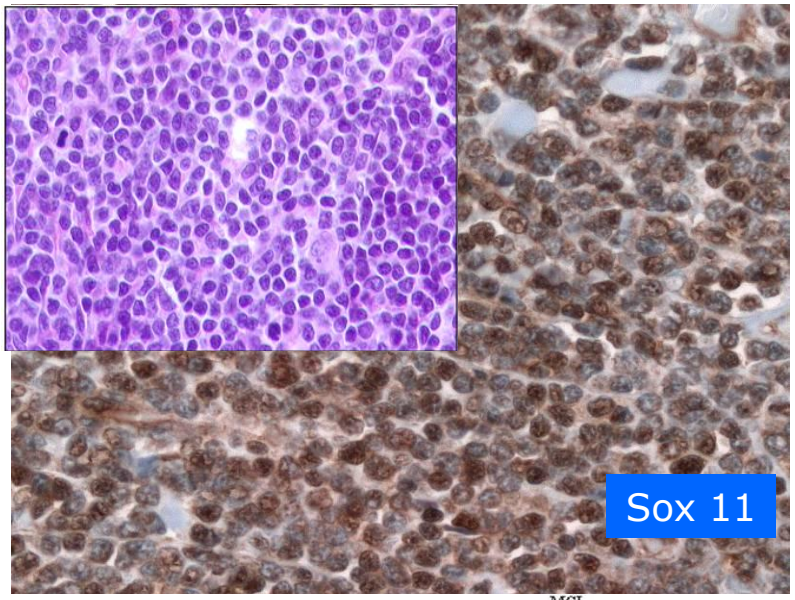
- LEF-1 is expressed normally in T-cells
- LEF-1 is expressed by B-cell precursors and CLL but not in mature B-cells
- LEF1 is a transcription factor involved in the Wnt signaling pathway

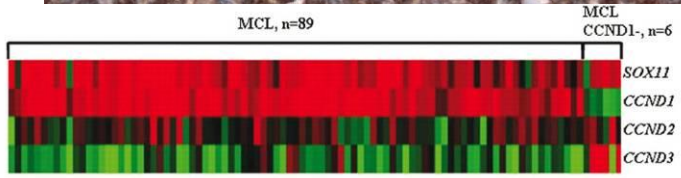


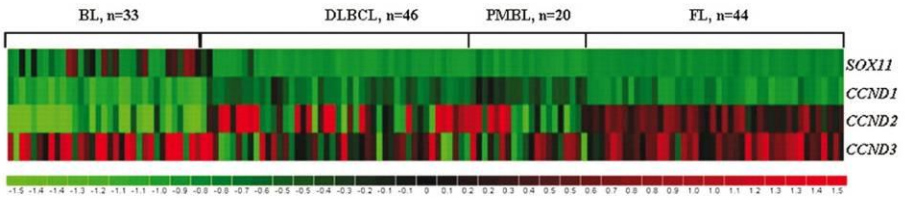
GUTIERREZ et al BLOOD, 21 OCTOBER 2010 • VOLUME 116, NUMBER 16

Sox 11 expression in Mantle cell lymphoma

- Most MCL are positive including the cases of cyclin D1 negative
  - (CycD1-,CD5+, Sox11+)
- Other „Low grade“ lymphomas are negative
- Some Burkitt lymphoma, lymphoblastic lymphomas and T-prolylmphocytic leukemias are postive

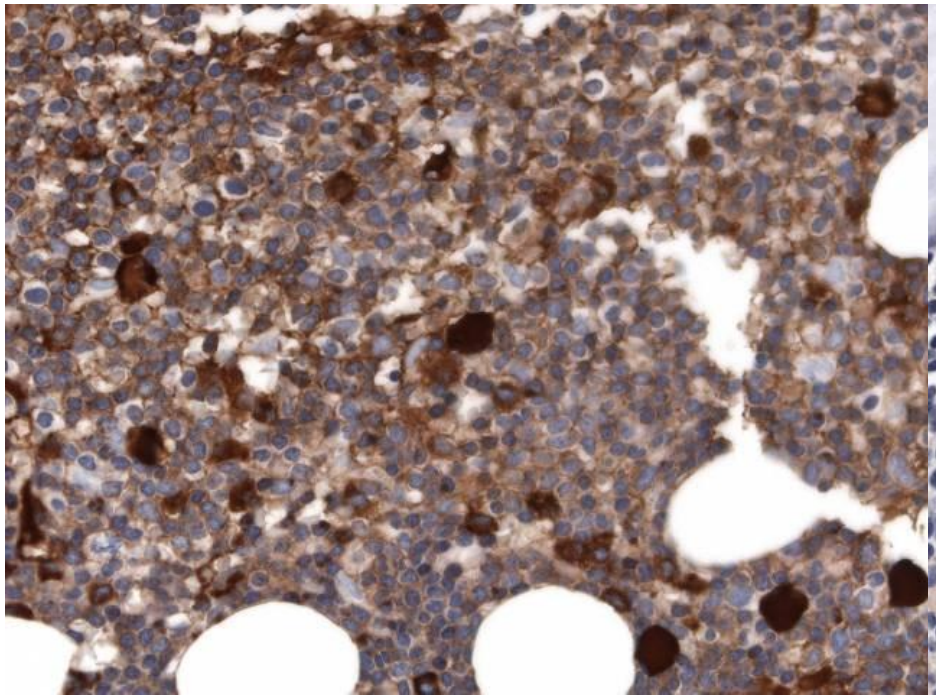




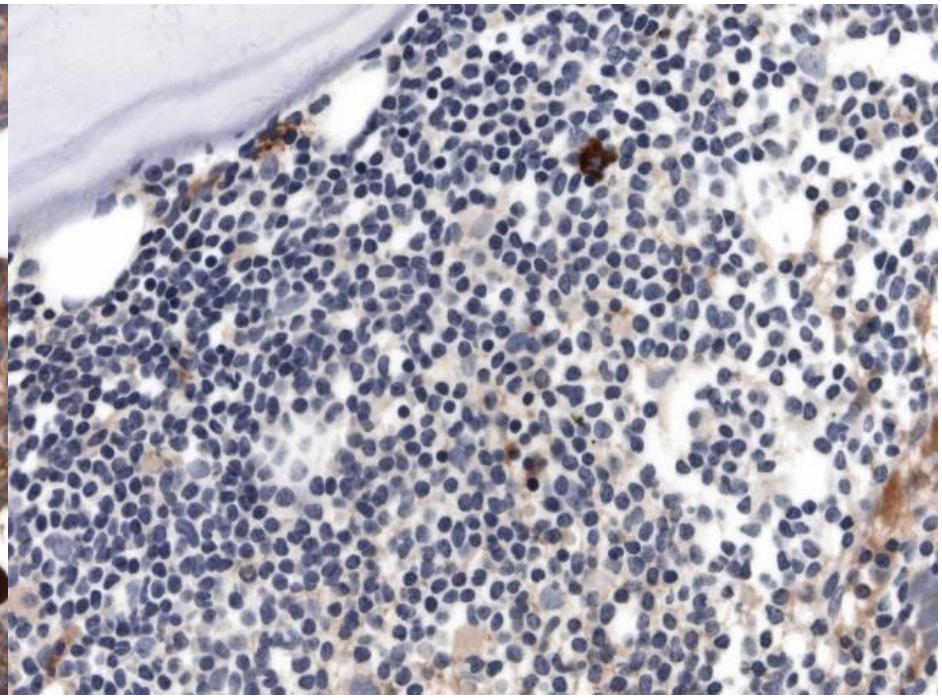


Mozos A et al. Haematologica 2009;94:1555-1562

Light chain restriction for clonality

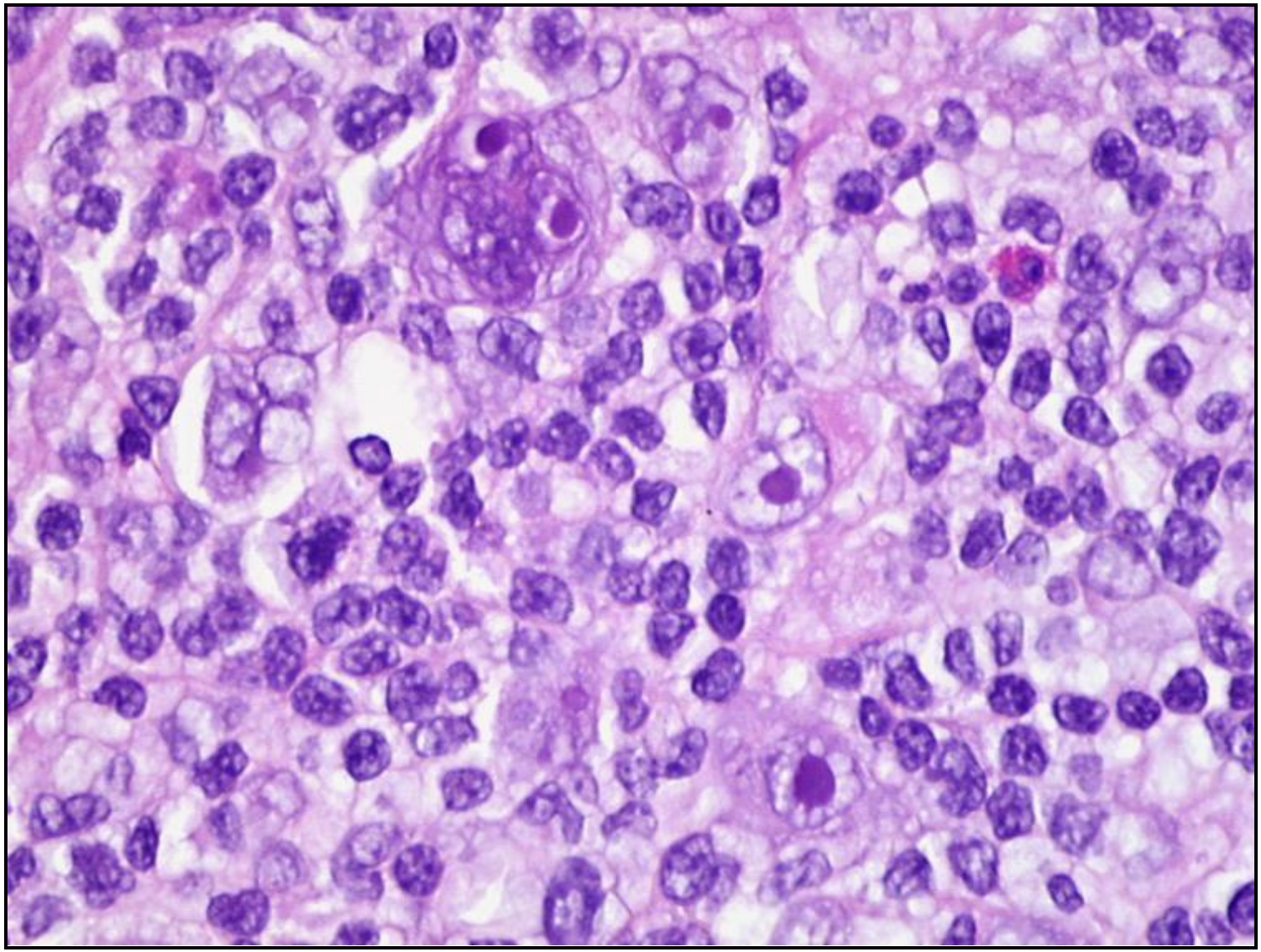


Kappa



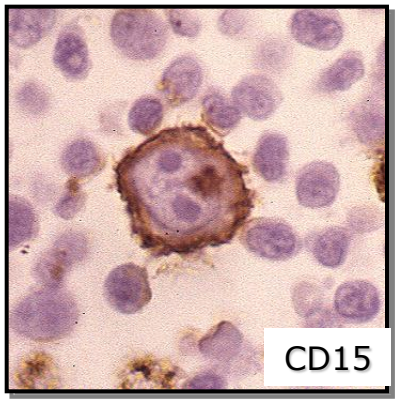
Lambda

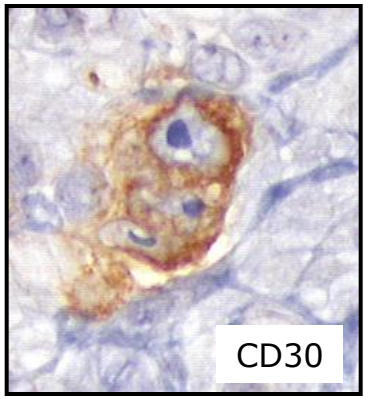
Accuracy in the diagnosis of CHL

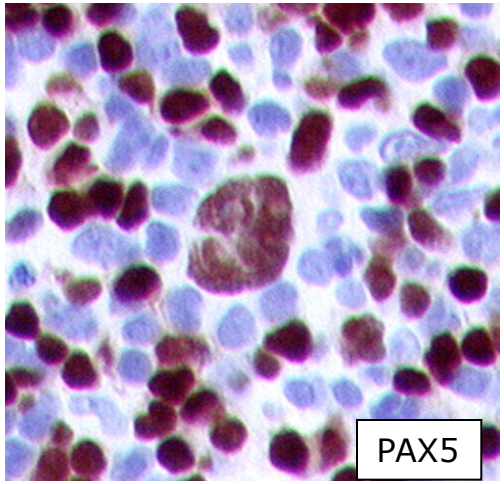


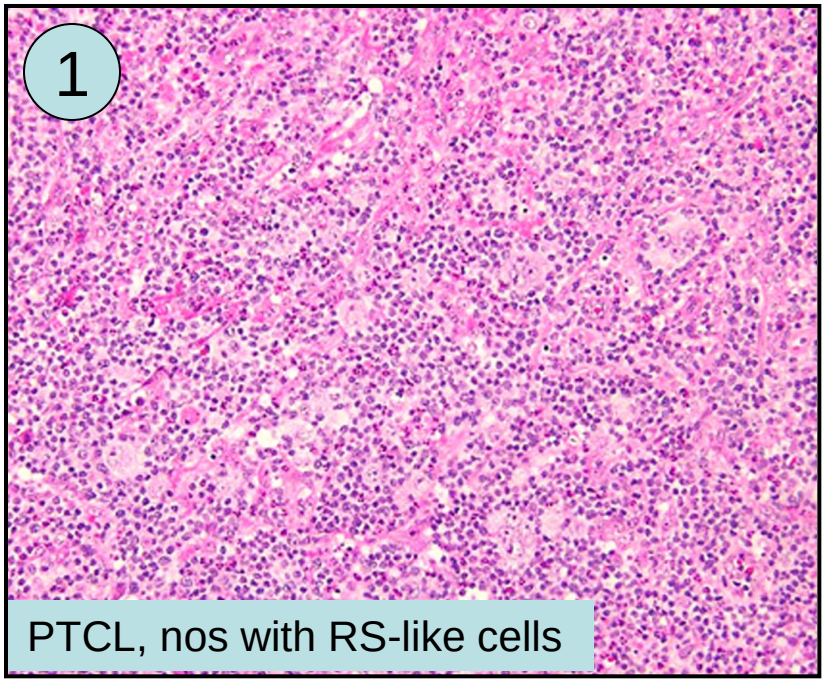
Accuracy in the diagnosis. Hodgkin lymphoma

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





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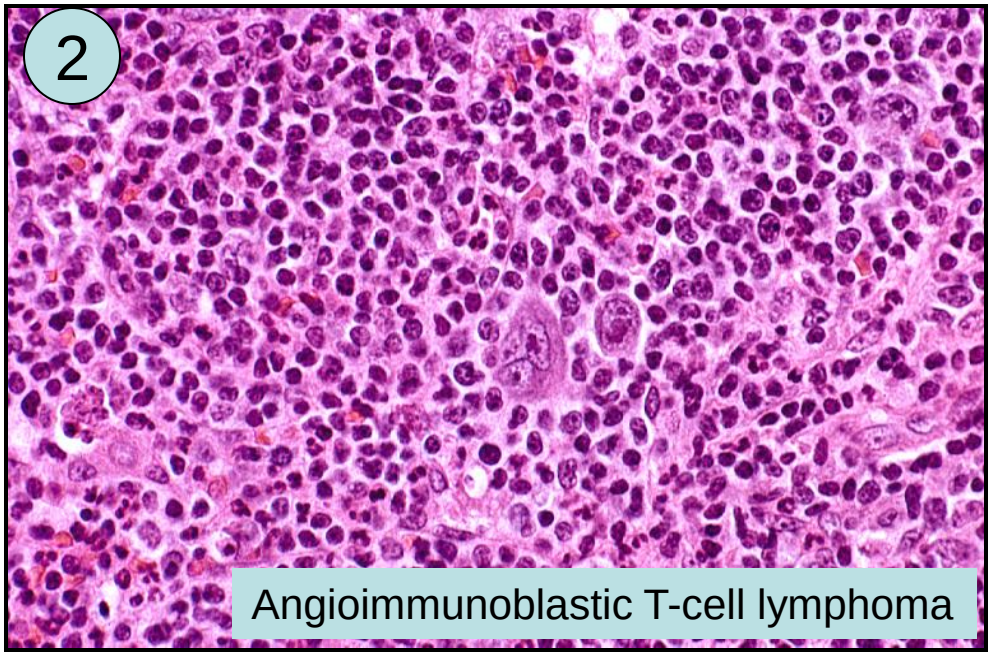
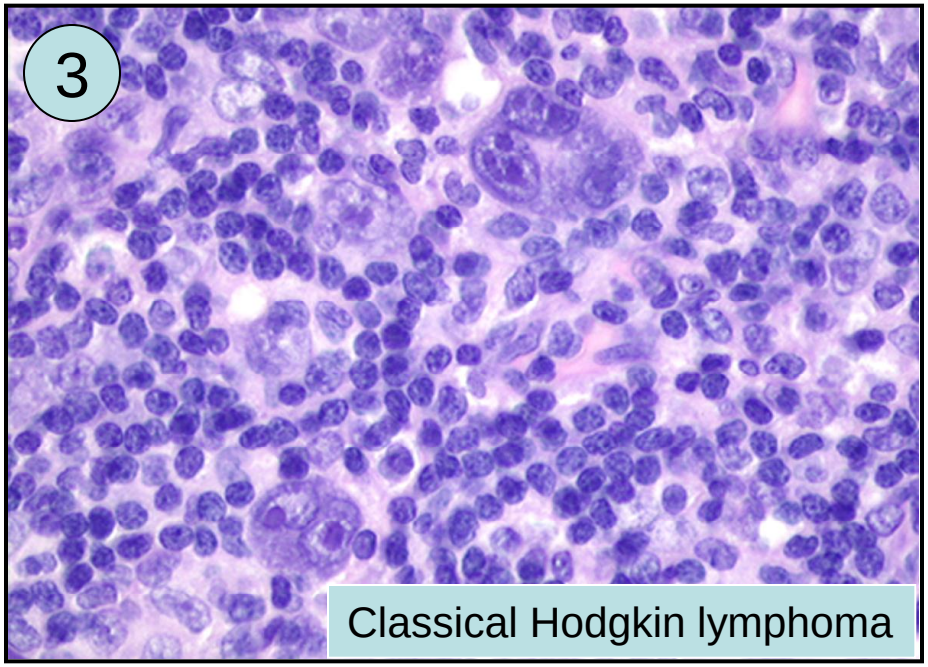
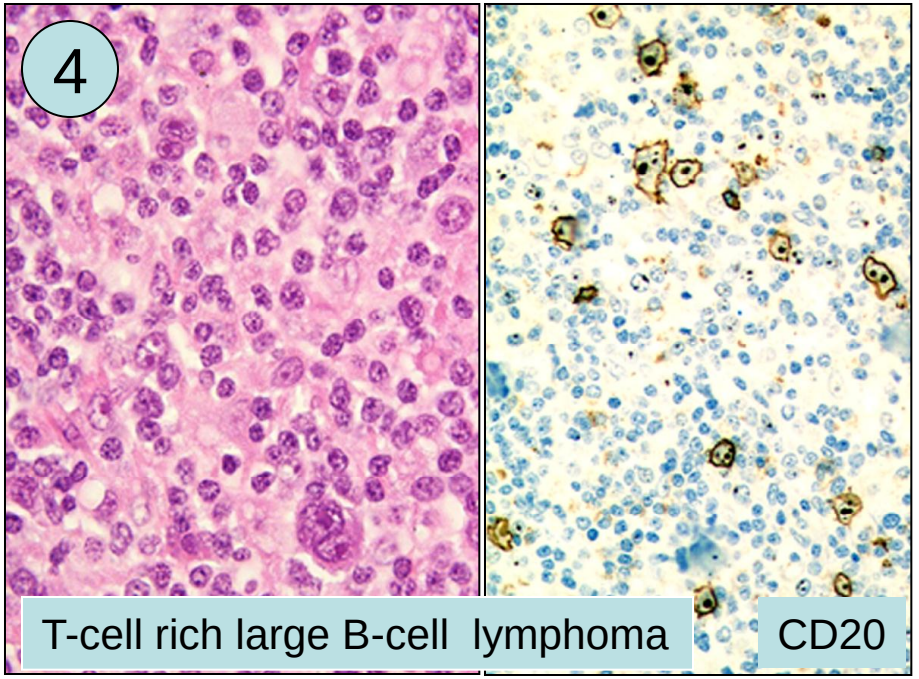
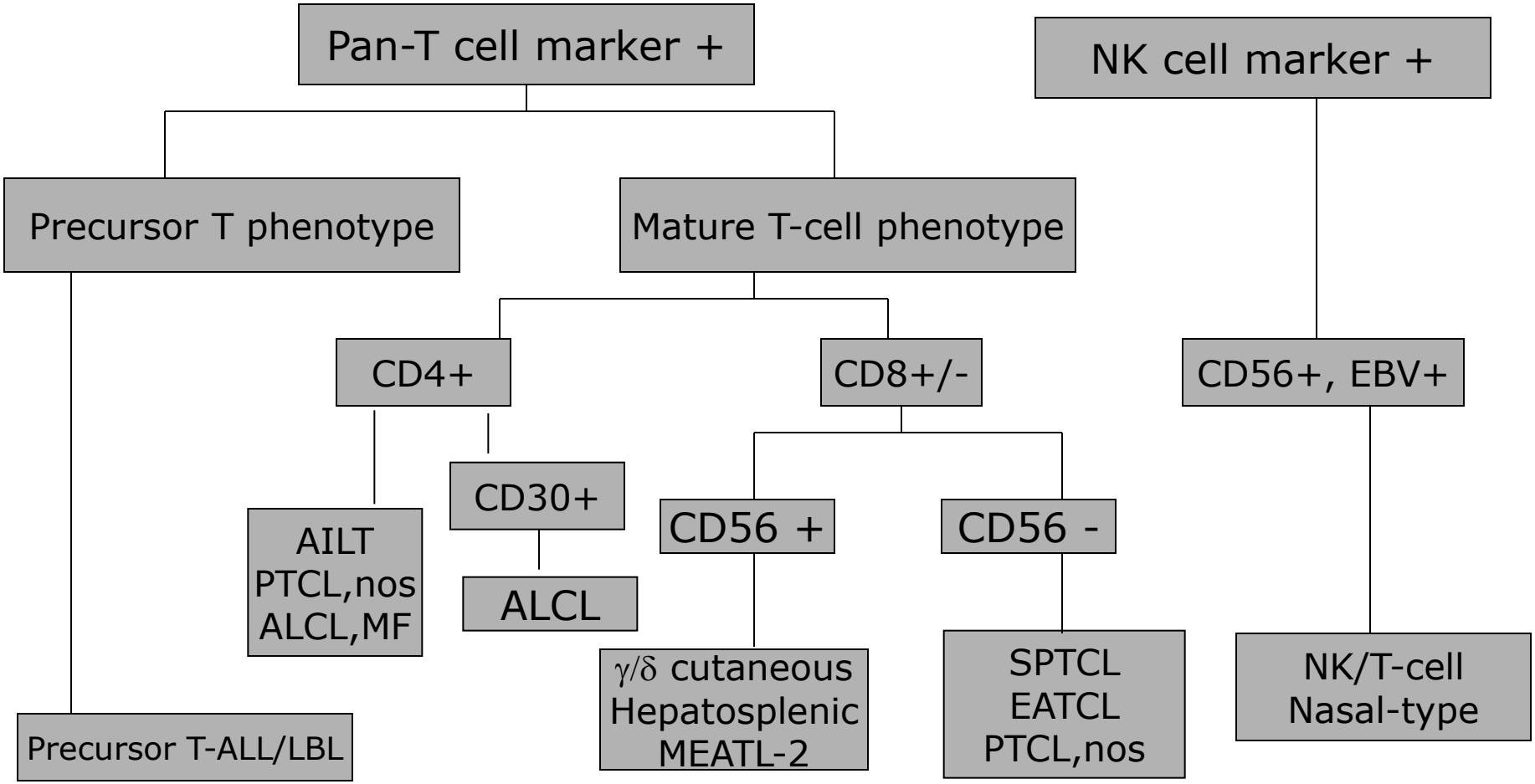
PTCL, nos with RS-like cells


2
Angioimmunoblastic T-cell lymphoma

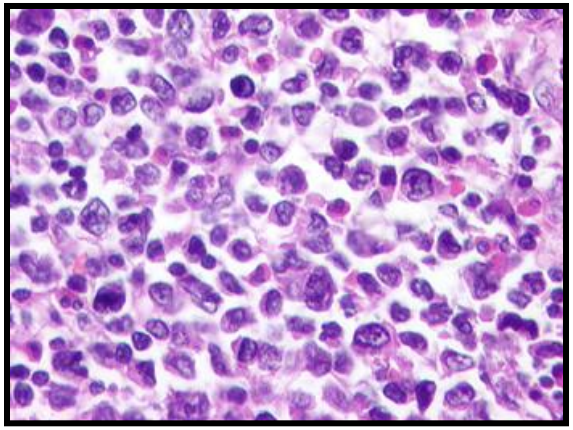

3
Classical Hodgkin lymphoma

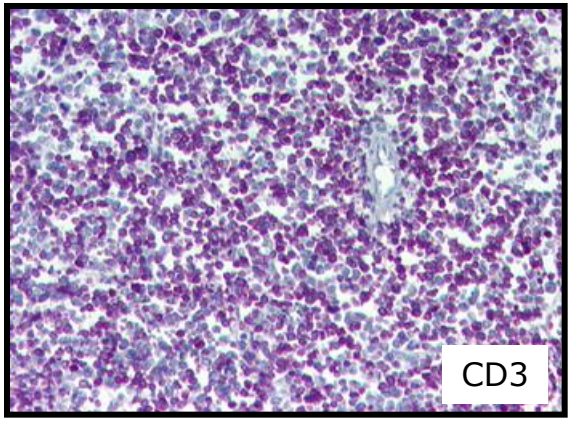

4
T-cell rich large B-cell lymphoma
CD20

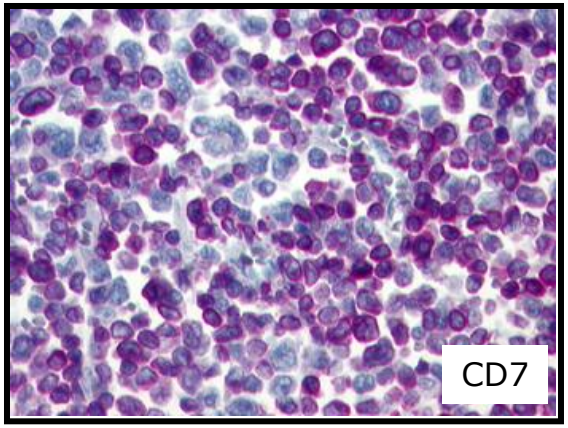
Diagnostic Accuracy in T/NK-cell lymphomas

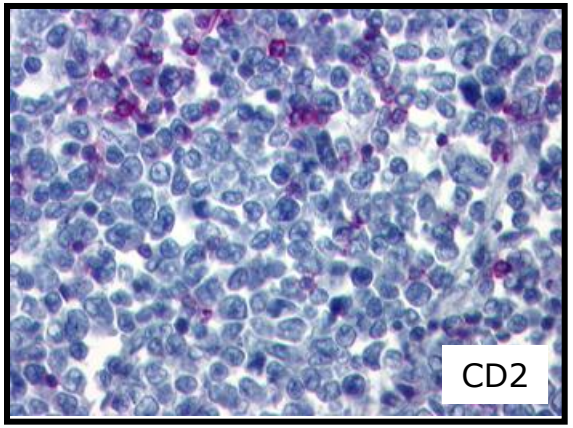


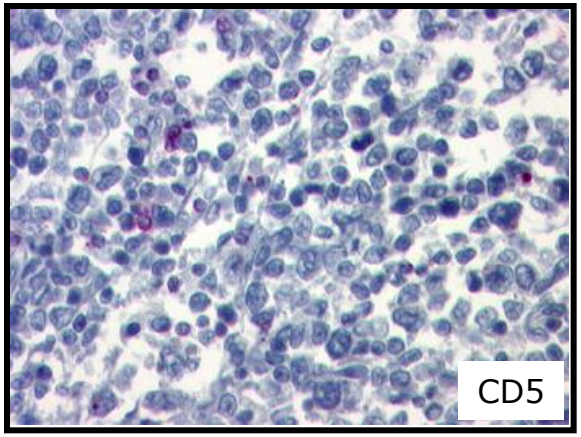
Diagnostic accuracy. T-cell NHL

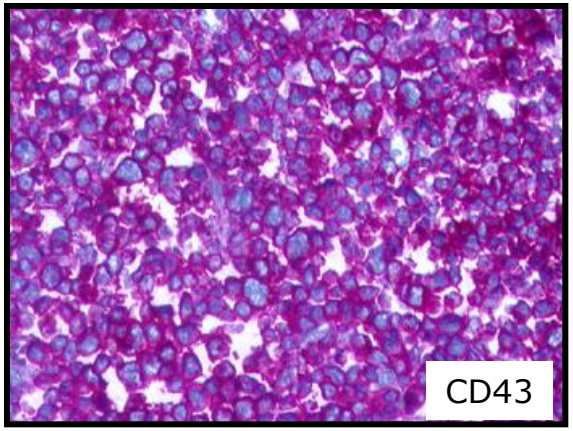






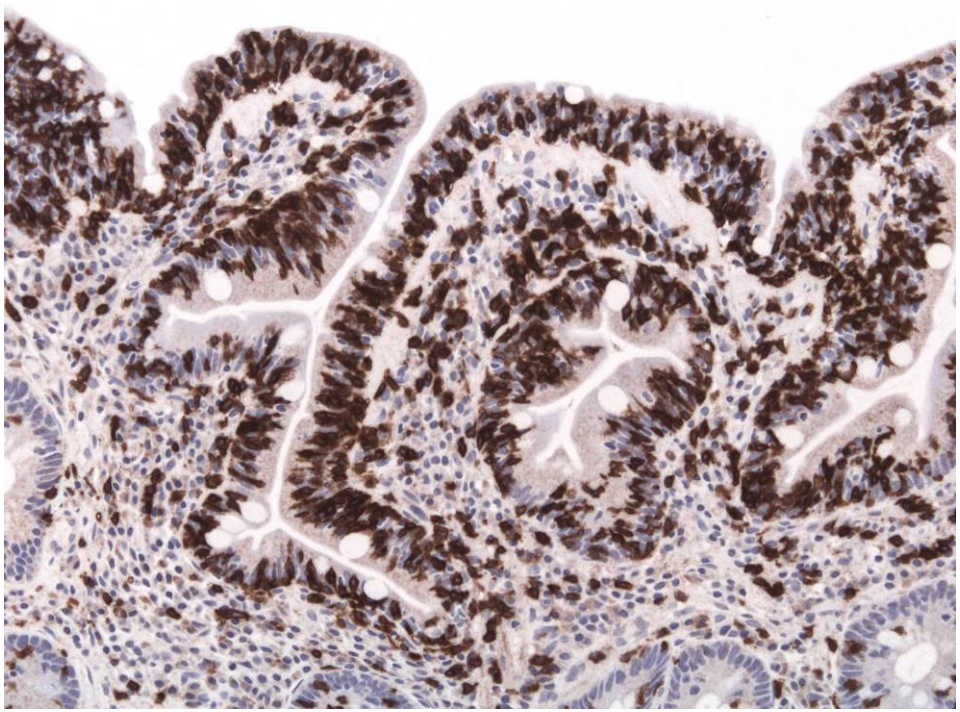




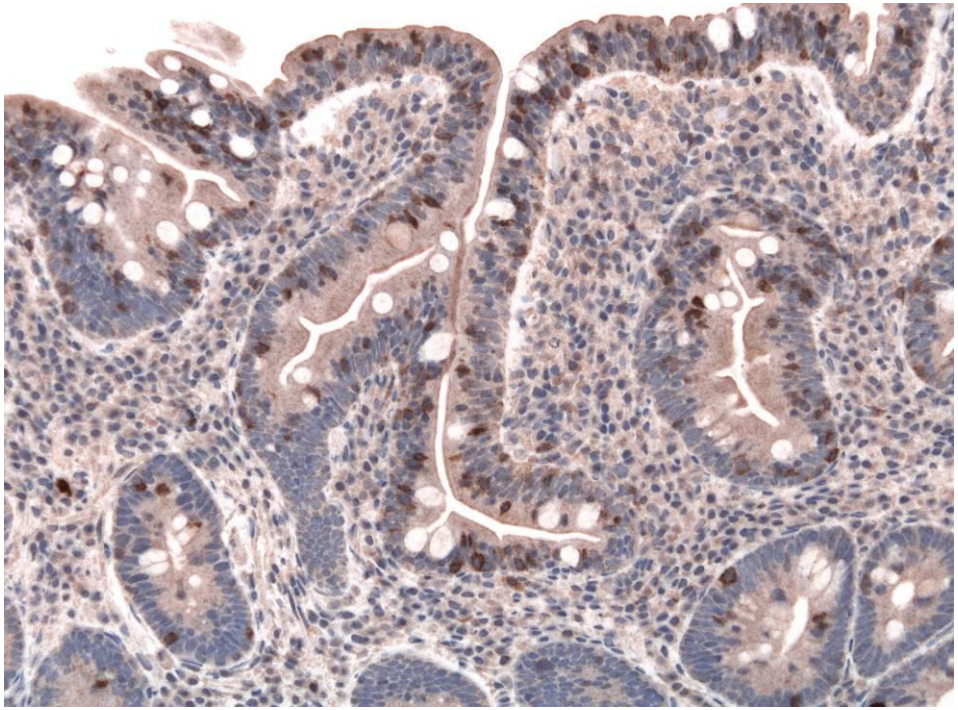


Aberrant phenotype: loss of one or more T-cell markers

Biomarkers of cell lineage/differentiation

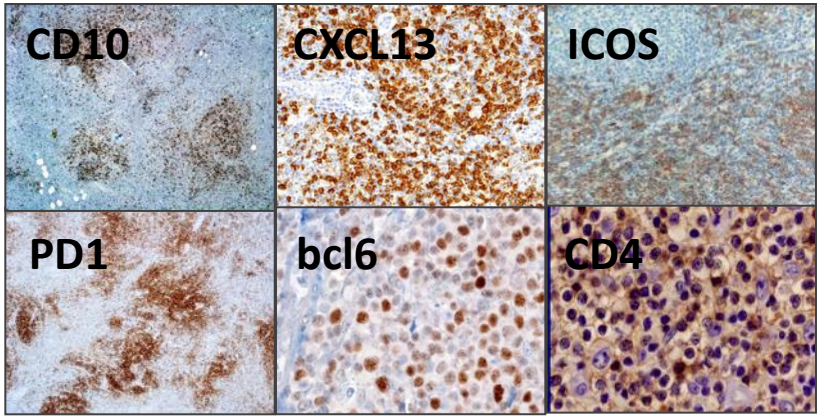


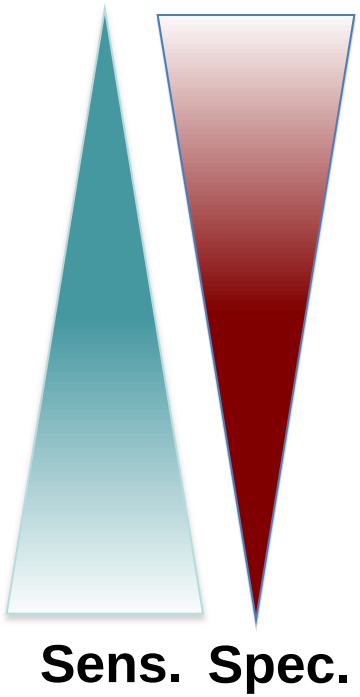
TCRαβ



TCRγ

T_{FH} cell markers for diagnostic use





ICOS
c-MAF
PD1

BCL6

CXCL13
CD10

Sens. Spec.

2016 updated WHO classification

- **IHC or FCM**
- Not a single marker is fully specific
- Expression of several T_{FH} markers can be found in activated reactive cells (ICOS, PD1)
- **In practice,**

  - at least, expression of 2 (3) TFH markers needed for TFH assessement

  - interprete in the pathological/clinical context

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

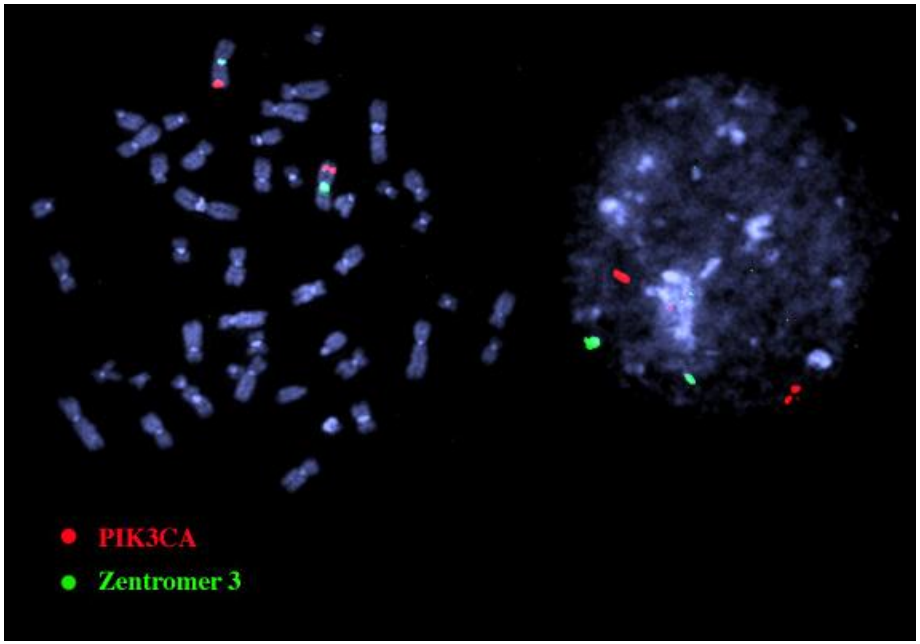
Techniques in molecular diagnosis

- 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

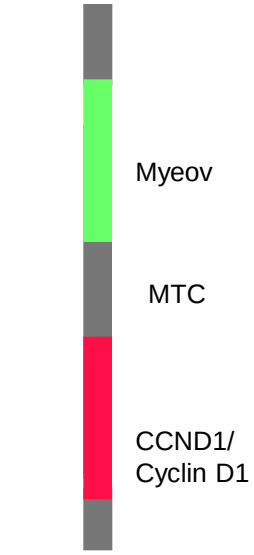


Metaphase Interphase

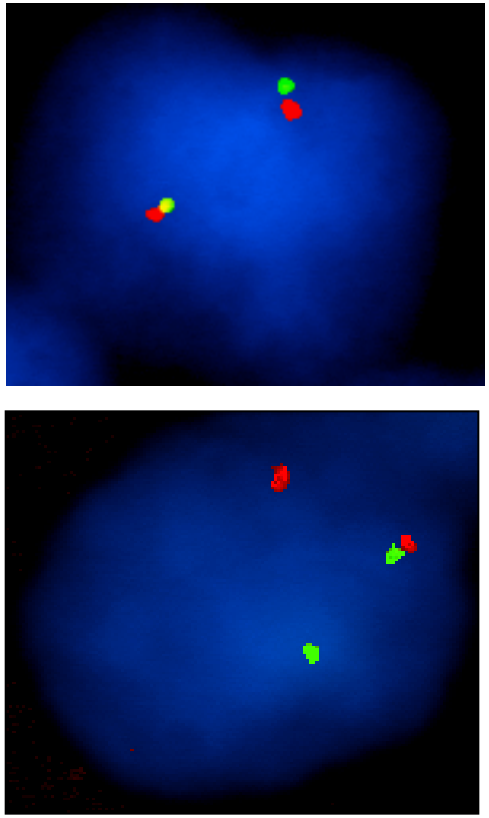
Haralambieva et al, J Pathol 2002, 198:163

Fluorescence in situ hybridization (FISH)

Break-apart FISH

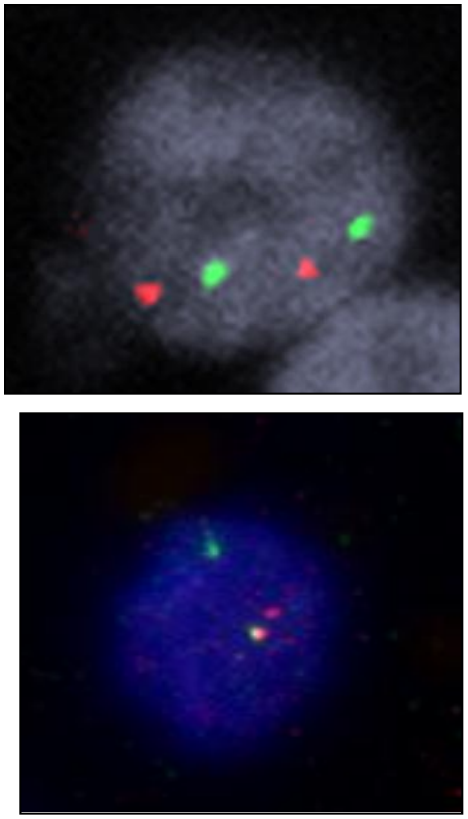


Chromosome 11



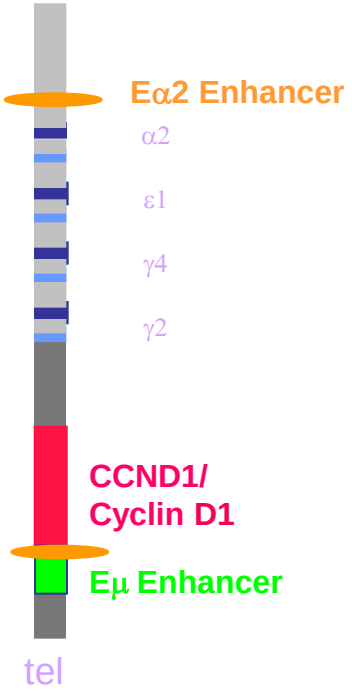
Break in chromosome 11

Fusion FISH



t(11;14)

Chromosome 11
Chromosome 14

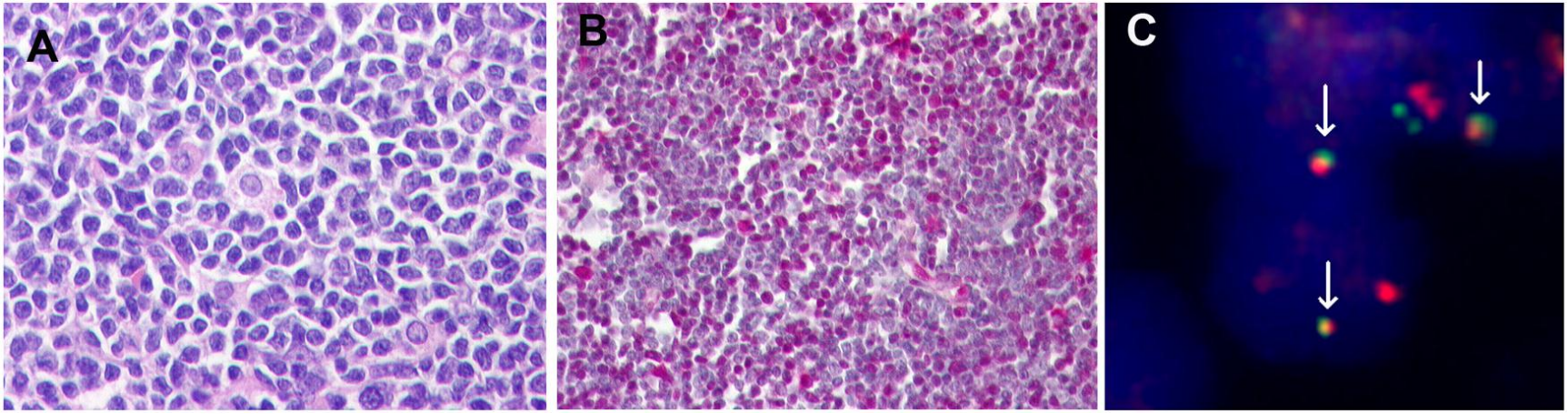


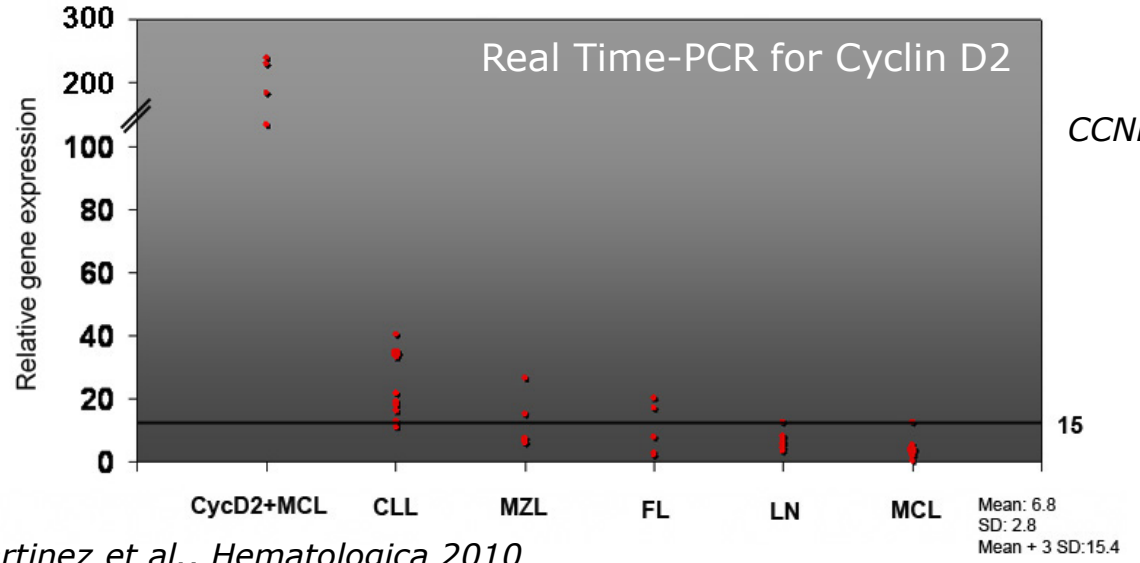
Chromosome 14

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)

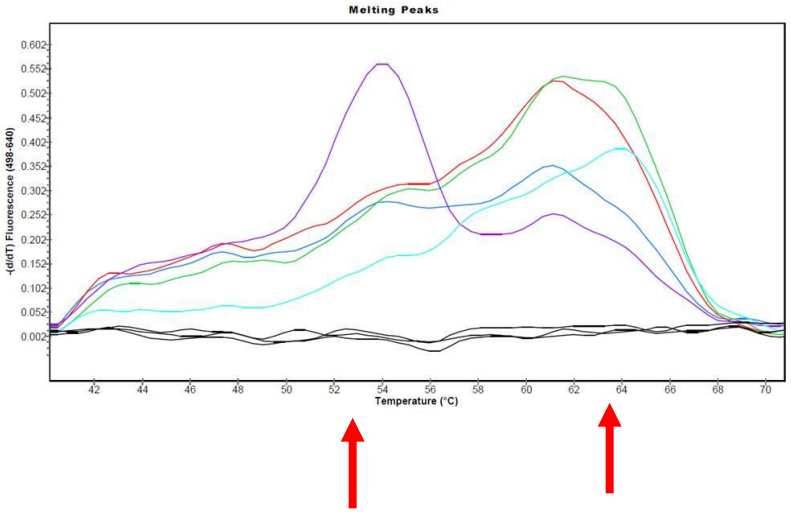
CCND2 (12p13) and *IGK* (2p12)

Quintanilla-Martinez et al., Hematologica 2010

Allelic specific PCR (mutation detection)

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

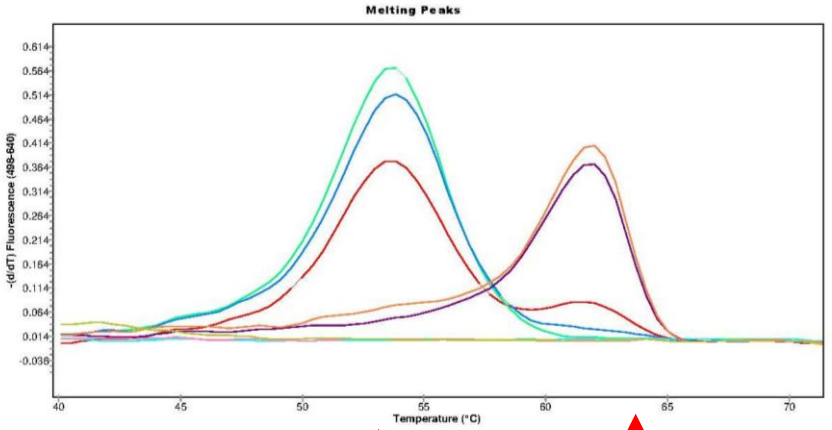
MYD88 mutation analysis (LPL)



Wild type

MYD88 **L265P** Mutation

RHOA mutation analysis (AITL)



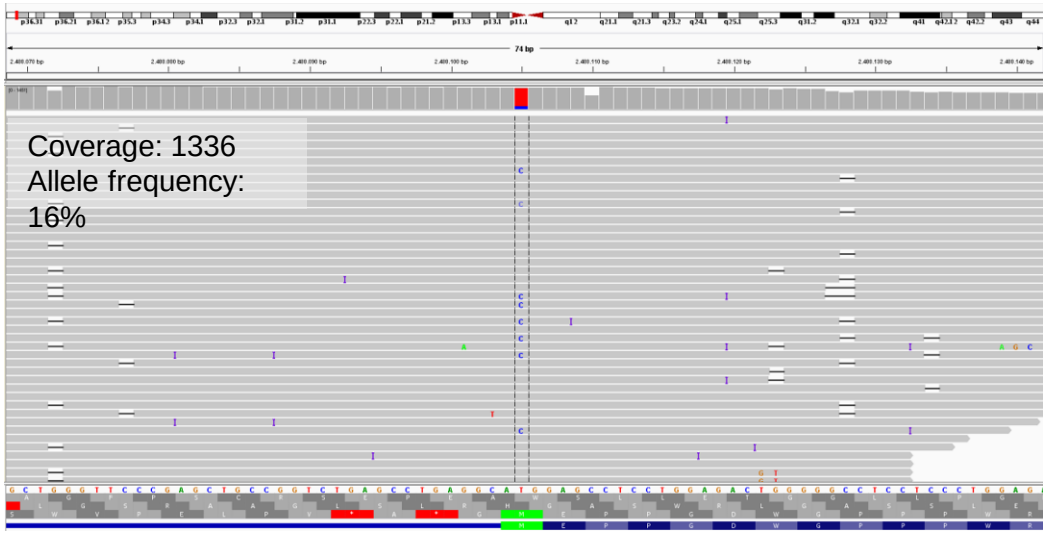
Wild type

RHOA **G17V** Mutation

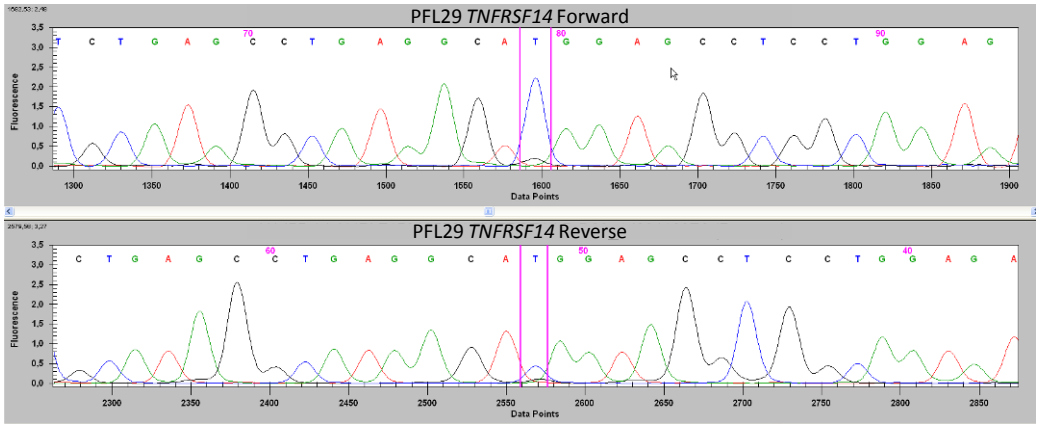
Schmidt J et al, BJH 2015, 169:795-803
Bonzheim I et al Blood 2015

TNFRSF14 mutations in PFL

A) Ion AmpliSeq Custom Panel



B) Validation Sanger sequencing



PFL29 *TNFRSF14*
p.Met1_Gln97del

Clinical impact of recurrently mutated genes on lymphoma diagnostics: state-of-the-art and beyond

Richard Rosenquist,[1] Andreas Rosenwald,[2] Ming-Qing Du,[3] Gianluca Gaidano,[4] Patricia Groenen,[5] Andrew Wotherspoon,[6] Paolo Ghia,[7] Philippe Gaulard,[8] Elias Campo,[9] Kostas Stamatopoulos,[10] on behalf of the European Research Initiative on CLL (ERIC) and the European Association for Haematopathology (EAHP)

Table 2. Categorization of gene mutations based on current evidence levels.

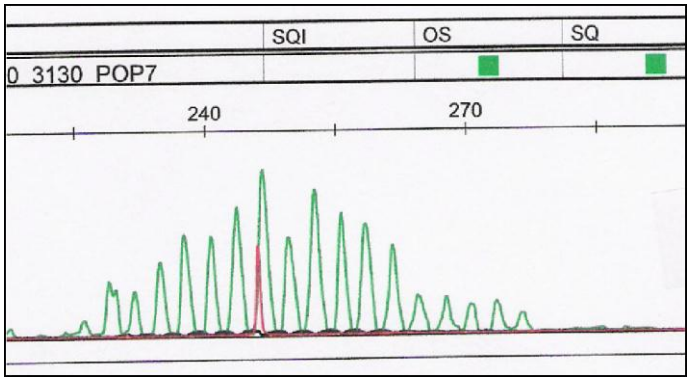
Category	Gene mutations
1. Immediate impact on patient care	*TP53* mutations (exons 4-10) in CLL
2. Diagnostic impact	MYD88^{L265P} mutation in WM/LPL BRAF^{V600E} mutation in HCL *KLF2* mutations in SMZL *ID3* and *TCF3* mutations in BL *STAT3* mutations in LGLL *RHOA*, *TET2*, *IDH2* and DNMT3A mutations in AITL and other T_{FH}-derived PTCL
3. Prognostic impact	*CLL*: *TP53*, *ATM*, *BIRC3*, *NFKBIE*, *NOTCH1*, *SF3B1* MCL: *TP53*, *NOTCH1*, *NOTCH2* mutations SMZL: *NOTCH2*, *TP53* mutations DLBCL: *TP53* mutation & MYC translocation NKTCL: *DDX3X* mutations
4. Potential clinical impact in the near future	Therapy response to BcR inhibitors: WM: *MYD88*, *CXCR4* mutations DLBCL: *CD79B* mutations (responsive) *CARD11*, MYD88 mutations (non-responsive) Resistance to BcR inhibitors: BTK^{C481S}, PCLG2 mutations New inhibitors under development: *EZH2*, *SF3B1* & *NOTCH1*

haematologica | 2016; 101(9)

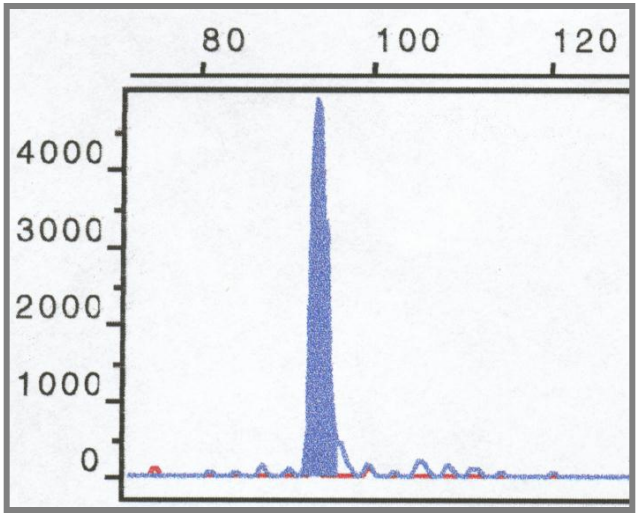
Clonality as marker of lymphoid neoplasia

- 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



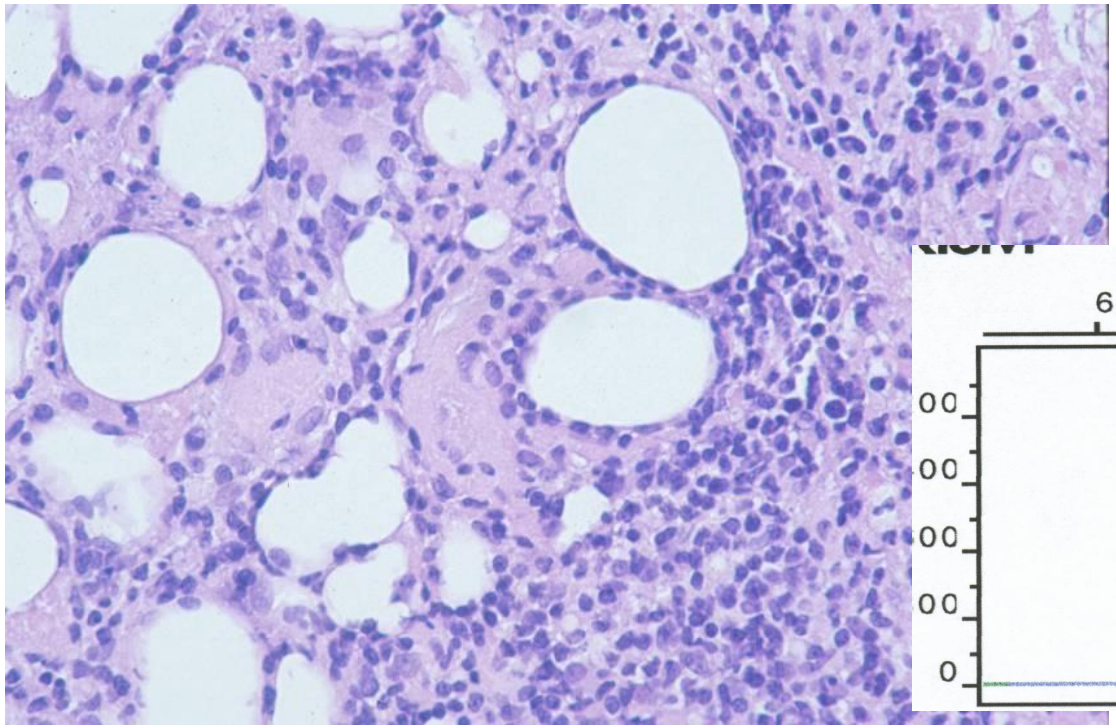
Automated fragment length analysis

Monoclonal peak FR3
Faint polyclonal background

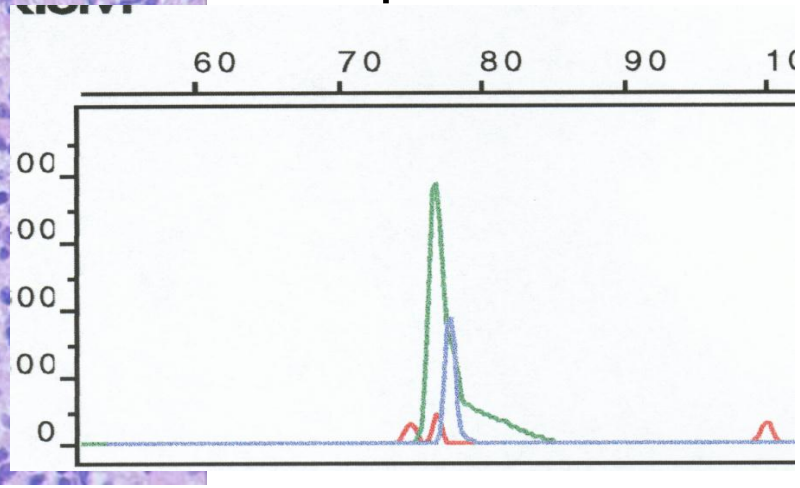
GeneScan

Benign vs. Malignant

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

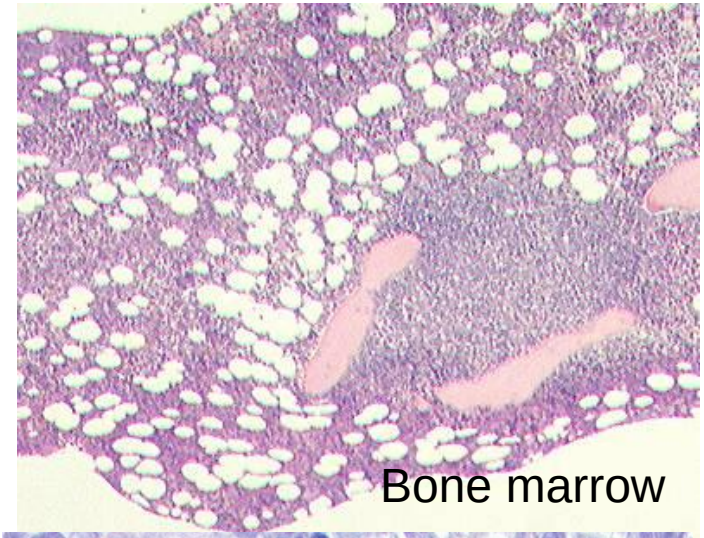


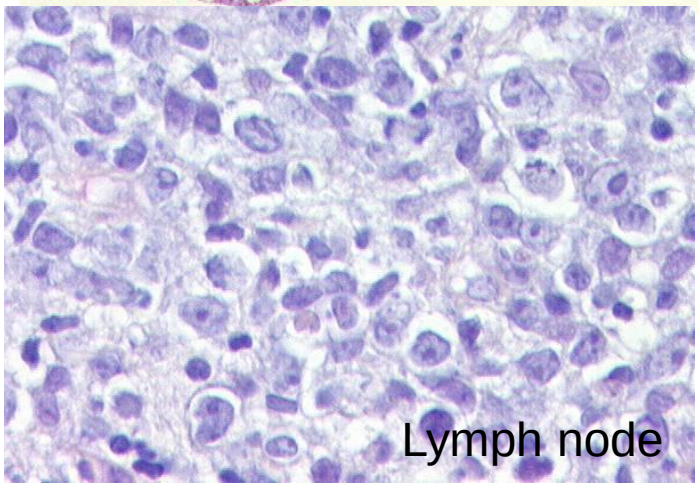
35-year-old woman,
Subcutaneous nodules
leukopenia

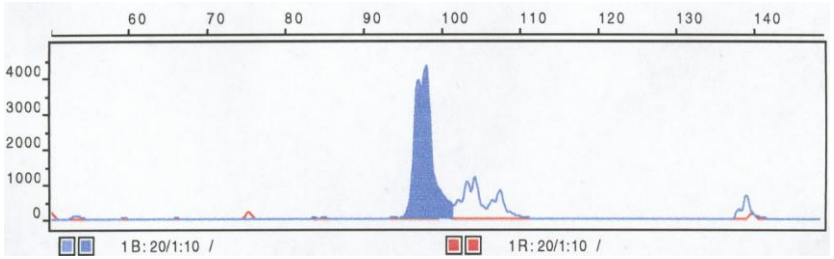


-Monoclonal TCRγ rearrangement

Discordant lymphomas

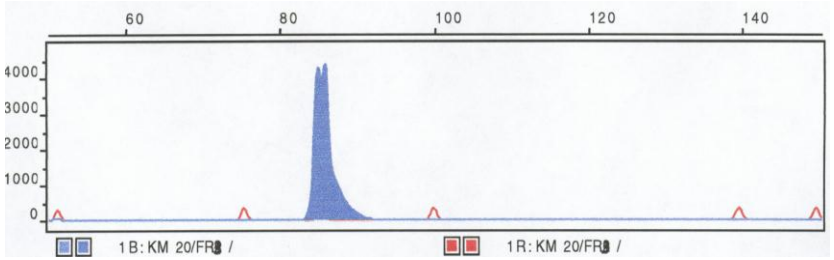






97 bp

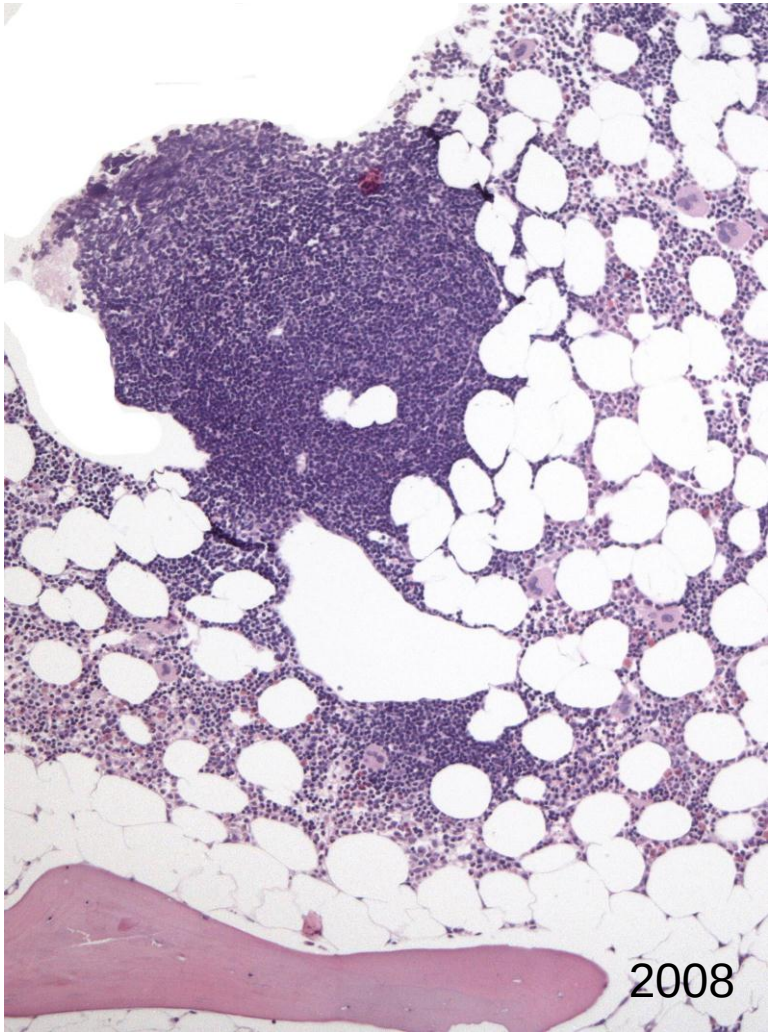
In 20% of the cases a second neoplasia is found!

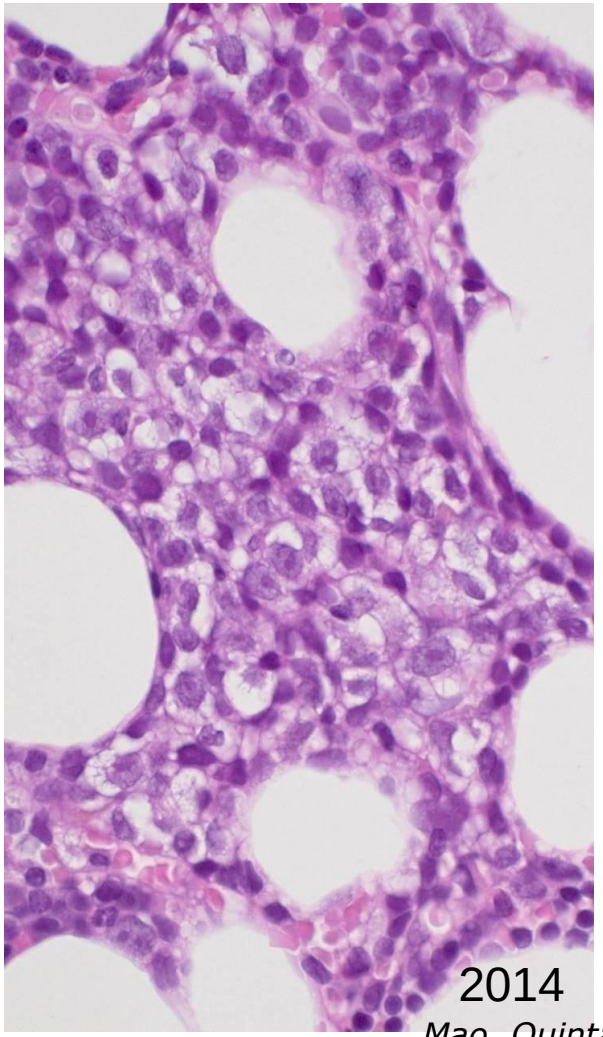


85 bp

Kremer et al, Lab Invest 2003

Richter´s transformation





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

Mao, Quintanilla-Martinez et al, AJSP 2007

Review Article

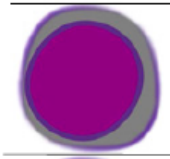
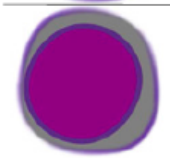
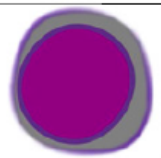
BLOOD, 25 FEBRUARY 2016 • VOLUME 127, NUMBER 8

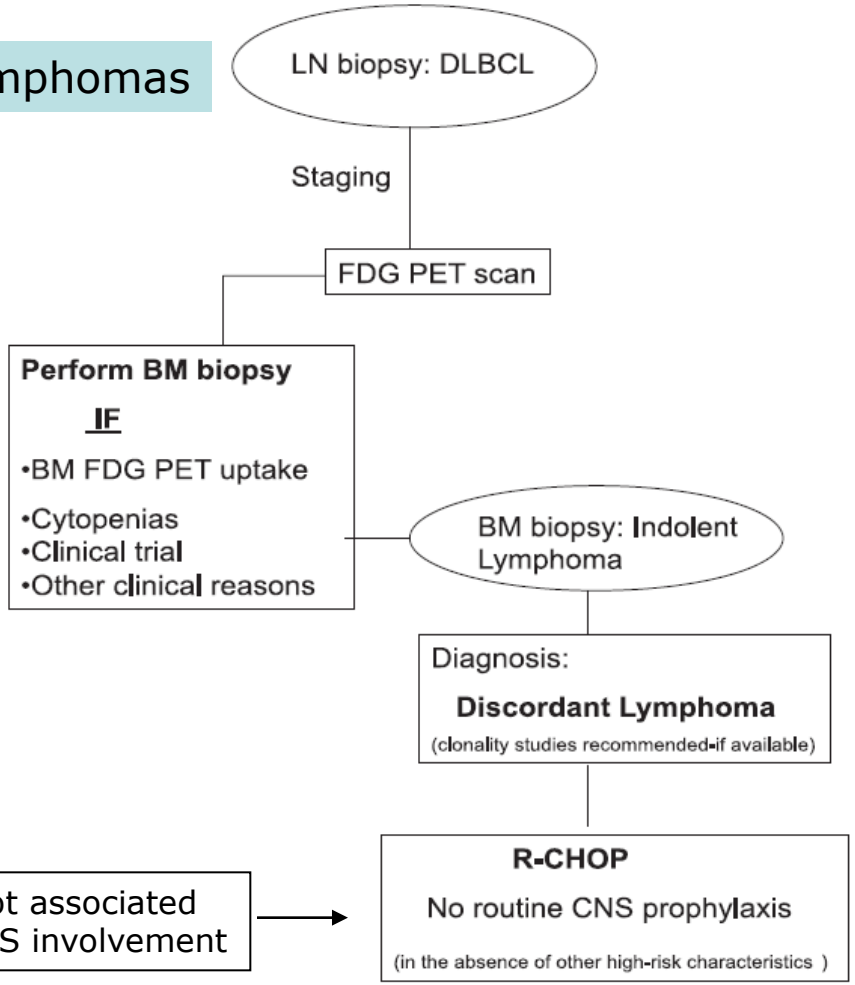
Discordant bone marrow involvement in non-Hodgkin lymphoma

Jennifer Brudno,[1,*] Tamar Tadmor,[2,3,*] Stefania Pittaluga,[4] Alina Nicolae,[4] Aaron Polliack,[5,†] and Kieron Dunleavy[1,†]

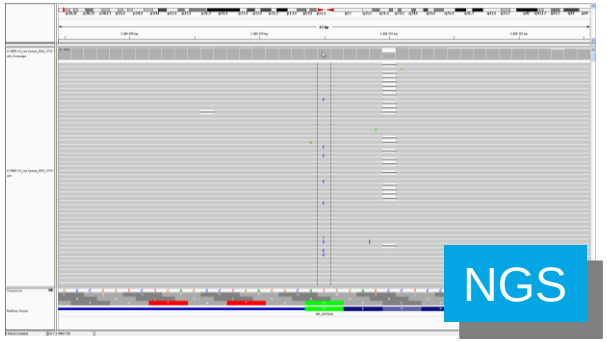
[1]Lymphoid Malignancies Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD; [2]Hematology Unit, Bnai Zion Medical Center and [3]The Ruth and Bruce Rappaport Faculty of Medicine, Technion Haifa, Haifa, Israel; [4]Hematopathology Section, Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, Bethesda, MD; and [5]Department of Hematology, Hadassah University, Hospital and Hebrew University Medical School, Jerusalem, Israel

Approach to the diagnosis of discordant lymphomas

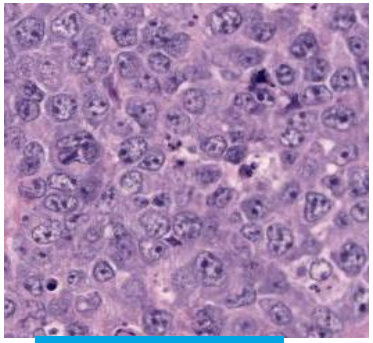
Lymph-node With DLBCL infiltration	**Bone-marrow** Infiltration with lymphoma=BMI	**Clonality studies**	**Histologically Concordant** vs. **Discordant**
		Clonally unrelated	BMI with different histology and different clonality studies = Discordant histology
		Clonally related	BMI with different histology but similar clonality studies = Discordant histology
		Clonally related	BMI with Concordant histology



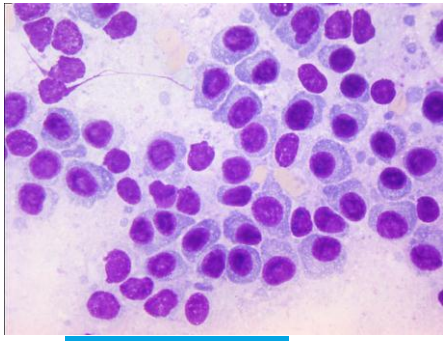
Discordant lymphoma is not associated with an increase risk of CNS involvement



NGS

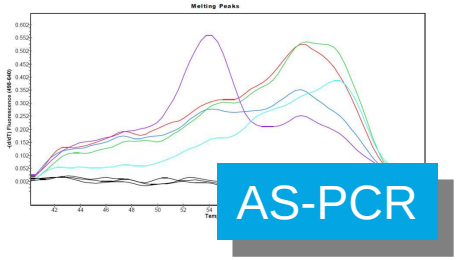


histology

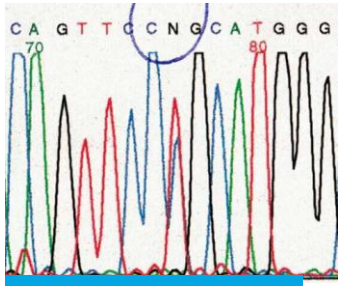


Cytology

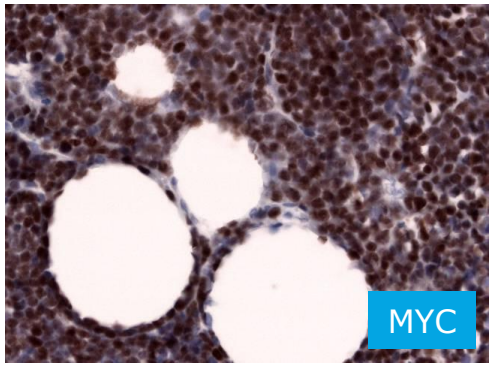
Molecular techniques



AS-PCR

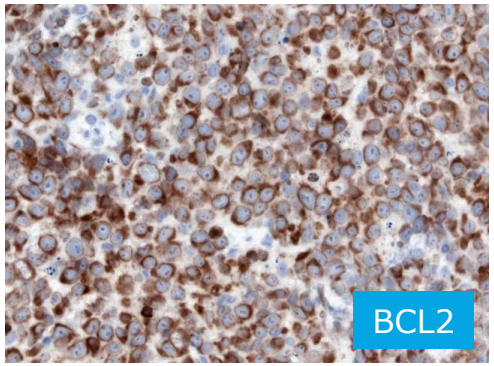


Sanger seq.

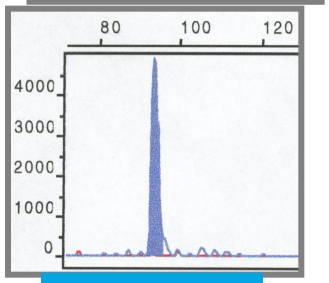


MYC

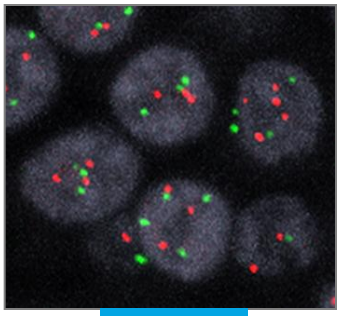
Immunohistochemistry



BCL2



Clonality



FISH