

# Diagnostic Molecular Pathology of Lymphoid Neoplasms

(Part II)  
Rational use of molecular testing in lymphomas

23<sup>rd</sup> Congress of The Arab Division  
International  
Academy of Pathology

Beirut, Lebanon

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why Major indications for molecular testing

why

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- diagnosis
  - neoplastic vs reactive (and beyond)

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  - neoplastic vs reactive (and beyond)
- classification
  - based upon the genetic lesion [sine qua non]

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  - in otherwise homogeneous diseases

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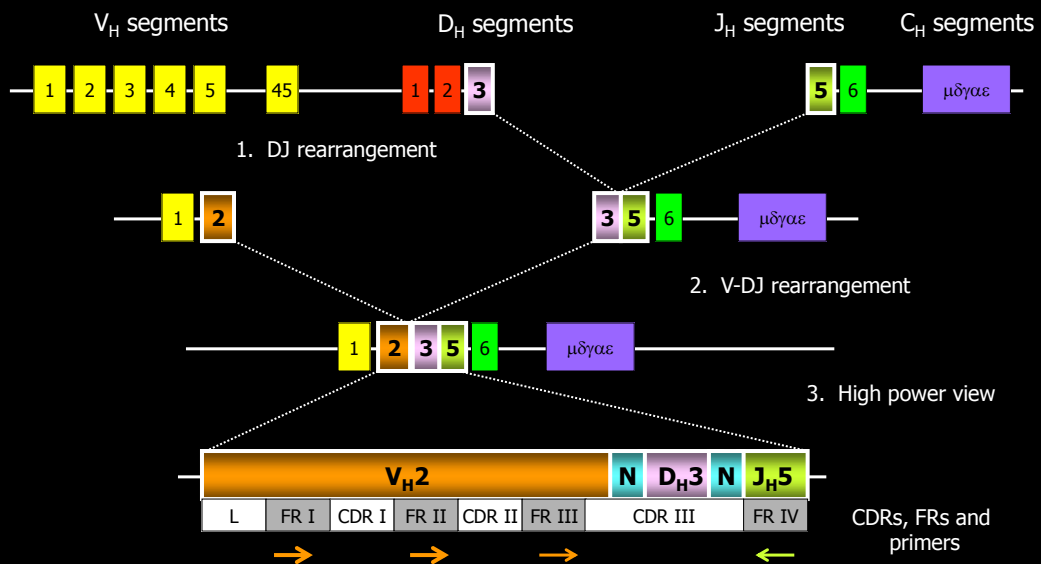
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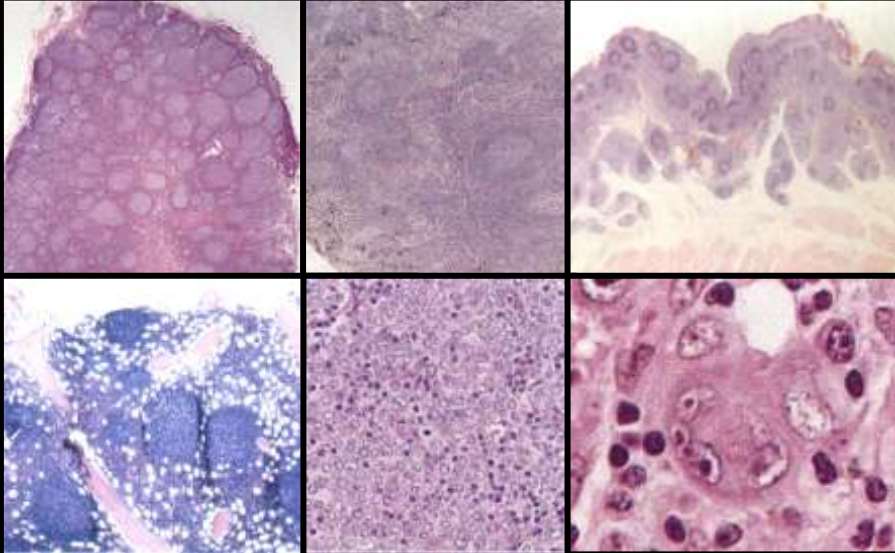
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  - neoplastic vs reactive (and beyond)
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- prognosis
  - in otherwise homogeneous diseases
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  - both response and early recurrence
- cryptic abnormalities
- technical reasons

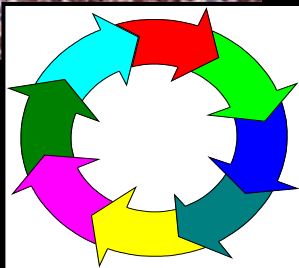
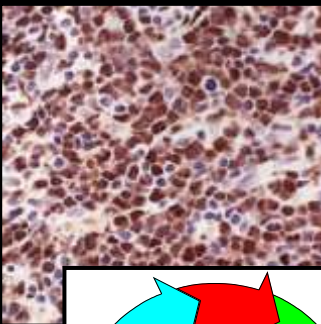
## IGH@ gene rearrangements and PCR



## Specific lymphoma categories ...



## Mantle cell lymphoma



- $t(11;14)(q13;q32)$ 
  - upregulates cyclinD1 expression
  - +s progression through cell cycle

- testing the defect:

DNA-PCR  
FISH  
Fibre-FISH  
IHC  
RT-PCR

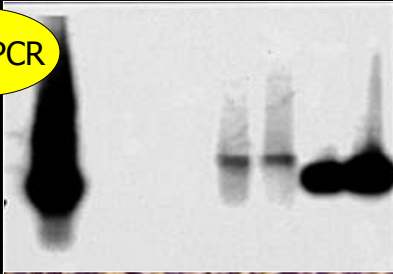
- ~ 10% cyclin D1 negative

?? still = mantle cell lymphoma ??

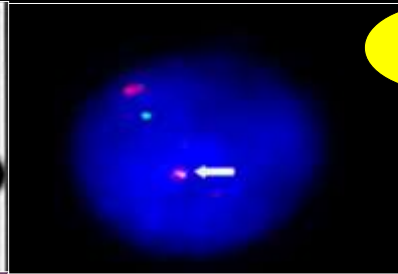
SOX11

## Cyclin D1 testing in mantle cell lymphoma

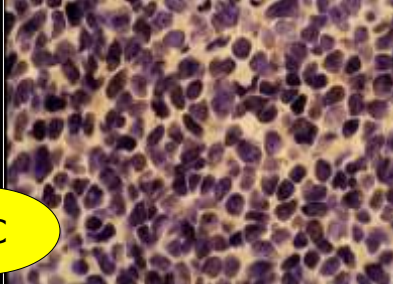
DNA-PCR



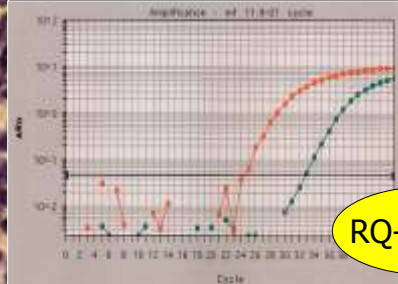
FISH



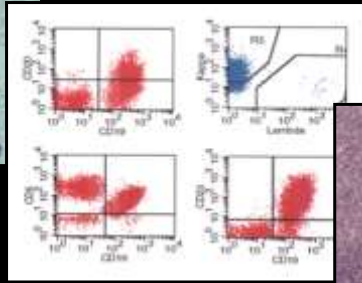
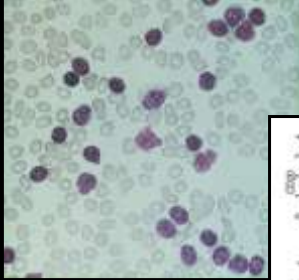
IHC



RQ-RT-PCR

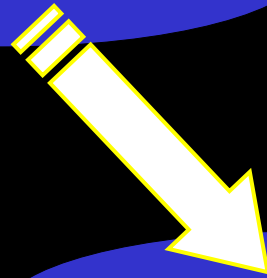


## Chronic lymphocytic leukemia/SLL



## CLL: Unraveling the heterogeneity

Conventional parameters



Novel parameters

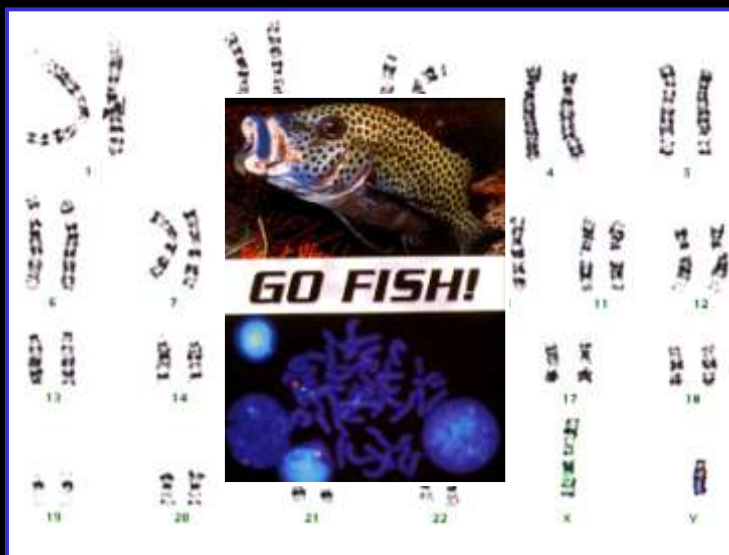
## CLL: Unraveling the heterogeneity

### Novel parameters

Cytogenetics

Cell of origin

## Cytogenetics





## Cytogenetics



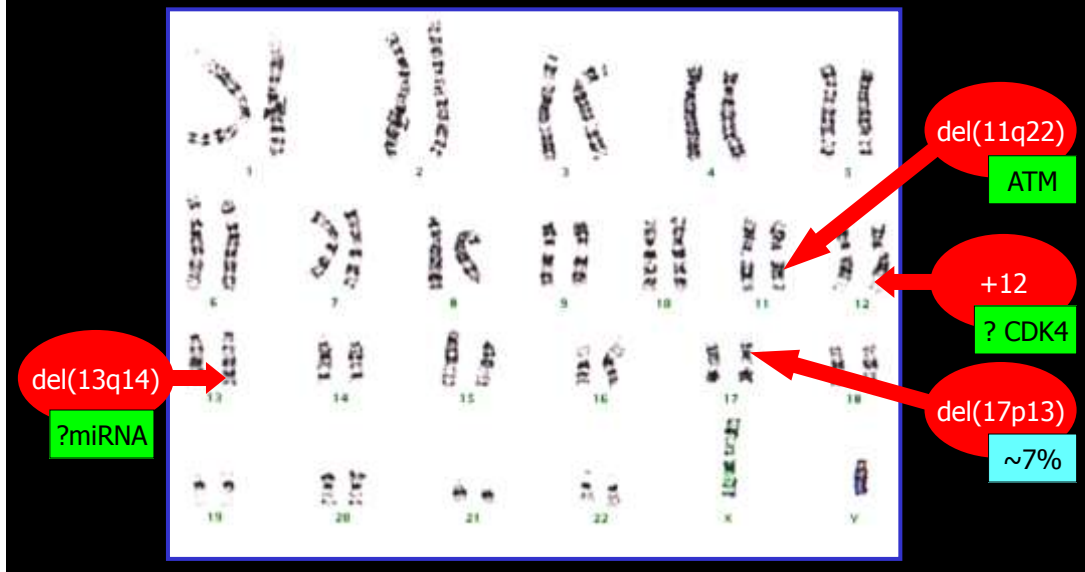
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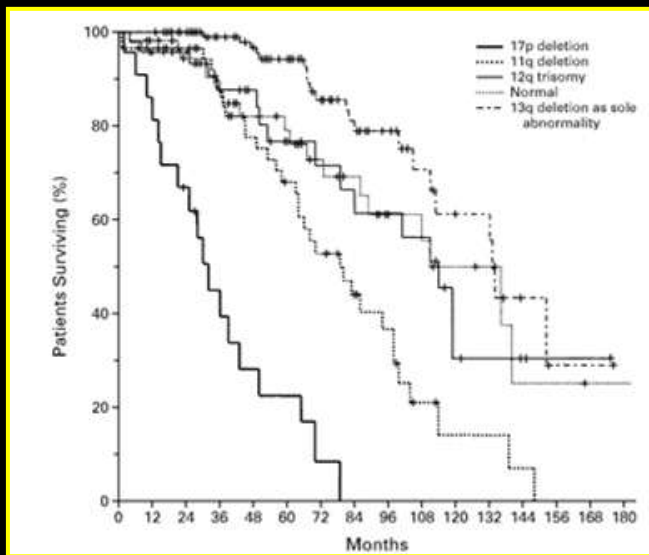
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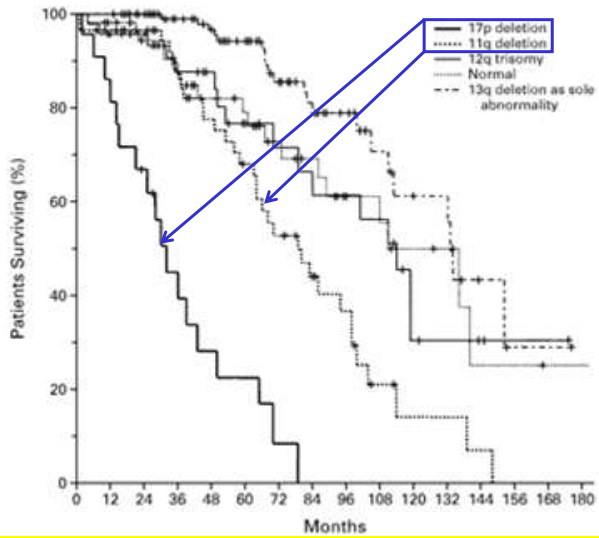


## Prognostication by cytogenetics



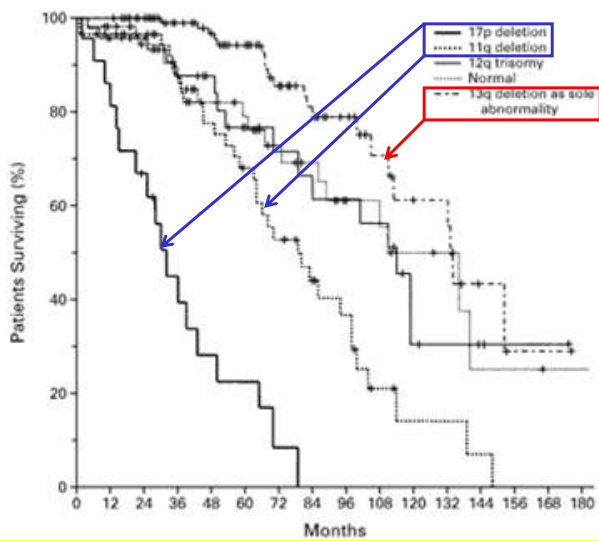
Dohner, H et al N Engl J Med 2000, 343:1910.  
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## Cell of origin

### The dogma

CLL is a neoplasm of naïve B-cells ...



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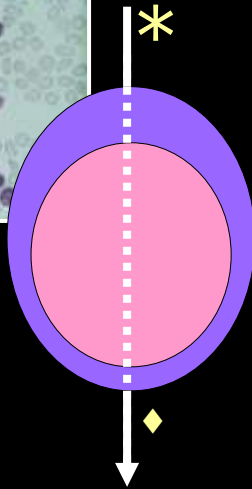
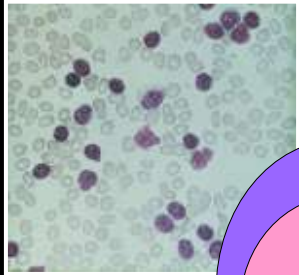
### The dogma

CLL is a neoplasm of naïve B-cells ...

... but a **KAR**ma has run over this **DOG**ma



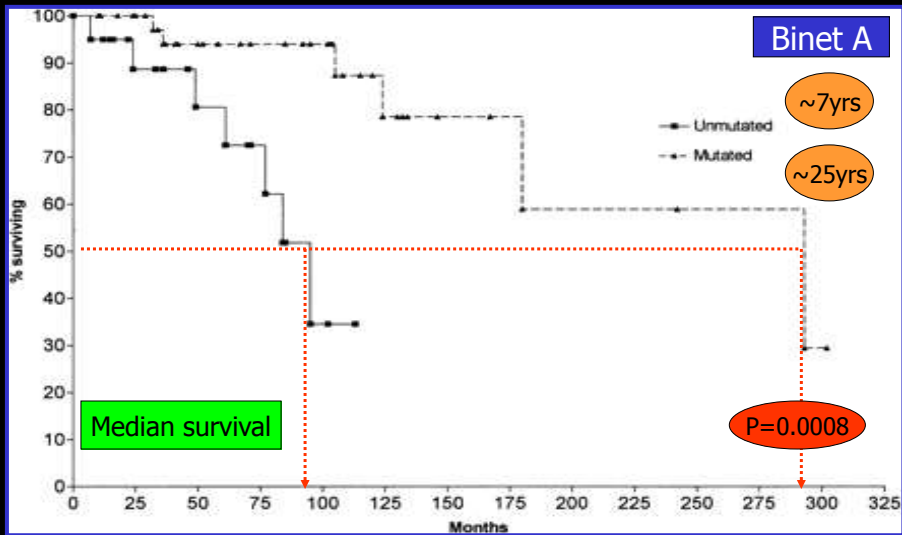
## Chronic lymphocytic leukemia



- \* **pre-GC**
  - non-mutated IgH
  - "naïve"
  - poorer prognosis

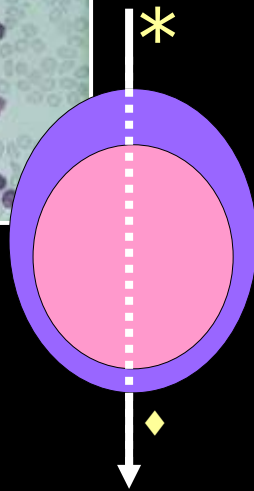
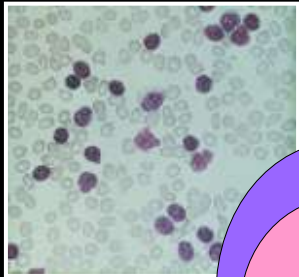
- ◆ **post-GC**
  - mutated IgH
  - memory
  - better prognosis

## Impact of SHM on prognosis



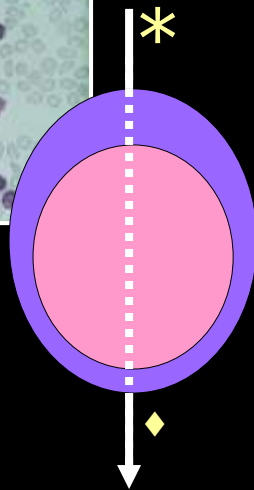
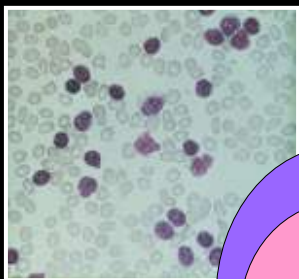
Hamblin, TJ Blood 1999, 94:1848-1854. Copyright © 1999 reproduced with permission of American Society of Hematology (ASH).

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  - (CD38+)
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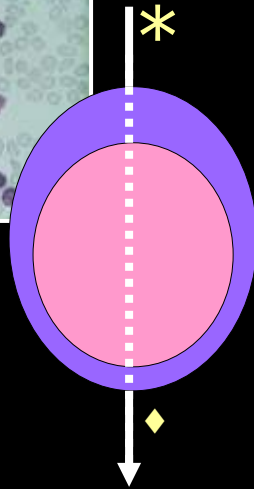
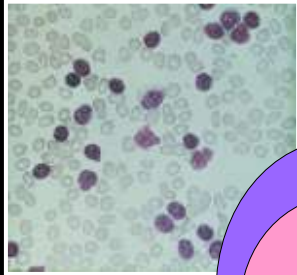
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↑ ZAP70

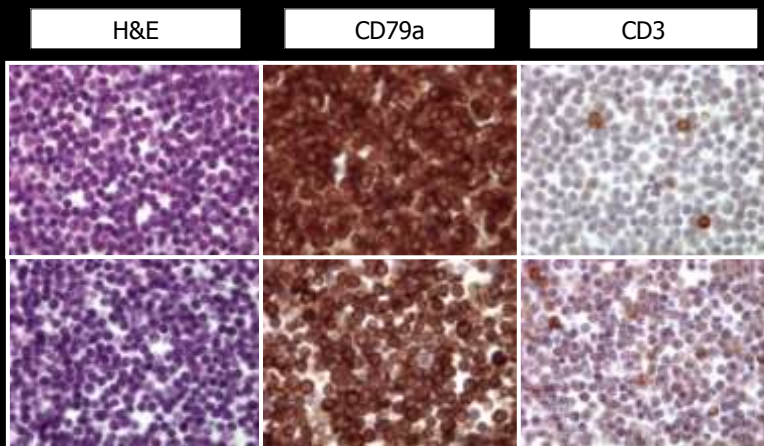
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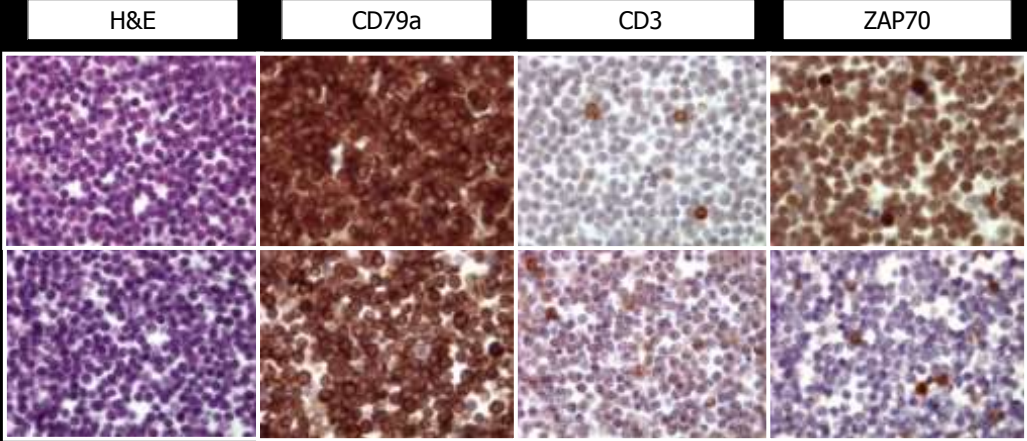
- ◆ **post-GC** — **?↓miR15/16**
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  - memory
  - better prognosis

## ZAP70 by IHC on PB

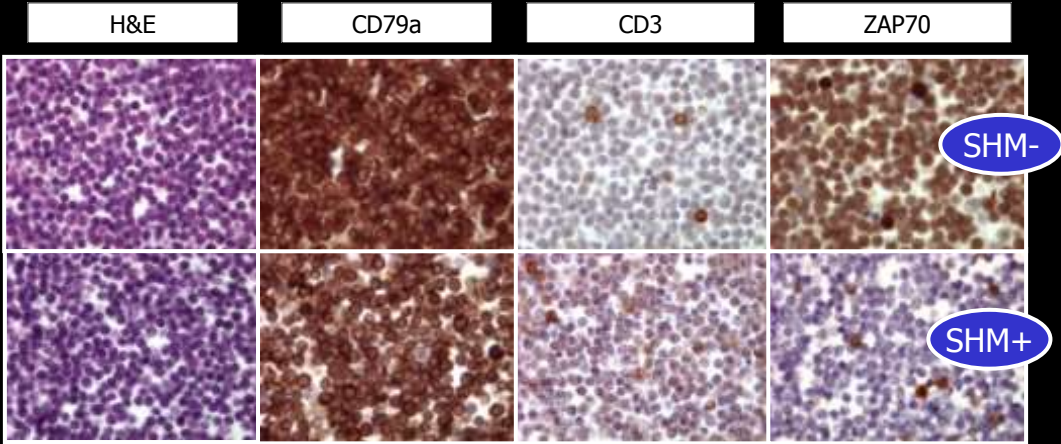




ZAP70 by IHC on PB



ZAP70 by IHC on PB

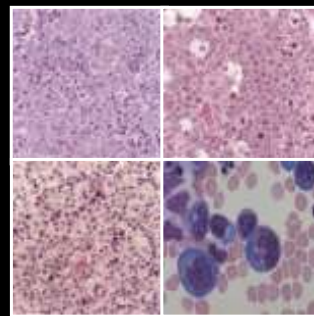




## Diffuse Large B-cell Lymphoma (DLBCL)

- **Morphologic:**

- centroblastic
- immunoblastic
- T-cell/histiocyte-rich
- anaplastic
- plasmablastic
- lymphomatoid granulomatosis type



- **Clinicopathologic:**

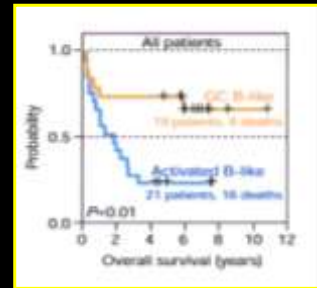
- primary mediastinal (thymic) large B-cell lymphoma
- primary CNS lymphoma
- primary effusion lymphoma
- primary cutaneous large B-cell lymphoma (of the leg!)
- intravascular large cell lymphoma

## DLBCL: Gene expression profiling



Separation of DLBCLs into two broad groups:

- germinal center
- activated B-cell



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	GCB-DLBCL	ABC-DLBCL
Frequency	~50%	~30%
Key genes	<i>CD10</i> <i>BCL-6</i> <i>A-MYB</i> <i>LMO2</i>	<i>PKCβ1</i> <i>Cyclin D2</i> <i>BCL-2</i> <i>IRF/MUM1</i>
Oncogenic events	t( <i>BCL-2</i> )	+NFκB
IGH SHM	Ongoing	Fixed
Centro:Immunoblastic	~20:1	~2:1
5-year survival	~60%	~35%
Median survival	~10 years	~2 years

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That's all very nice and impressive, but ...

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So, what's a humble, information-overloaded pathologist to do?

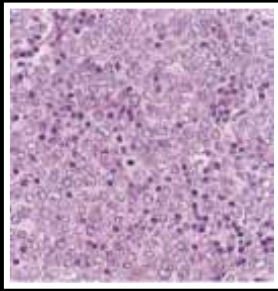
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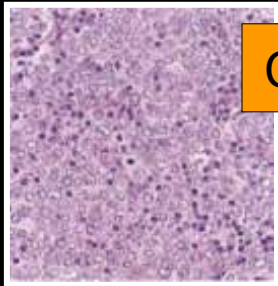
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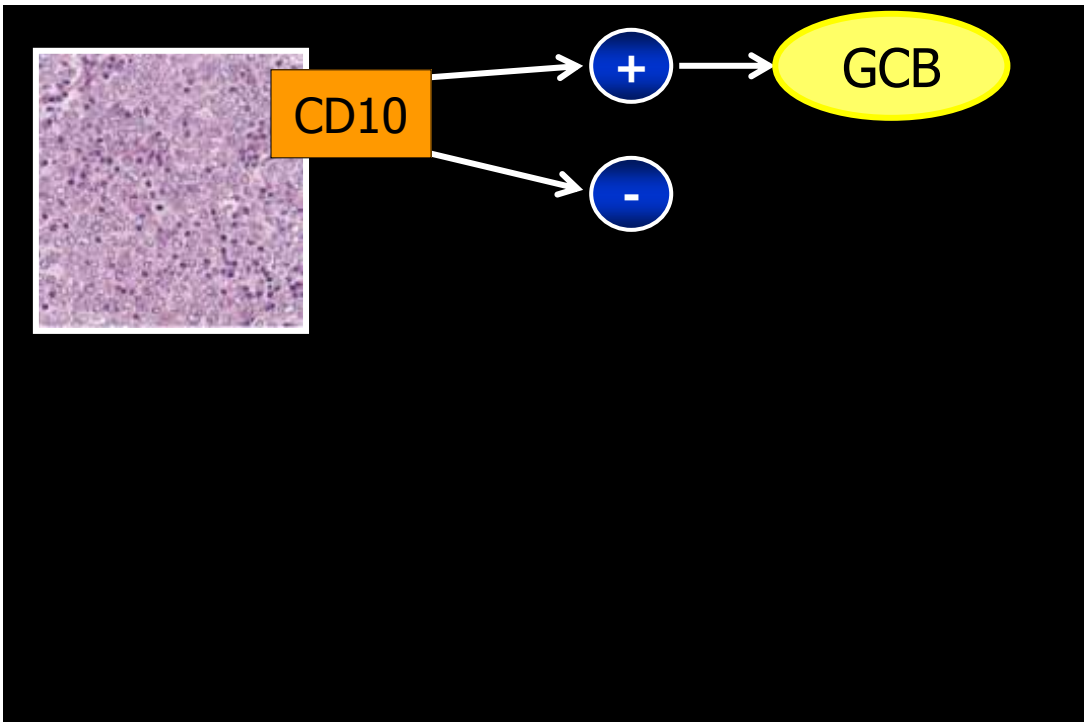
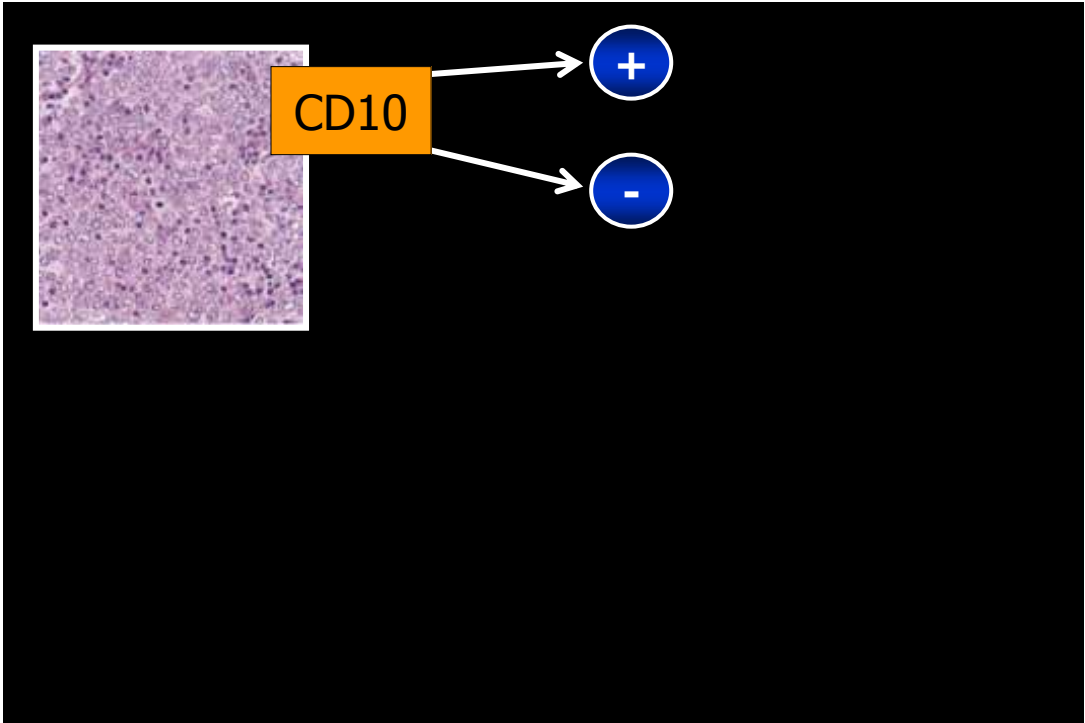
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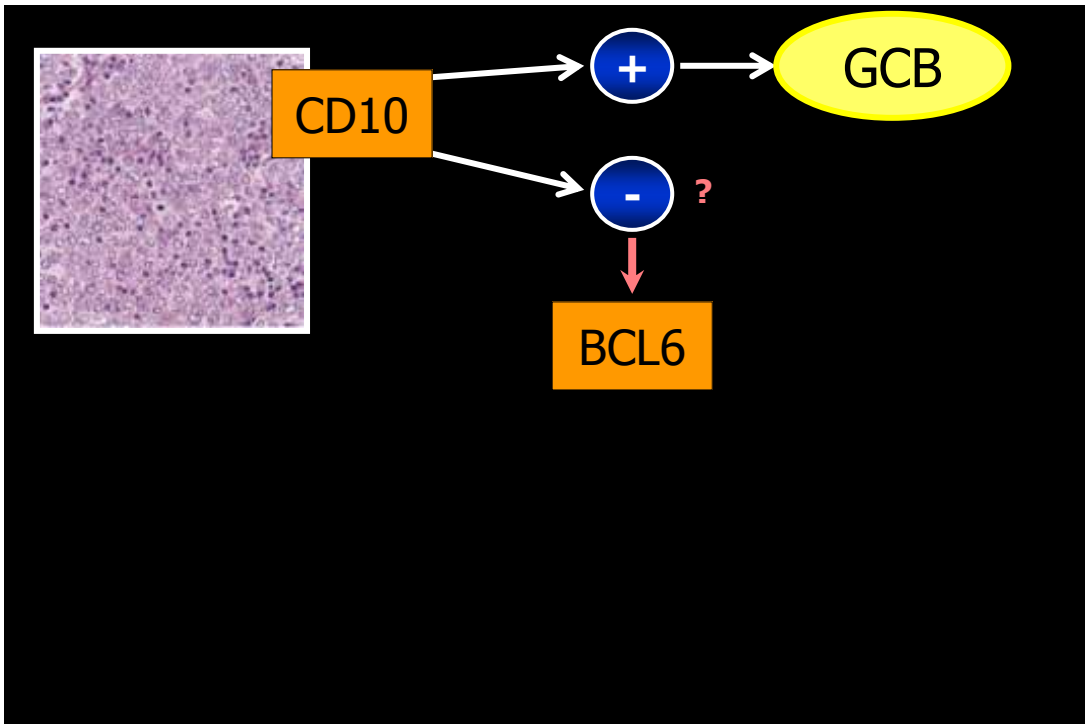
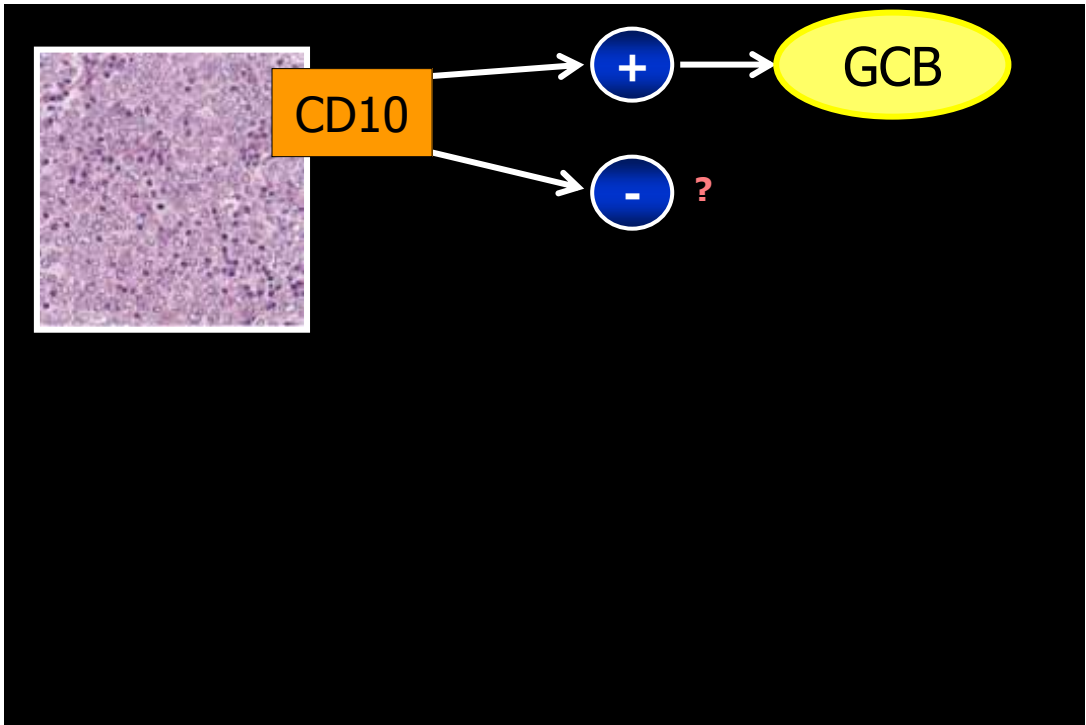
- Wait ...
  - ... for the dust to settle ...
  - ... and it might be ...
  - ... and use IHC ... (and only 3 markers at that!)



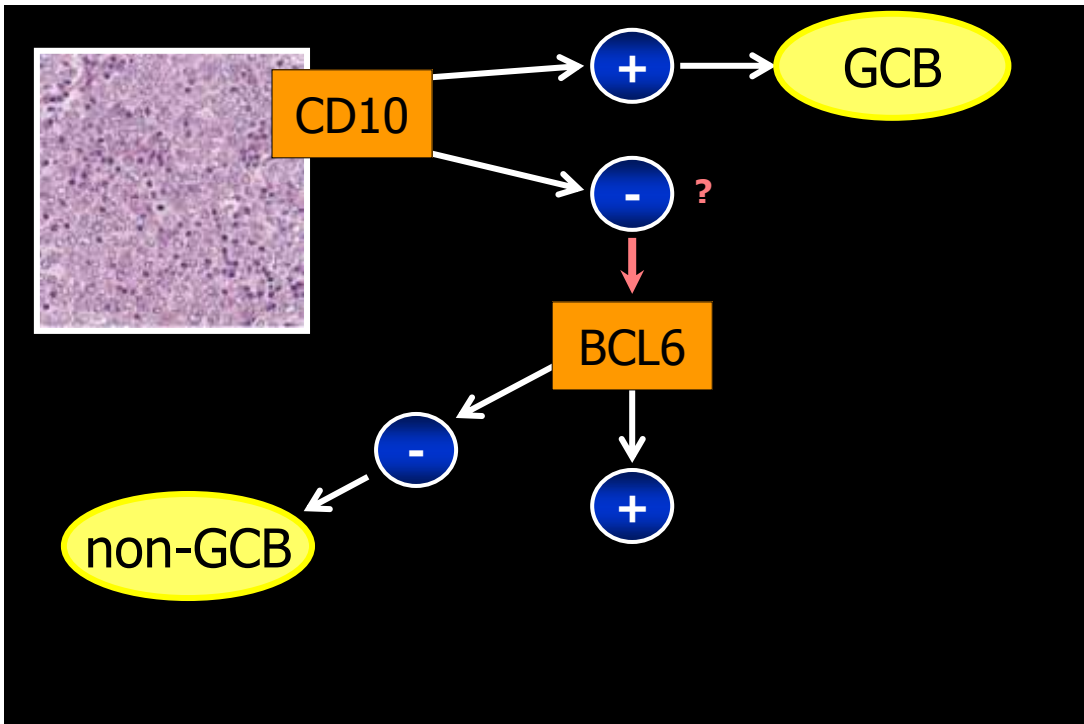
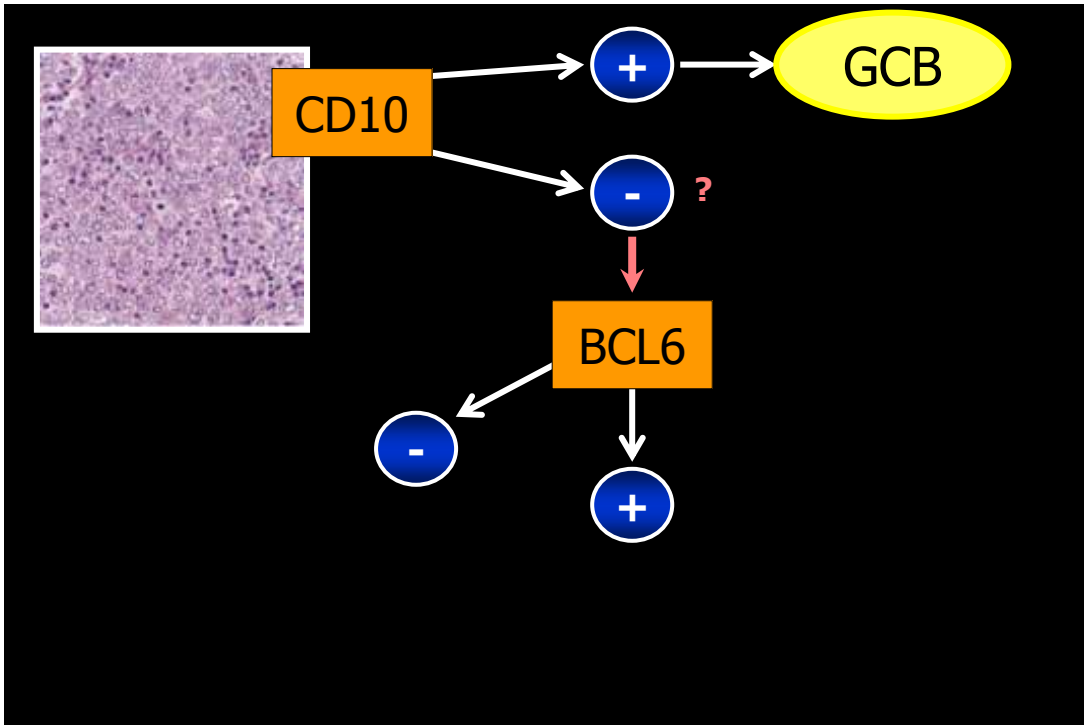
CD10

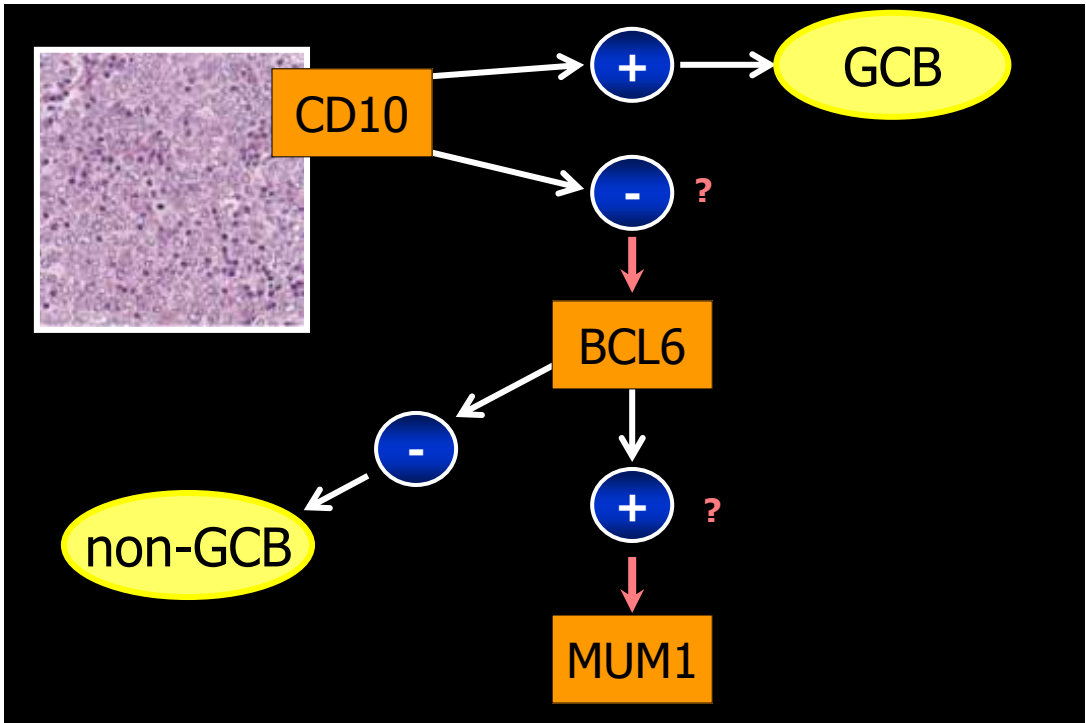
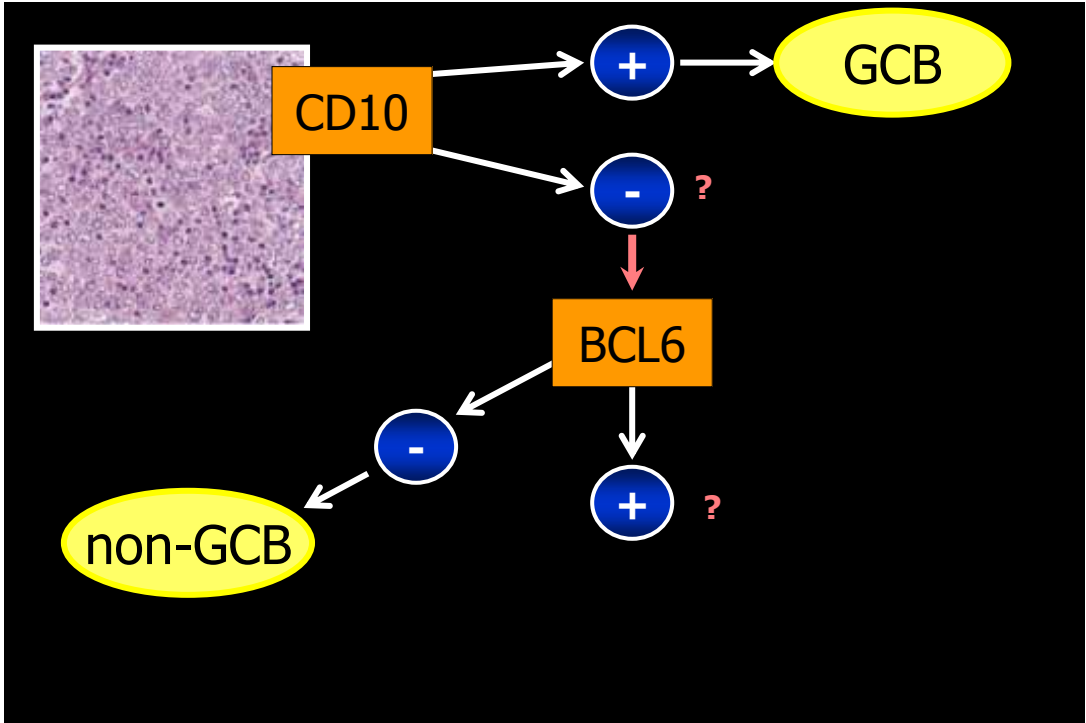


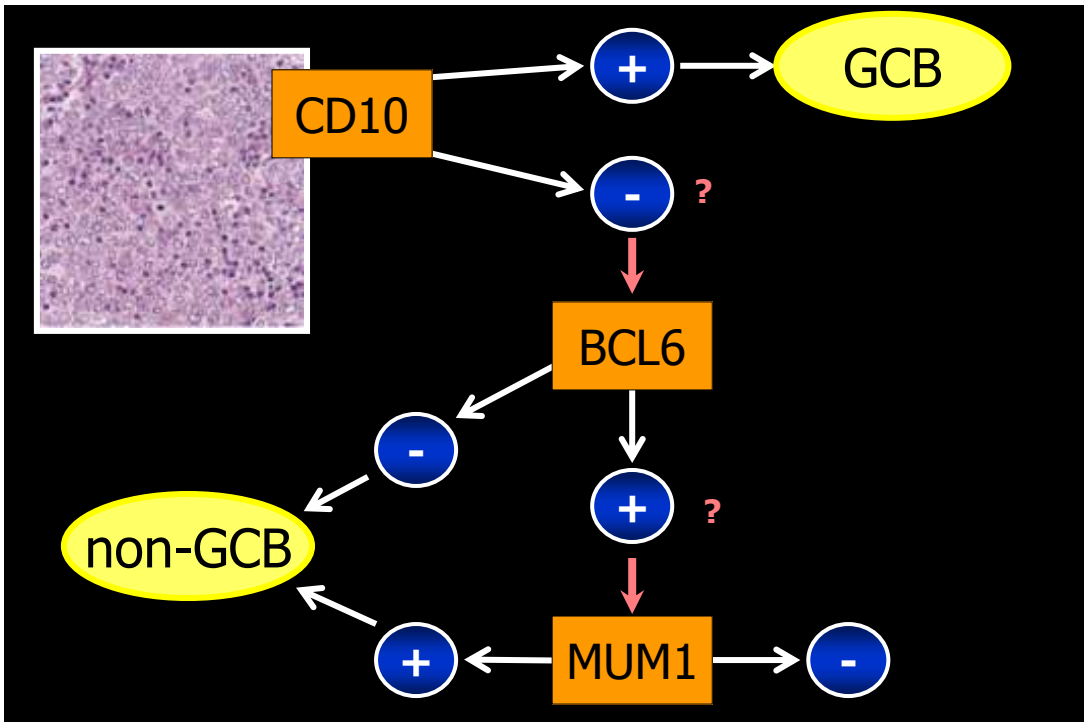
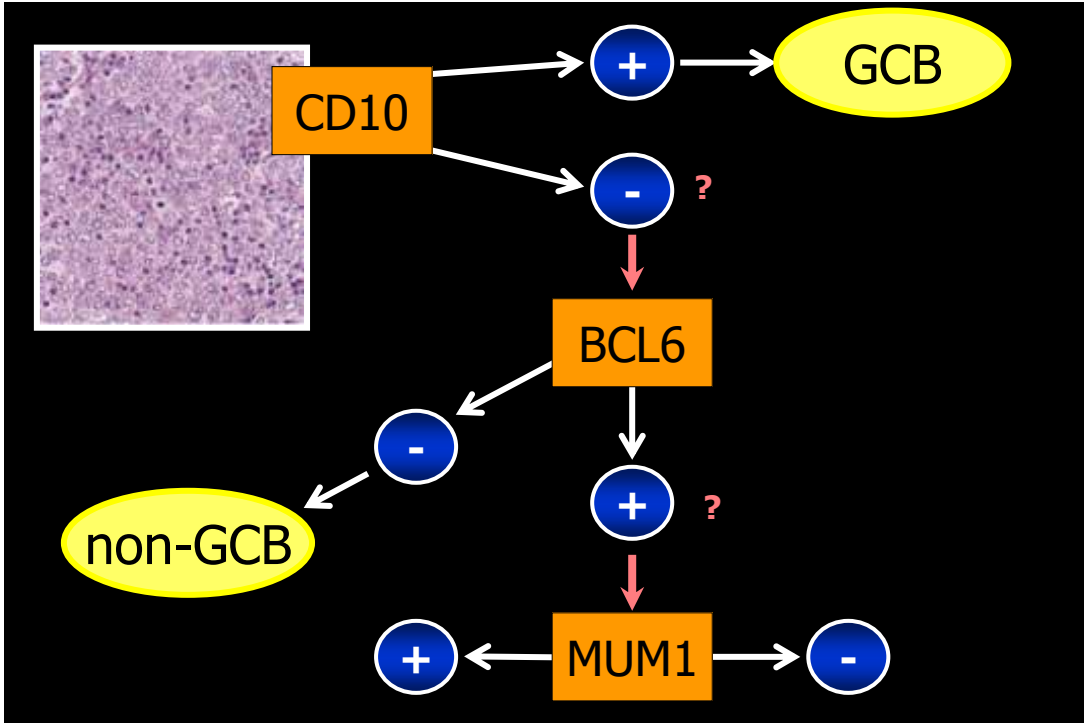


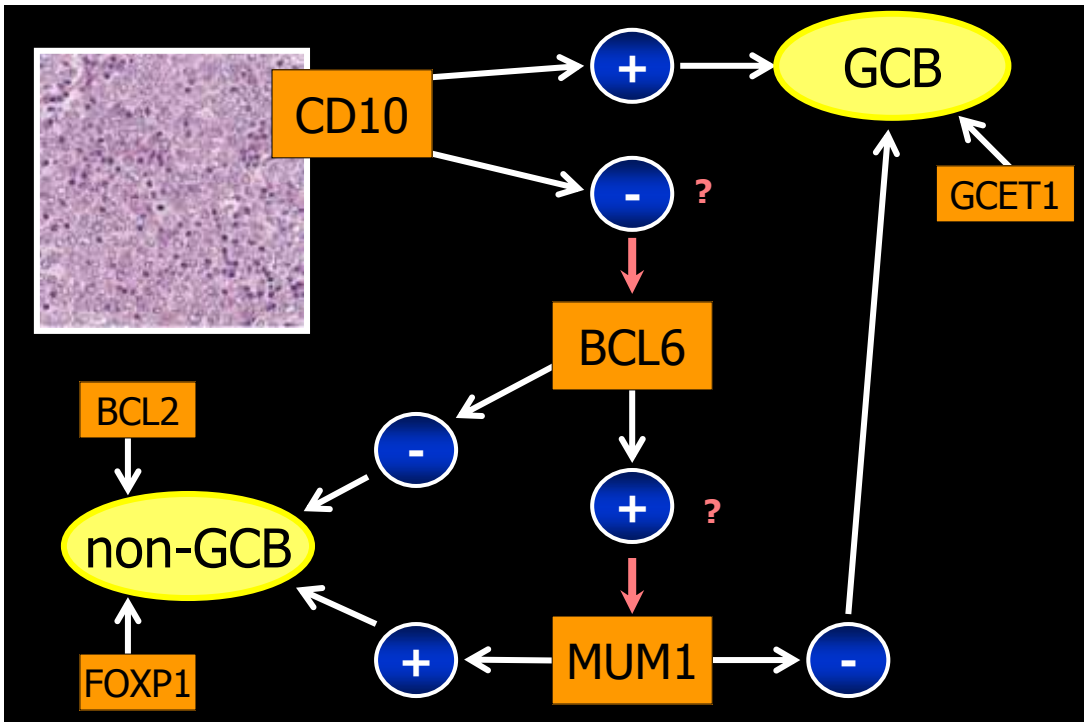
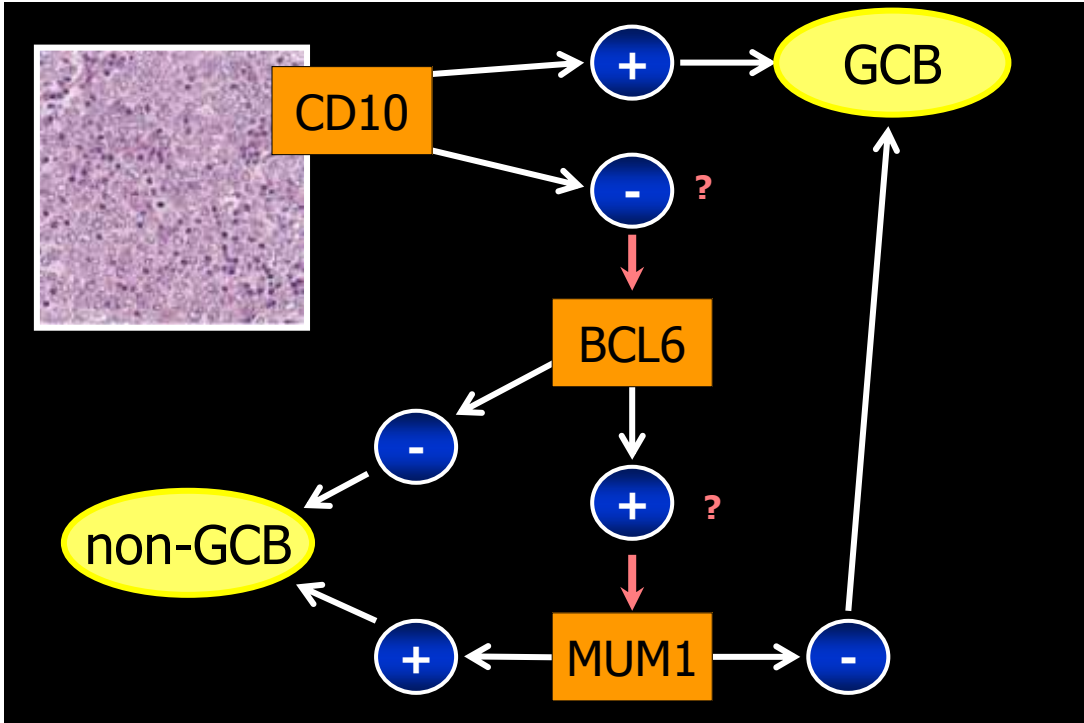


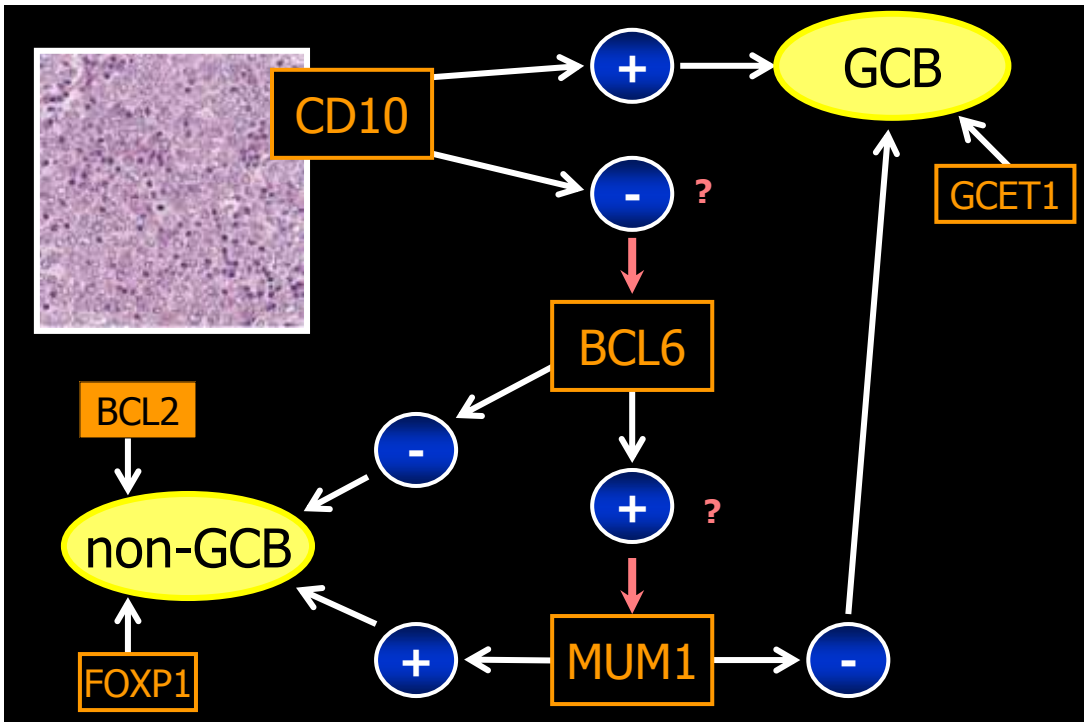
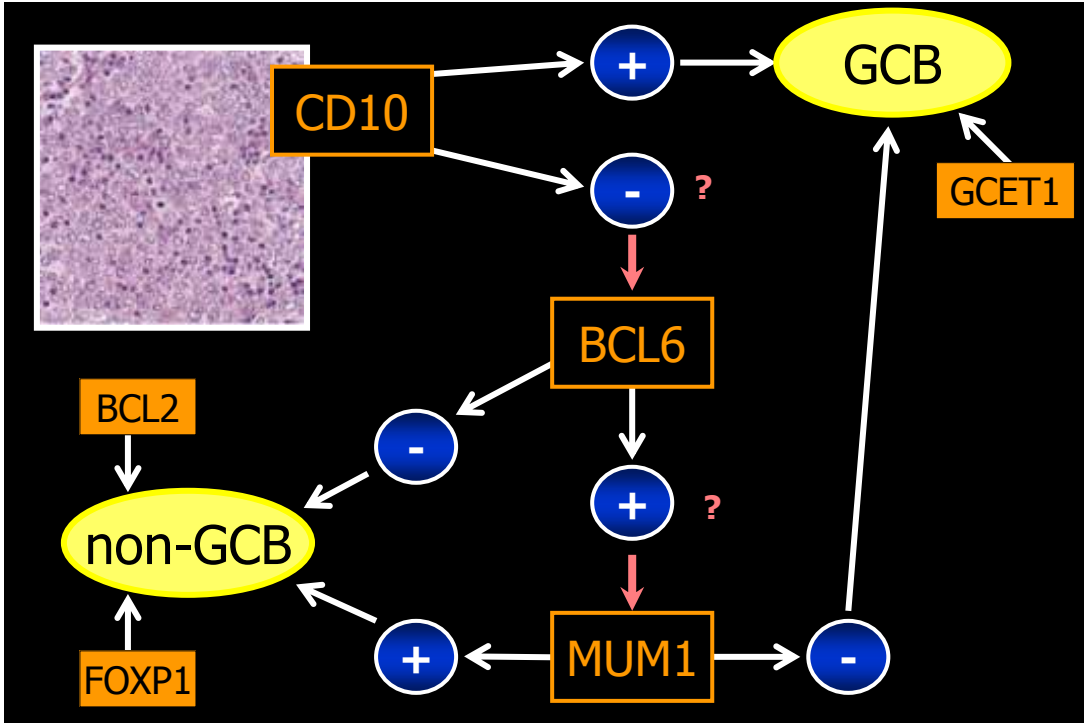














## Diffuse large B-cell lymphoma: what's new in WHO (2008)

Incorporation of borderline categories:

EBV positive DLBCL of the elderly:

## Diffuse large B-cell lymphoma: BCLUWFIBDLBCLABL

Characteristic	BL	DLBCL
<b>Morphology</b>		
only small-medium sized cells	yes	no
only large cells	no	common
mixture	no	rare
<b>Proliferation (Ki67)</b>		
>90% and homogeneous	yes	rare
<90% or heterogeneous	no	common
<b>BCL2 expression</b>		
negative/weak	yes	sometimes
strong	no	sometimes

## Diffuse large B-cell lymphoma: BCLUWFIBDLBCLABL

Characteristic	BL	DLBCL
<b>Genetic features</b>		
<i>MYC</i> rearrangement	yes	rare
<i>IG/MYC</i>	yes	rare
non- <i>IG/MYC</i>	no	rare
<i>BCL2</i> + but <i>MYC</i> -	no	sometimes
<i>BCL6</i> + but <i>MYC</i> -	no	sometimes
double hit	no	rare
<i>MYC</i> -simple karyotype	yes	rare
<i>MYC</i> -complex karyotype	rare	rare

Diffuse large B-cell lymphoma: BCLUWFIBDLBCLABL

BL

DLBCL





## Pitfalls and caveats: Ig and TCR PCR

"False" positives

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- "clonal dermatitis"

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- Preanalytic variables
- Technical
  
- Biologic

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### False negatives

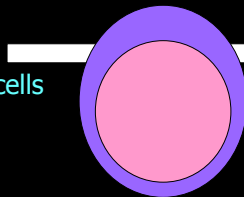
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- Technical
  - consensus primers
  - using CDR3 IgH primers only
- Biologic

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### False negatives

- Preanalytic variables [degradation, fixation, representative sampling]
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pre GC/  
precursor B-cells



intra/post GC

## Pitfalls and caveats: Ig and TCR PCR

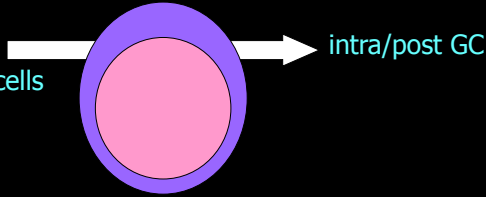
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consensus primers  
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pre GC/  
precursor B-cells



- partial DJ
- oligoclonal  
(~1/3 precursor B-ALL)
- ongoing rearrangements at relapse

## Pitfalls and caveats: Ig and TCR PCR

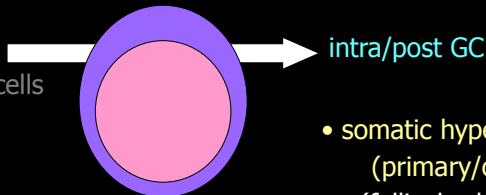
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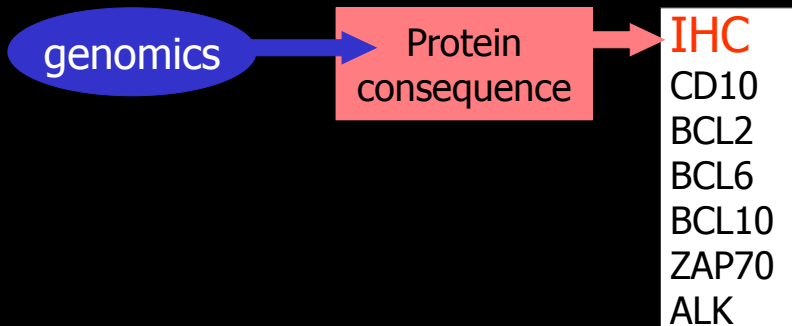
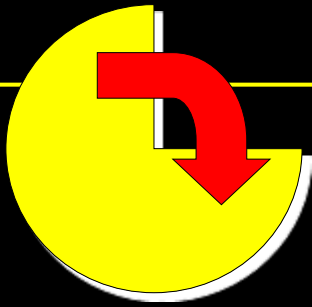
pre GC/  
precursor B-cells



- partial DJ
- oligoclonal  
(~1/3 precursor B-ALL)
- ongoing rearrangements at relapse

- somatic hypermutation  
(primary/ongoing)  
(follicular lymphoma,  
myeloma)
  - IgH deletions  
(~1/10 lymphomas)

Coming full circle?



The last slide ...

- Powerful ... but one piece of the puzzle



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- Positive result: not always = neoplastic

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## The last slide ...

- Powerful ... but one piece of the puzzle
- Positive result: not always = neoplastic
- Negative result: not always = not neoplastic
- Integrate: with morphologic, immunophenotypic, clinical data
- Decision to perform/ability to interpret: contextual
- More rational, biologically-based diagnosis:  
→ more appropriate, targeted Rx

## Any questions ...

