

Diagnostic Molecular Pathology of Lymphoid Neoplasms

(Part I)
Antigen receptor gene rearrangements and some "indolent" lymphomas

23rd Congress of The Arab Division
International Academy of Pathology
Beirut, Lebanon
Tuesday November 29, 2011: Pre-congress workshop

Adam Bagg
University of Pennsylvania
Philadelphia, USA

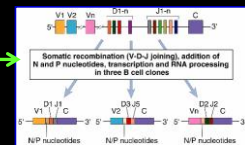
Molecular targets

Molecular targets

- Rearrangements
 - physiologic
 - pathologic

Molecular targets

- Rearrangements
 - physiologic
 - pathologic



Molecular targets

- Rearrangements
 - physiologic
 - pathologic

creation of a novel chimeric gene

A B

C

t(9;22) ⇒ bcr-abl

qualitative

upregulated/overexpression of a protooncogene

A B

A B

t(8;14) ⇒ Igh + c-myc

quantitative

Molecular targets

- Rearrangements
 - physiologic
 - pathologic

creation of a novel chimeric gene

A B

C

t(9;22) ⇒ bcr-abl

qualitative

upregulated/overexpression of a protooncogene

A B

A B

t(8;14) ⇒ Igh + c-myc

quantitative

homogeneity vs heterogeneity

 present vs absent

Molecular targets

- Rearrangements
 - physiologic
 - pathologic
- Mutations

creation of a novel chimeric gene

A B

C

t(9;22) ⇒ bcr-abl

qualitative

upregulated/overexpression of a protooncogene

A B

A B

t(8;14) ⇒ Igh + c-myc

quantitative

homogeneity vs heterogeneity

 present vs absent

Molecular targets

- Rearrangements
 - physiologic
 - pathologic
- Mutations
- Additions
- Losses
 - deletions
 - silencing

creation of a novel chimeric gene

A B

C

t(9;22) ⇒ bcr-abl

qualitative

upregulated/overexpression of a protooncogene

A B

A B

t(8;14) ⇒ Igh + c-myc

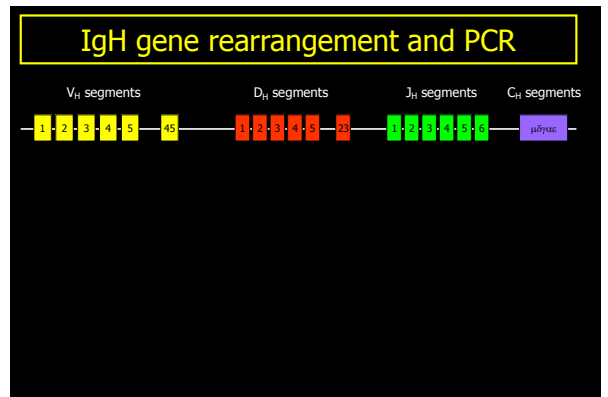
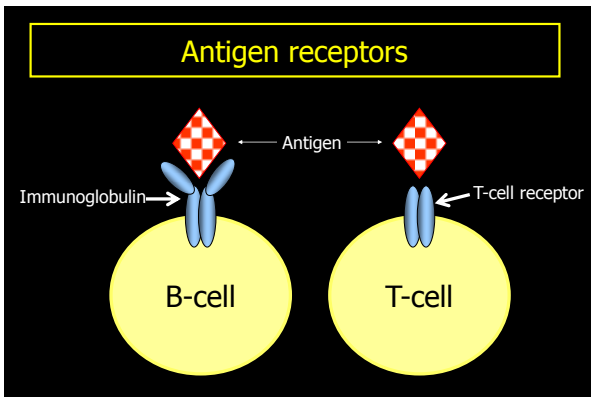
quantitative

homogeneity vs heterogeneity

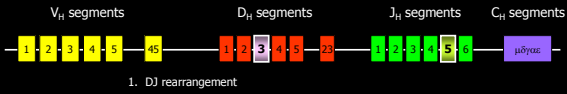
 present vs absent



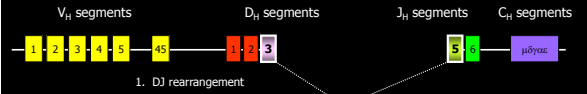
Antigen receptor
(Ig and TCR)
gene rearrangements



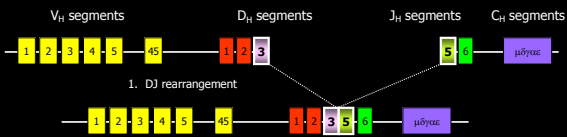
IgH gene rearrangement and PCR



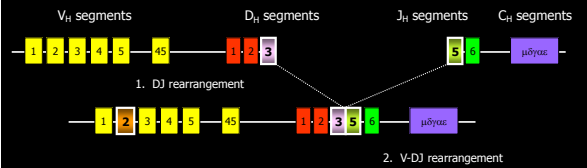
IgH gene rearrangement and PCR



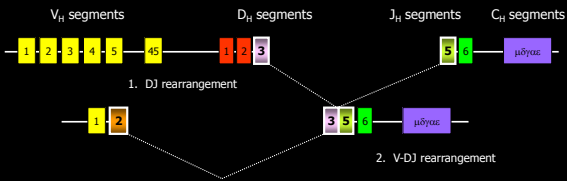
IgH gene rearrangement and PCR



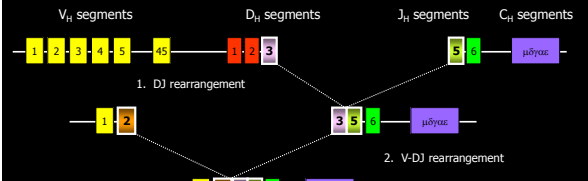
IgH gene rearrangement and PCR



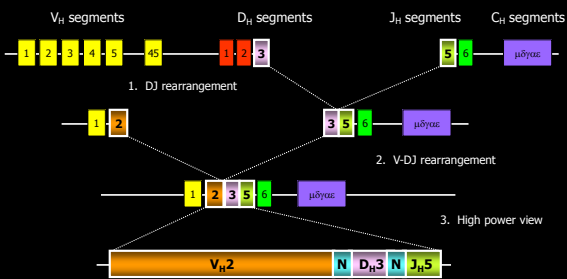
IgH gene rearrangement and PCR



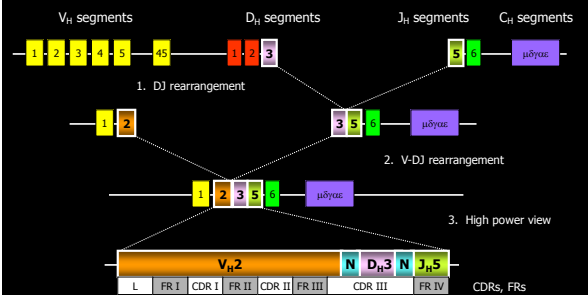
IgH gene rearrangement and PCR

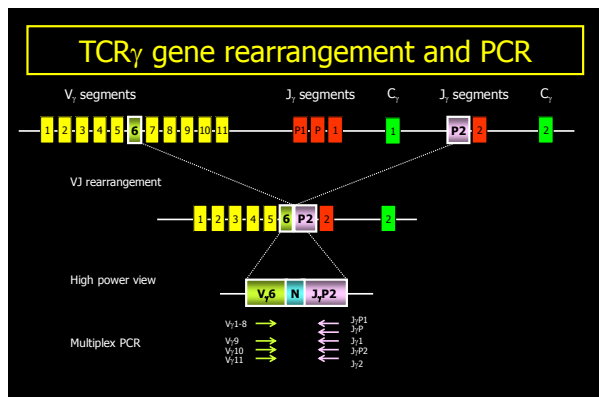
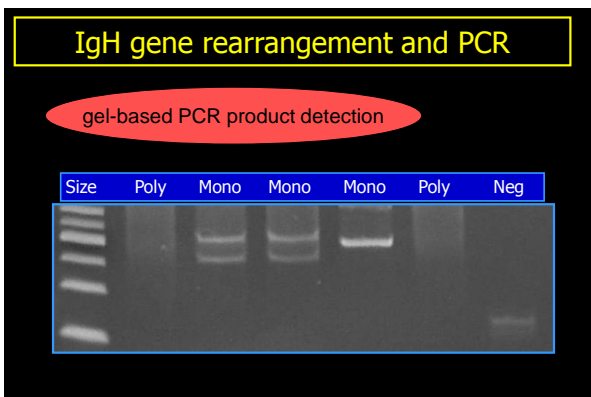
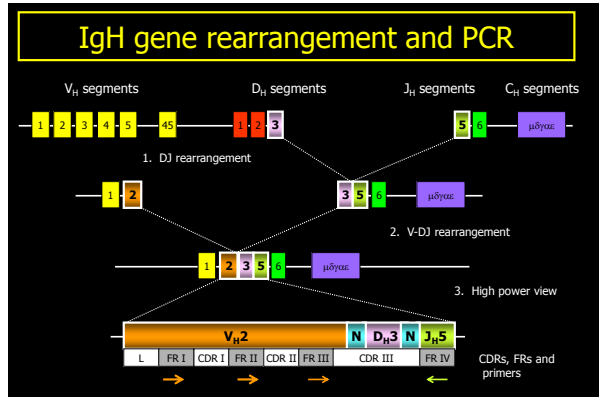
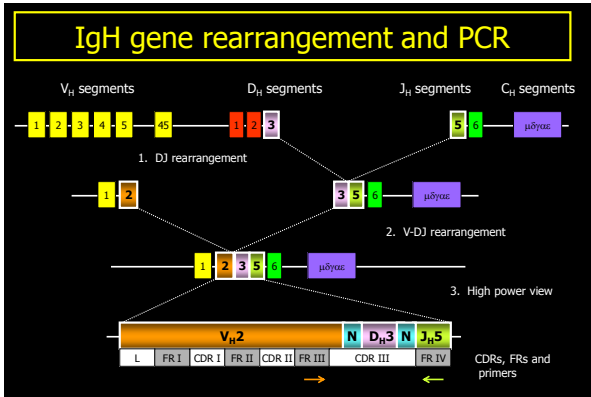


IgH gene rearrangement and PCR



IgH gene rearrangement and PCR





TCR γ gene rearrangement and PCR

- Atypical T-cell lymphoproliferations



TCR γ gene rearrangement and PCR

- Atypical T-cell lymphoproliferations



capillary electrophoresis-based PCR product detection

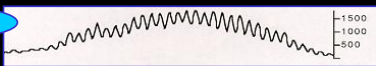
TCR γ gene rearrangement and PCR

- Atypical T-cell lymphoproliferations



capillary electrophoresis-based PCR product detection

reactive



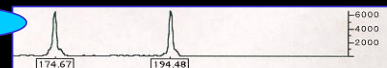
TCR γ gene rearrangement and PCR

- Atypical T-cell lymphoproliferations



capillary electrophoresis-based PCR product detection

neoplastic





Other methods for AR GR ...

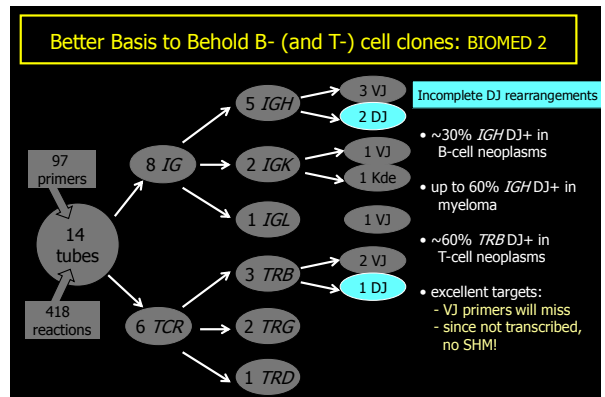
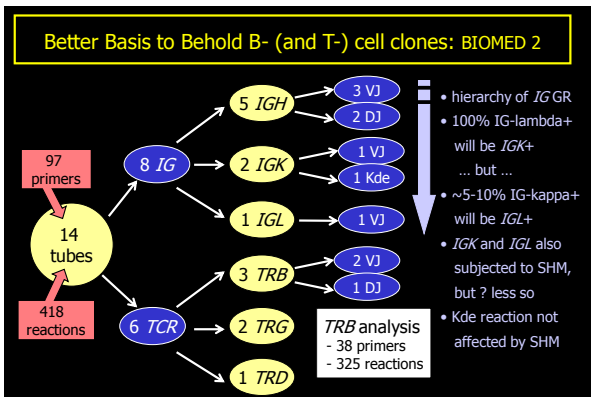
Detection

- heteroduplex analysis
- SSCP
- DGGE
- microchip
- melting curve
- multichannel electrophoresis
- ligase chain reaction

IGH PCR: usually straight-forward
TRG PCR: more of an issue

Interpretation

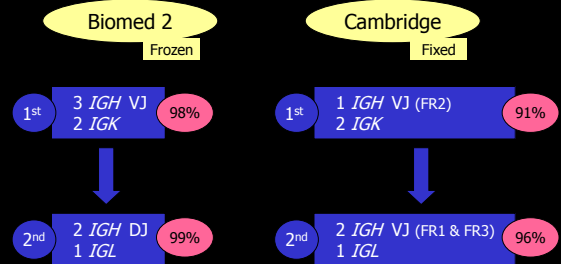
- relative peak height
- relative peak ratio
- height ratio
- peak height ratio
- best-fit normal distribution algorithm



Better Basis to Behold B- (and T-) cell clones: BIOMED 2

	Biomed	Cambridge
Tissue		
T-cell lymphomas		
B-cell lymphomas		
Pre-GC B (MCL)		
GC B (FL)		

BIOMED 2 *IG* – proposed algorithms



Antigen receptor gene rearrangements

Useful in the following situations:

Antigen receptor gene rearrangements

Useful in the following situations:

- atypical lymphoproliferations

Antigen receptor gene rearrangements

Useful in the following situations:

- atypical lymphoproliferations
- limited tissue

Antigen receptor gene rearrangements

Useful in the following situations:

- atypical lymphoproliferations
- limited tissue
- equivocal immunophenotype

Antigen receptor gene rearrangements

Useful in the following situations:

- atypical lymphoproliferations
- limited tissue
- equivocal immunophenotype
- T-cell lymphoproliferations

Antigen receptor gene rearrangements

Useful in the following situations:

- atypical lymphoproliferations
- limited tissue
- equivocal immunophenotype
- T-cell lymphoproliferations
- baseline for MRD

Antigen receptor gene rearrangements

Useful in the following situations:

- atypical lymphoproliferations
- limited tissue
- equivocal immunophenotype
- T-cell lymphoproliferations
- baseline for MRD
- BM: ↑ precursor B-cells

Antigen receptor gene rearrangements

Useful in the following situations:

- atypical lymphoproliferations
- limited tissue
- equivocal immunophenotype
- T-cell lymphoproliferations
- baseline for MRD
- BM: ↑ precursor B-cells

But not that helpful in:

Antigen receptor gene rearrangements

Useful in the following situations:

- atypical lymphoproliferations
- limited tissue
- equivocal immunophenotype
- T-cell lymphoproliferations
- baseline for MRD
- BM: ↑ precursor B-cells

But not that helpful in:

- diagnosing specific entities

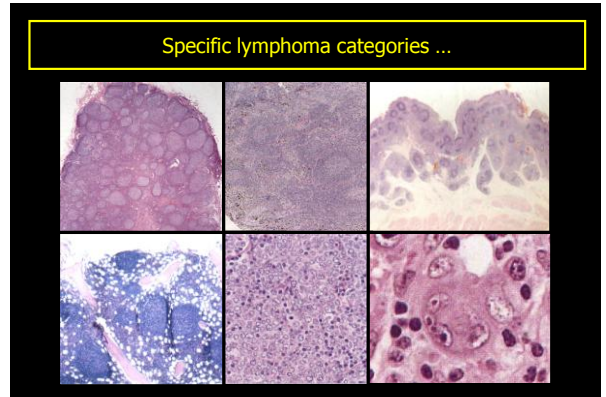
Antigen receptor gene rearrangements


Useful in the following situations:

- atypical lymphoproliferations
- limited tissue
- equivocal immunophenotype
- T-cell lymphoproliferations
- baseline for MRD
- BM: ↑ precursor B-cells

But not that helpful in:

- diagnosing specific entities
- unraveling the heterogeneity



Follicular lymphoma in a 

Definition: germinal center B-cell
(not unique to follicular lymphoma)

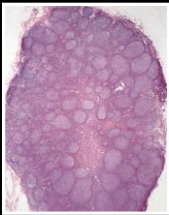
Epidemiology: ~35% of lymphomas
2nd commonest lymphoma

Clinically: nodal, disseminated

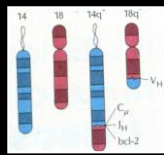
Morphology: follicular growth; small cleaved and large cells

Immunophen: CD10+, BCL6+, CD43- monoclonal B-cells

Course: usually indolent, ~30% large cell transformation

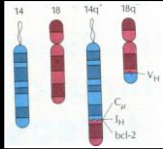


Follicular lymphoma: t(14;18) and BCL2



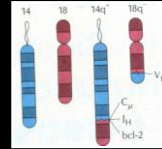
The diagram shows chromosomes 14 and 18. Chromosome 14 has a blue band labeled 14q, and chromosome 18 has a red band labeled 18q. A reciprocal translocation is shown where a segment from 14q moves to 18q, and a segment from 18q moves to 14q. The resulting chromosomes are labeled 14q11 and 18q21. The BCL2 gene is shown on the 18q chromosome, and the IgH gene is shown on the 14q chromosome. The BCL2 gene is now under the control of the IgH promoter, leading to overexpression of BCL2.

Follicular lymphoma: t(14;18) and BCL2



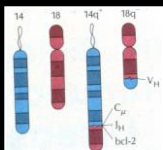
- only ~85% t(14;18)+

Follicular lymphoma: t(14;18) and BCL2

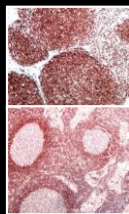


- only ~85% t(14;18)+
- not all t(14;18)+ cases = FL

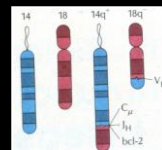
Follicular lymphoma: t(14;18) and BCL2



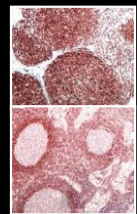
- only ~85% t(14;18)+
- not all t(14;18)+ cases = FL
- FL vs RFH (IHC ✓)
- FL vs other SBCL (IHC ✗)



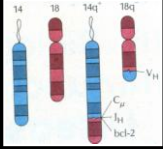
Follicular lymphoma: t(14;18) and BCL2



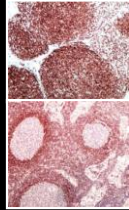
- only ~85% t(14;18)+
- not all t(14;18)+ cases = FL
- FL vs RFH (IHC ✓)
- FL vs other SBCL (IHC ✗)
- rare cases:
 - t(14;18)+; IHC-
 - point mutation



Follicular lymphoma: t(14;18) and BCL2

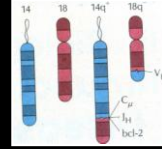


- only ~85% t(14;18)+
- not all t(14;18)+ cases = FL
- FL vs RFH (IHC ✓)
- FL vs other SBCL (IHC ✗)
- rare cases:
 - t(14;18)+; IHC-
 - point mutation

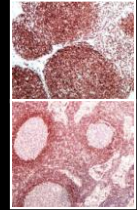


- t(14;18) PCR preferable to IGH PCR

Follicular lymphoma: t(14;18) and BCL2



- only ~85% t(14;18)+
- not all t(14;18)+ cases = FL
- FL vs RFH (IHC ✓)
- FL vs other SBCL (IHC ✗)
- rare cases:
 - t(14;18)+; IHC-
 - point mutation



- t(14;18) PCR preferable to IGH PCR

- of the ~15% t(14;18)-negative cases:

↑ copies chromosome 18/BCL-2 → BCL2+
t(BCL6) → BCL2- (MUM1+, grade 3)

BCL2 gene rearrangements: what method?

BCL2 on 18q21

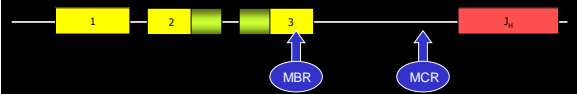
IGH on 14q32

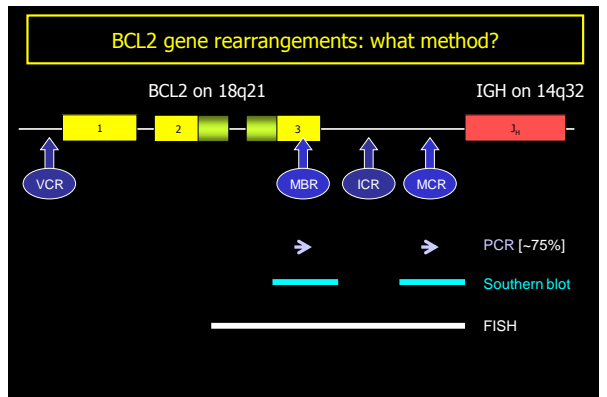
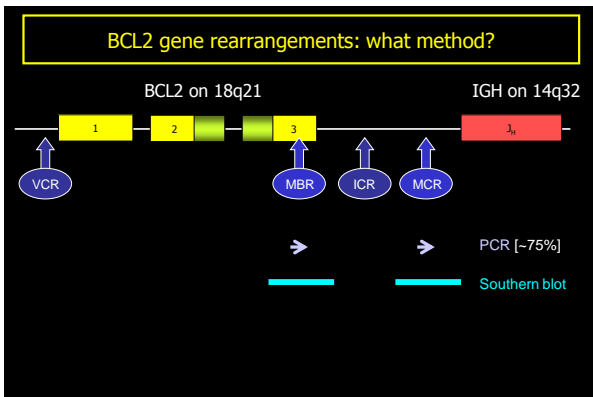
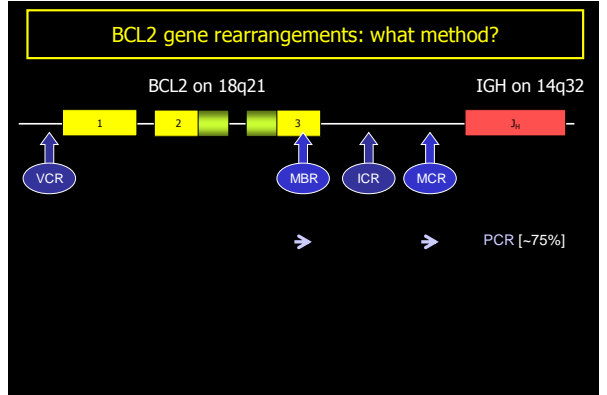
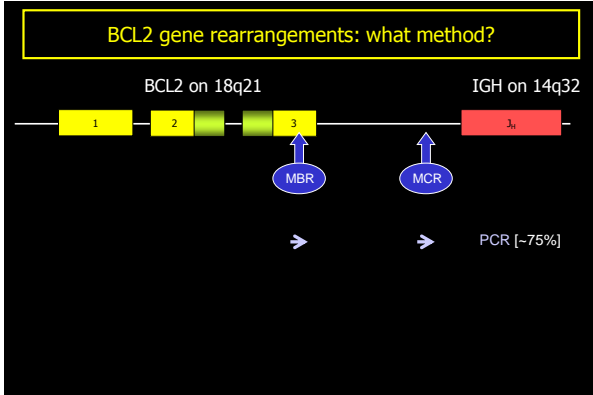


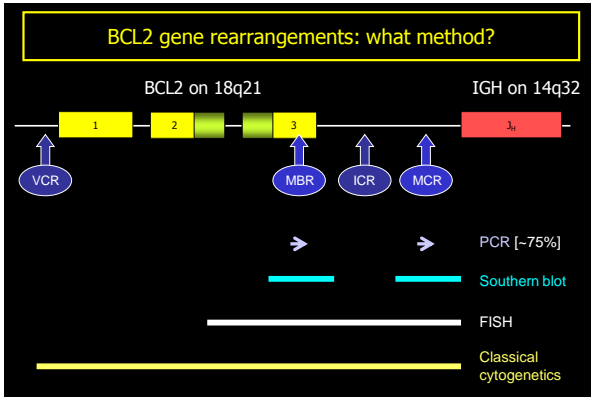
BCL2 gene rearrangements: what method?


BCL2 on 18q21

IGH on 14q32







Extranodal marginal zone lymphomas in a 

Definition: marginal zone, epithelium
acquired MALT

Epidemiology: ~6% of lymphomas
chronic inflammation: infection, autoimmune

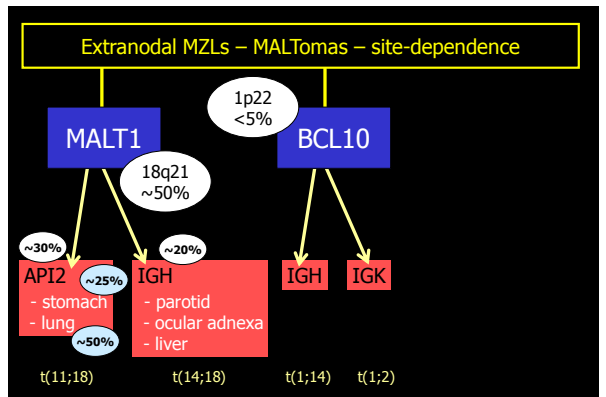
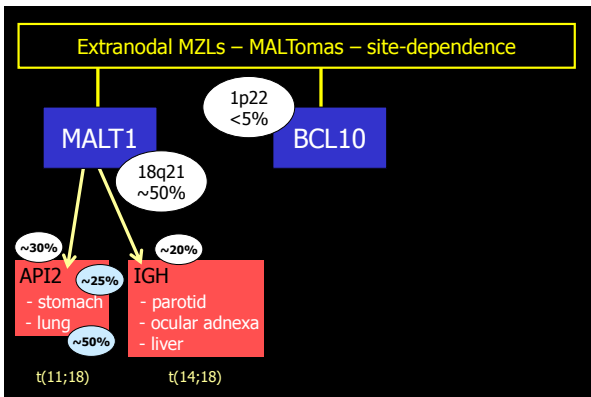
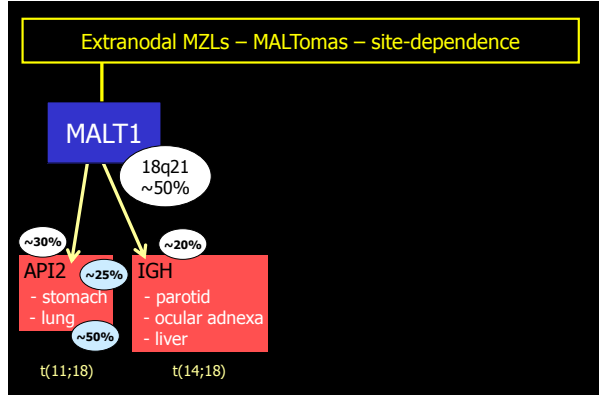
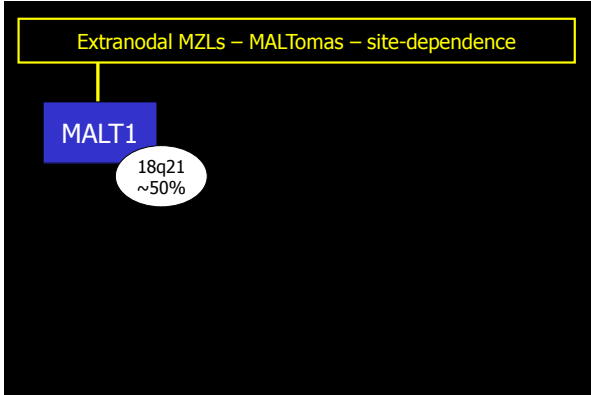
Clinically: stomach, many other, typically localized

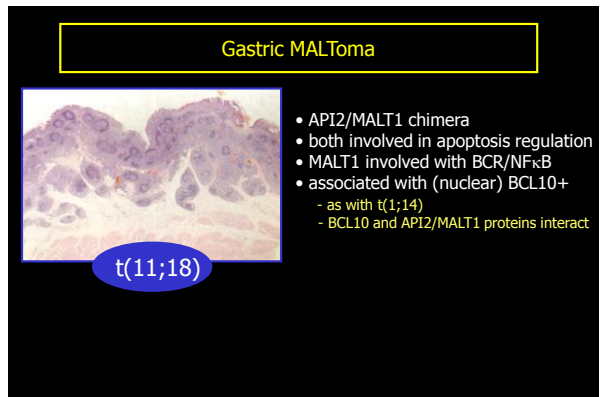
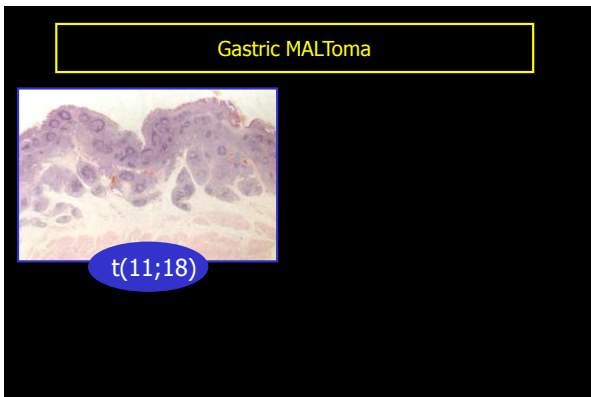
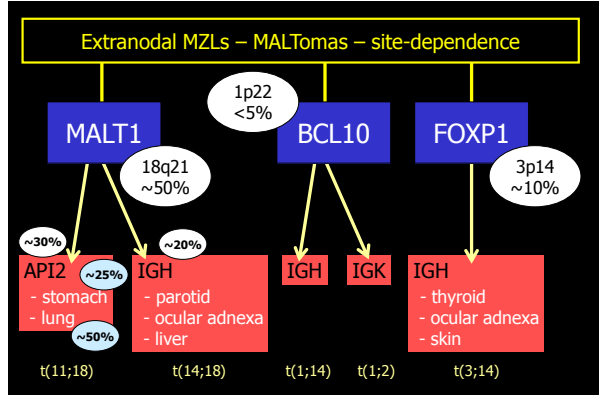
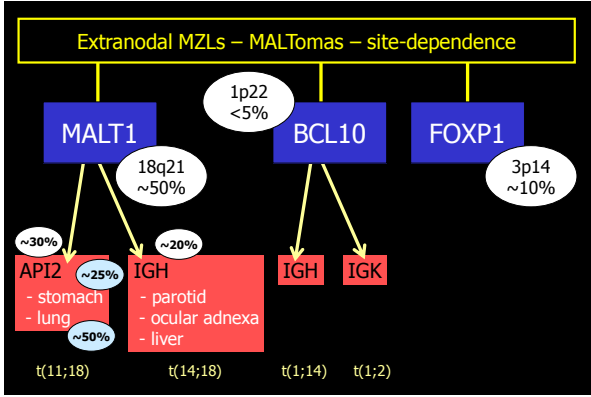
Morphology: small-to-medium sized, follicular colonization

Immunophen: non-specific monoclonal B-cells

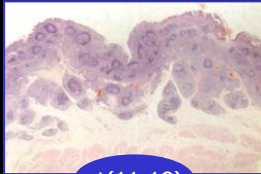
Course: generally indolent, can transform

Extranodal MZLs – MALTomas – site-dependence





Gastric MALToma



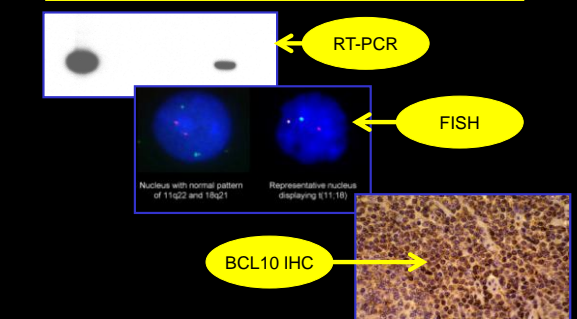
- API2/MALT1 chimera
- both involved in apoptosis regulation
- MALT1 involved with BCR/NFκB
- associated with (nuclear) BCL10+
 - as with t(1;14)
 - BCL10 and API2/MALT1 proteins interact

t(11;18)

• → H. Pylori independence

Therapeutic implication:
antibiotics no help

Testing for the t(11;18) translocation



RT-PCR

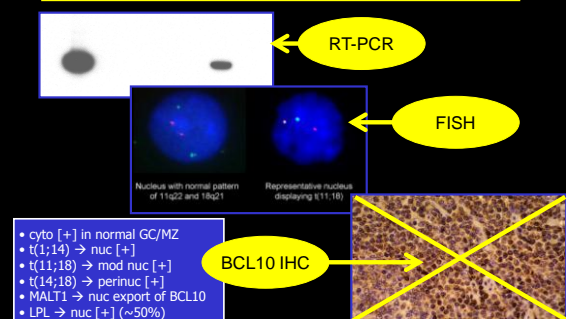
FISH

Nucleus with normal pattern of 11q22 and 18q21

Representative nucleus displaying t(11;18)

BCL10 IHC

Testing for the t(11;18) translocation



RT-PCR

FISH

Nucleus with normal pattern of 11q22 and 18q21

Representative nucleus displaying t(11;18)

BCL10 IHC

- cyto [+] in normal GC/MZ
- t(1;14) → nuc [+]
- t(11;18) → mod nuc [+]
- t(14;18) → perinuc [+]
- MALT1 → nuc export of BCL10
- LPL → nuc [+] (~50%)

