

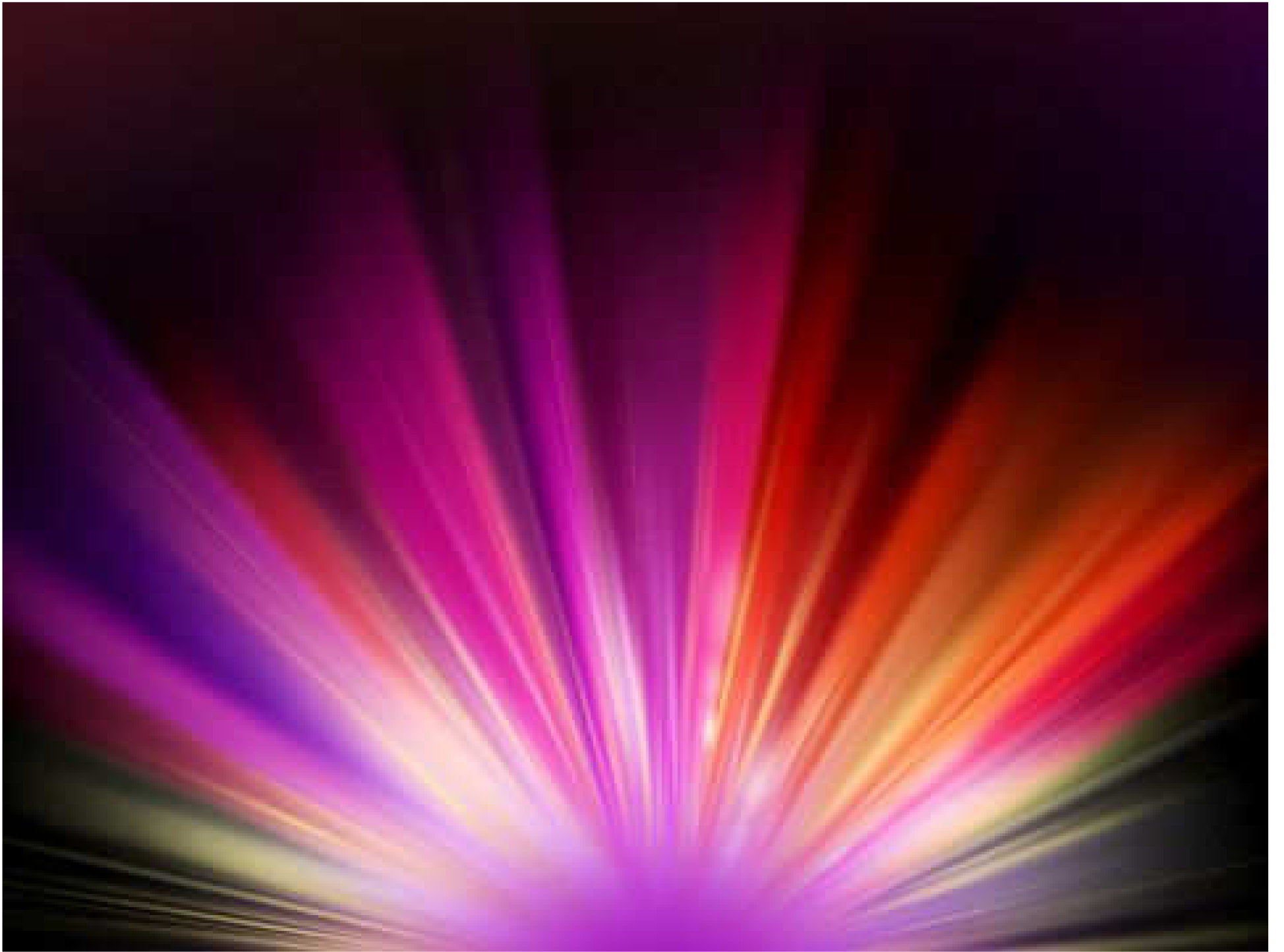


Semmelweis Symposium
2015

Molecular diagnostics of lymphoma

András Matolcsy

1st. Department of Pathology and Experimental Cancer Research
Semmelweis University
Budapest
Hungary



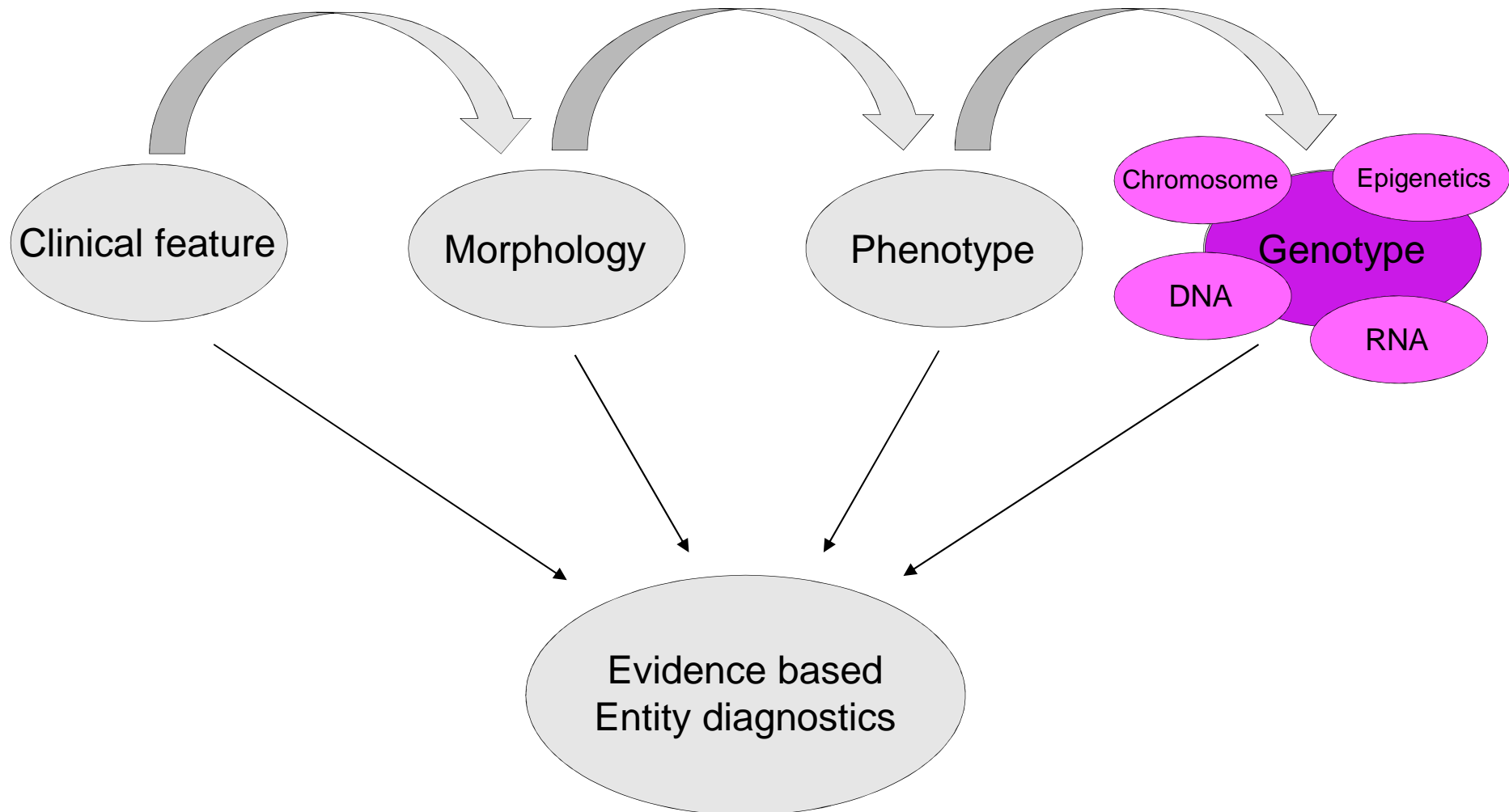








The place of molecular analysis in lymphoma diagnostics





The role of molecular analysis in lymphoma diagnostics

- Determine clonality of the lymphoid cells
- Determine subtype of lymphoma
- Determine prognosis of lymphoma
- Determine effectiveness of treatment (MRD)

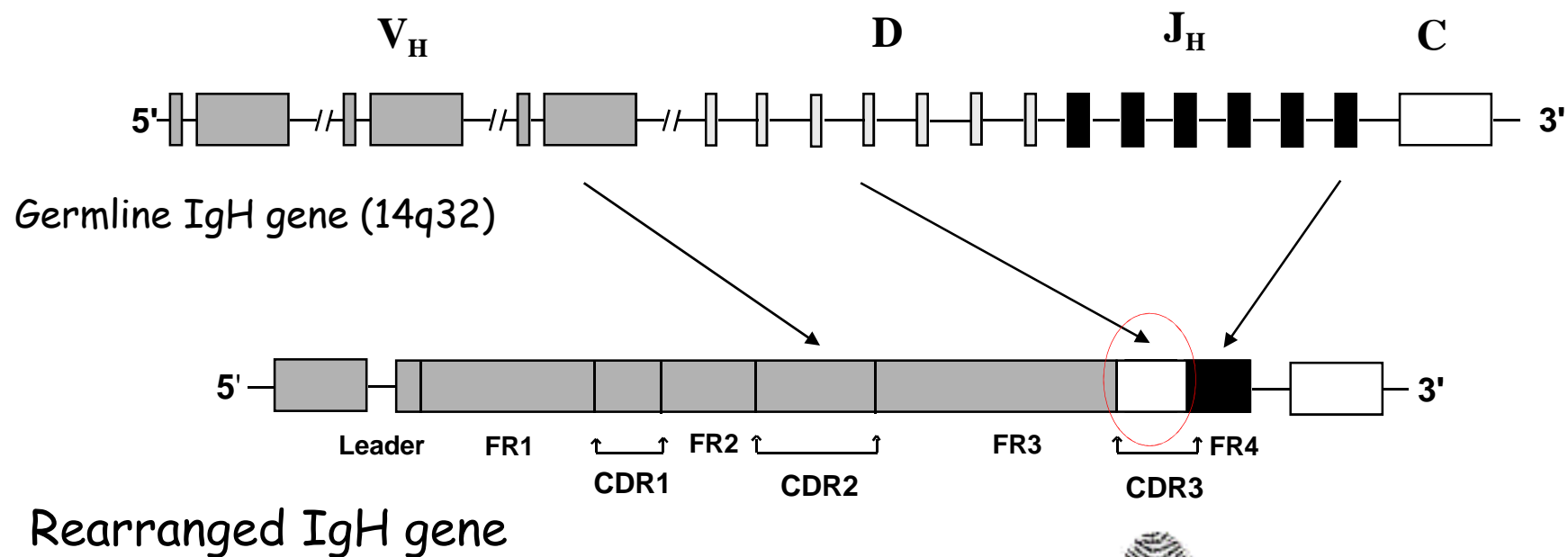


The role of molecular analysis in lymphoma diagnostics

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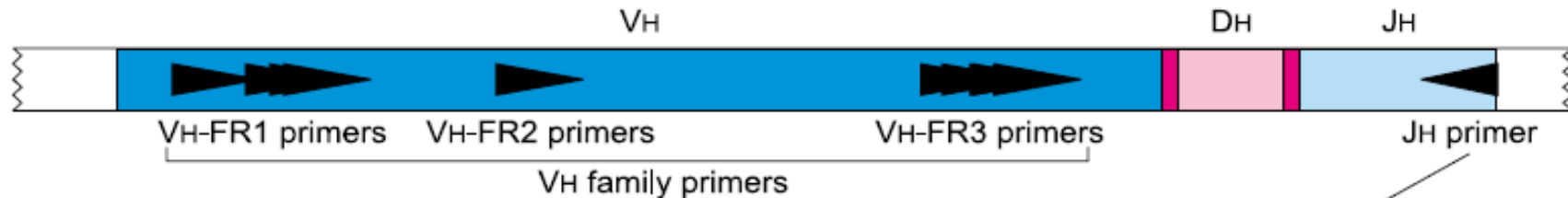


Immunoglobulin heavy chain (IgH) gene rearrangement





PCR analysis of IgH gene rearrangement (BIOMED 2)



IGH tube A

	5'	3'
VH1-FR1 (1-2) (-252)	GGCCTCAGTGAAGGTCTCCTGCAAG	
VH2-FR1 (2-5) (-284)	GTCTGGTCTACGCTGGTGAAACCC	
VH3-FR1 (3-7) (-256)	CTGGGGGGTCCCTGAGACTCTCCTG	
VH4-FR1 (4-4) (-256)	CTTCGGAGACCCTGTCCCTCACCTG	
VH5-FR1 (5-51) (-255)	CGGGGAGTCTCTGAAGATCTCCTGT	
VH6-FR1 (6-1) (-263)	TCGCAGACCCTCTCACTCACCTGTG	

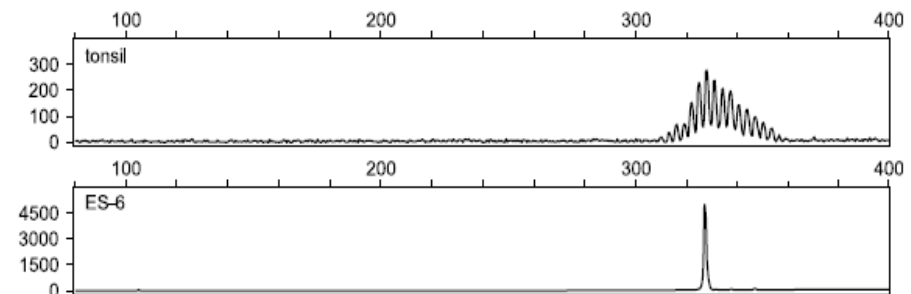
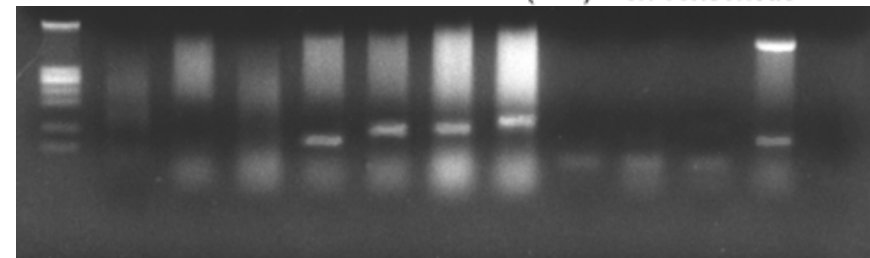
IGH tube B

VH1-FR2 (1-2) (-192)	CTGGGTGCGACAGGCCCTGGACAA
VH2-FR2 (2-5) (-190)	TGGATCCGTCAGCCCCAGGGAAGG
VH3-FR2 (3-7) (-189)	GGTCCGCCAGGCTCCAGGGAA
VH4-FR2 (4-4) (-188)	TGGATCCGCCAGCCCCAGGGAAGG
VH5-FR2 (5-51) (-190)	GGGTGCGCCAGATGCCCGGGAAAGG
VH6-FR2 (6-1) (-194)	TGGATCAGGCAGTCCCCATCGAGAG
VH7-FR2 (7) (-192)	TTGGGTGCGACAGGCCCTGGACAA

IGH tube C

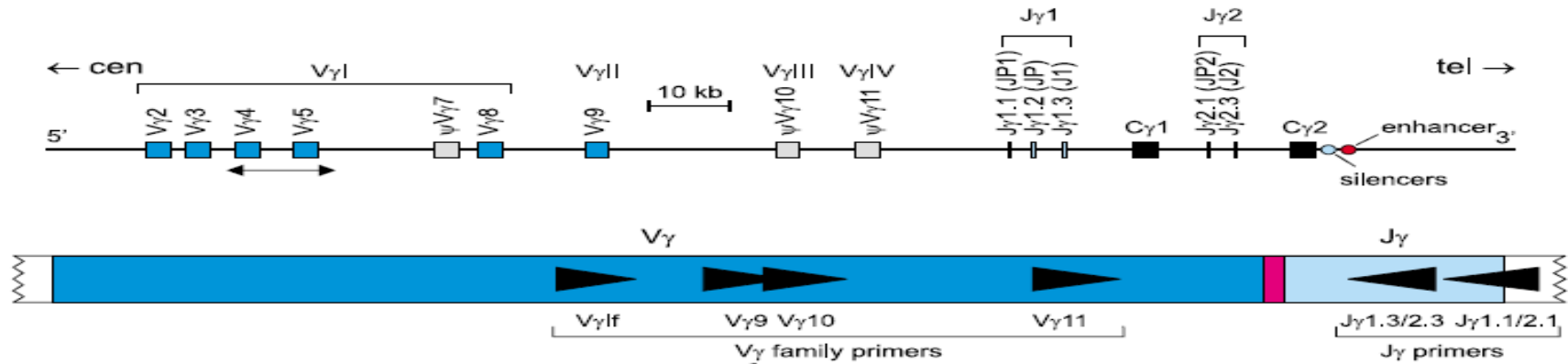
VH1-FR3 (1-2) (-55)	TGGAGCTGAGCAGCCTGAGATCTGA
VH2-FR3 (2-5) (-54)	CAATGACCAACATGGACCCTGTGGA
VH3-FR3 (3-7) (-57)	TCTGCAAATGAACAGCCTGAGAGCC
VH4-FR3 (4-4) (-48)	GAGCTCTGTGACCGCCGCGGACACG
VH5-FR3 (5-51) (-69)	CAGCACCGCCTACCTGCAGTGGAGC
VH6-FR3 (6-1) (-63)	GTTCTCCCTGCAGCTGAAGTCTGTG
VH7-FR3 (7) (-69)	CAGCACGGCATATCTGCAGATCAG

3' IGH tubes A, B, and C
5' CCAGTGGCAGAGGAGTCCATTC (+57) JH consensus





PCR analysis of TCR gene rearrangement (BIOMED 2)



TCRG tube A

V_γ1f (-178) 5' GGAAGGCCCCACAGCRTCTT 3'
 V_γ10 (-126) AGCATGGGTAAGACAAGCAA

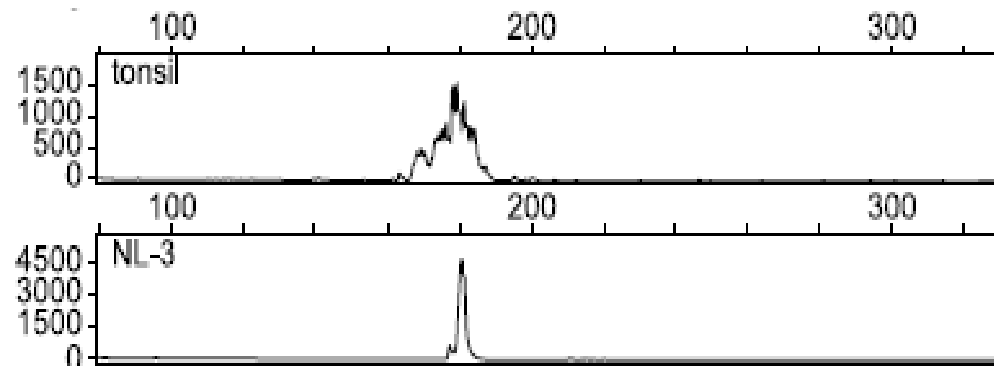
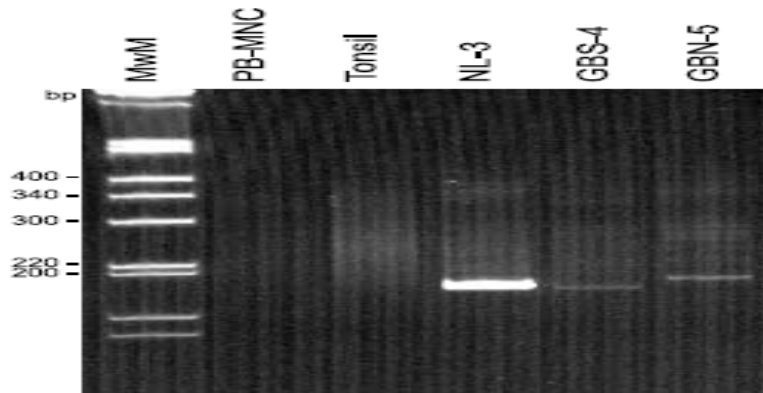
TCRG tube B

V_γ9 (-141) CGGCACTGTCAGAAAGGAATC
 V_γ11 (-58) CTTCCACTTCCACTTTGAAA

3' CGAGTATCATTGAAGCGGACCATT 5'
 GAGAAACCGTCACCTTGTGTG

TCRG tubes A and B

(+64) J_γ1.1/2.1 (JP1/2)
 (+38) J_γ1.3/2.3 (Jγ1/2)





Application of clonality analysis in lymphoma diagnostics

Targeted genes for clonality analysis

IgH, Igκ, Igλ
TCRβ, TCRγ, TCRδ

Application of Ig/TCR clonality analysis

Tumor versus reactive lesions

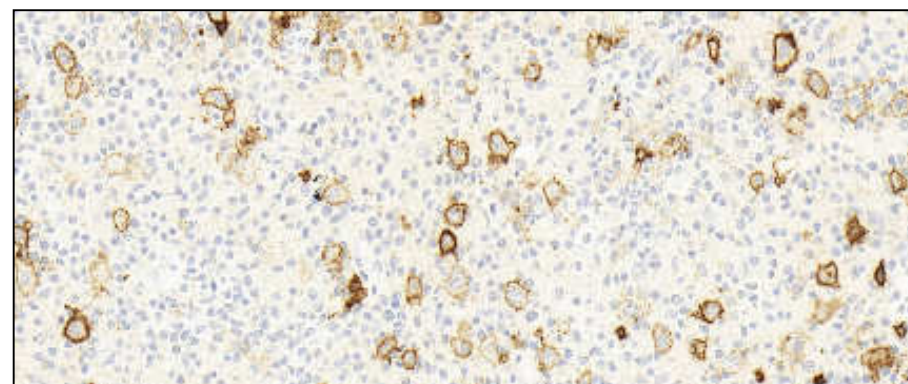
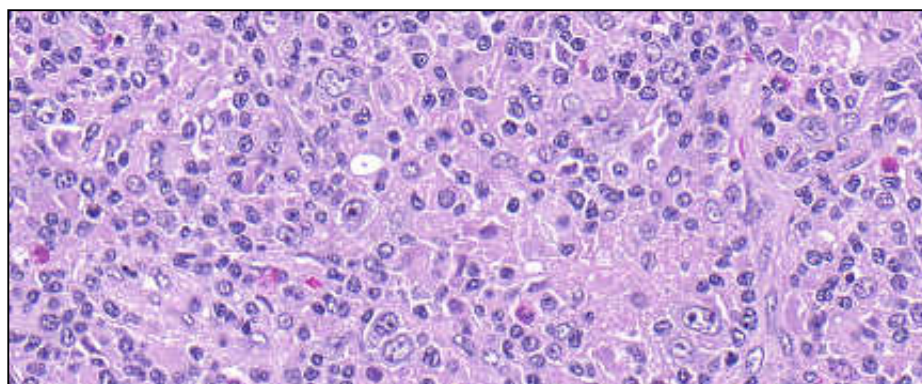
Lineage (B- versus T-cell) determination (ambiguous lineage)

Clonal relationship between different lesions

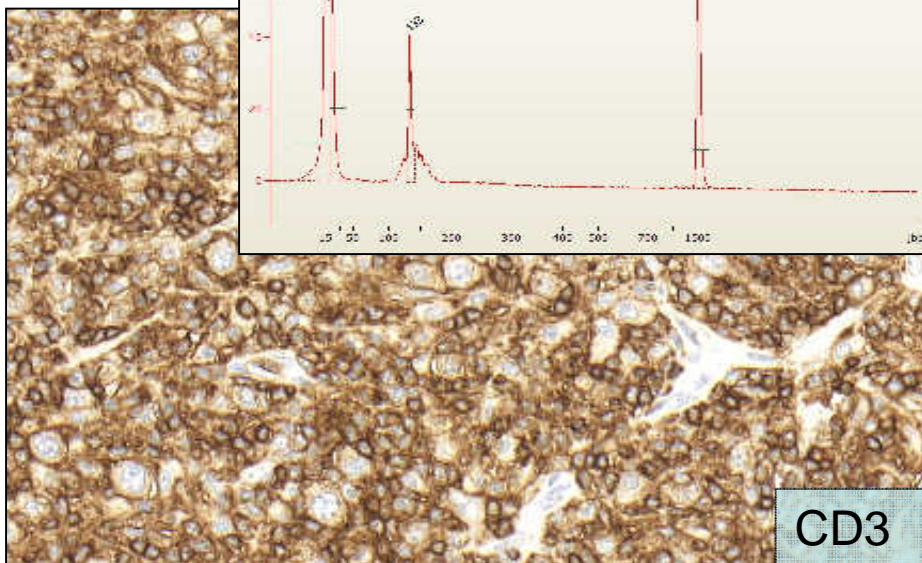
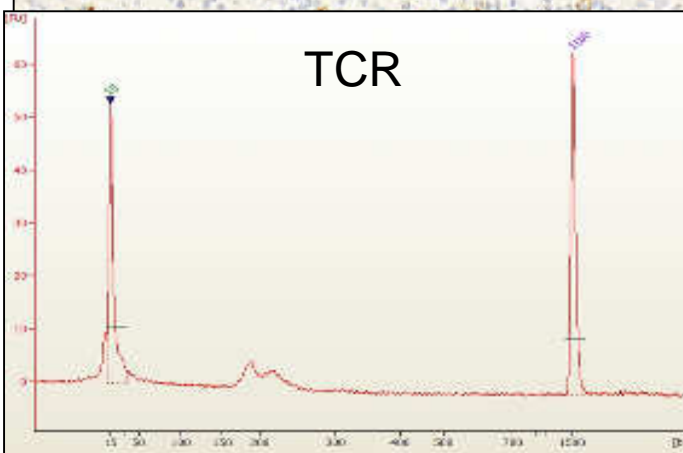
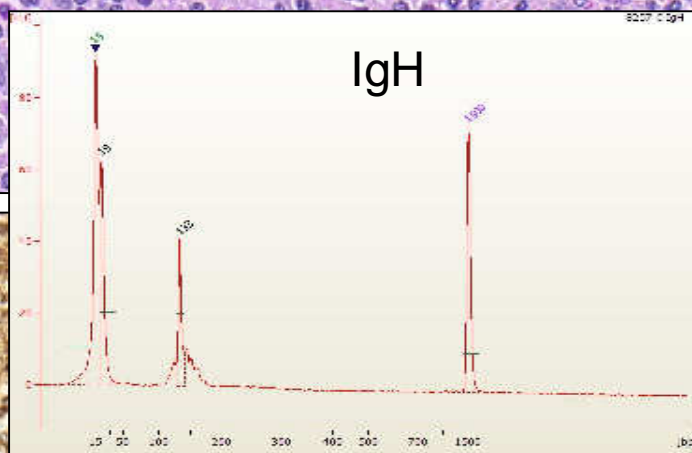
Clonal relationship between original and relapsed disease



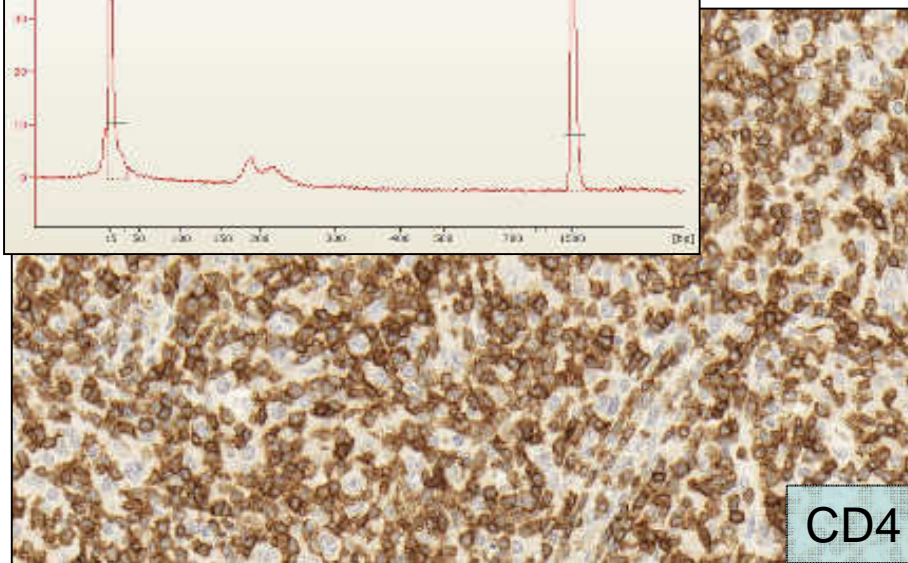
Clonality analysis of lymphoma with ambiguous lineage



CD20



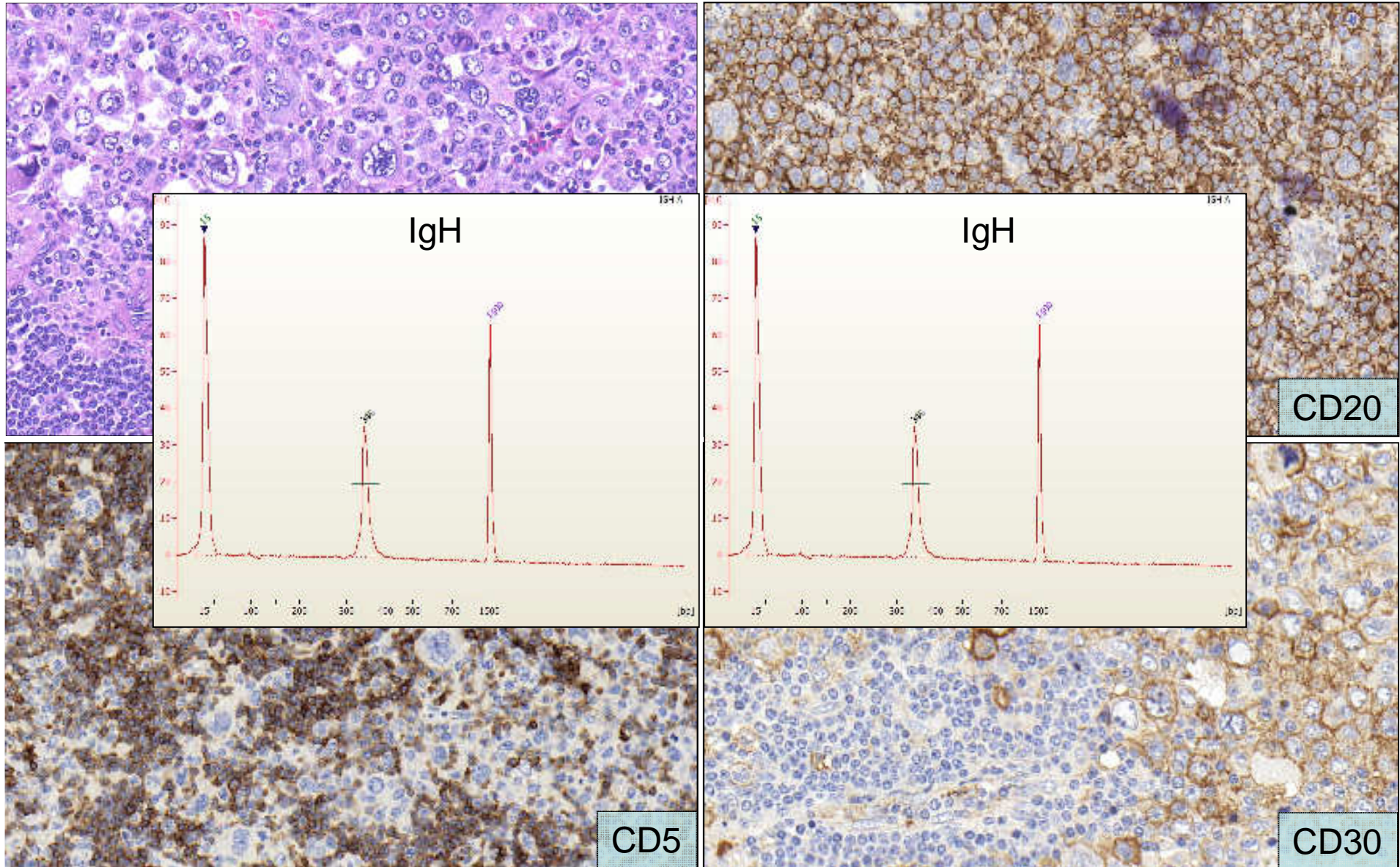
CD3



CD4



Clonality analysis on progressed CLL





Pitfalls of Ig/TCR clonality analysis

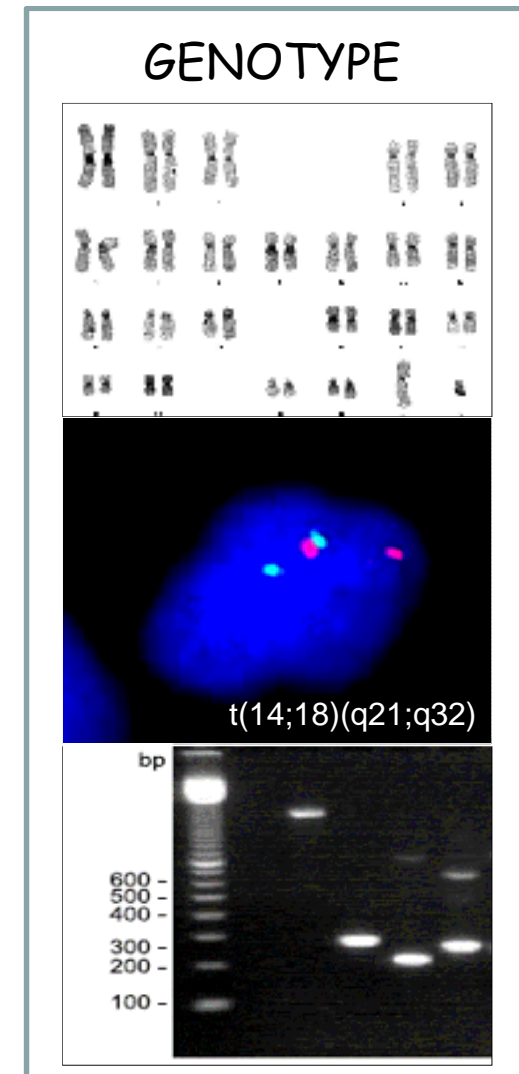
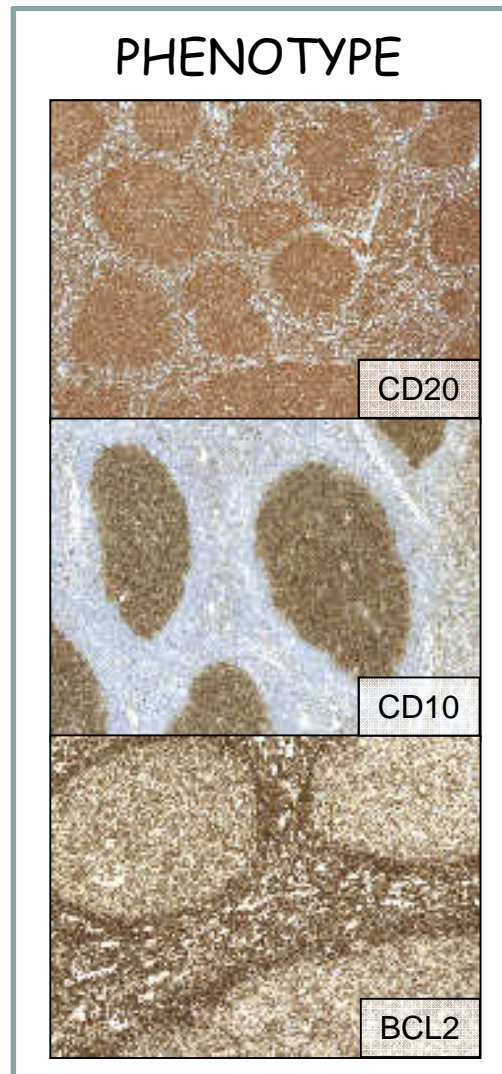
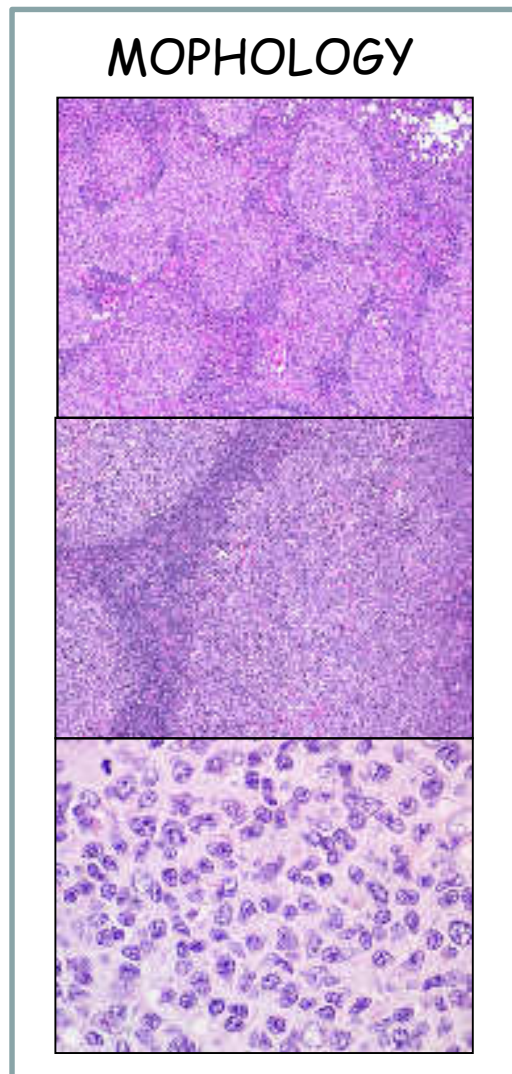
- Fals negativity: Few neoplastic B- or T-cells in sample
Poor DNA quality
Somatic hypermutation in antigen receptors of neoplastic cells
- Fals positivity: Oligoclonality in reactive lesions
Selective amplification of a reactive subclone



The role of molecular analysis in lymphoma diagnostics

- Determine clonality of the lymphoid cells
- **Determine subtype of lymphoma**
- Determine prognosis of lymphoma
- Determine effectiveness of treatment (MRD)

WHO classification of lymphomas (entity classification)



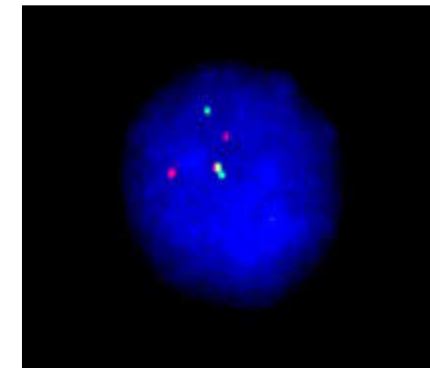
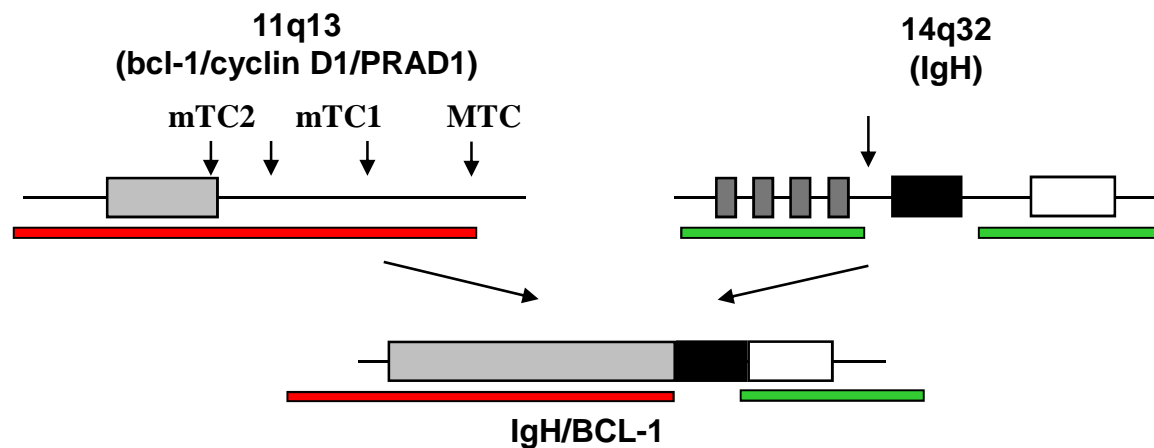
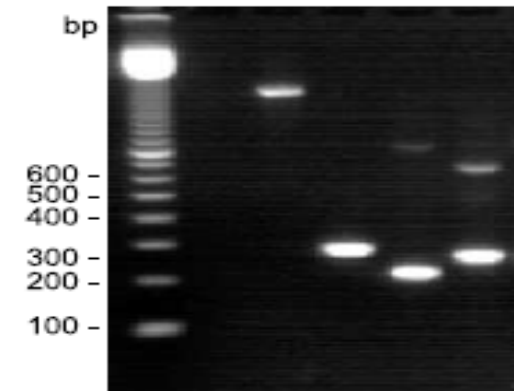
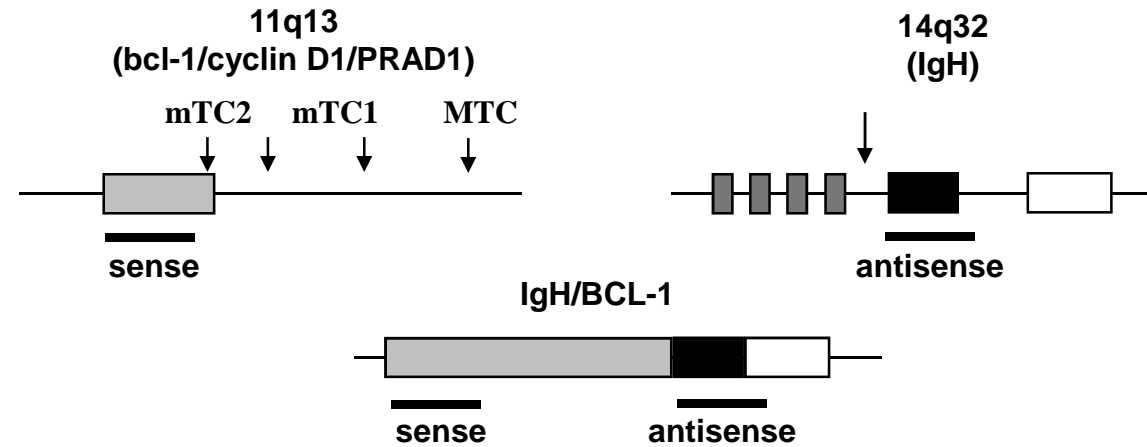


Reccurent genetic lesions in different lymphomas

Lymphoma	Translocation/mutation	Genes involved
Follicular lymphoma	t(14;18)(q21;q32)	<i>IgH/BCL2</i>
Mantle cell lymphoma	t(11;14)(q13;q32)	<i>CCND1/IgH</i>
Burkitt lymphoma	t(8;14)(q24;q32)	<i>cMYC/IgH</i>
	t(2;8)(p11;q24)	<i>IgK/cMYC</i>
	t(8;22)(q24;q11)	<i>cMYC/IgL</i>
Anaplastic large cell lymphoma	t(2;5)(p23;q35)	<i>ALK1/NPM</i>
	t(1;2)(q25;p23)	<i>TPM3/ALK1</i>
	inv(2)(p23;q35)	<i>ALK1/ATIC</i>
	t(2;3)(p23;q21)	<i>ALK1/TFG</i>
	t(2;17)(p23;q23)	<i>ALK1/CLTC</i>
	t(2;X)(p23;q11)	<i>ALK1/MSN</i>
	t(2;19)(p23;p13)	<i>ALK1TPM4</i>
Gastric MALT lymphoma	t(11;18)(q21;q22)	<i>API2/MLT1</i>
Diffuse large B-cell lymphoma	t(14;18)(q21;q32)	<i>IgH/BCL2</i>
	t(8;14)(q24;q32)	<i>cMYC/IgH</i>
	3q27	<i>BCL6</i>
Hairy cell leukaemia	7q34	<i>BRAF (V600G)</i>



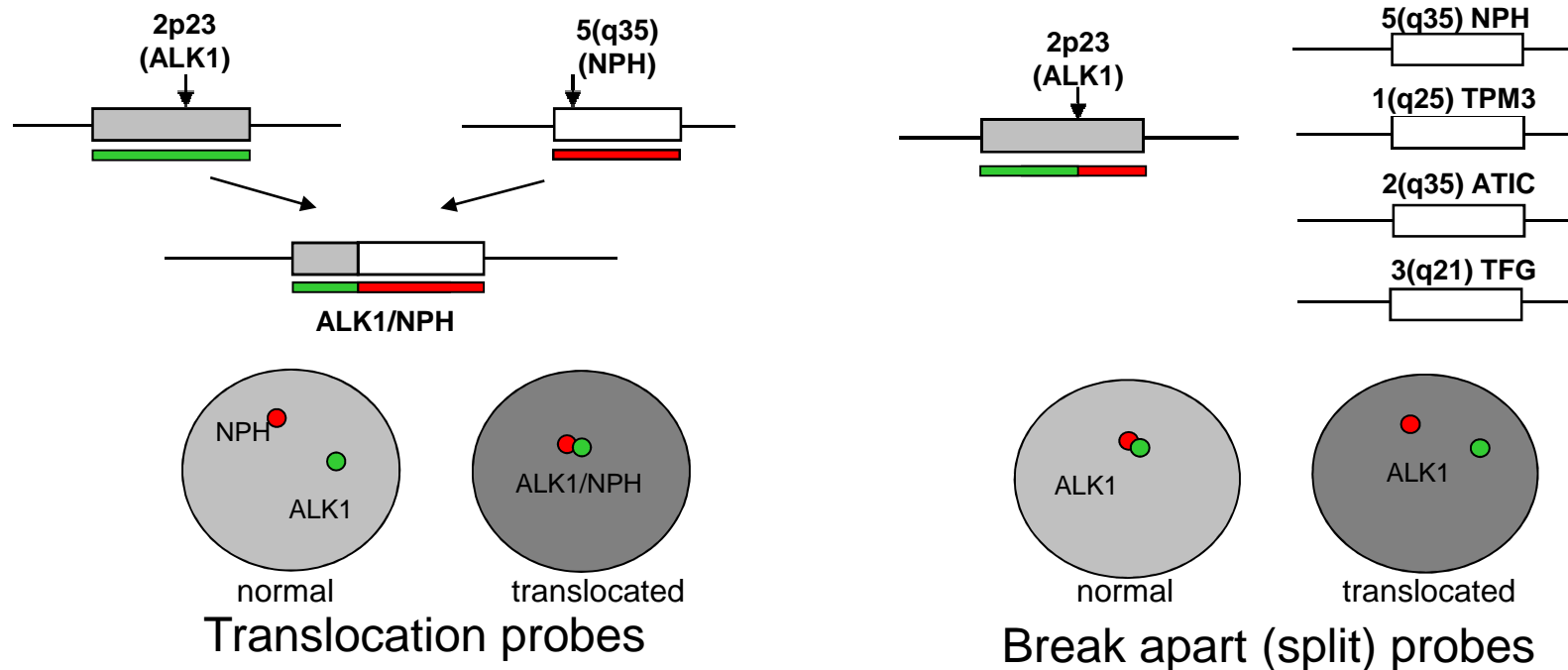
Detection of recurrent genetic lesions in lymphomas





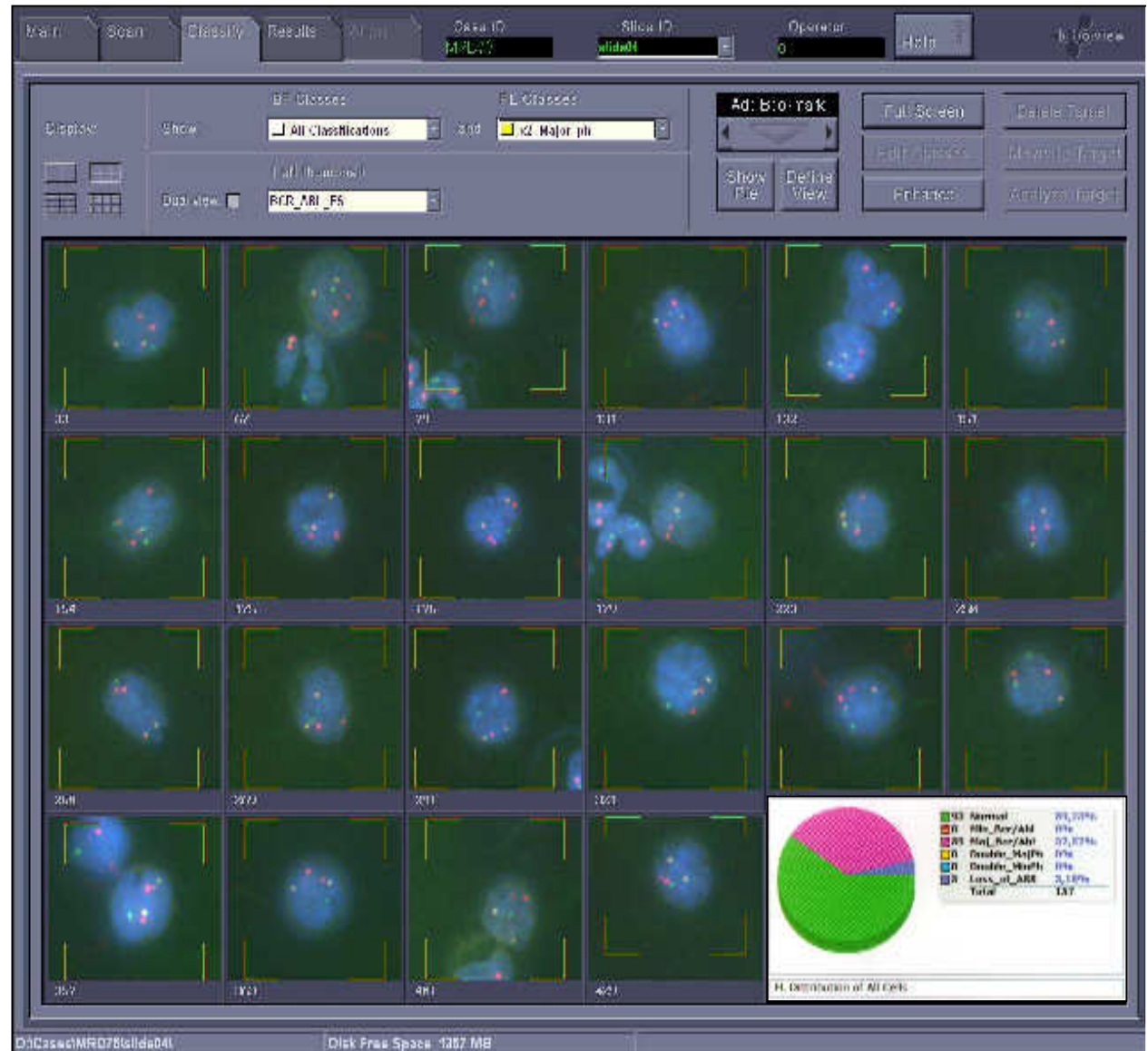
FISH strategies for detection of genetic lesions in lymphomas

Lymphoma	Translocation/mutation	Genes involved
Anaplastic large cell lymphoma	t(2;5)(p23;q35)	<i>ALK1/NPM</i>
	t(1;2)(q25;p23)	<i>TPM3/ALK1</i>
	inv(2)(p23;q35)	<i>ALK1/ATIC</i>
	t(2;3)(p23;q21)	<i>ALK1/TFG</i>
	t(2;17)(p23;q23)	<i>ALK1/CLTC</i>
	t(2;X)(p23;q11)	<i>ALK1/MSN</i>
	t(2;19)(p23;p13)	<i>ALK1/TPM4</i>





Automated FISH analysis



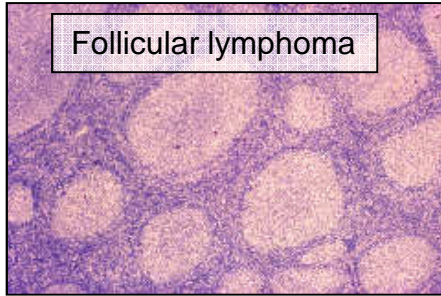


Reccurent genetic lesions with oncoprotein overexpression

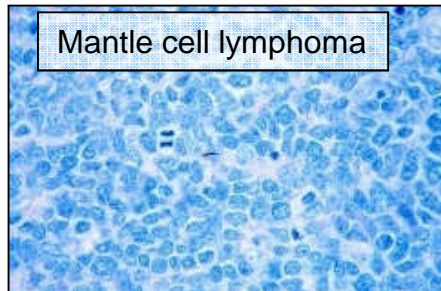
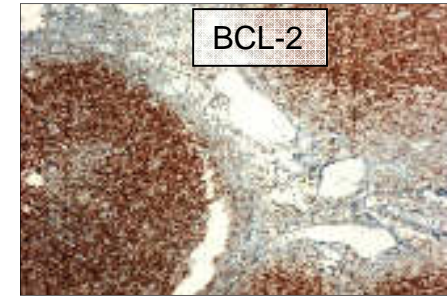
Lymphoma	Translocation/mutation	Genes involved
Follicular lymphoma	t(14;18)(q21;q32)	<i>IgH/BCL2</i>
Mantle cell lymphoma	t(11;14)(q13;q32)	<i>CCND1/IgH</i>
Burkitt lymphoma	t(8;14)(q24;q32)	<i>cMYC/IgH</i>
	t(2;8)(p11;q24)	<i>IgK/cMYC</i>
	t(8;22)(q24;q11)	<i>cMYC/IgL</i>
Anaplastic large cell lymphoma	t(2;5)(p23;q35)	<i>ALK1/NPM</i>
	t(1;2)(q25;p23)	<i>TPM3/ALK1</i>
	inv(2)(p23;q35)	<i>ALK1/ATIC</i>
	t(2;3)(p23;q21)	<i>ALK1/TFG</i>
	t(2;17)(p23;q23)	<i>ALK1/CLTC</i>
	t(2;X)(p23;q11)	<i>ALK1/MSN</i>
	t(2;19)(p23;p13)	<i>ALK1TPM4</i>
Gastric MALT lymphoma	t(11;18)(q21;q22)	<i>API2/MLT1</i>
Diffuse large B-cell lymphoma	t(14;18)(q21;q32)	<i>IgH/BCL2</i>
	t(8;14)(q24;q32)	<i>cMYC/IgH</i>
	3q27	<i>BCL6</i>
Hairy cell leukaemia	7q34	<i>BRAF (V600G)</i>



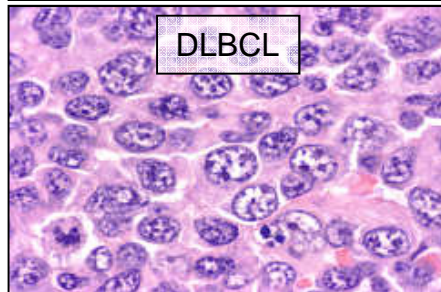
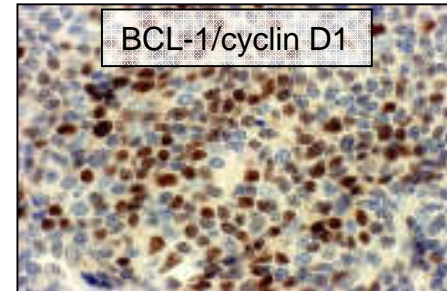
Genetic alterations with oncoprotein overexpression



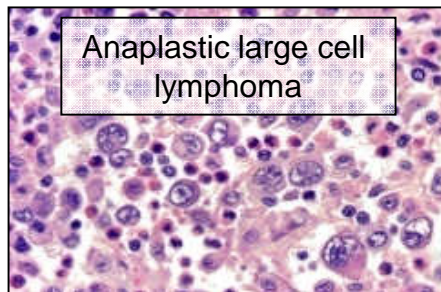
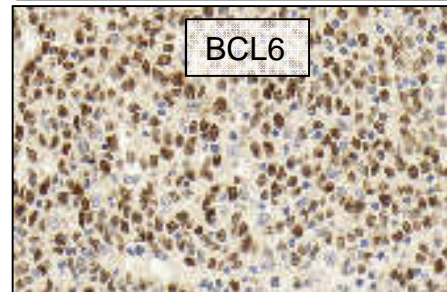
t(14;18)(q21;q32)
IgH/BCL2



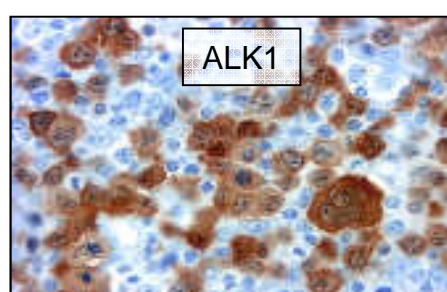
t(11;14)(q13;q32)
CCND1/IgH



t(3q27; v)
v/BCL6

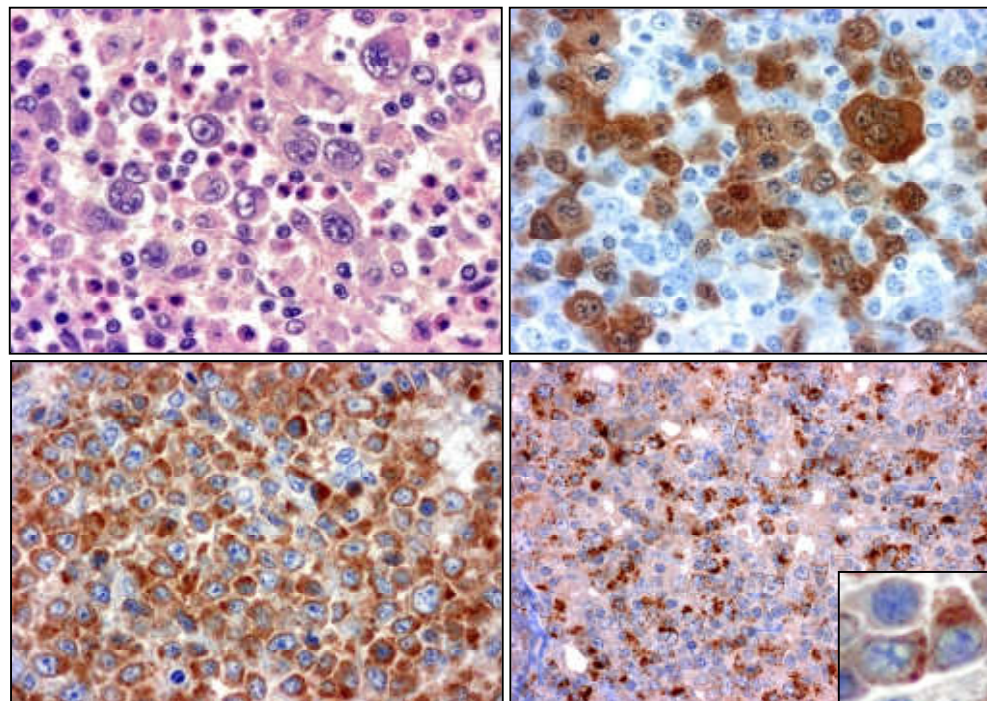


t(2;5)(p23;q35)
ALK1/NPM



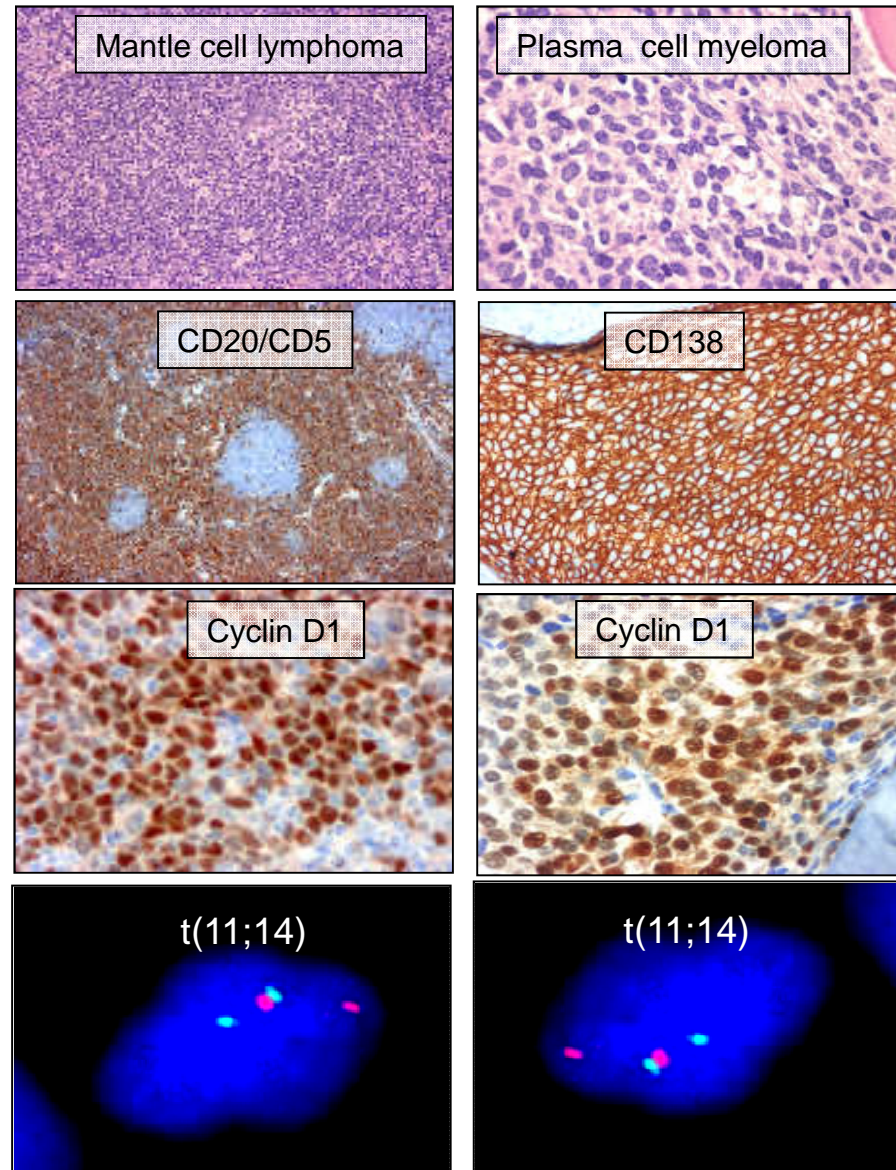
Patterns of ALK1 positivity in anaplastic large cell lymphoma

Translocation/mutation	Genes involved	ALK1 positivity
t(2;5)(p23;q35)	<i>ALK1/NPM</i>	nucleus, cytoplasm
t(1;2)(q25;p23)	<i>TPM3/ALK1</i>	cytoplasm
inv(2)(p23;q35)	<i>ALK1/ATIC</i>	cytoplasm
t(2;3)(p23;q21)	<i>ALK1/TFG</i>	cytoplasm
t(2;17)(p23;q23)	<i>ALK1/CLTC</i>	cytoplasm (granular)
t(2;X)(p23;q11)	<i>ALK1/MSN</i>	membrane
t(2;19)(p23;p13)	<i>ALK1TPM4</i>	cytoplasm



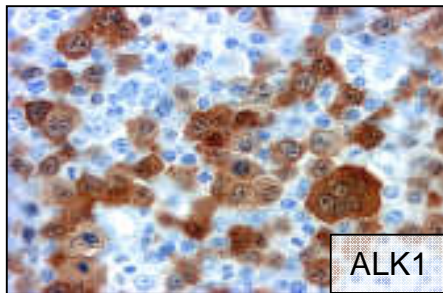


Overlapping oncoprotein overexpression in different lymphomas

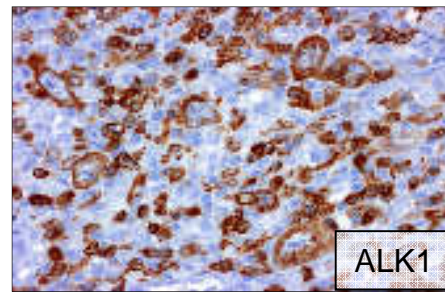




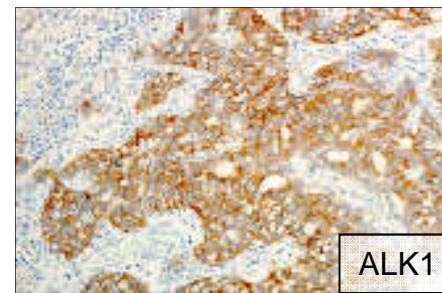
Overlapping oncoprotein overexpression in different neoplasms



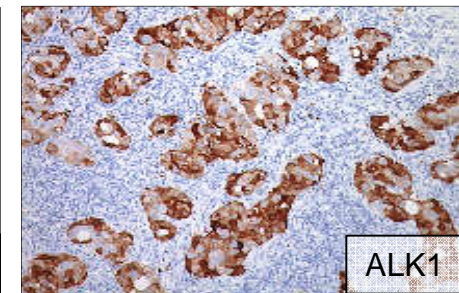
Anaplastic large cell lymphoma



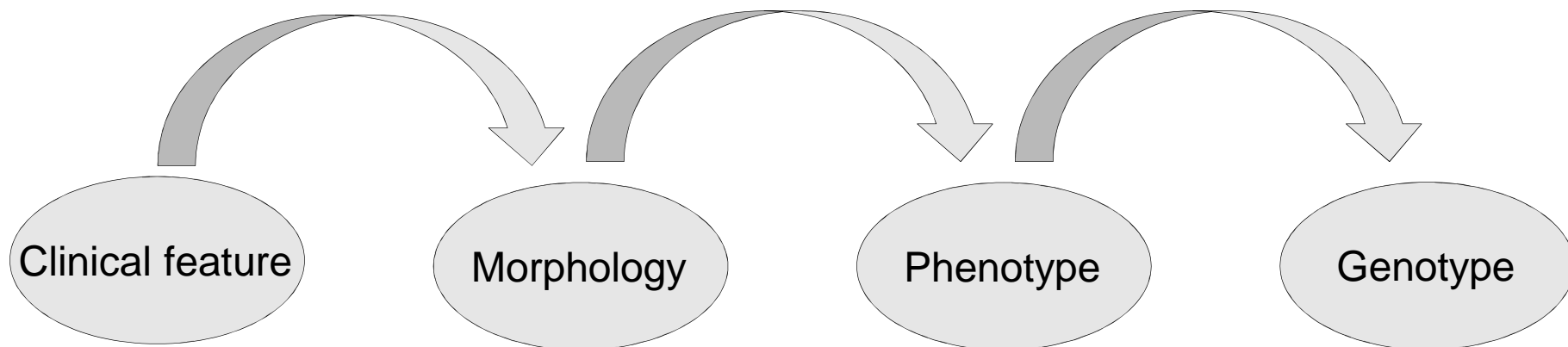
Diffuse large B cell lymphoma



Lung cancer



Gastric cancer



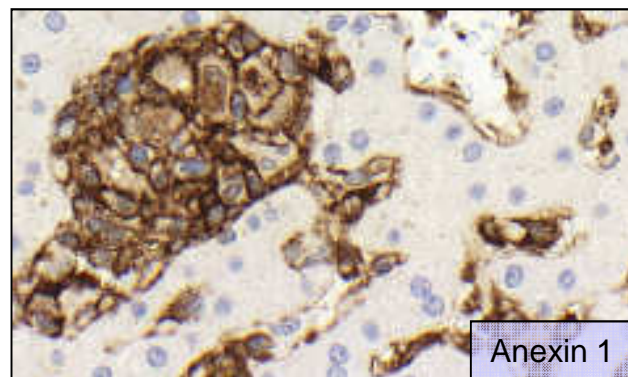
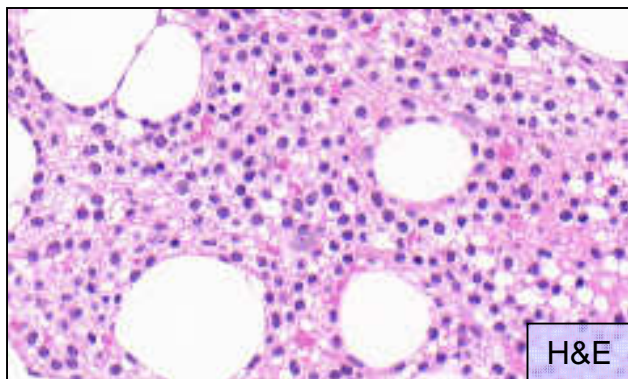
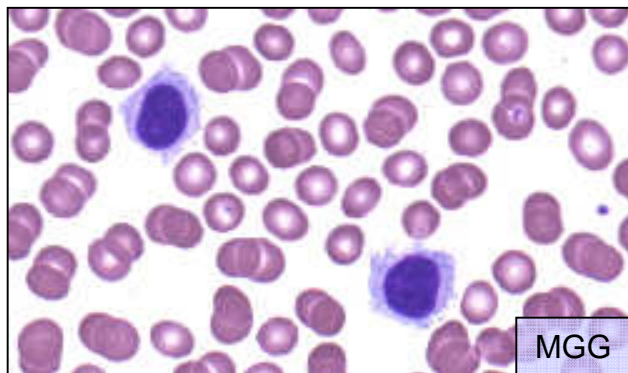


Reccurent genetic lesions in different lymphoma entities

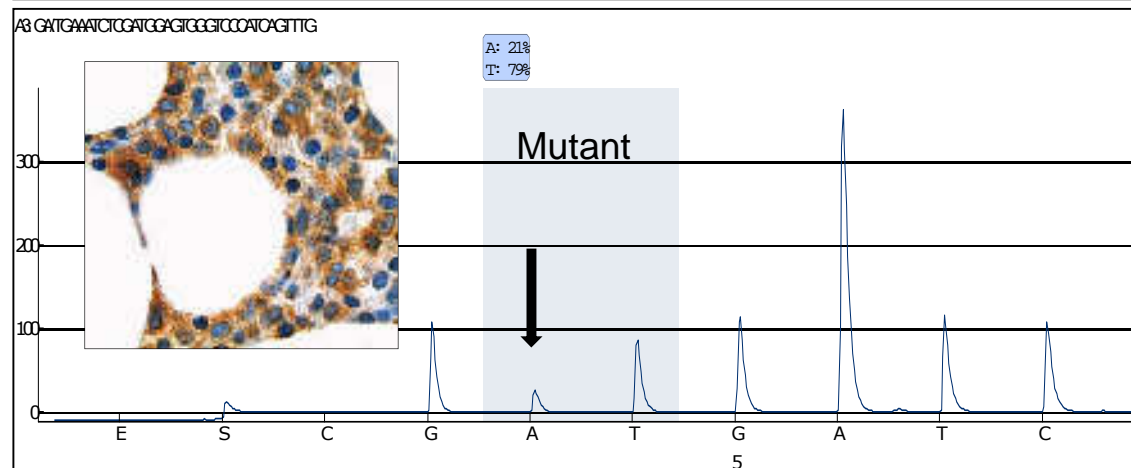
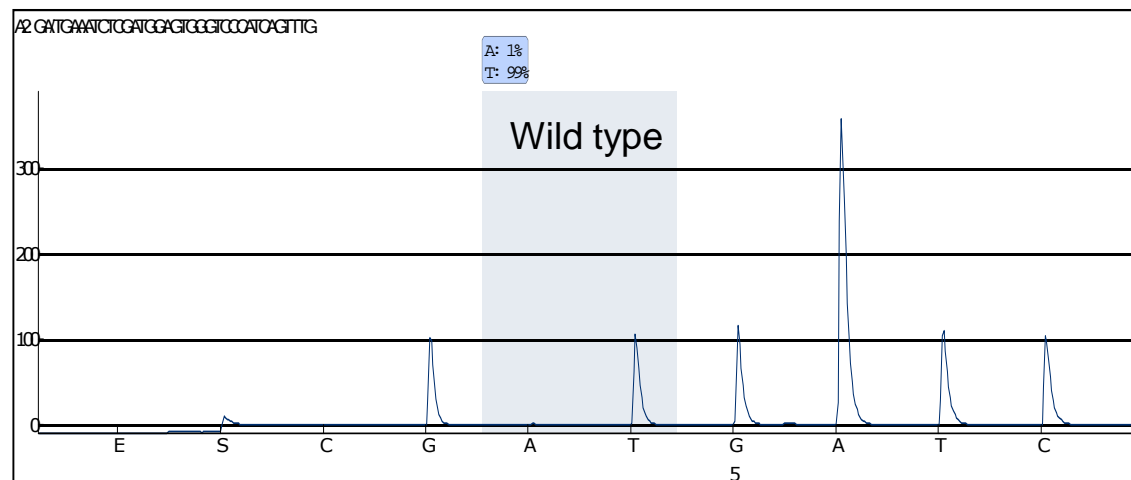
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Mantle cell mymphoma	t(11;14)q13;q32)	<i>CCND1/IgH</i>
Burkitt lymphoma	t(8;14)(q24;q32)	<i>cMYC/IgH</i>
	t(2;8)(p11;q24)	<i>IgK/cMYC</i>
	t(8;22)(q24;q11)	<i>cMYC/IgL</i>
Anaplastic large cell lymphoma	t(2;5)(p23;q35)	<i>ALK1/NPM</i>
	t(1;2)(q25;p23)	<i>TPM3/ALK1</i>
	inv(2)(p23;q35)	<i>ALK1/ATIC</i>
	t(2;3)(p23;q21)	<i>ALK1/TFG</i>
	t(2;17)(p23;q23)	<i>ALK1/CLTC</i>
	t(2;X)(p23;q11)	<i>ALK1/MSN</i>
	t(2;19)(p23;p13)	<i>ALK1TPM4</i>
Gastric MALT lymphoma	t(11;18)(q21;q22)	<i>API2/MLT1</i>
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	t(8;14)(q24;q32)	<i>cMYC/IgH</i>
	3q27	<i>BCL6</i>
Hairy cell leukaemia	7q34	<i>BRAF (V600G)</i>



Molecular diagnostics of hairy cell leukaemia

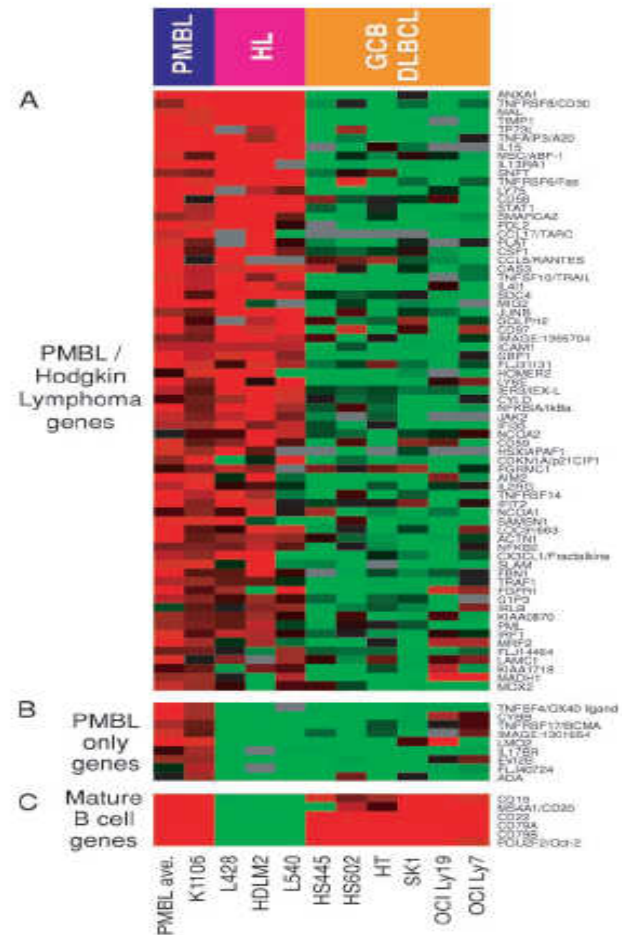
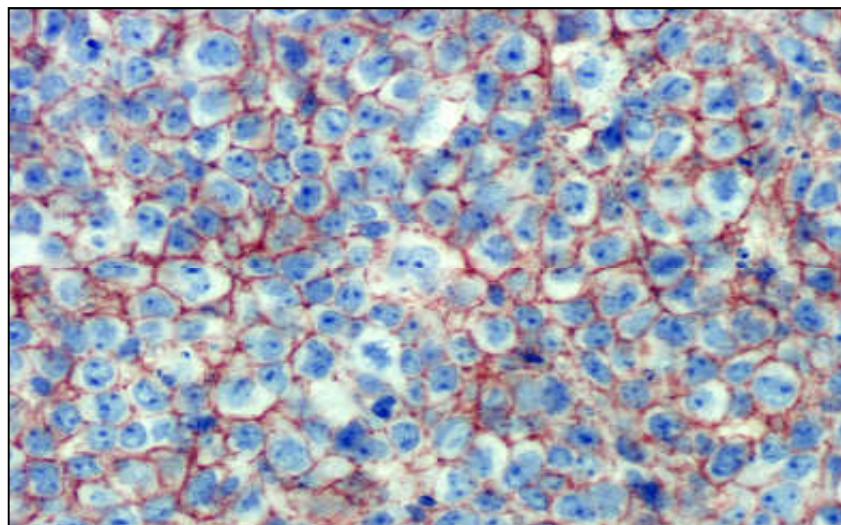
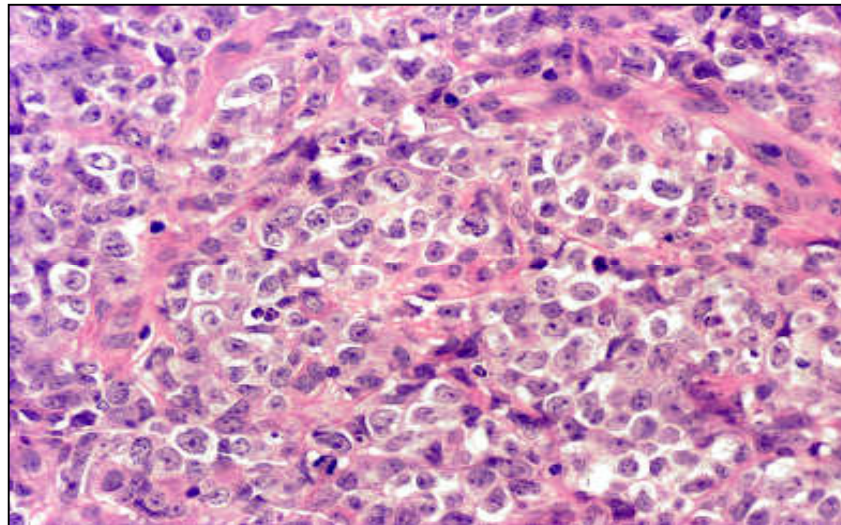


BRAF G600V



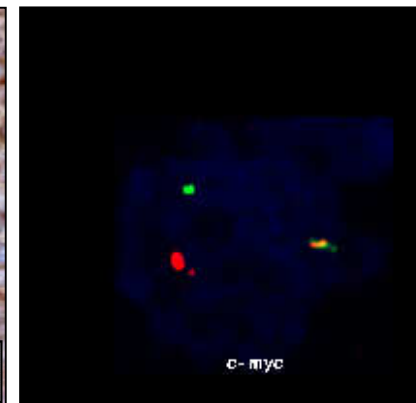
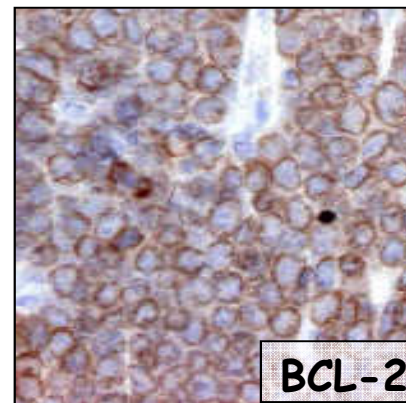
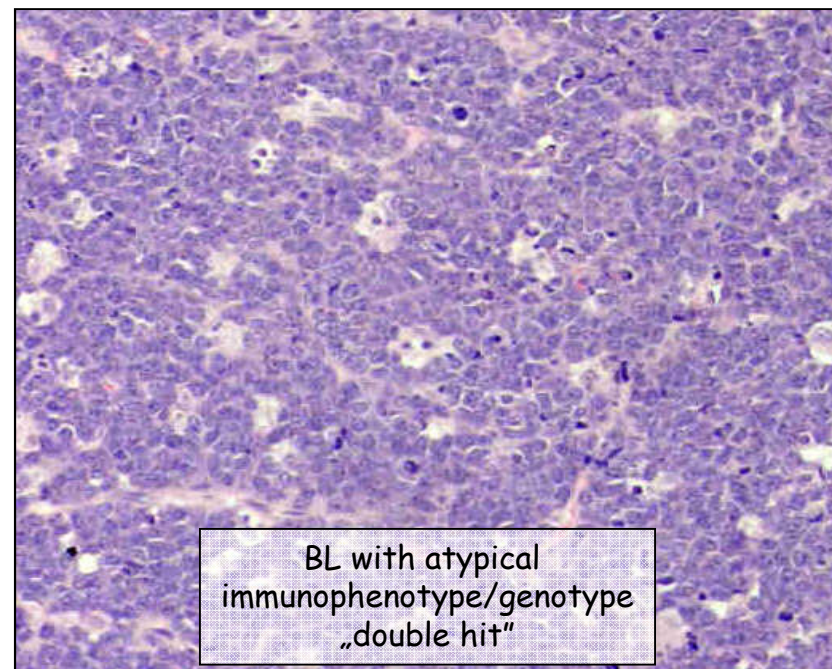
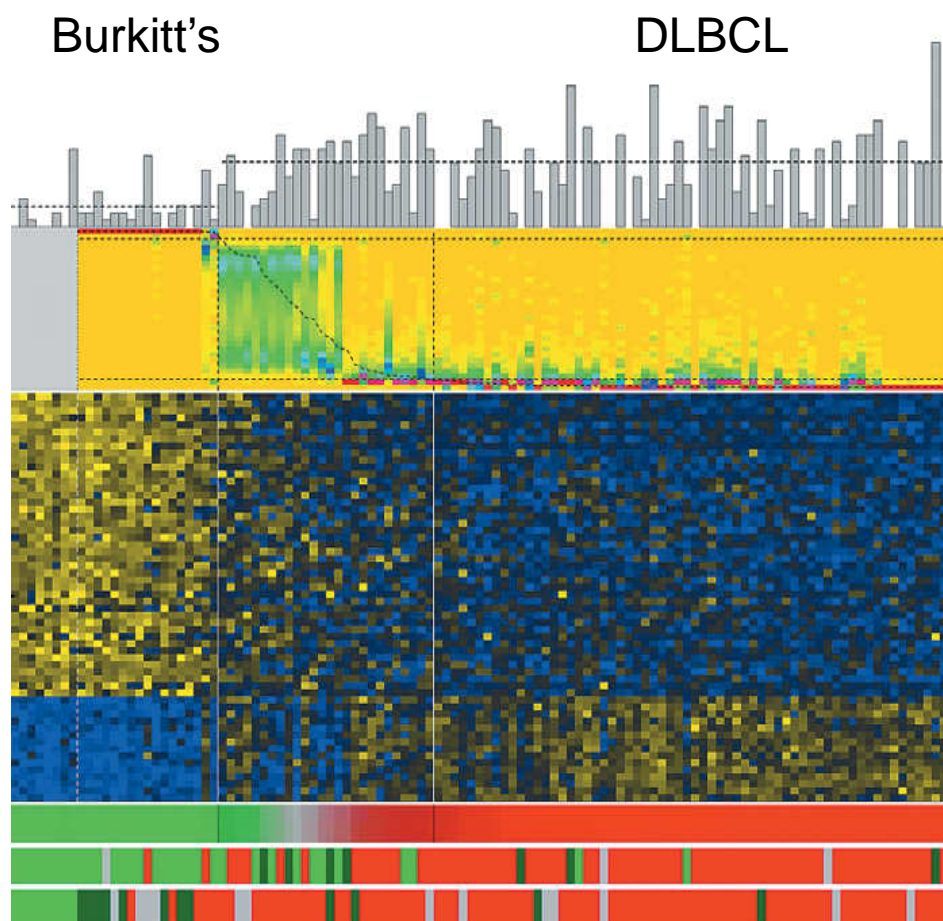


Expressional profile analysis of primary mediastinal large B-cell lymphomas





Expressional profile analysis of Burkitt's lymphoma





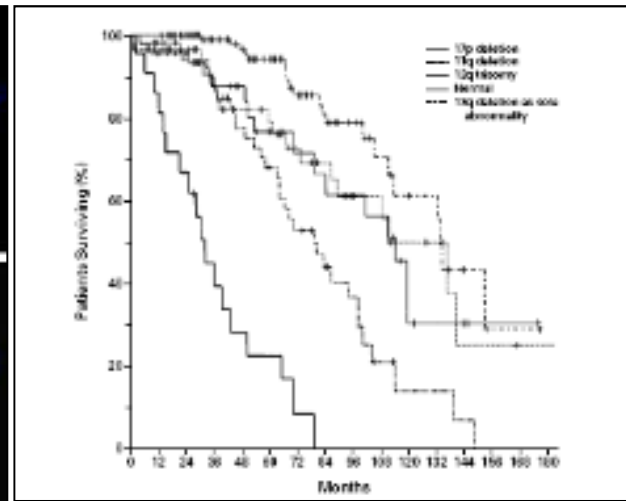
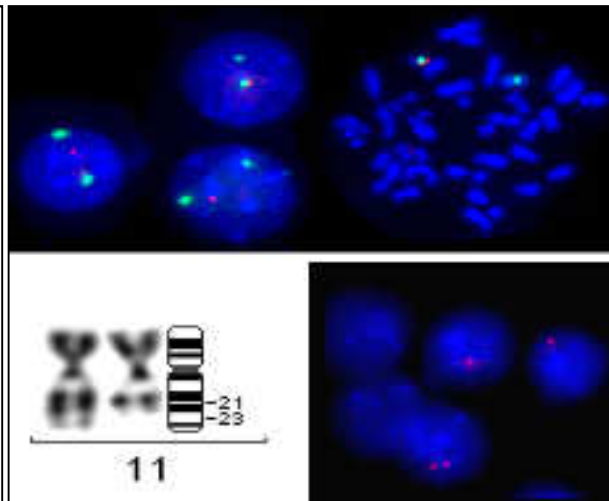
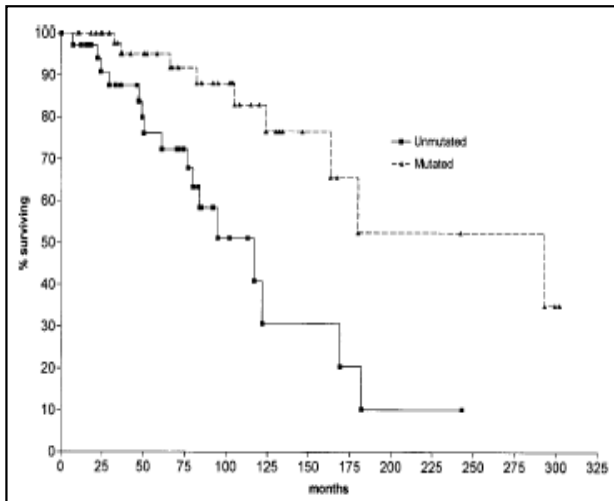
The role of molecular analysis in lymphoma diagnostics

- Determine clonality of the lymphoid cells
- Determine subtype of lymphoma
- **Determine prognosis of lymphoma**
- Determine effectiveness of treatment (MRD)



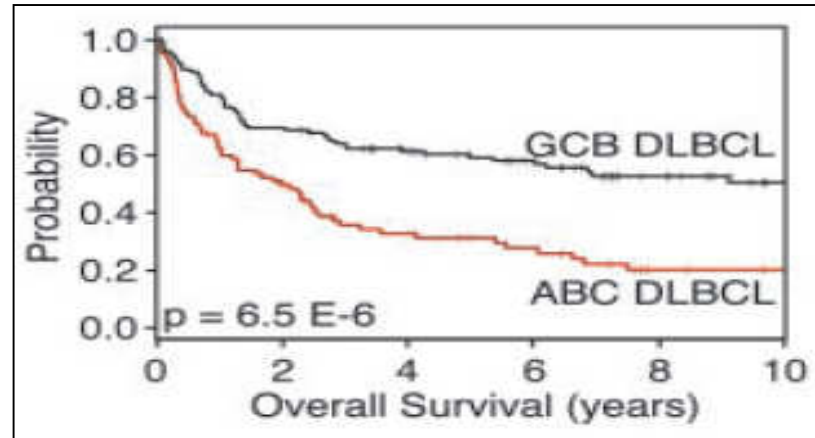
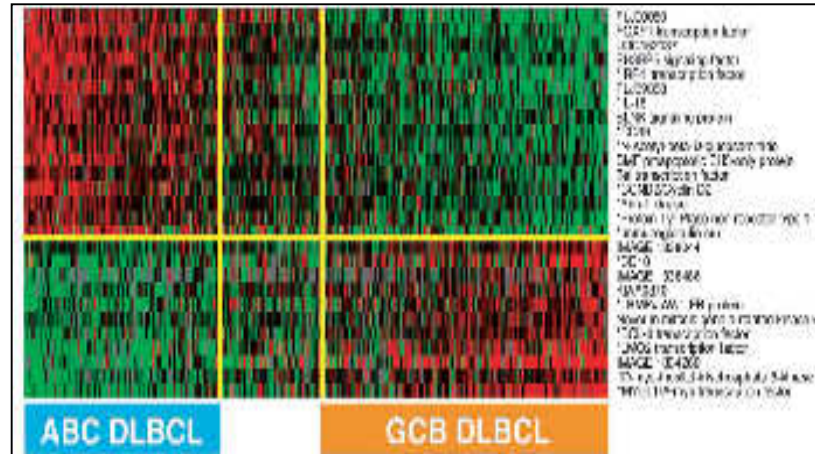
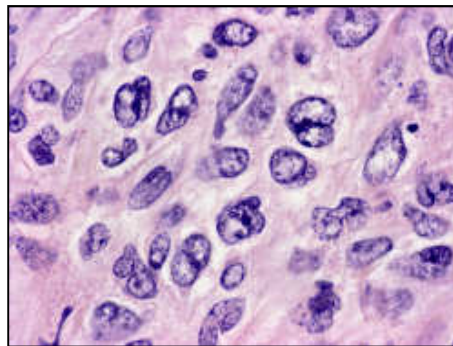
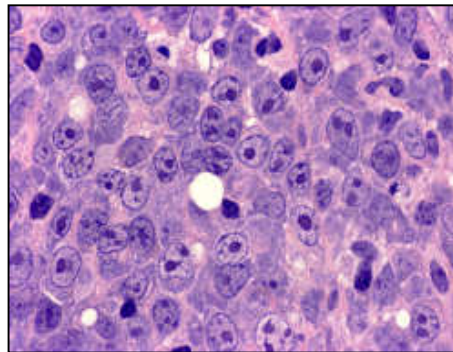
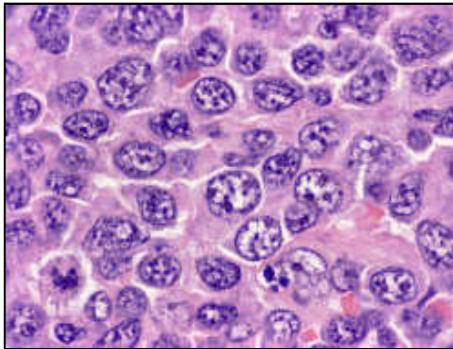
Prognostic groups of chronic lymphocytic leukaemia/lymphocytic lymphoma

		CDR1		CDR2	
VH251 Case 1	EVQLVQSGAEVKKPGESLKISCKGSGYSFT	SYWIG	WVRQMPGKGLEWVG	IIYPGDS DTRYSPSFQG	QVTISADKSISTAYLQWSSLKASDTAMYYCAR
DP-42 Case 2	EVQLVETGGGLIQPGGSLRLSCAASGFTVS	SNYMS	WVRQAPGKGLEWVS	VIY-SGGSTYYADSVKG	RFTISRDN SKNTLYLQMN SLRAEDTAVYYCAR
DP-14 Case 3	QVQLVQSGAEVKKPGASVKV SCKASGYTFTT.....	SNGIS .Y...	WVRQAPGQGLEWVG	WISAYNGNTNYAQKLQG ...T..D.....F..	RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR
VH4.21 Case 4	QVQLQQWGAGLLKPSETLSLTCAVYGGSF SGF..A....	GYYS	WIRQPPGKGLEWIG	EII-HSGSTNYNPSLKS ..N-.....	RVTISVDTSKNQFSLKLSVTAADTAVYYCAR ...V.....K.L.....N.....
VH26 Case 5	EVQLLES GGLVQPGGSLRLSCAASGFTFSV.....K	SYAMS N....	WVRQAPGKGLEWVS	AISGGSGSTYYADSVKG G.GA..FQ.F.P..LR.	RFTISRDN SKNTLYLQMN SLRAEDTAVYYCAKTR...S..V.....M...V.

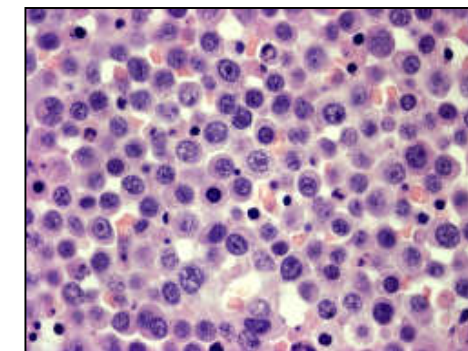
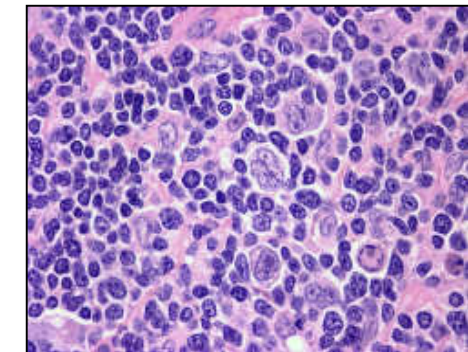
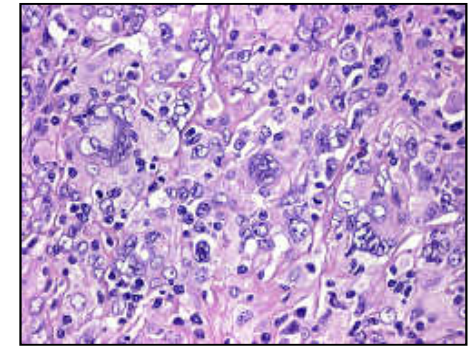




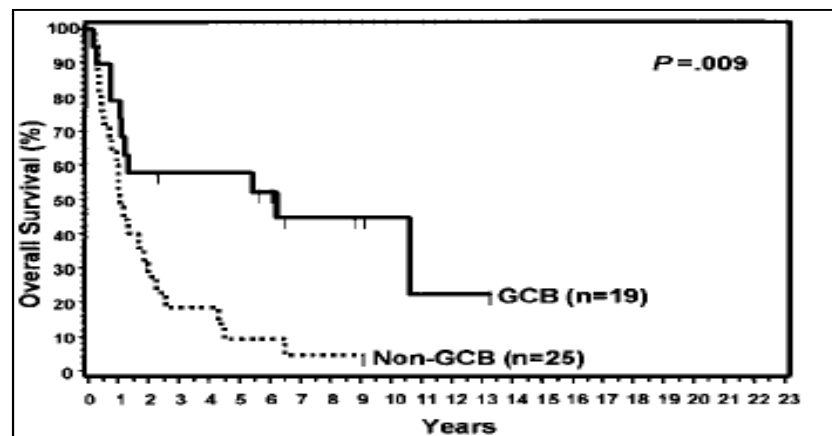
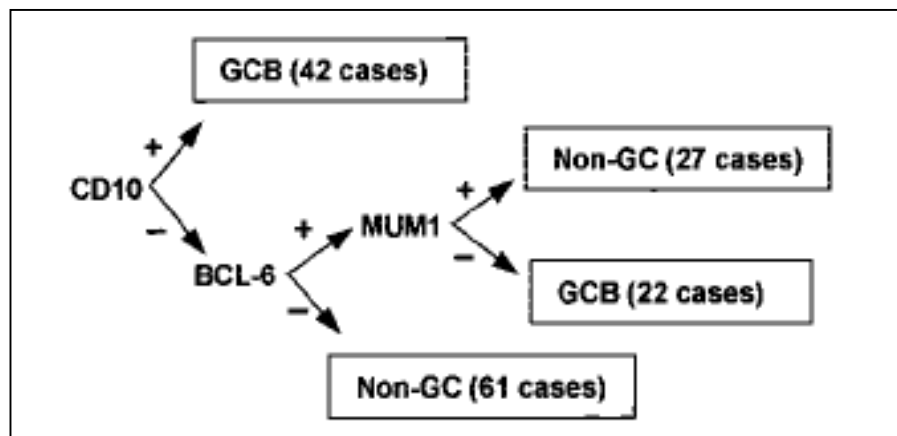
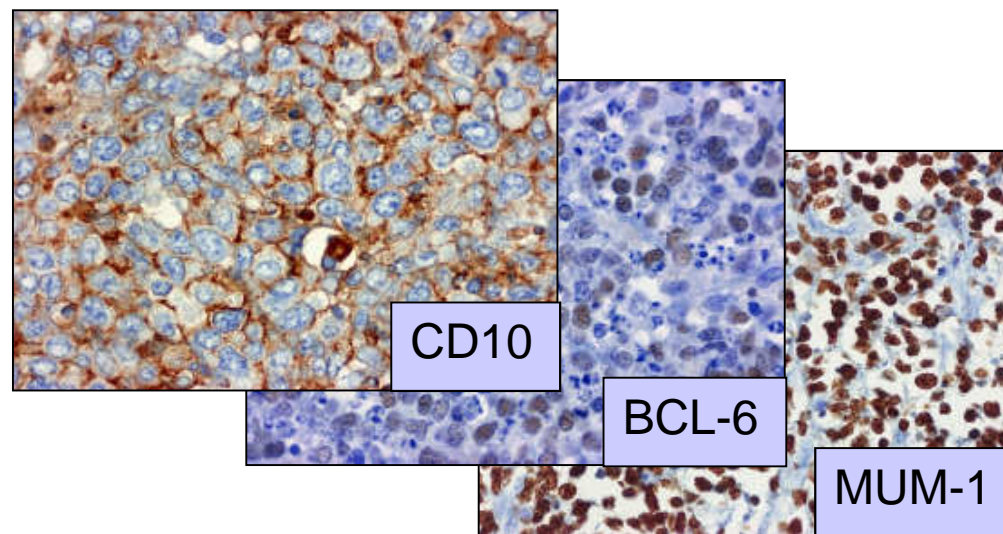
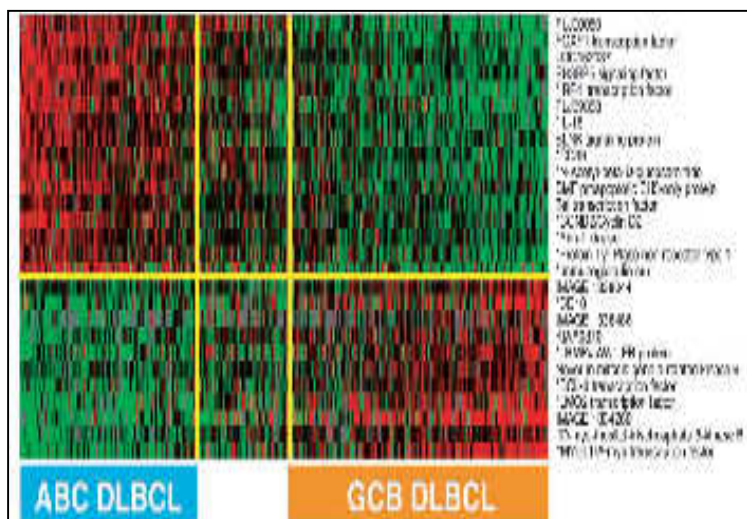
Prognostic groups of DLBCL based on expressional profile analysis



PNAS 2003, 100:9991

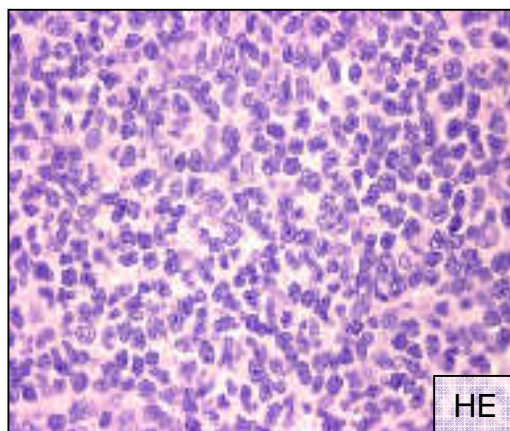


Diagnostic application of the results of expressional profile analysis in DLBCL

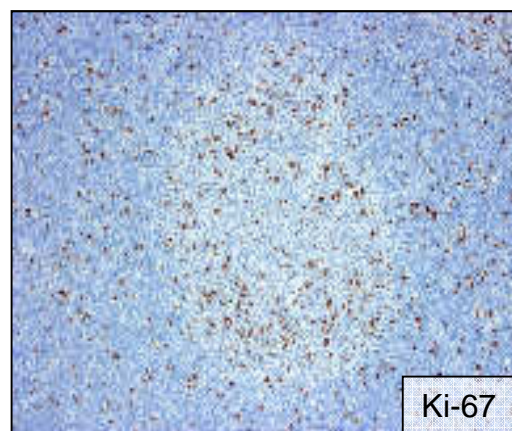




Prognostic factors of follicular lymphoma based on expressional profile analysis

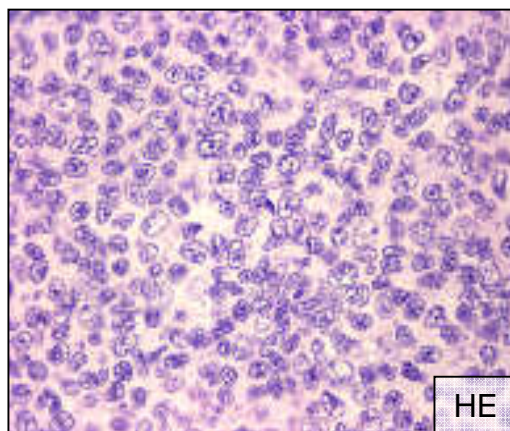


HE

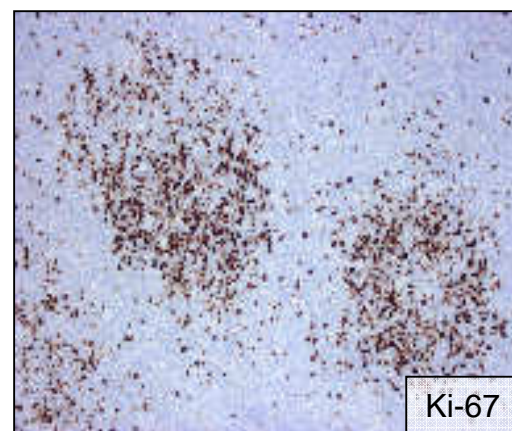


Ki-67

Follicular lymphoma, grade 1

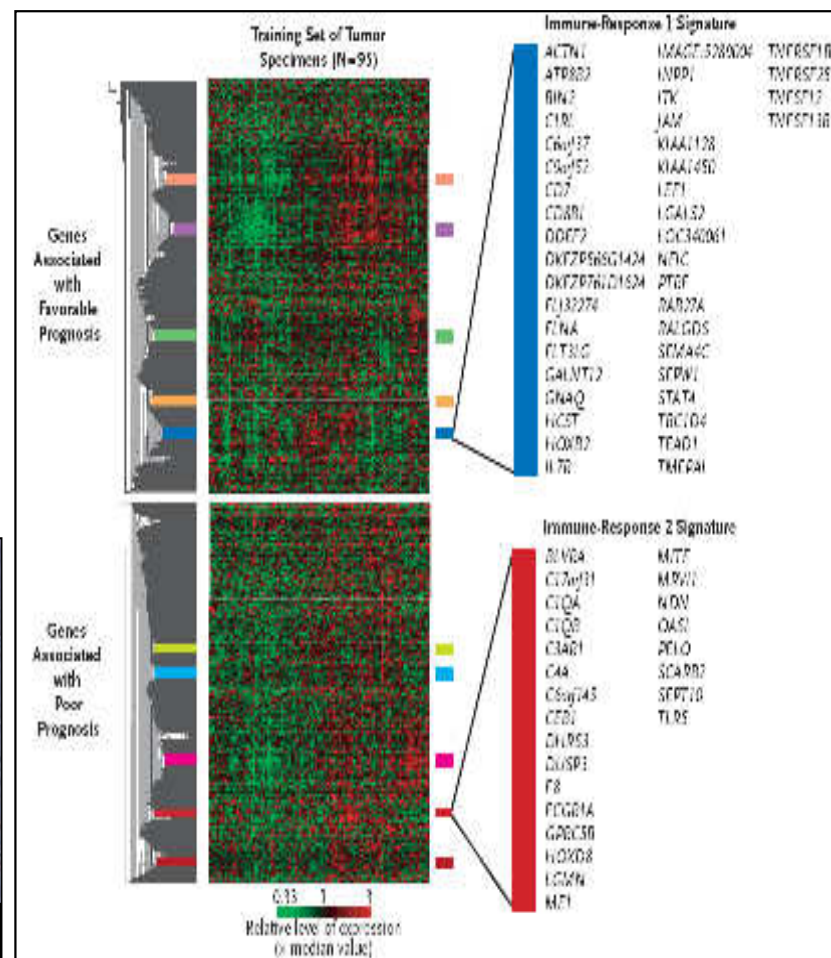


HE



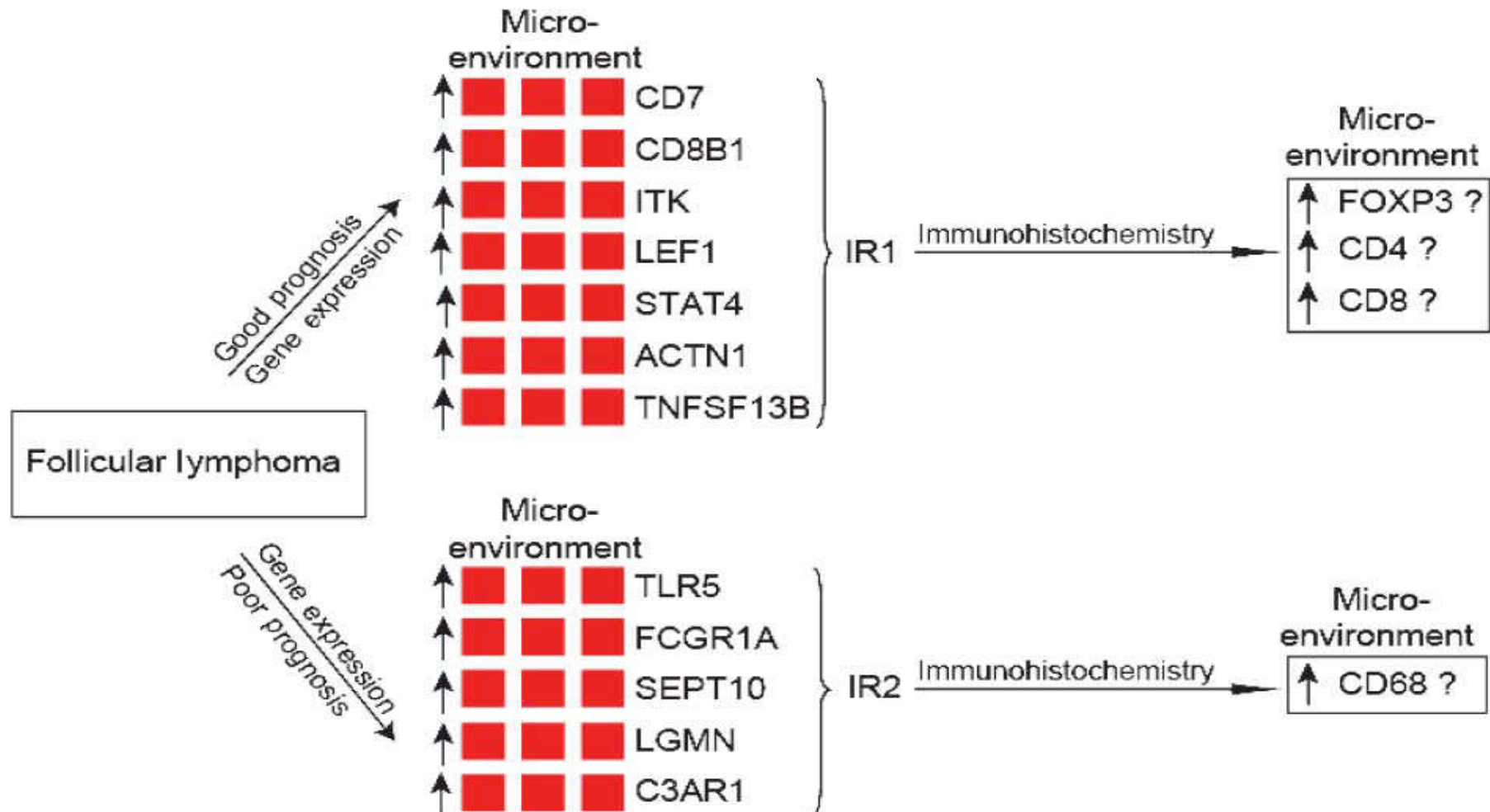
Ki-67

Follicular lymphoma, grade 3





Prognostic factors of follicular lymphoma based on expressional profile analysis



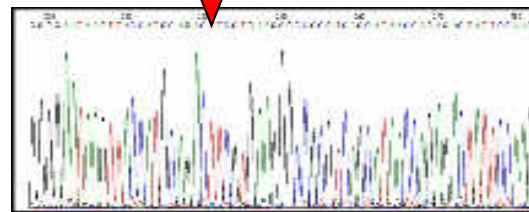
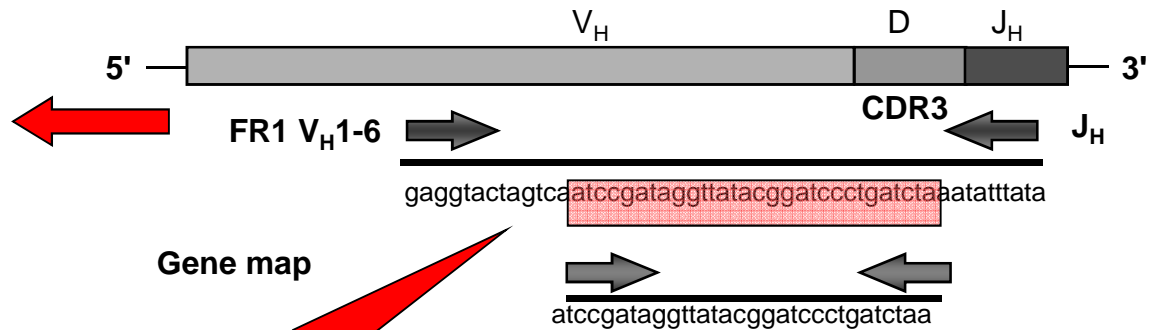
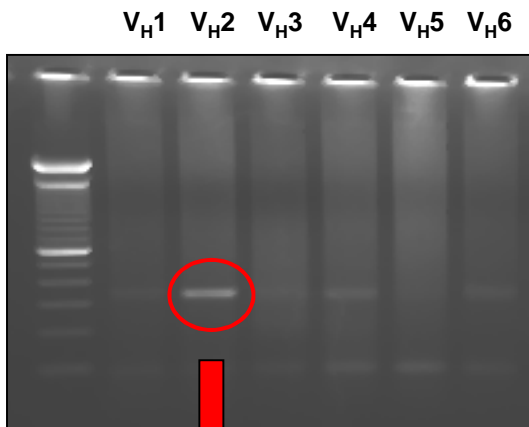
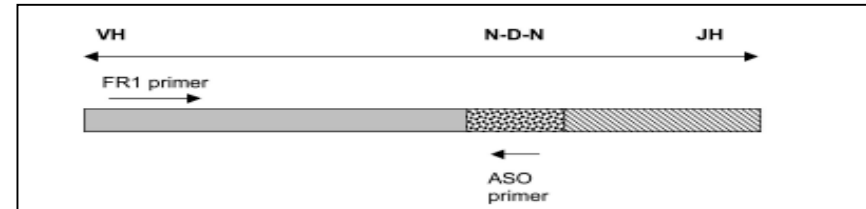
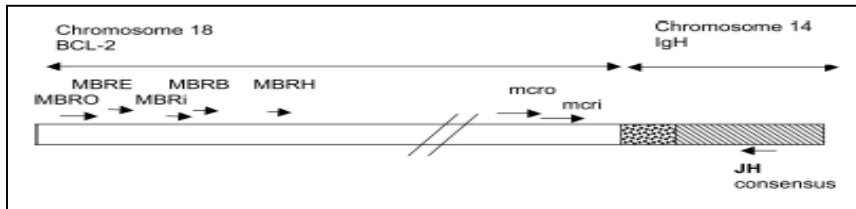


The role of molecular analysis in lymphoma diagnostics

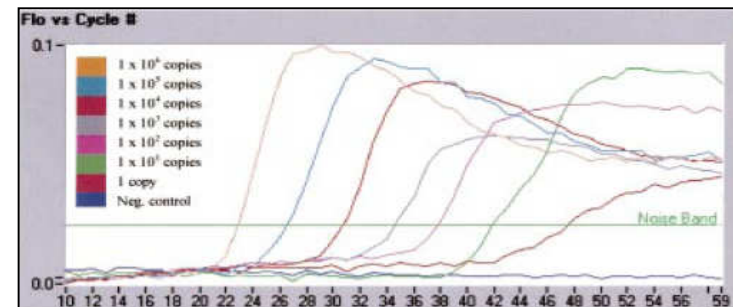
- Determine clonality of the lymphoid cells
- Determine subtype of lymphoma
- Determine prognosis of lymphoma
- Determine effectiveness of treatment (MRD)



Real-time quantitative allele-specific oligonucleotide PCR (RQ-ASO PCR) for assessing MRD in lymphoma



DNA sequencing

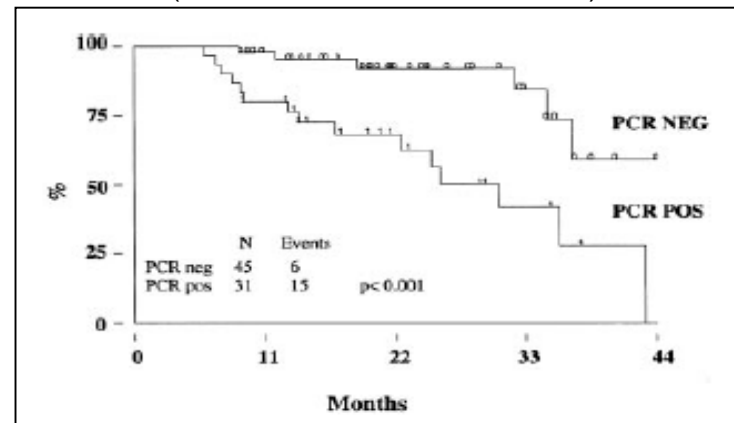


Serially diluted neoplastic cells

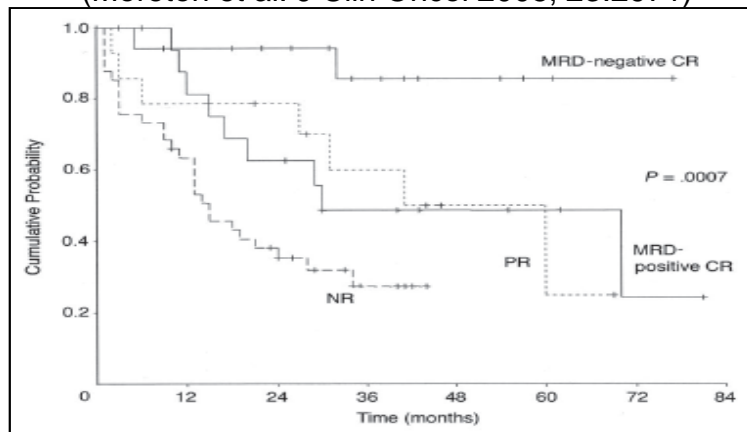


MRD detection following immunochemotherapy of different lymphomas

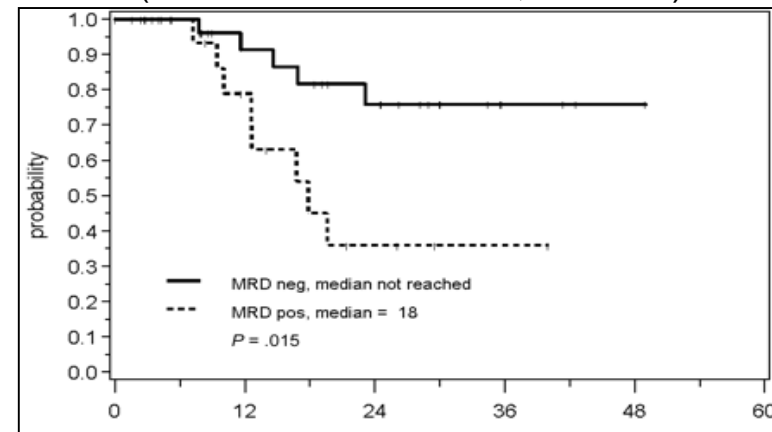
CHOP and Rituximab therapy in FL
(Rambaldi et al. 2002, 99:856)



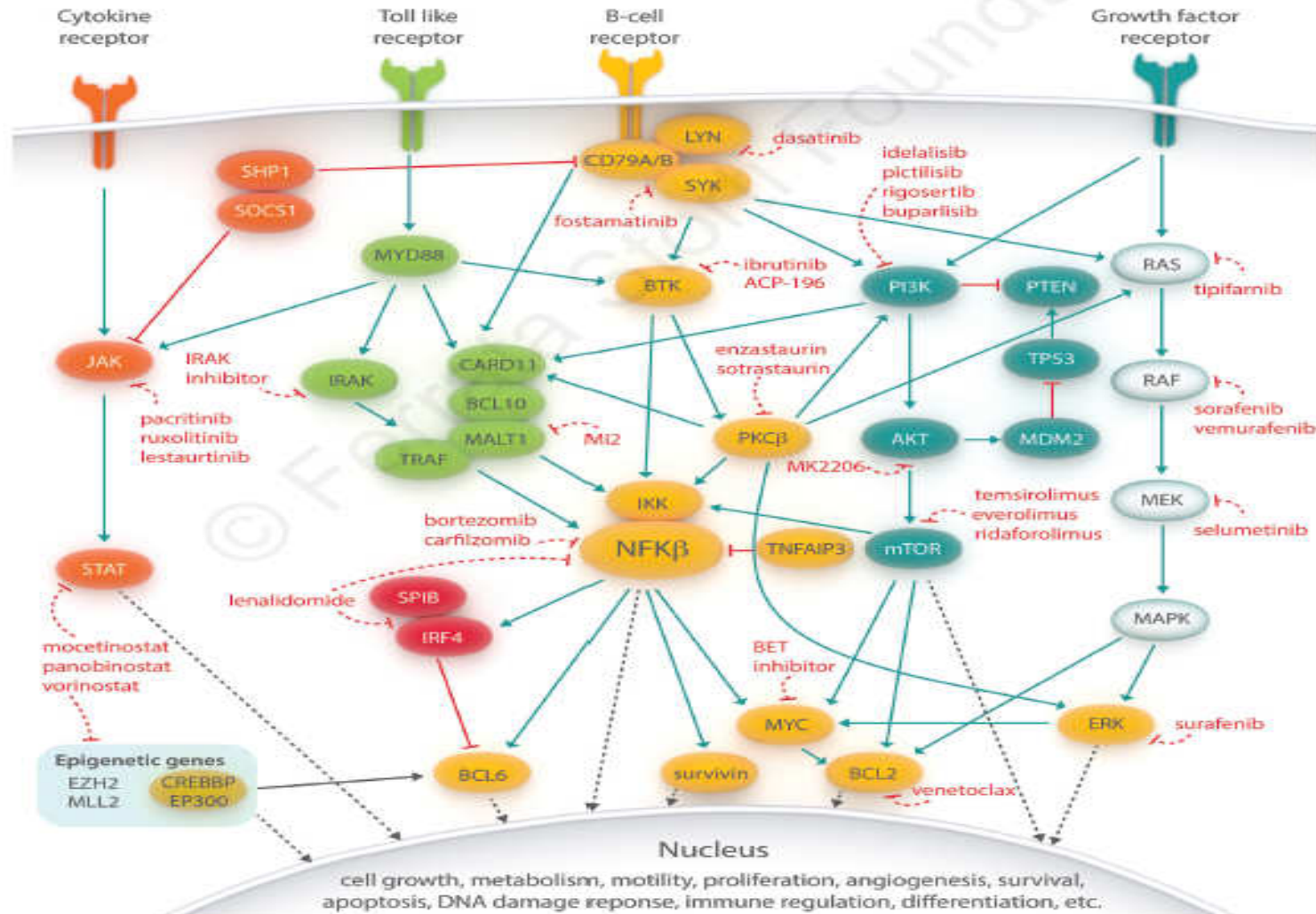
Alemtuzumab therapy in CLL
(Moreton et al. J Clin Oncol 2005, 23:2971)



Immunochemotherapy in MCL
(Pott et al. Blood. 2010, 22:3215)



The future of lymphoma diagnostics





The effect of molecular analysis in lymphoma diagnostics

