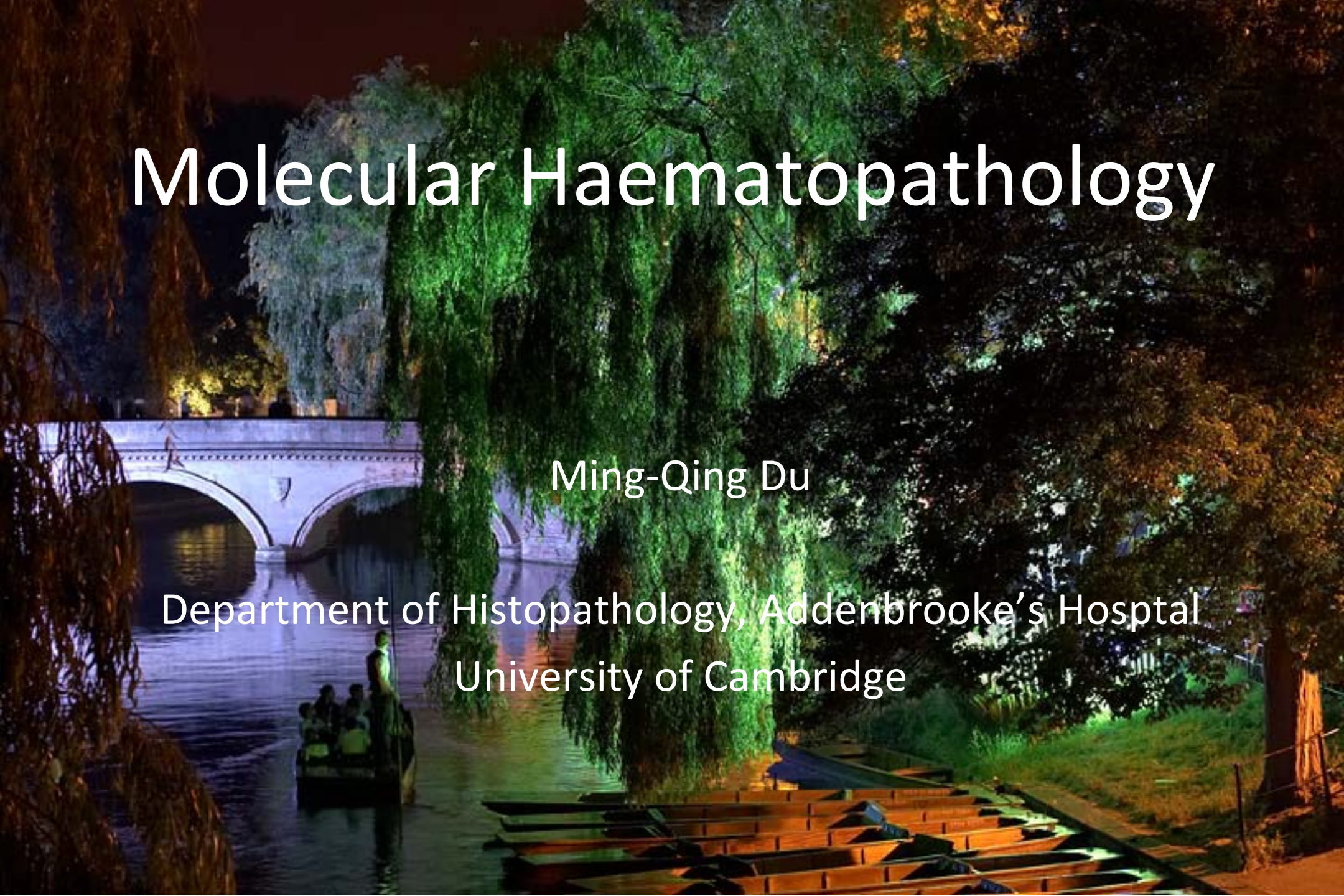


Molecular Haematopathology

Ming-Qing Du

Department of Histopathology, Addenbrooke's Hosptal
University of Cambridge

Molecular Haematopathology: value

- Diagnosis:
  - Benign vs malignant
  - Disease monitoring
  - Sub-classification
- Prognosis
- Treatment stratification

Molecular Haematopathology: **role** of **histopathologist**

- Sample quality
  - "tumour" content in remaining tissue blocks

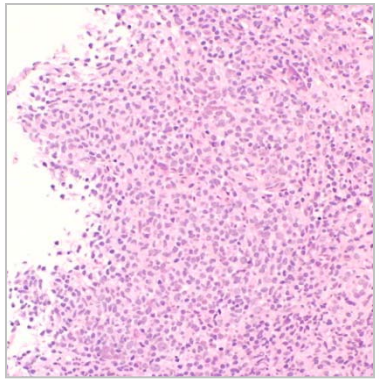
    >10% for clonality analysis

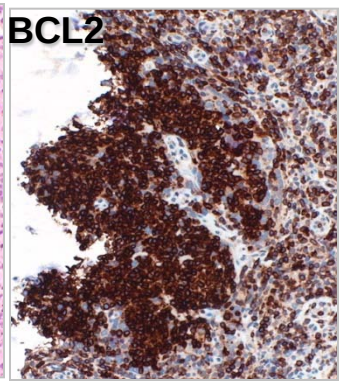
    ? microdissection to enrich suspected cells

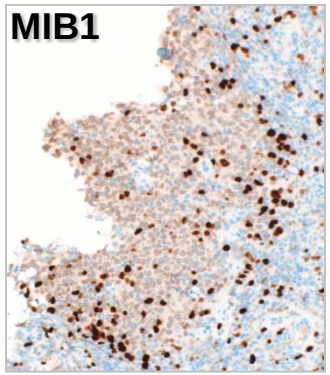
    preliminary histological opinion
  - indicating area of lymphoma or suspected lesion

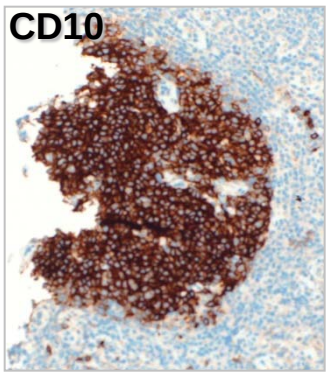
    interphase FISH & analysis of lymphoma cells

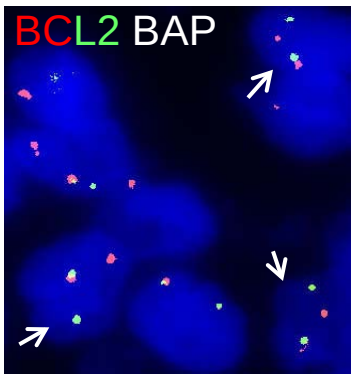
In situ follicular neoplasia

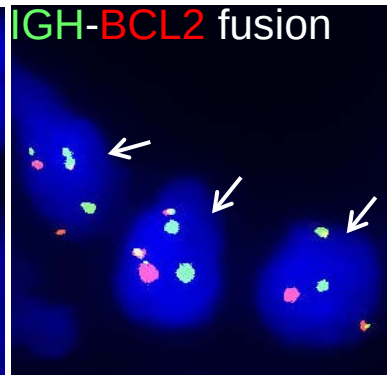


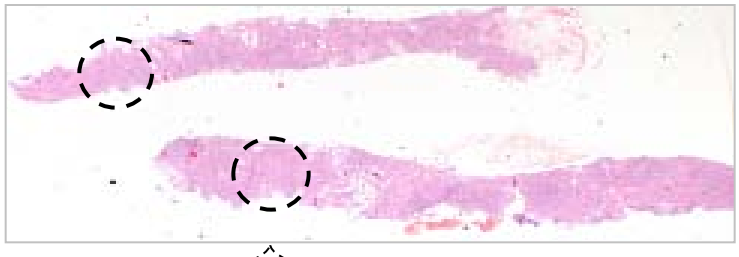










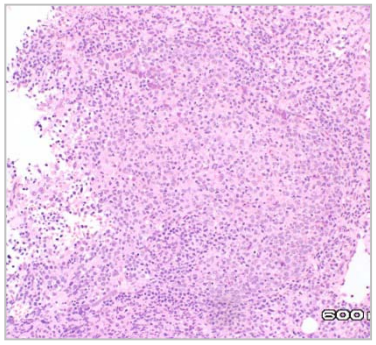


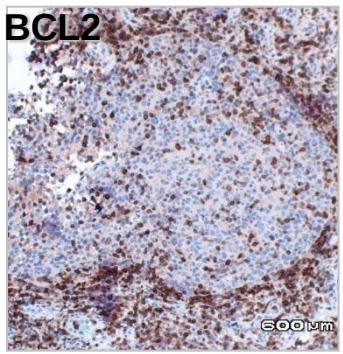
M1066,

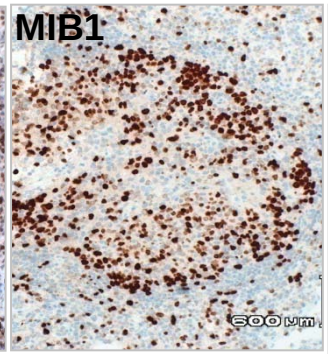
46/female, left axillary LN core biopsy

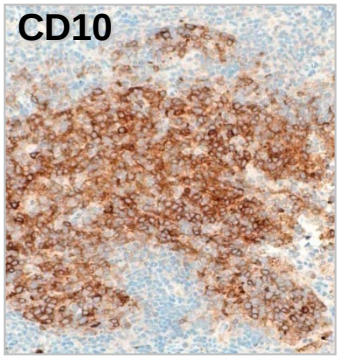
B cell clonality analysis = polyclonal

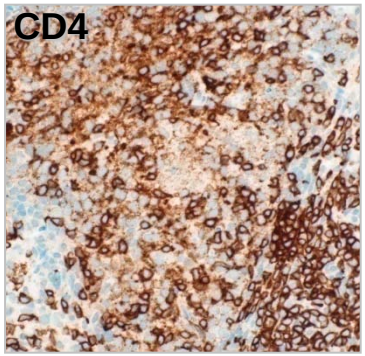
(LN excision biopsy: a few follicles showing features of FL)

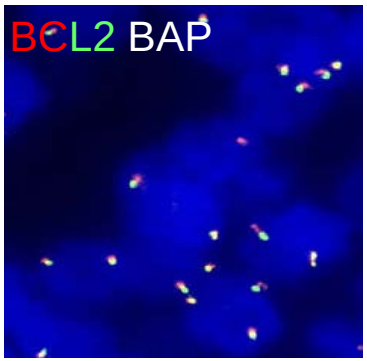












Molecular Haematopathology: **role** of **histopathologist**

- Appropriate molecular tests
  - clear need of molecular test

    suspicious, but not diagnostic lymphoma

    cutaneous T-cell infiltrate suspected for T cell lymphoma

    celiac disease refractory to gluten free diet

    LPD associated with immunodeficiency

    …….
  - clear impact if results informative

    diagnosis, classification, prognosis, treatment stratification

Molecular Haematopathology: **role** of **histopathologist**

Histopathologist

Clinical Scientist

- Sample quality
- Appropriate molecular tests
- Results interpretation in the context of clinicopathological findings
- Integrated report

- Appropriate test & method
- Test sensitivity
- Test specificity
- False negative
- False positive

Molecular Haematopathology:
Diagnosis: benign vs malignant

- B and T cell clonality
- Somatic genetic changes

B & T cell clonality: BIOMED-2 protocol

IG gene rearrangements

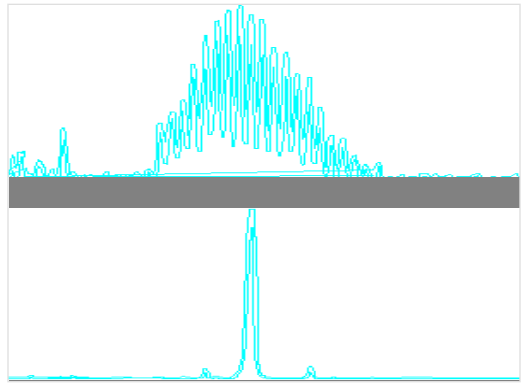
locus	PCR reaction
IGH	5
IGK	2
IGL	1

TCR gene rearrangement

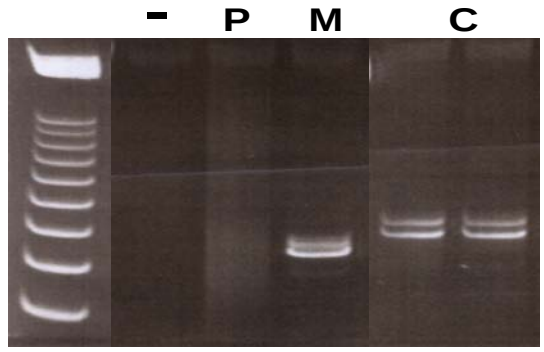
locus	PCR reaction
TCRG	2
TCRB	3

A single
PCR condition

GeneScan

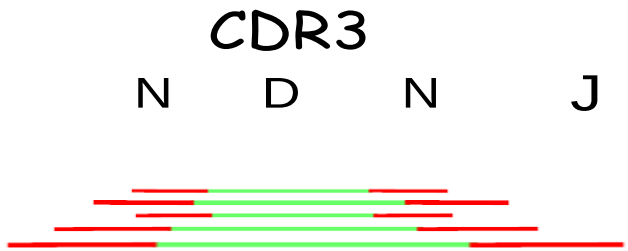


Heteroduplex treatment
& PAGE



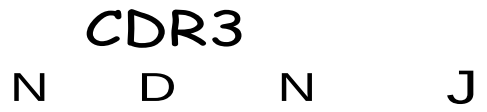
PCR Products analyses : GeneScan or Heteroduplex analysis/PAGE?

IGH VH-JH
TCR β
TCRδ



Heteroduplex & GeneScan are equally suitable, with the exception of TCRδ, which is better by heteroduplex

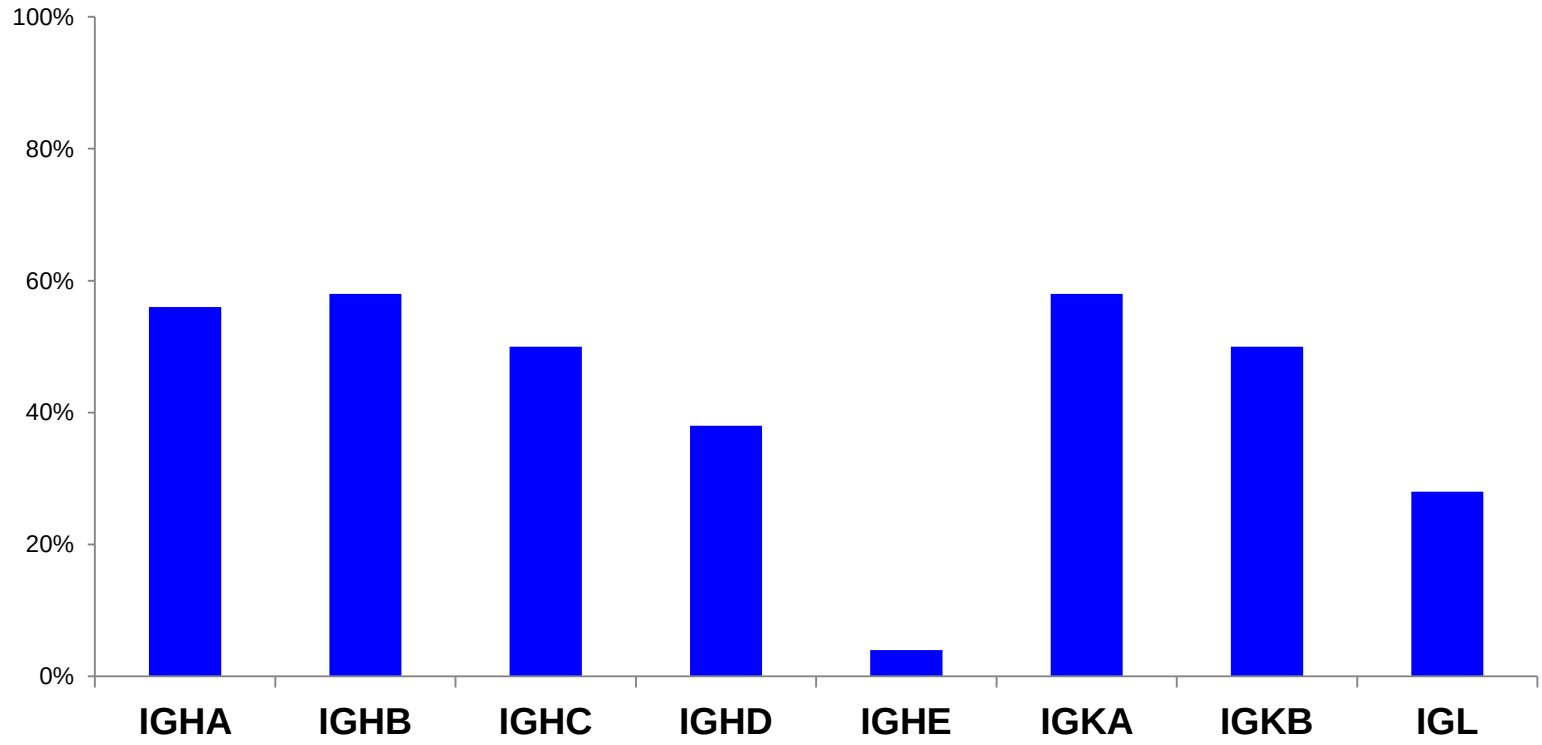
IGH DH-JH



IGK
IGL
TCRγ

Heteroduplex better than GeneScan

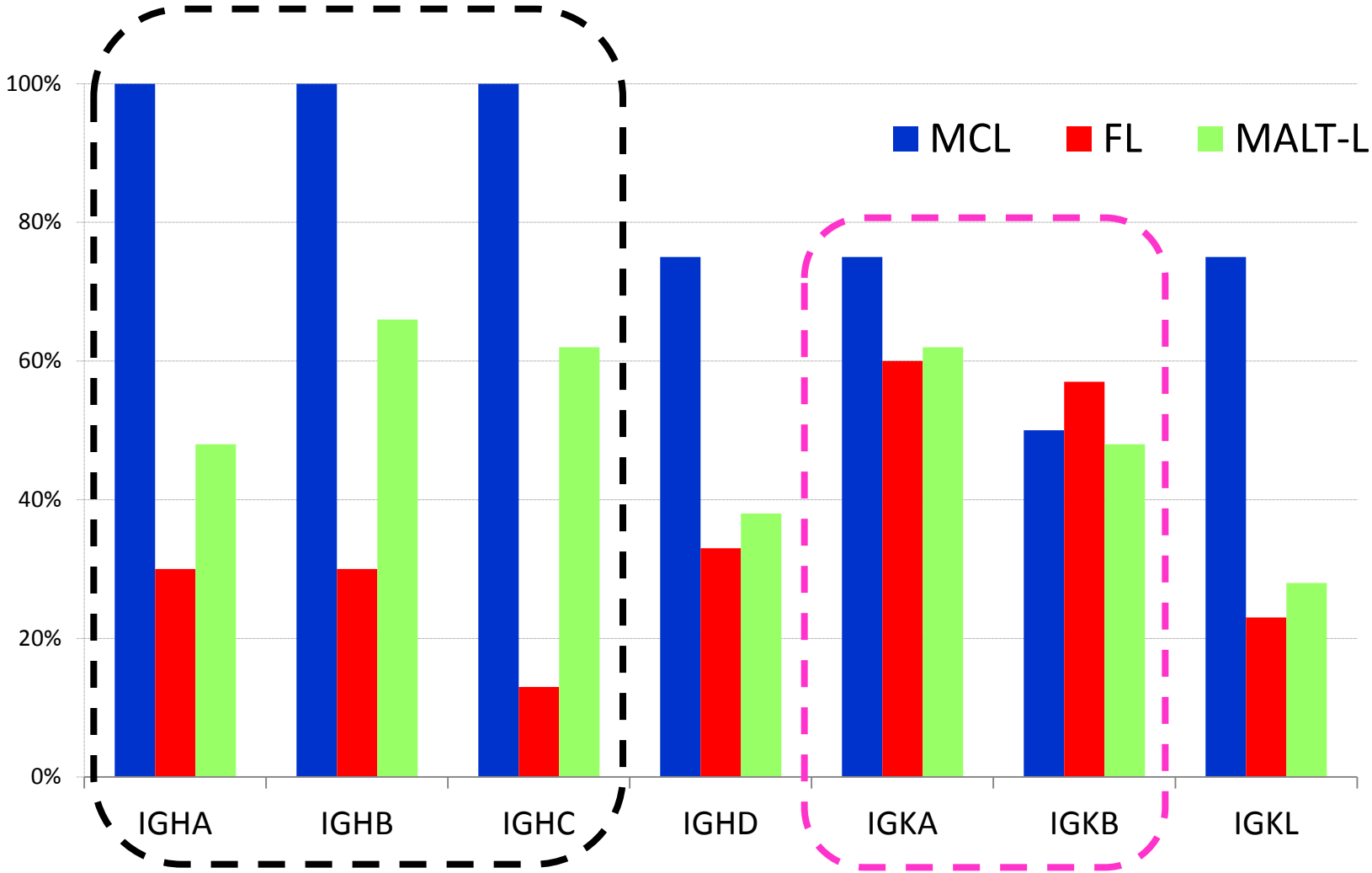
BIOMED-2 B-cell clonality: efficacy depends on PCR reactions



The sole use of any individual reaction is of limited value!

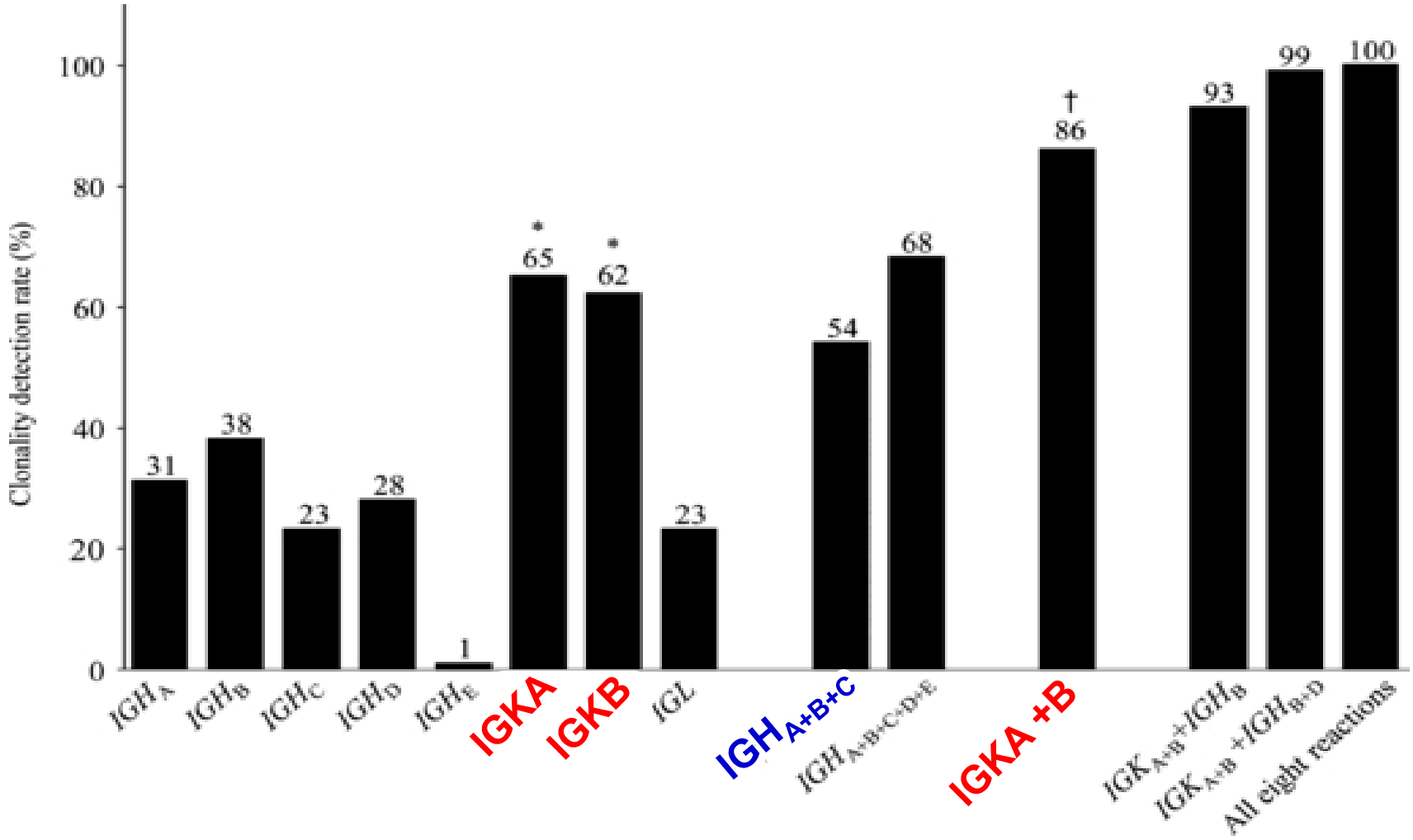
Liu et al BJH, 2007

BIOMED-2 B-cell clonality: efficacy depends on lymphoma subtypes



Liu et al BJH, 2007

IGK PCR essential for clonality analysis of FL



Payne et al BJH 2011; 155: 84-92

Failure of IGH PCR in FL due to mutations abolishing primer binding

Case 4

IGHV1-2

FR1 primer

..ggcctcagtgaaggtctcctgcaaggc

gg----------

FR2 primer

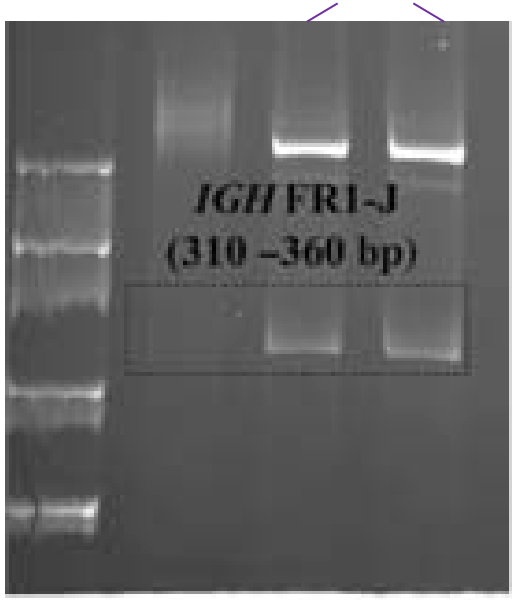
ctgggtgcgacaggcccctggactagg

----------a---

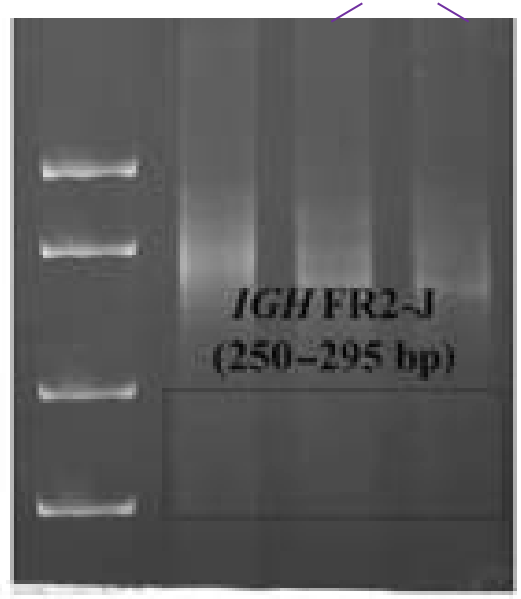
FR3 primer

atggagatgagccggctgacgtctgac

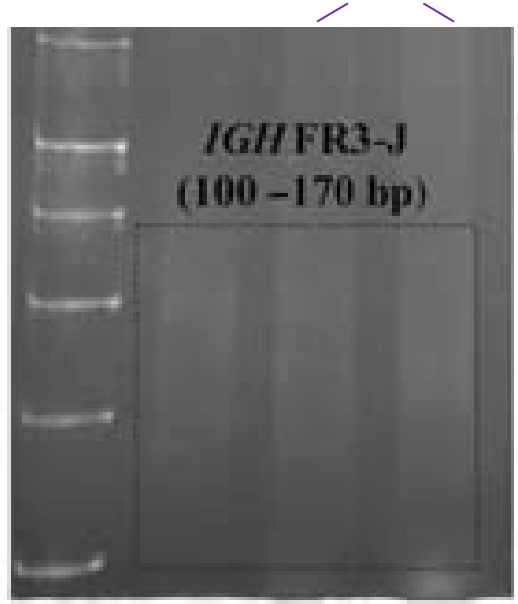
-----c-----a------ga----



IGH_A (clonal)



IGH_B (not clonal)



IGH_C (not clonal)

Payne et al BJH 2011; 155: 84-92

BIOMED-2 B-cell clonality: strategy for routine application

PCR combination	Clonal rearrangement (%)	
	by >1 PCR	by ≥ 2PCR
$IGH_B + IGK_{A+B}$	91%	58%
If not clonal ↓		
$+ IGH_{A+C+D}$	99%	79%
If not clonal ↓		
$+ IGHL \& IGH_E$	100%	80%

Liu et al BJH, 2007

BIOMED-2 T-cell clonality: strategy for routine application

PCR combination	Clonal rearrangement (%)	
	by >1 PCR	by ≥ 2PCR
$TCRG_{A+B}$	94%	30%
If not clonal ↓		
+ $TCRB_{A+B}$	98%	73%
If not clonal ↓		
+ $TCRB_C$ + TCR_D	100%	82%

Liu et al BJH, 2007

False negative

- Inadequate sample
  - few lymphocytes
  - poor DNA quality
- Technical limitations
  - <10% clonal cells
  - Inappropriate PCR combination
  - Incomplete inclusion of all PCR tubes
- Nature of lymphoma
  - Hodgkin's lymphoma
  - GC and post-GC lymphoma
  - NK cell lymphoma

False Positive

- Inadequate sample
  - poor quality DNA
  - limited lymphoid cells
- GI & skin biopsies: oligoclonal T-cells
- Technical issues
  - Inadequate methods and inappropriate interpretation
  - Occasional non-specific PCR product
  - Cross lineage antigen receptor rearrangement

    TCR gene rearrangement in B-ALL

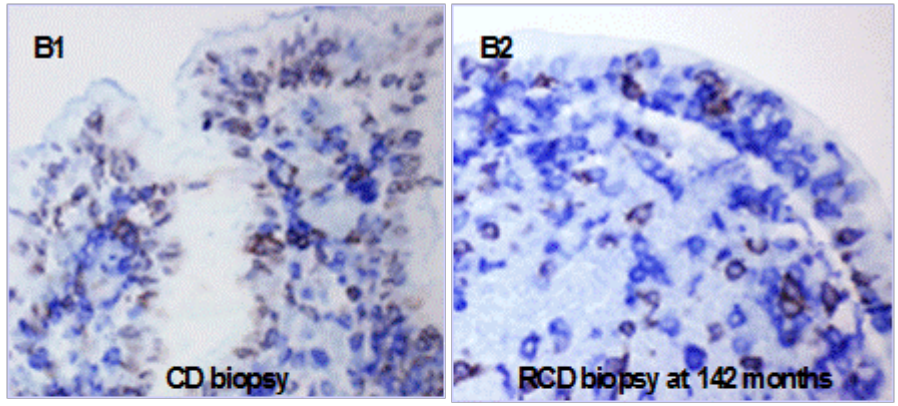
    IG gene rearrangement in T-ALL
- Reactive / inflammatory pathology:

  HP gastritis, EBV infection, active celiac disease, Sjogren's syndrome

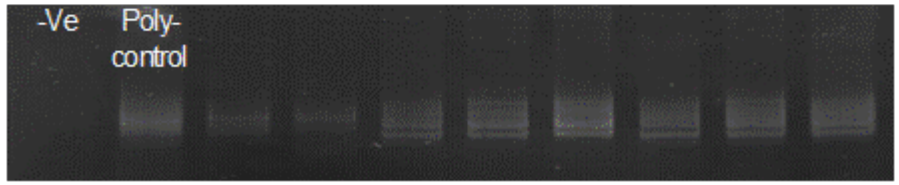
Molecular Haematopathology:
Diagnosis: disease monitoring

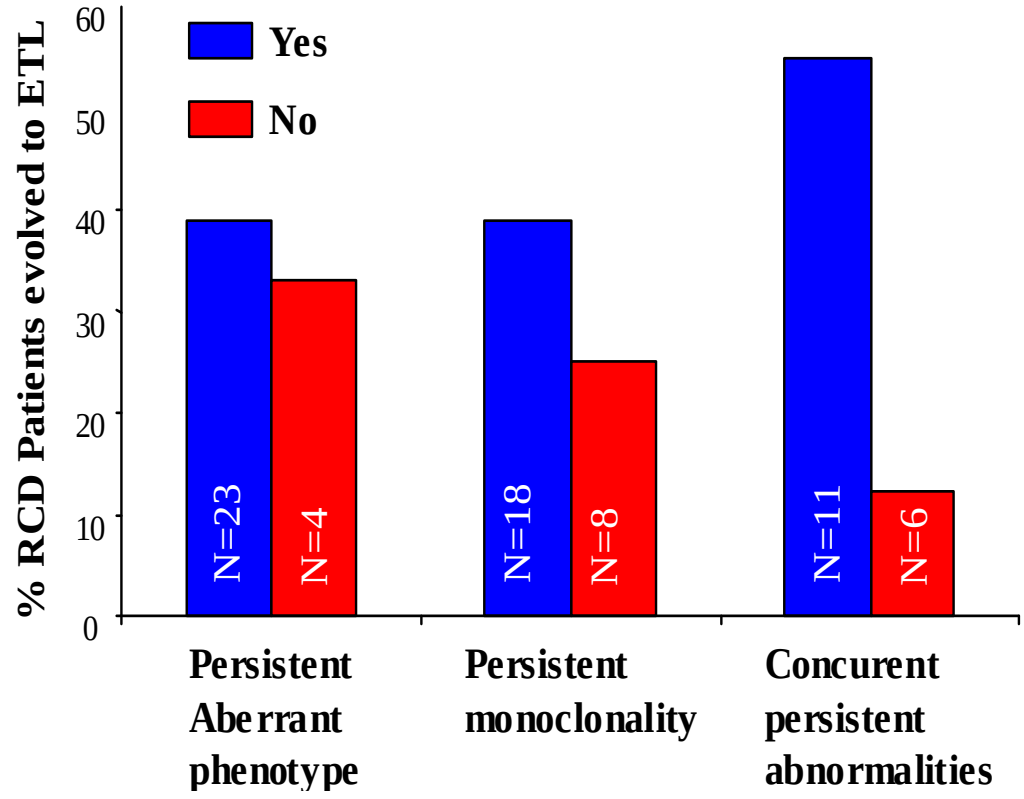
- Persistent reactive lesions suspected lymphoma
  - CD-RCD-EATL sequence
  - Cutaneous T cell infiltrates
  - PTLD
  - …
- Clonal relationship of synchronous & metachronous lymphomas

Clonality analysis in RCD/EATL surveillance



Diagnosis	CD	RCD						
Follow-up (months)	0	31	41	120	130	142	149	161
% $CD3^+CD8^-$ IEL/T-cells	3	19	17	24	23	**45**	**95**	**53**
Clonality	P	P	C=	C=	C=	C=	C=	C=



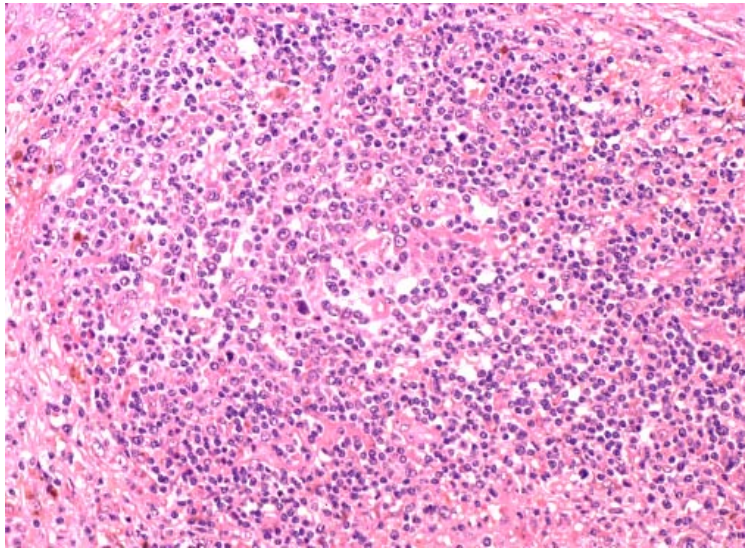


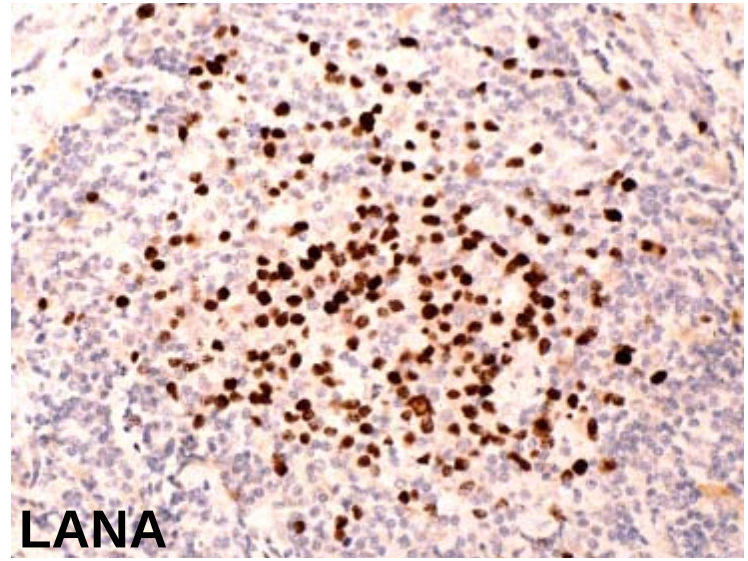
- Transient monoclonality or aberrant IEL immunophenotype: CD with GFD non-compliance;
- Persistent identical monoclonality and aberrant IEL immunophenotype: a feature of RCD;
- Persistence of both the abnormalities in RCD: high risk for EATL.
- Importance of continuous monitoring of both IEL immunophenotype and clonality in diagnosis and follow up of RCD.

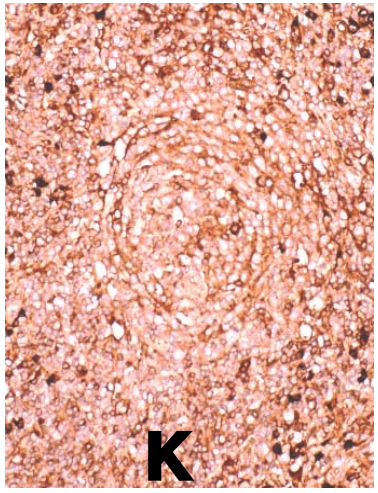
Liu et al Gut 2010

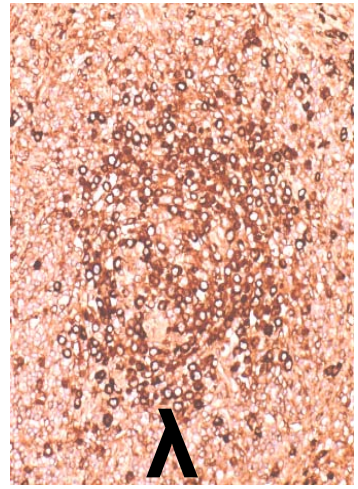
Paradoxical findings: light chain IHC vs IG PCR

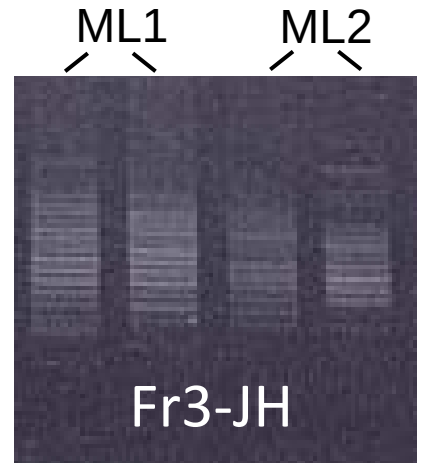
KSHV associated LPD in MCD







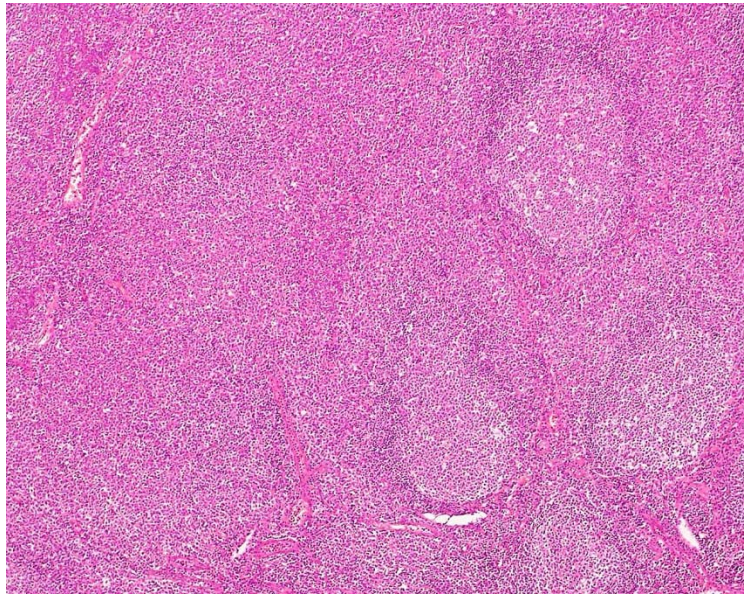


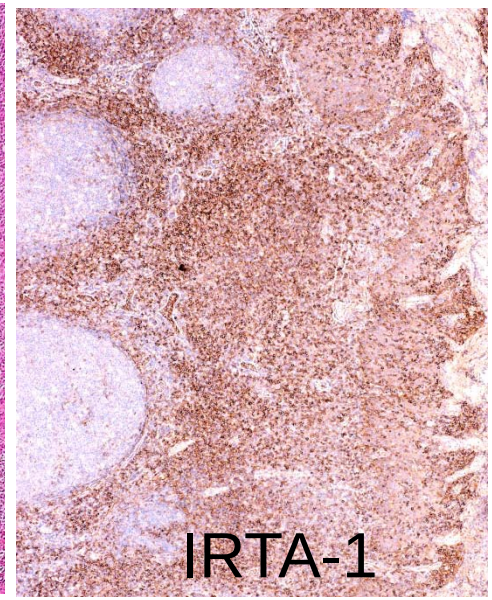


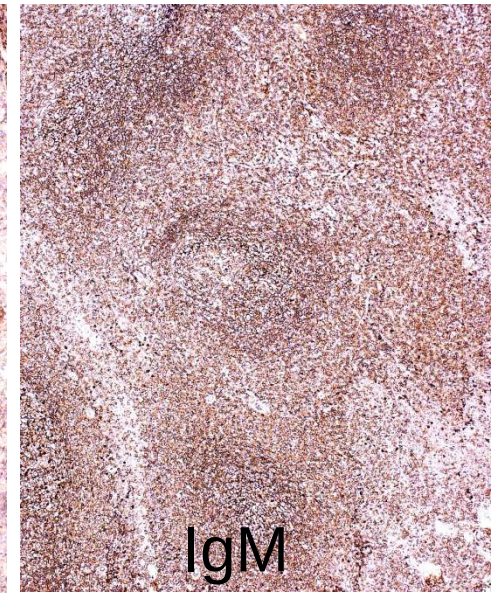
Du MQ et al Blood 2001

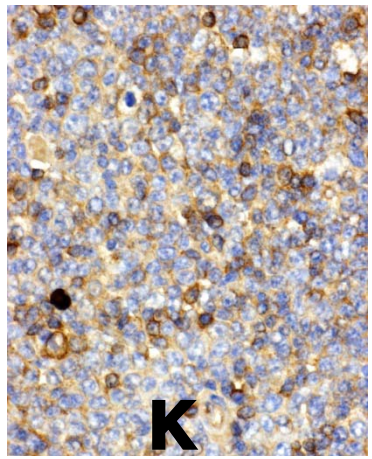
Paradoxical findings: light chain IHC vs IG PCR

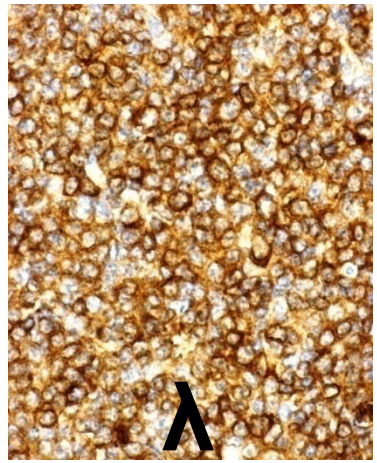
Atypical marginal zone hyperplasia in children

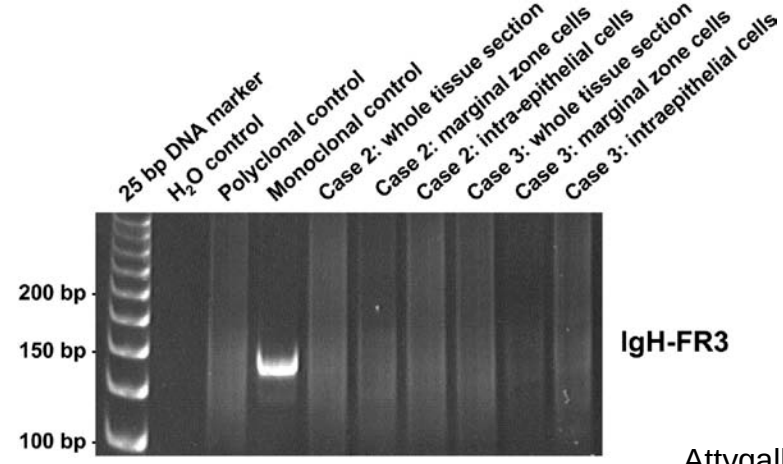












Attygalle et al. Blood 2004

Molecular Haematopathology:
Diagnosis: sub-classification

- Chromosome translocation
  - t(14;18)/IGH-BCL2: FL
  - t(11;14)/CCND1-IGH: MCL
  - t(8;14)/MYC-IGH BL
  - t(11;18)/API2-MALT1: MALT lymphoma
  - t(2;5)(p23;35)/NPM-ALK ALCL
- Genomic CNVs
  - 7q32 deletion: SMZL
- Somatic mutations
  - BRAF HCL
  - MYD88 LPL

Incidence and specificity

Lymphoma type	Ch. translocation	Frequency	The gene involved
Follicular lymphoma	t(14;18)(q32;q21)	~90%	BCL2/IGH
Mantle cell lymphoma	t(11;14)(q13;q32)	~95%	CCND1/IGH
Burkitt lymphoma	t(8;14)(q24;q32)	100%	MYC/IGH
MALT lymphoma	t(11;18)(q21;q21) t(1;14)(p22;q32) t(14;18)(q32;q21)	0-40% 5% 5%	API2-MALT1 BCL10/IGH MALT1/IGH
Anaplastic large cell lymphoma	t(2;5)(p23;35) t(1;2)(q25;p23)	70-80% 10-20%	NPM-ALK TPM3-ALK
SMZL	7q32 deletion	30%	?

Burkitt lymphoma and DLBCL: differential diagnosis at genetic level

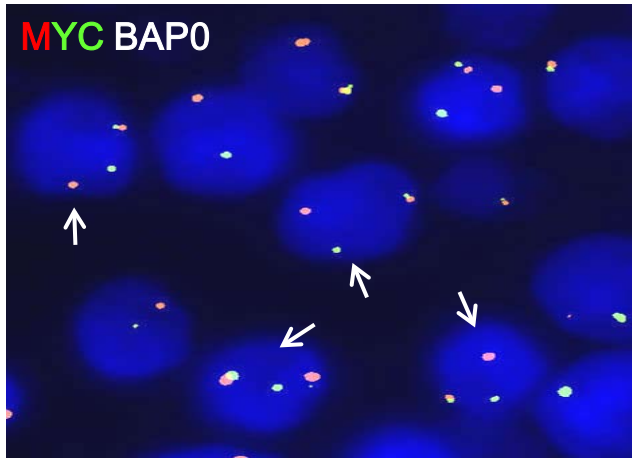
	BL	DLBCL (GC phenotype + high proliferation index)
Genetic changes	MYC translocation associated with IG gene MYC BAP +ve MYC-IG fusion +ve	-/+ MYC translocation frequently associate with non-IG genes MYC BAP +ve MYC-IG fusion often –ve
	Lack or simple cytogenetic/genomic changes BCL2 BAP: no CNV BCL6 BAP: no CNV	Presence of complex cytogenetic/genomic changes BCL2: often gain at 18q21 BCL6: often gain at 3q27
	Lack of other translocations BCL2 BAP: no translocation BCL6 BAP: no translocation	-/+ other translocations BCL2 BAP: -/+ translocation BCL6 BAP: -/+ translocation

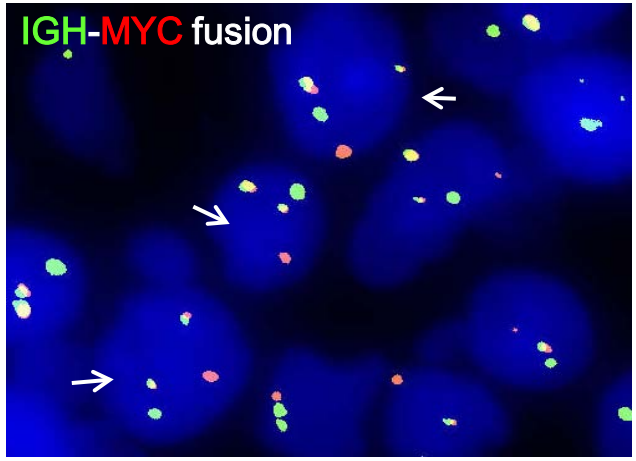
M0911

6 year/male, small bowel

Diffuse infiltrate of intermediate sized cells, starry-sky,

CD20+, CD10+, BCL6+, BCL2+, Ki67~100%, TdT-





BCL2 BAP: no abnormalities

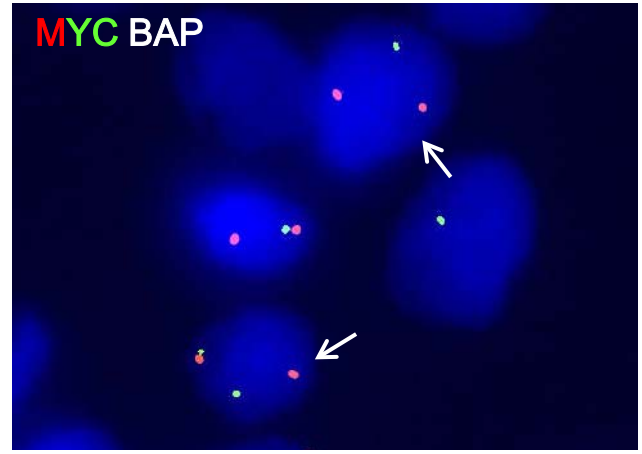
BCL6 BAP: no abnormalities

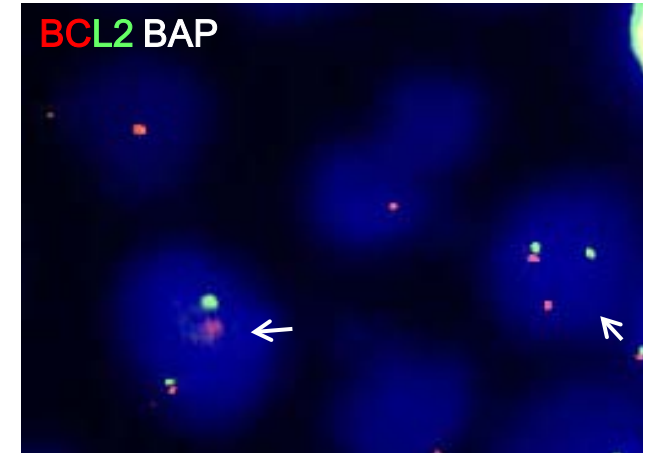
M1269

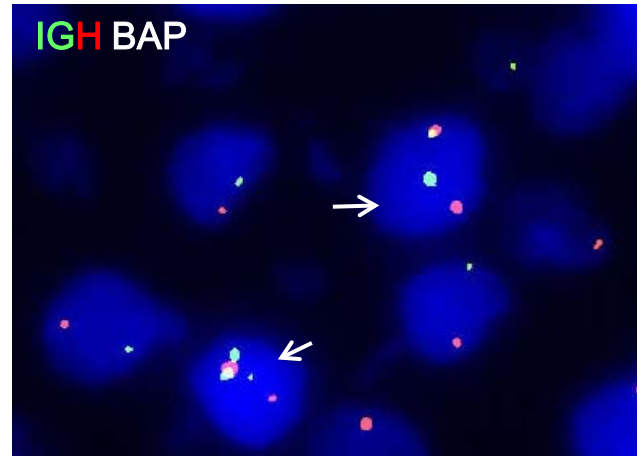
83/female, LN

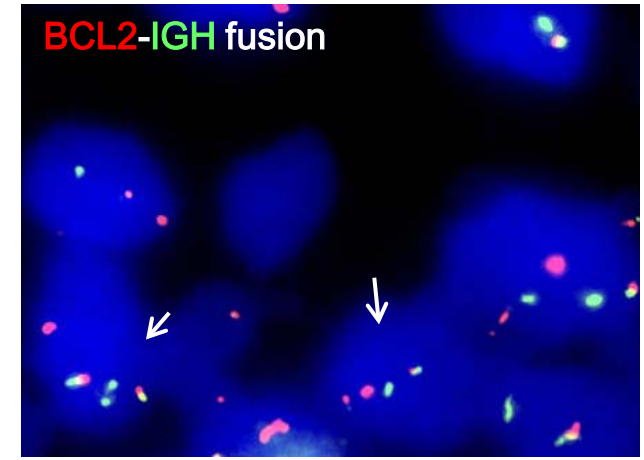
Diffuse infiltrate of large B cells,

CD20+, CD10+, BCL6+, BCL2+, Ki67~90%,





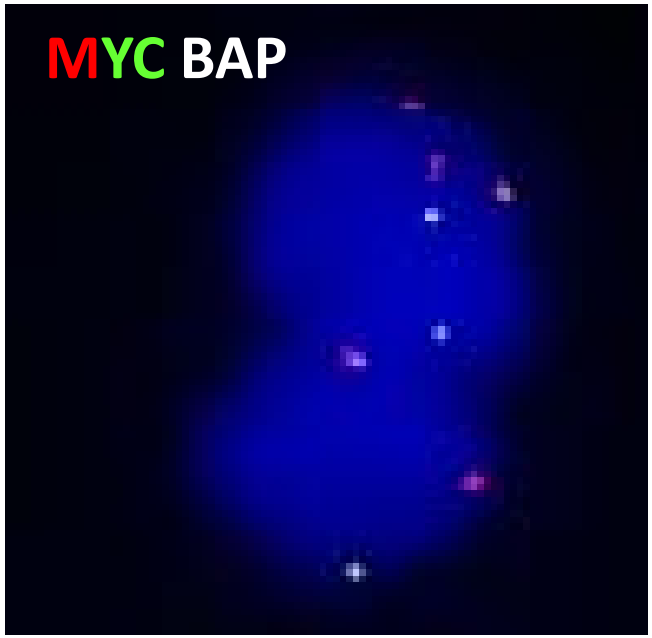




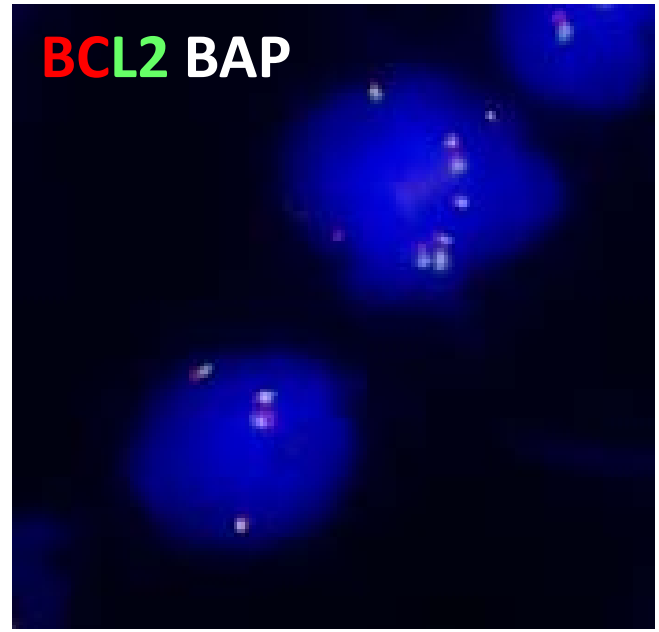
MYC-IGH fusion: no abnormalities

MYC translocation associates with CNV at BCL2 and BCL6 in a DLBCL

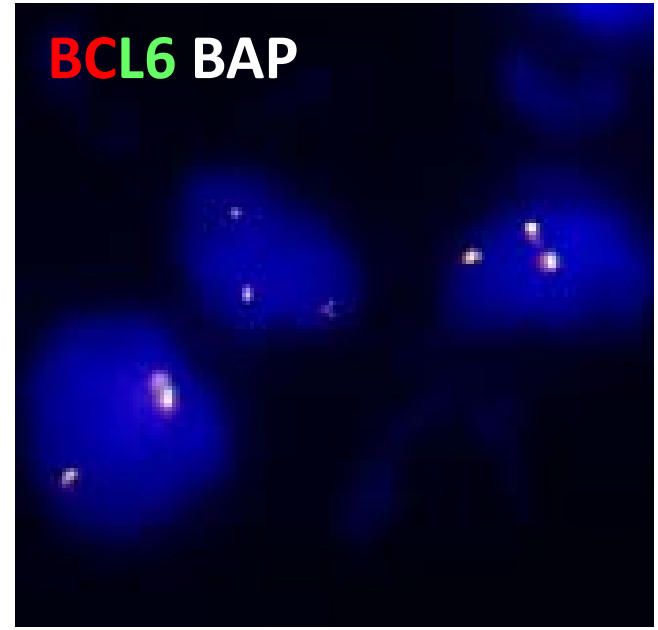
M3657



1C 2G 2R



4-8C

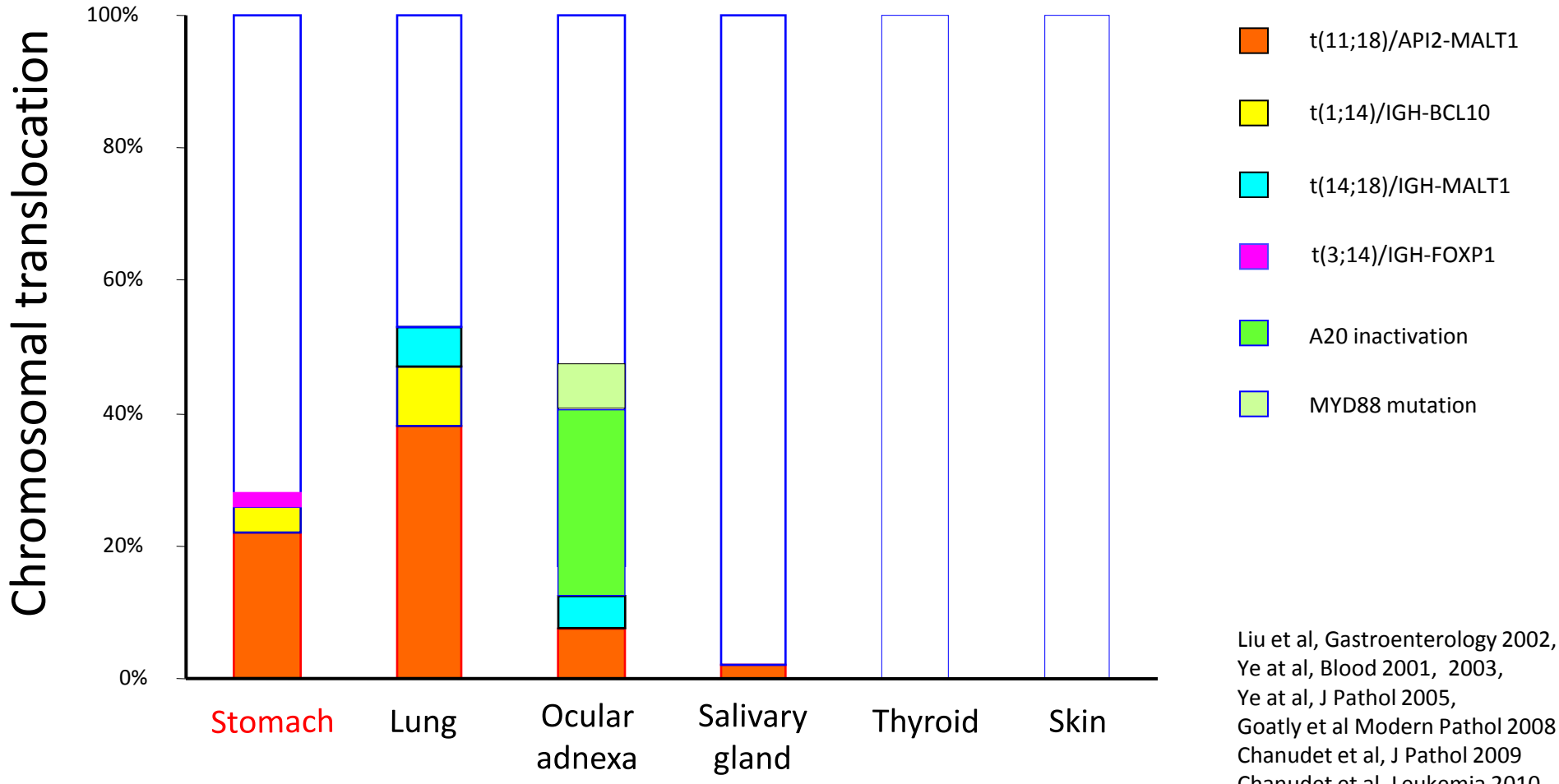


3C

Molecular Haematopathology:
Prognosis and treatment stratification

- MALT lymphoma
  - t(11;18)/API2-MALT1
  - t(1;14)/BCL10-IGH
- CLL
  - TP53 deletion/mutation
  - IGH mutation
- DLBCL:
  - MYC/BCL2 double hit
  - TP53 mutation
  - COO subtypes

Frequencies of known genetic abnormalities in MALT lymphoma



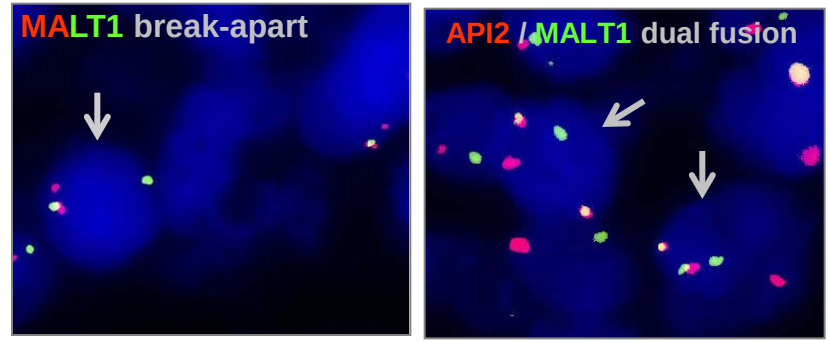
Liu et al, Gastroenterology 2002,
Ye at al, Blood 2001, 2003,
Ye at al, J Pathol 2005,
Goatly et al Modern Pathol 2008
Chanudet et al, J Pathol 2009
Chanudet et al, Leukemia 2010
Bi et al, Haematologica 2012

Gastric MALT lymphoma: treatment stratification by molecular subtyping

Gastric MALT lymphoma

↓

Analyses of MALT1 and BCL10 translocation



Positive

↓

H pylori eradication

Chemotherapy or radiation therapy

Negative

↓

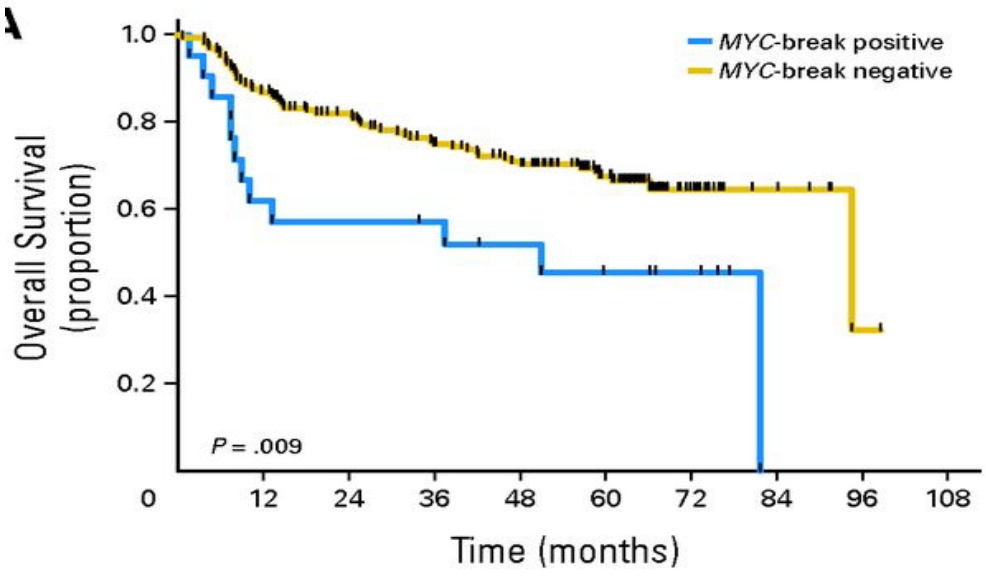
H pylori eradication, cured in 80%

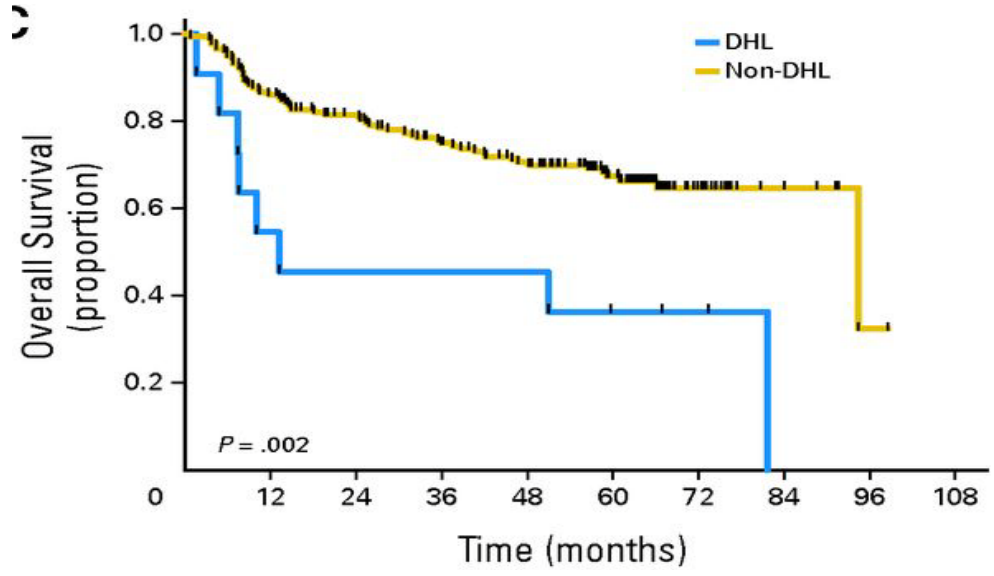
Liu et al Gastroenterology 2002; Ruskoné-Fourmestraux et al Gut 2011

DLBCL: MYC translocation

~10%, associated with complex pattern of genomic profile

~5% being double-hit (MYC + BCL2 trans)



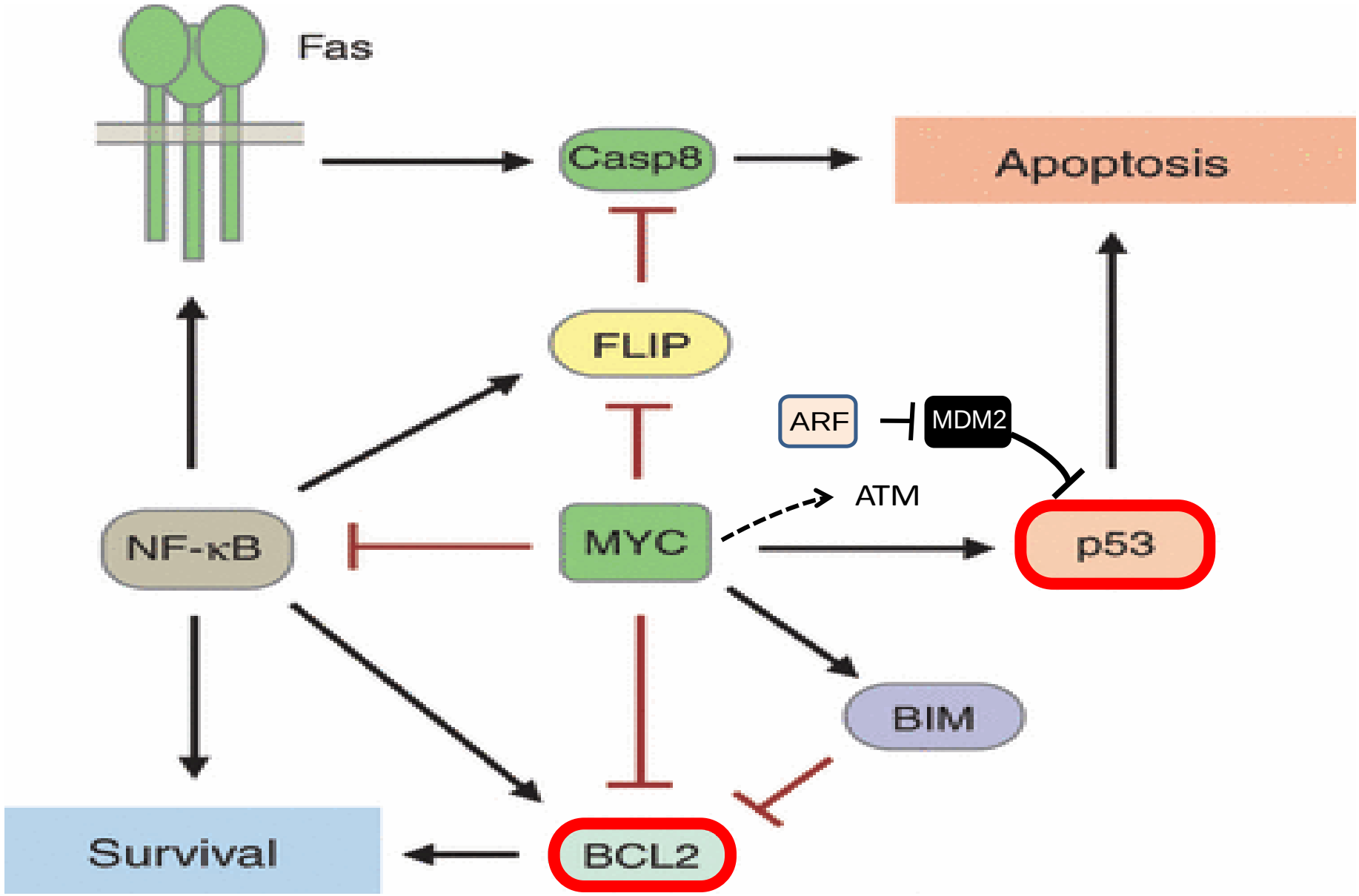


Green et al JCO 2012
Johnson et al JCO 2012
Barrans et al JCO 2010
Savage et al Blood 2009
Johnson et al Blood 2009

MYC: biological function

- Promoting cell-cycle progression
- Regulating ribosome biogenesis and protein synthesis
- Increase energy metabolism
- Regulating miRNA (miR 17-92) expression
- Increase stem cell renewal
- Inducing apoptosis

MYC and apoptosis

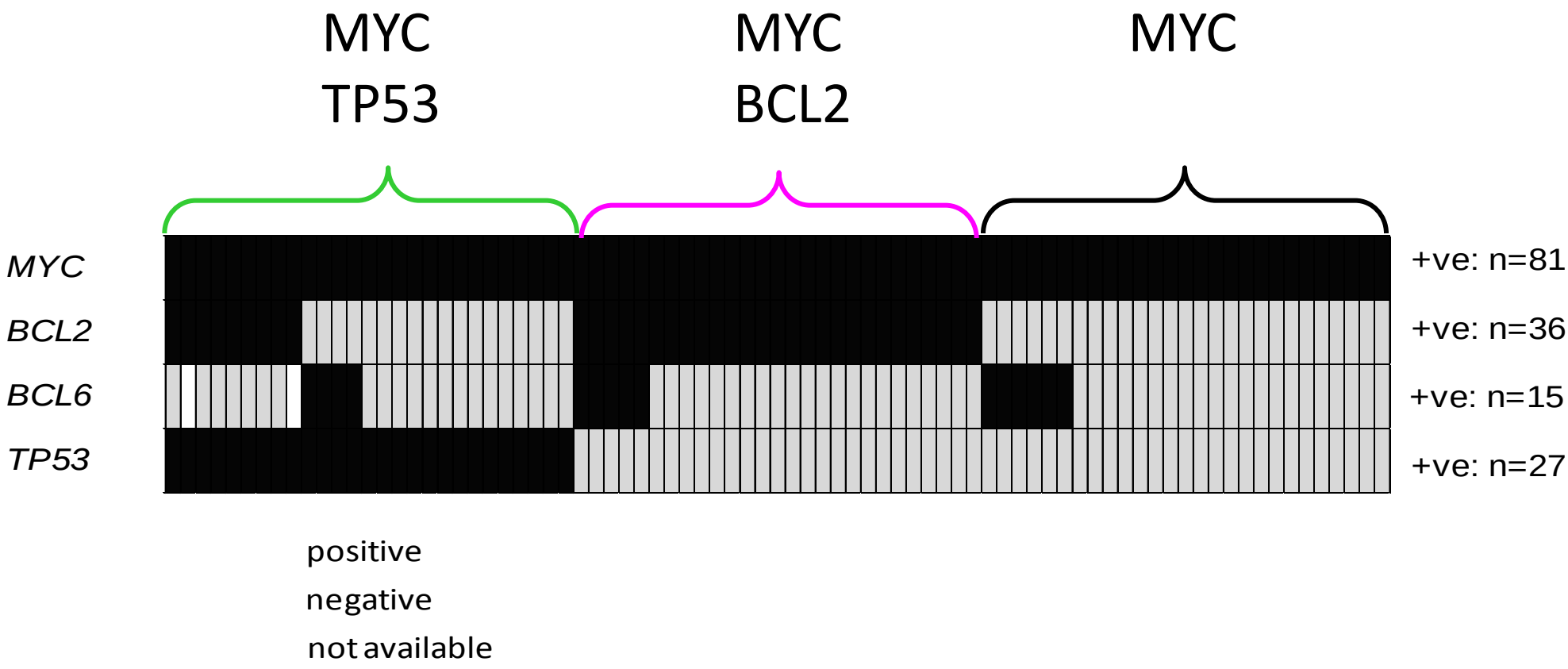


Klapproth and Wirth, BJH 2010: 149; 484-497

MYC and TP53: oncogenic cooperation

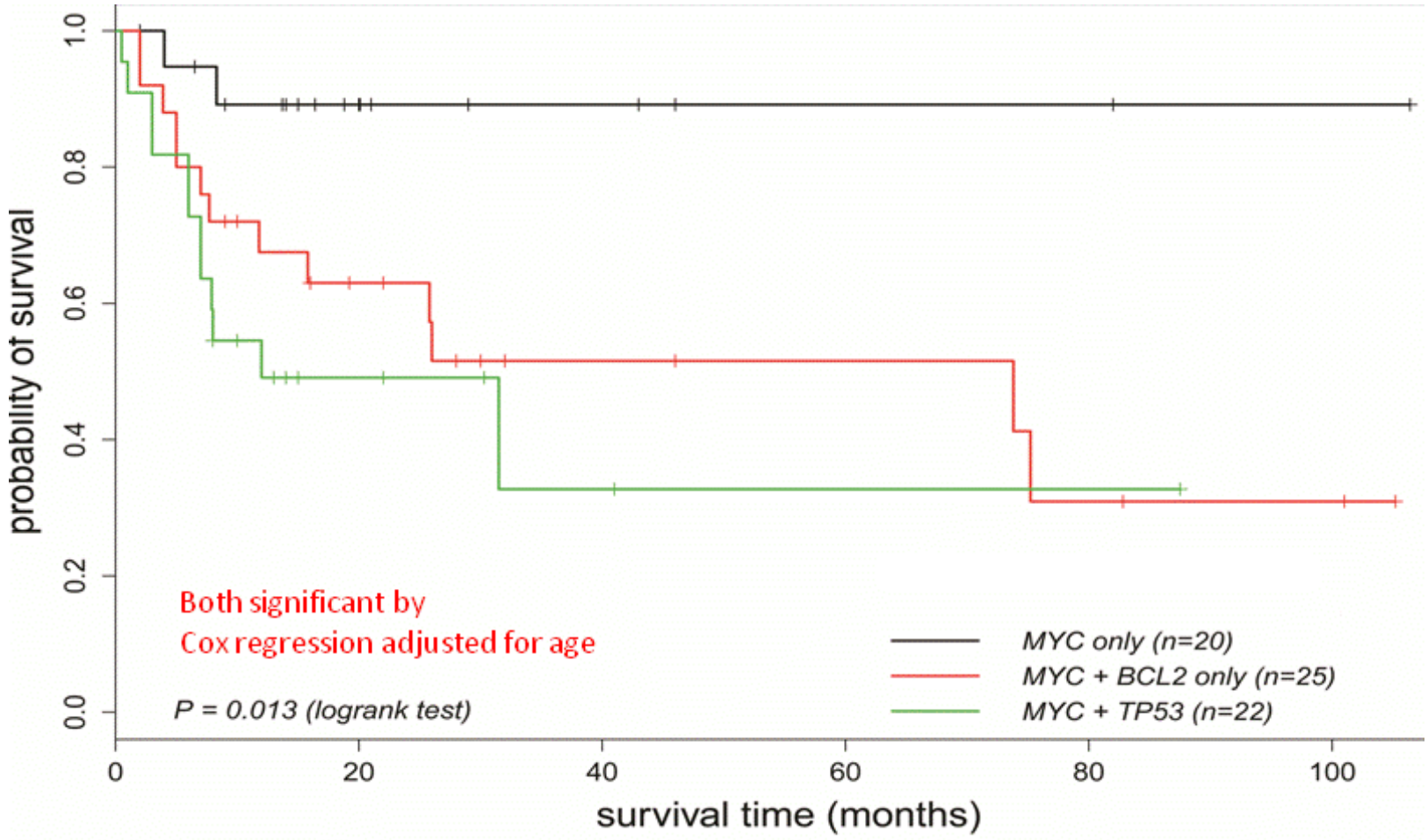
- Majority of lymphomas in Eμ-MYC mice harbour abnormalities in ARF1-MDM2-TP53
- Rapid lymphoma development in double Eμ-Myc and *p53*$^{+/-}$ mice
- Human Burkitt lymphoma
  - TP53 mutation in 40-50%
  - CDKN2A (INK4a/ARF) deletion in ~6%

TP53 mutation, BCL2 and BCL6 translocation in MYC trans+ve DLBCL



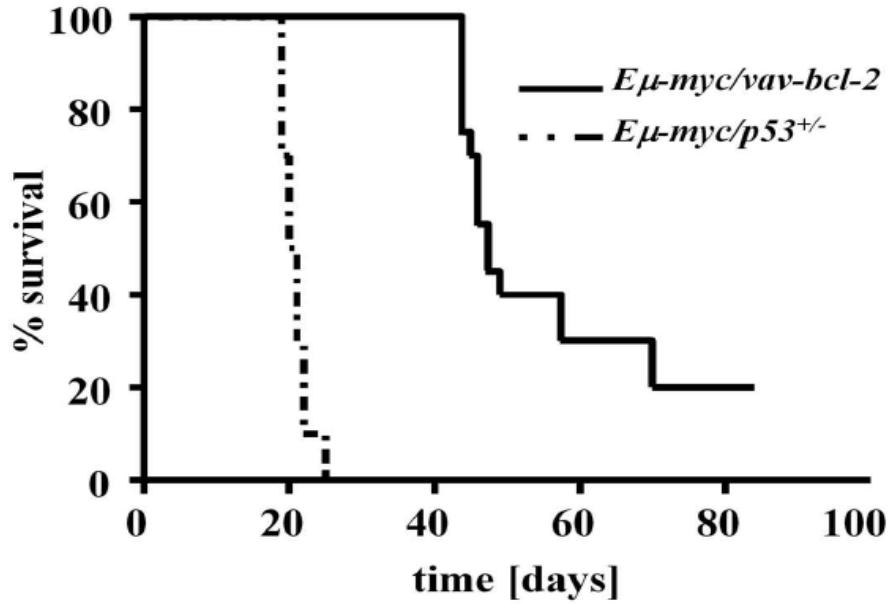
Clipson et al J Path Clin Res 2015

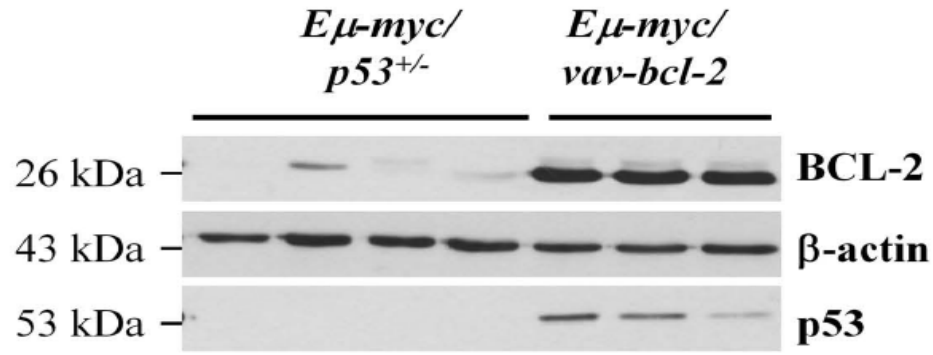
Prognosis of MYC trans+ve DLBCL according to the second genetic hit



Clipson et al J Path Clin Res 2015; Schiefer et al Medicine 2015

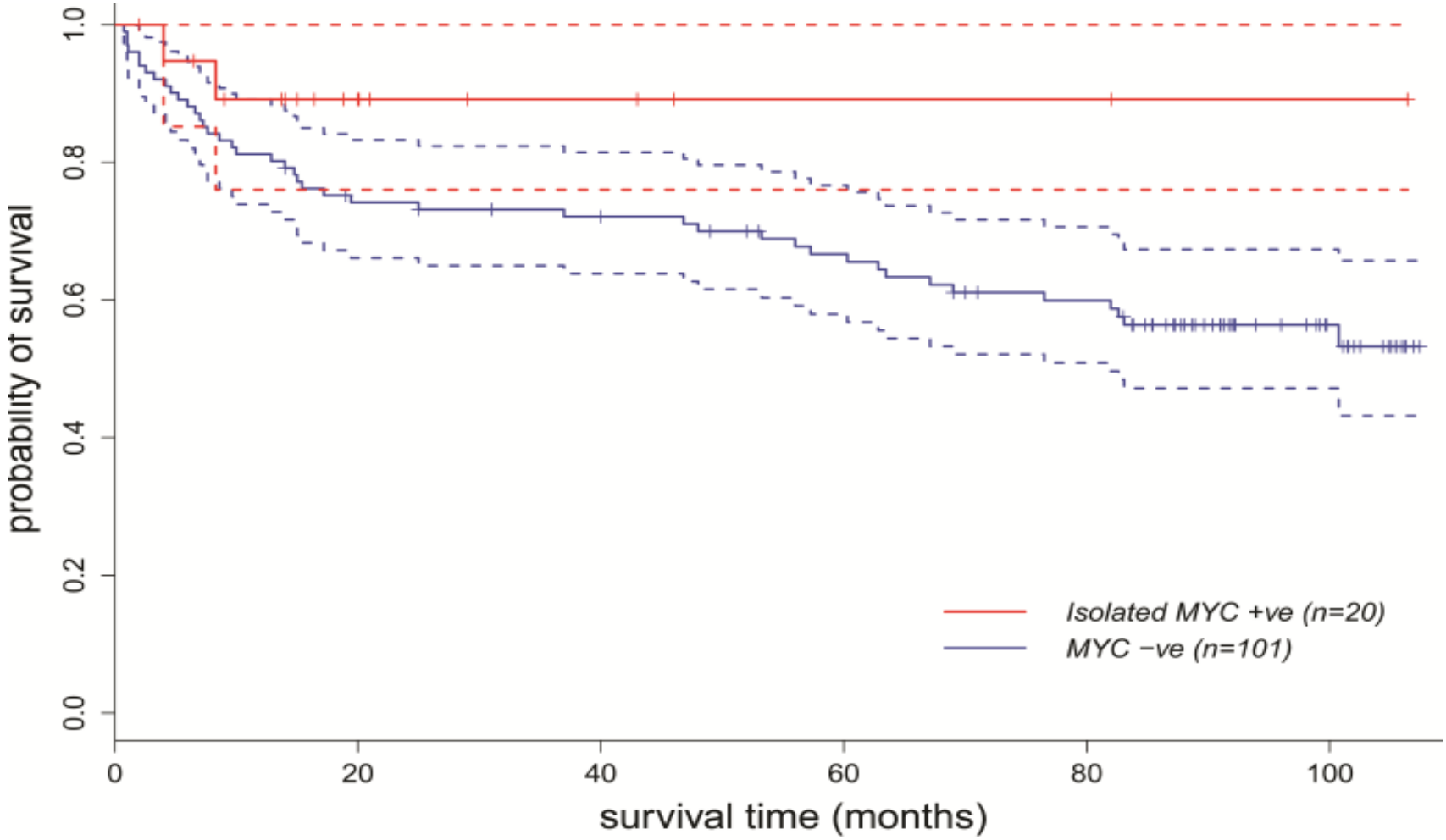
Eμ-MYC mice:
survival influenced by the second hit



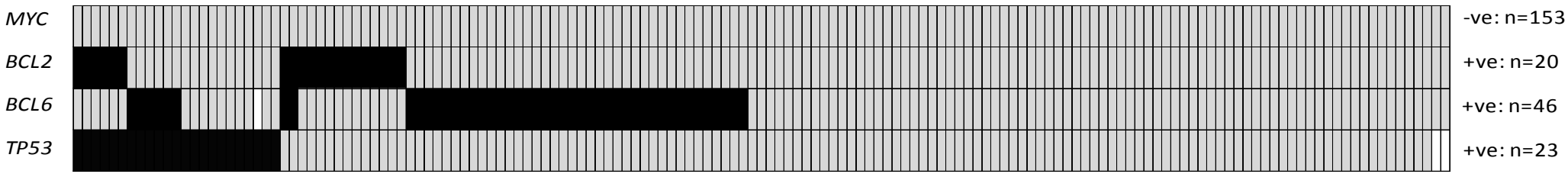


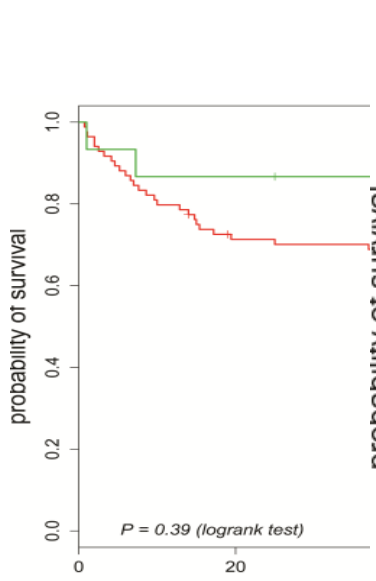
Schuster C et al Blood 2011

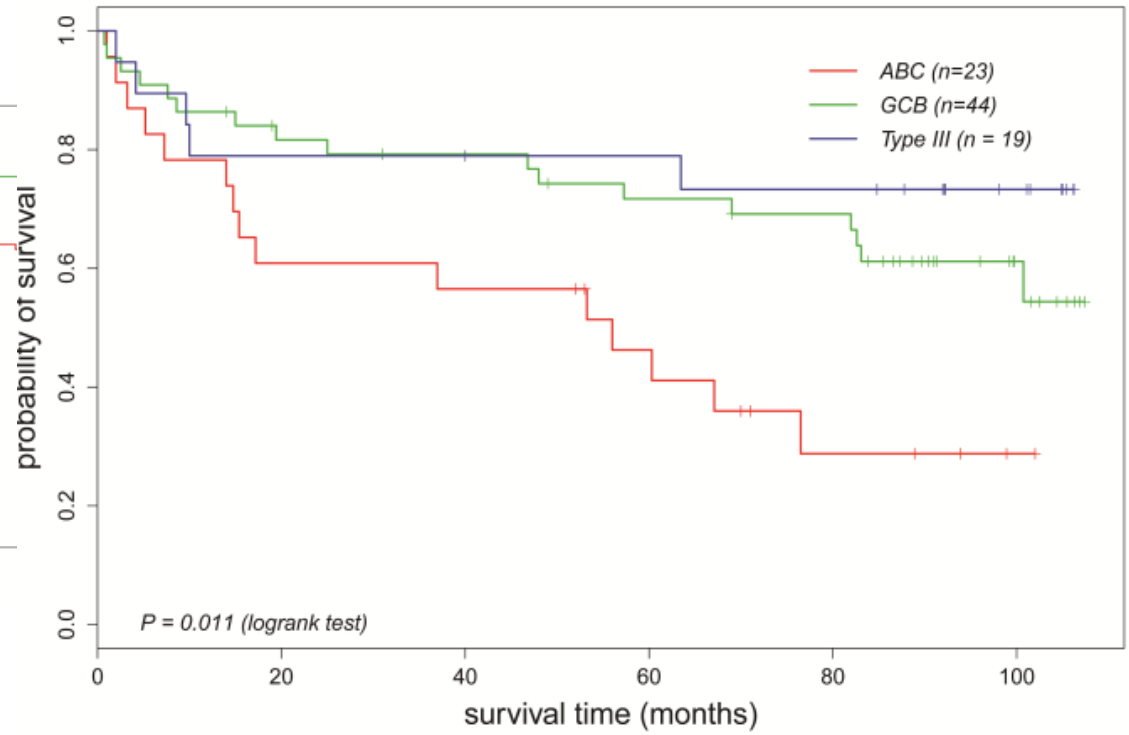
Overall survival of DLBCL with isolated MYC translocation

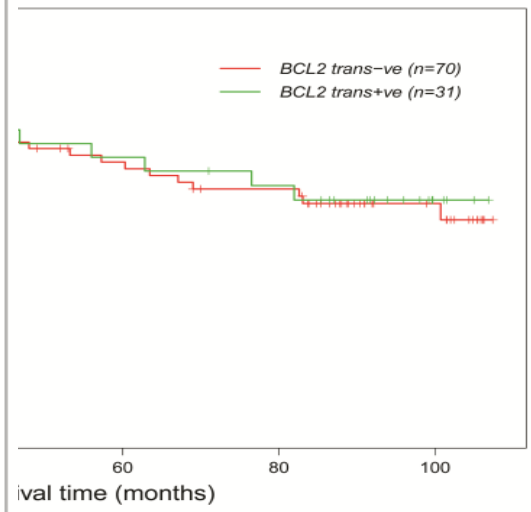


TP53 mutation, BCL2 and BCL6 translocation in MYC trans-ve DLBCL









Clipson et al J Path Clin Res 2015

MYC translocation in prognosis of DLBCL

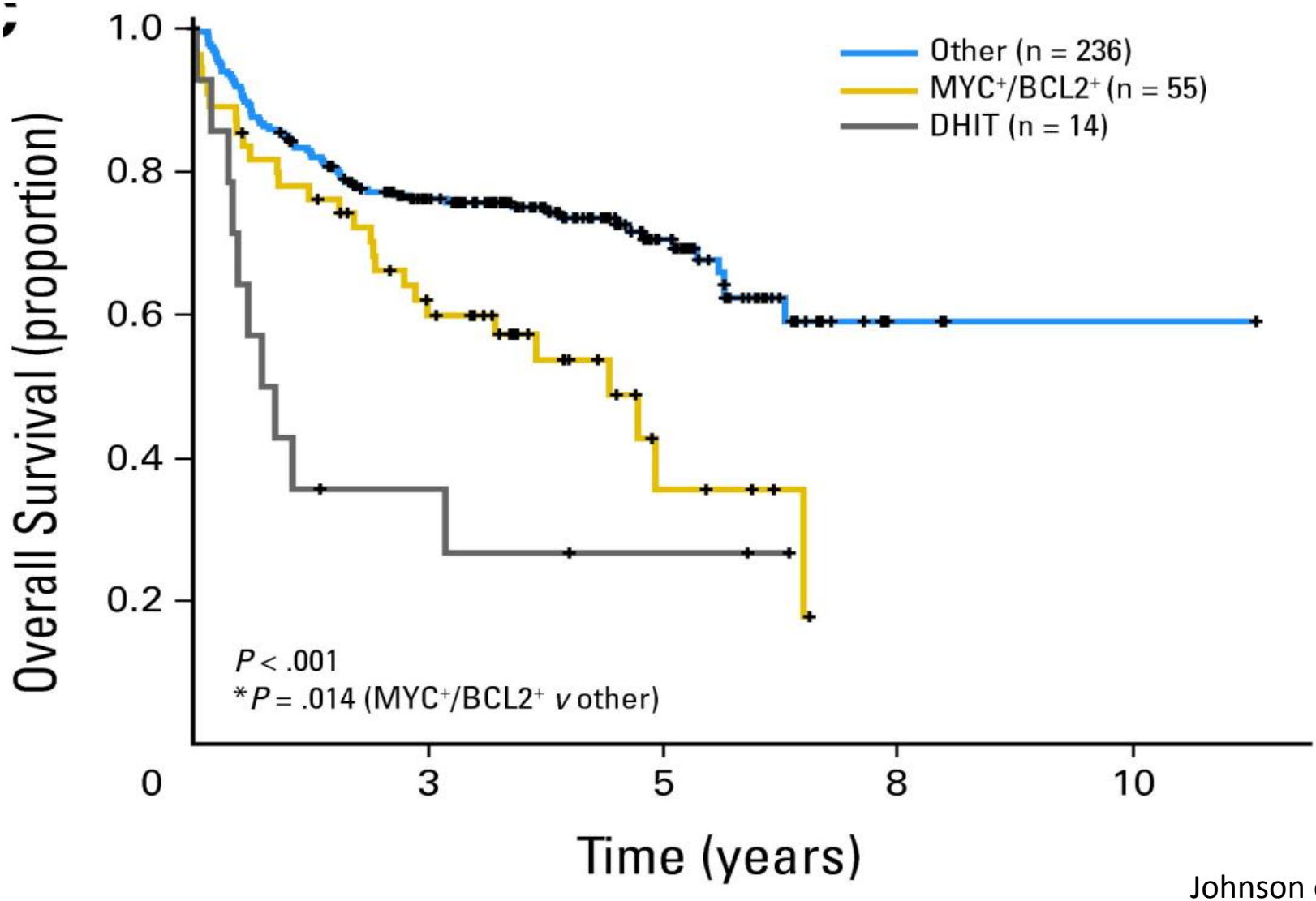
- Double hit DLBCL
  - MYC translocation + TP53 mutation
  - MYC + BCL2 translocation
- Double hit DLBCL show significant worse prognosis
- Distinguish double-hit DLBCLs from those with an isolated *MYC* translocation.

Double hit DLBCL: detection by IHC?

- MYC & BCL2 IHC
  - MYC/BCL2 double-hit
  - MYC/BCL2 over-expression, but no translocation
- MYC & TP53 IHC:
  - Not detecting *TP53* frameshift/nonsense changes

Potential issues: variability in staining
subjective
cutoff value
false negative / positive

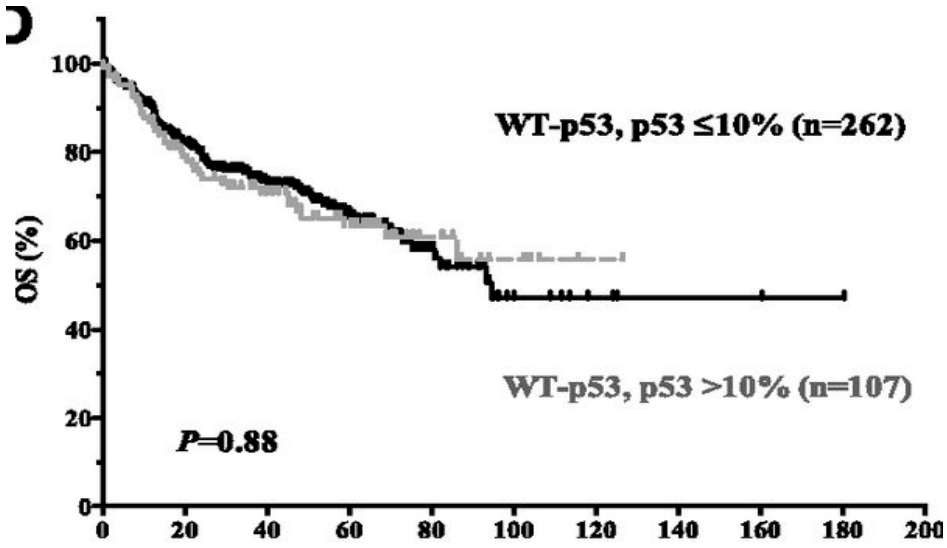
MYC/BCL2 double-hit vs MYC/BCL2 co-expression

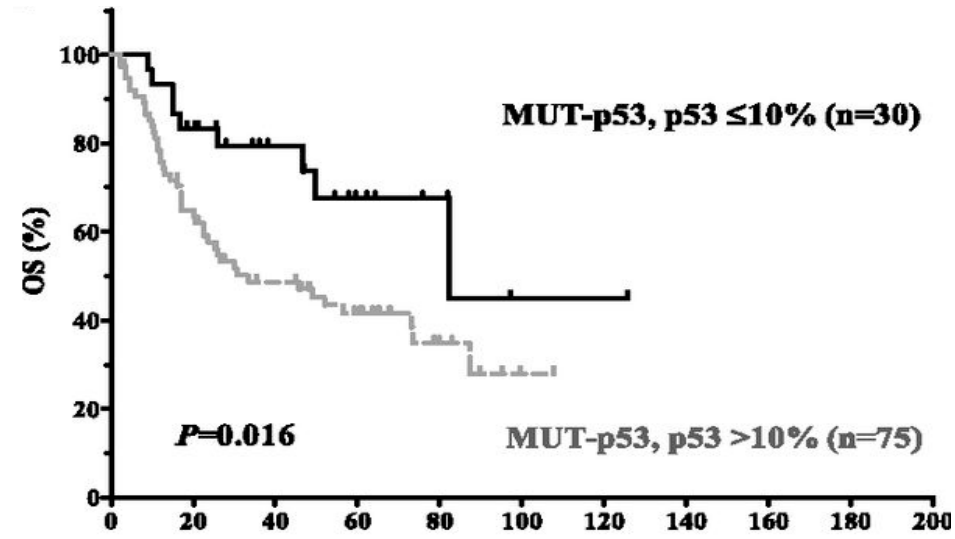


Johnson et al. JCO 2012

DLBCL: prognostic value of TP53 staining

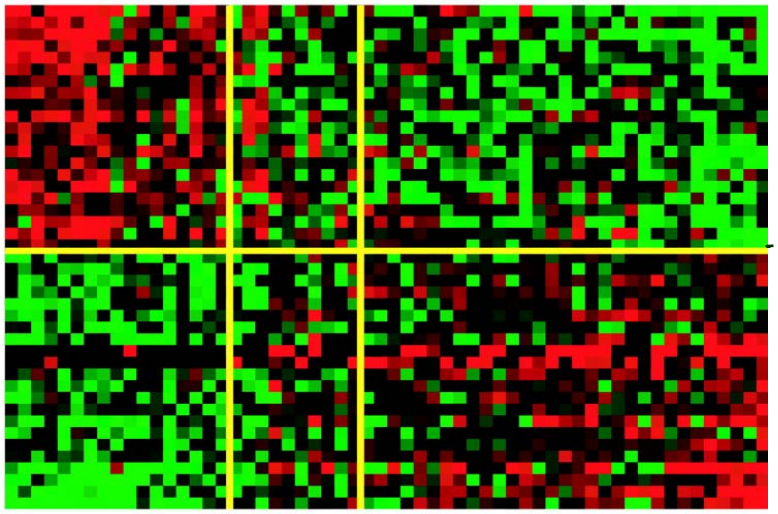
	Overall	WT-p53	MUT-p53
Number of MDM2$^-$ patients	285	216	69
Number of MDM2$^+$ patients	193	156	37
Mean MDM2 expression (% cells)	22%	24%	17%
Number of p53$^-$ patients	292	262	30
Number of p53$^+$ patients	182	107	75
Mean p53 expression (% cells)	23%	14%	54%



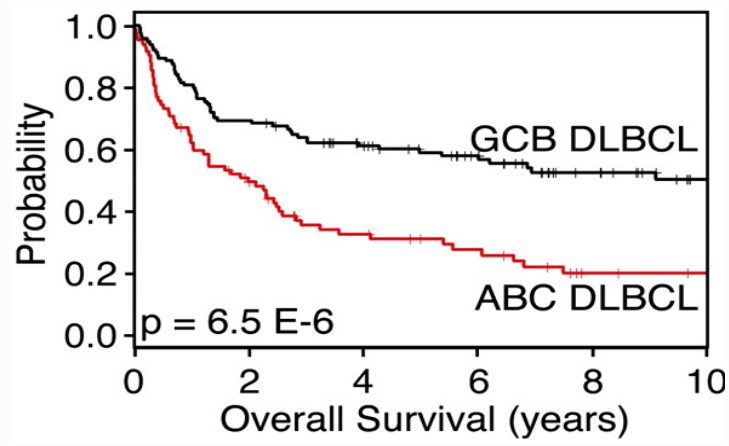


Xu-Monette Z Y et al. Blood 2013

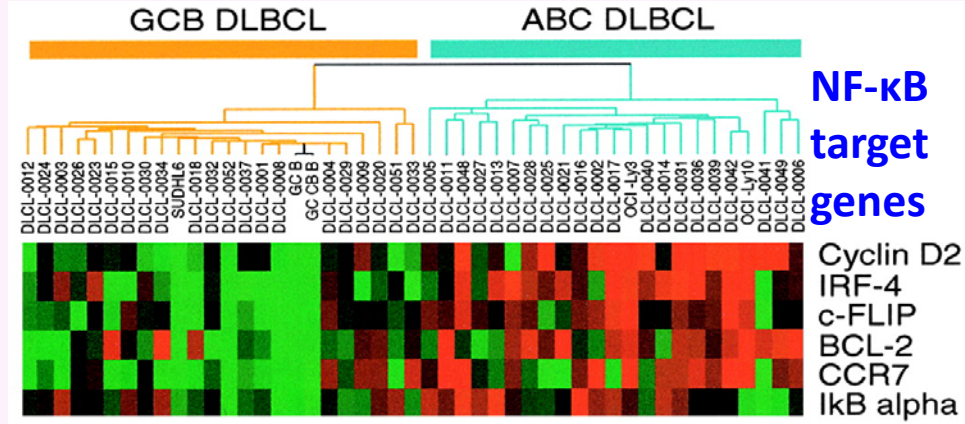
Diffuse large B-cell lymphoma (DLBCL)



Clinical utility



Molecular mechanism



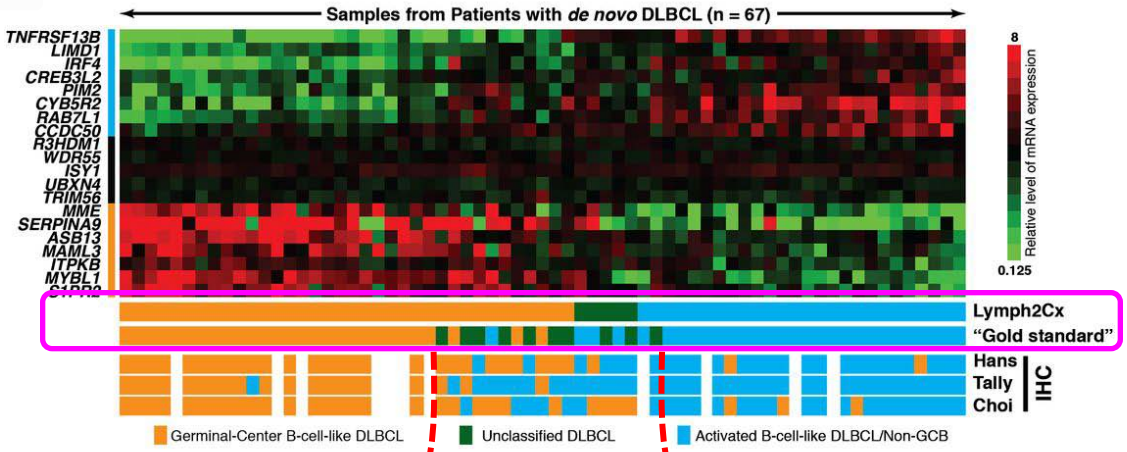
Wright G et al. PNAS 2003;100:9991-9996

COO subtyping using FFPE tissue

- IHC: lacks reproducibility and low efficacy in survival separation;

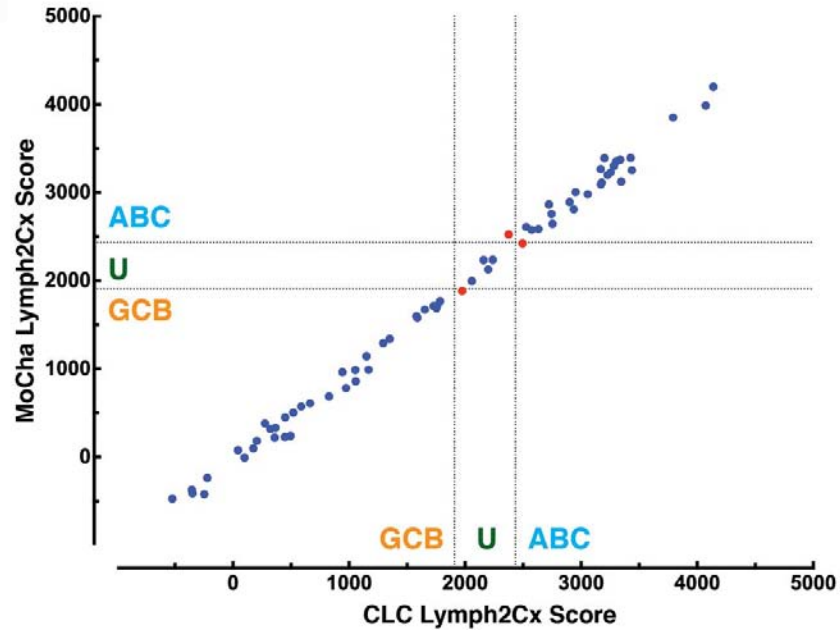
- Quantitation of classifier gene mRNA expression
  - Quantitative nuclease protection assay
  - Affymetrix HG133 plus 2 platform
  - Illumina DASL assay /DAC classifier
  - Nanostring technology
  - qRT-PCR with Fluidigm Biomark HD system

COO subtyping by GEP using FFPE tissue:



"molecular grey zone"

Concordance between paired FFPE and FF tissues = ~85%

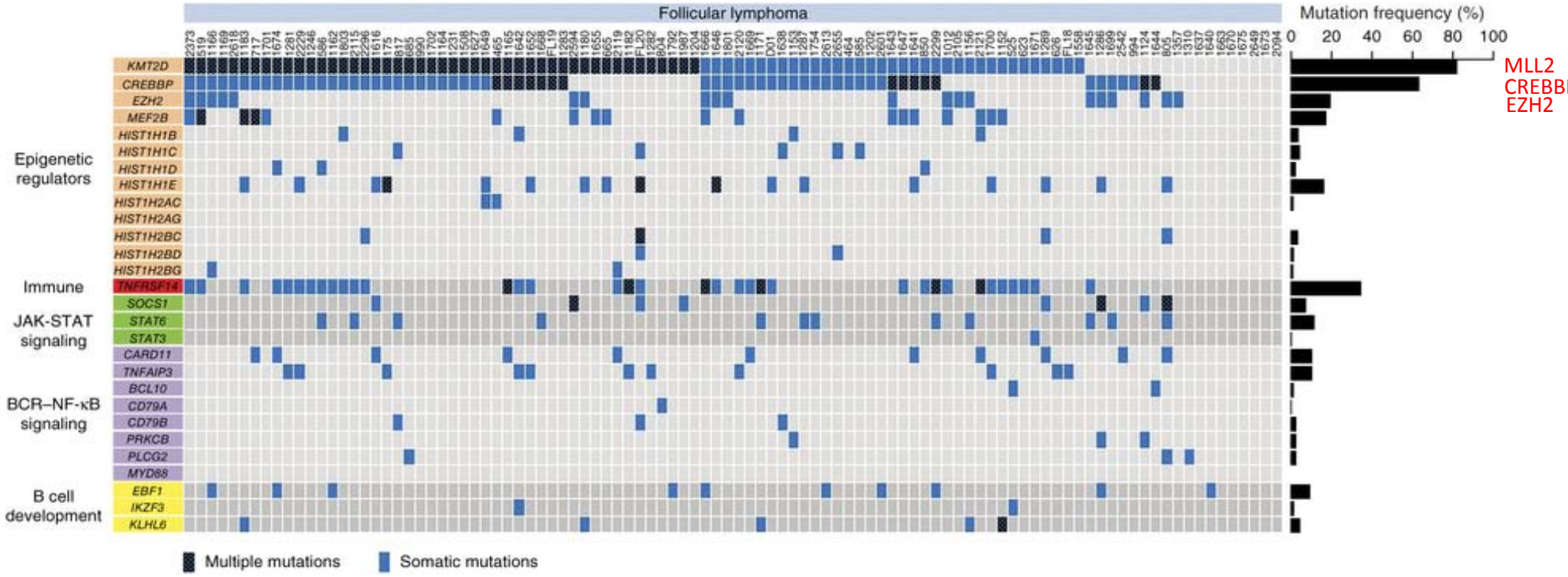


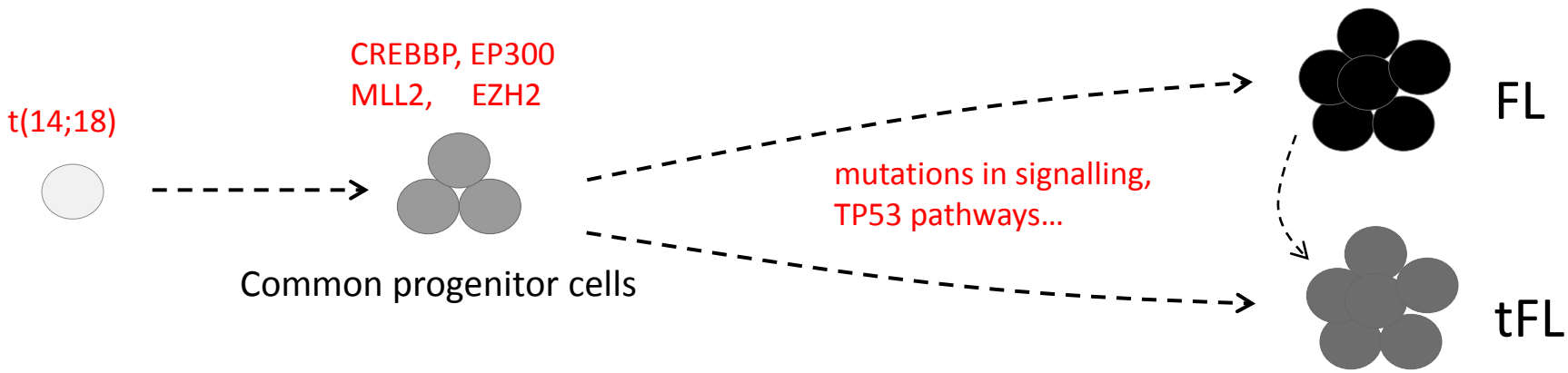
highly concordant between labs = 98%

Scott et al Blood 2014

Mutation discoveries by NGS and their potential clinical utility

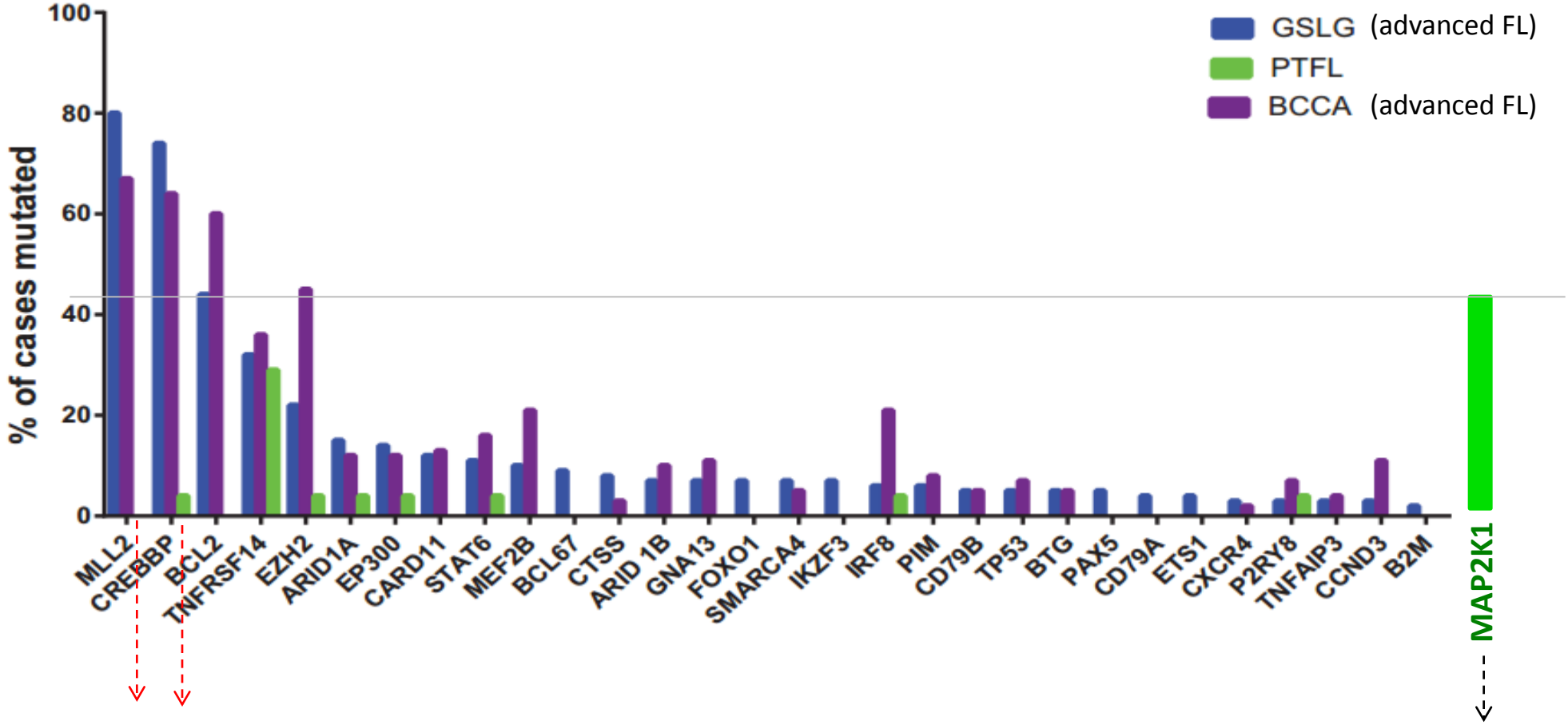
FL: mutation profile





Green et al Blood 2013; Pasqualucci et al Cell Rep 2014, Okosun et al Nat Genet 2014

Paediatric-type nodal FL: mutation profile



MLL2 CREBBP

↓↓ in PTFL

MAP2K1

↑↑ in PTFL

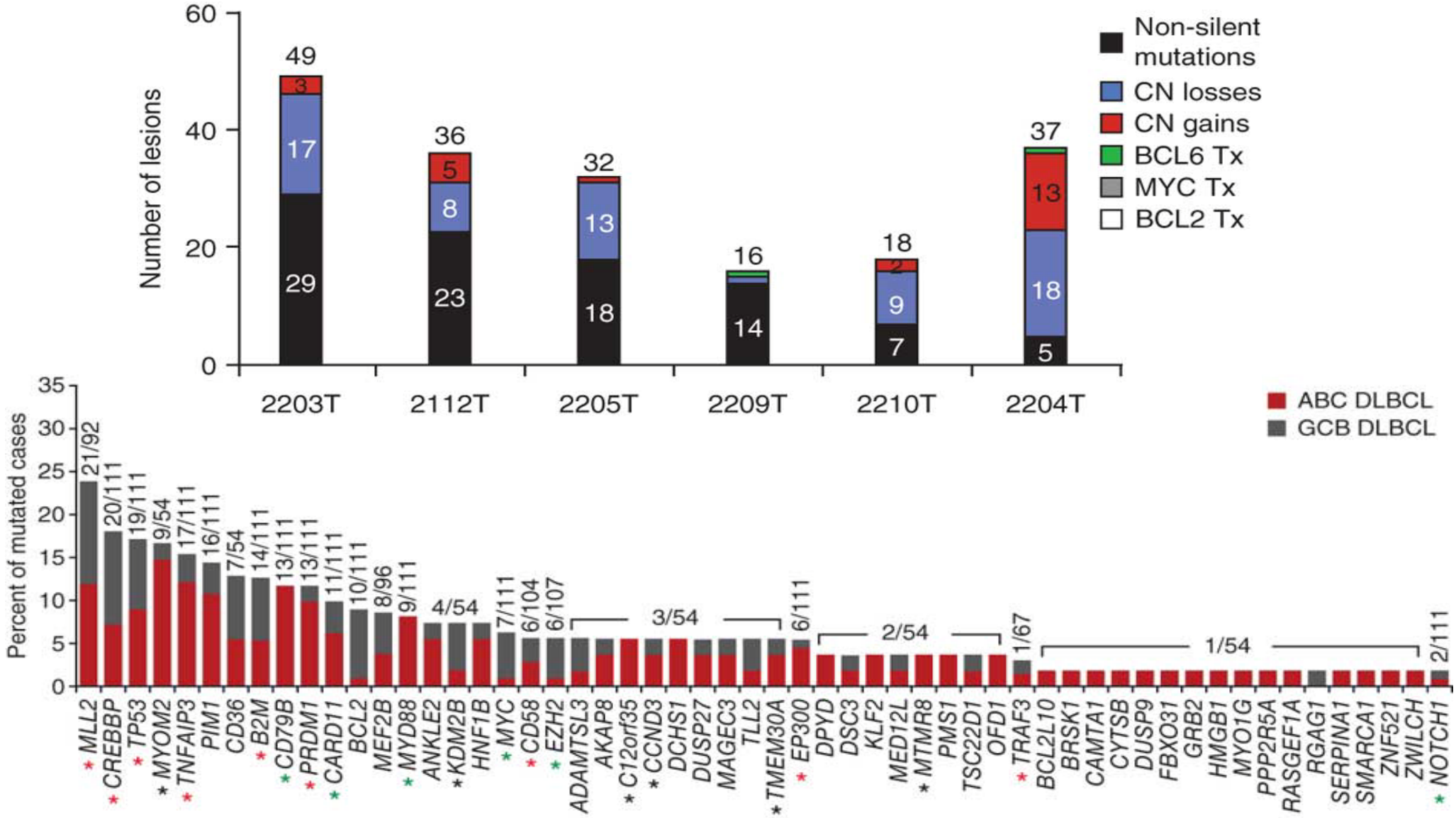
Louissaint Jr et al Blood 2016; Ozawa et al MP 2016

FL: genetic changes in differential diagnosis

Genetic changes	PTNFL	t(14;18)-ve FL	t(14;18)+ve FL
t(14;18)	-ve	-ve	+ve
MAP2K1	43%	n/a	?0
TNFRSF14	29-51%	36%	18-46%
KMT2D(MLL2)	0-16%	36%	67-82%
CREBBP	~3%	45%	33-64%
EZH2	0-3%	18%	7-20%

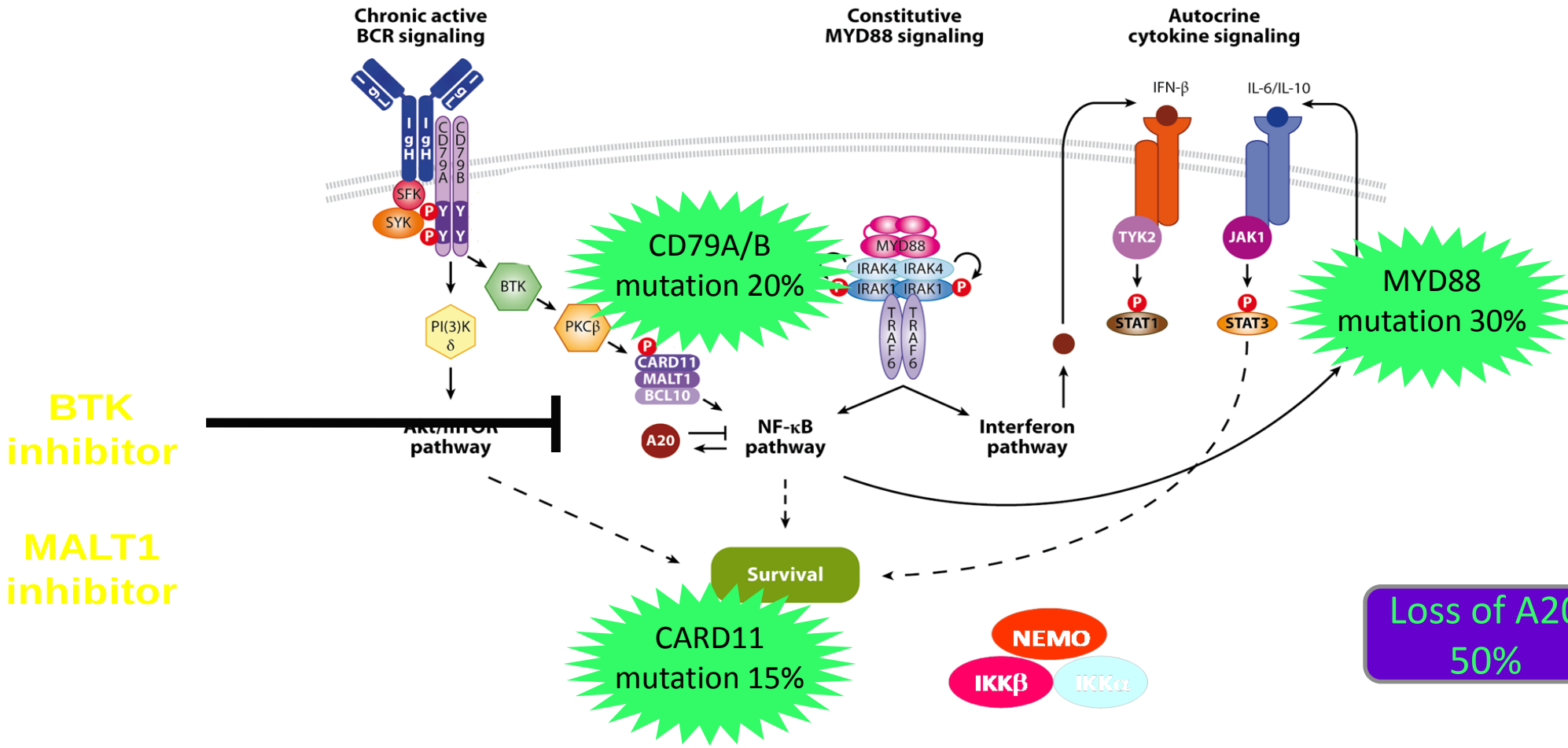
Louissaint et al Blood 2016; Schmidt et al Blood 2016

DLBCL: coding genome analyses

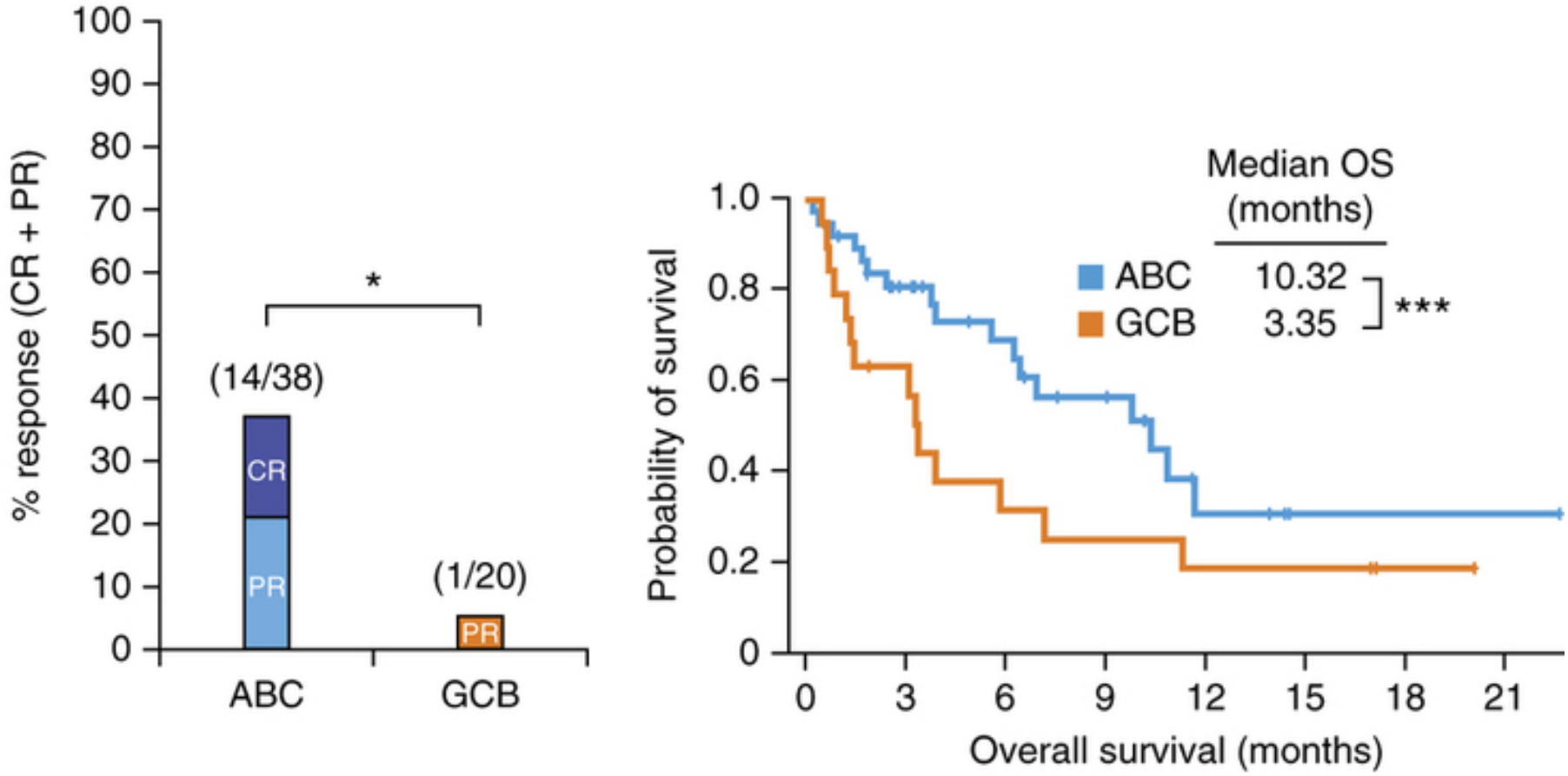


Pasqualucci et al, Nature 2011; Nat Genet 2011

Targeted therapy in ABC-DLBCL

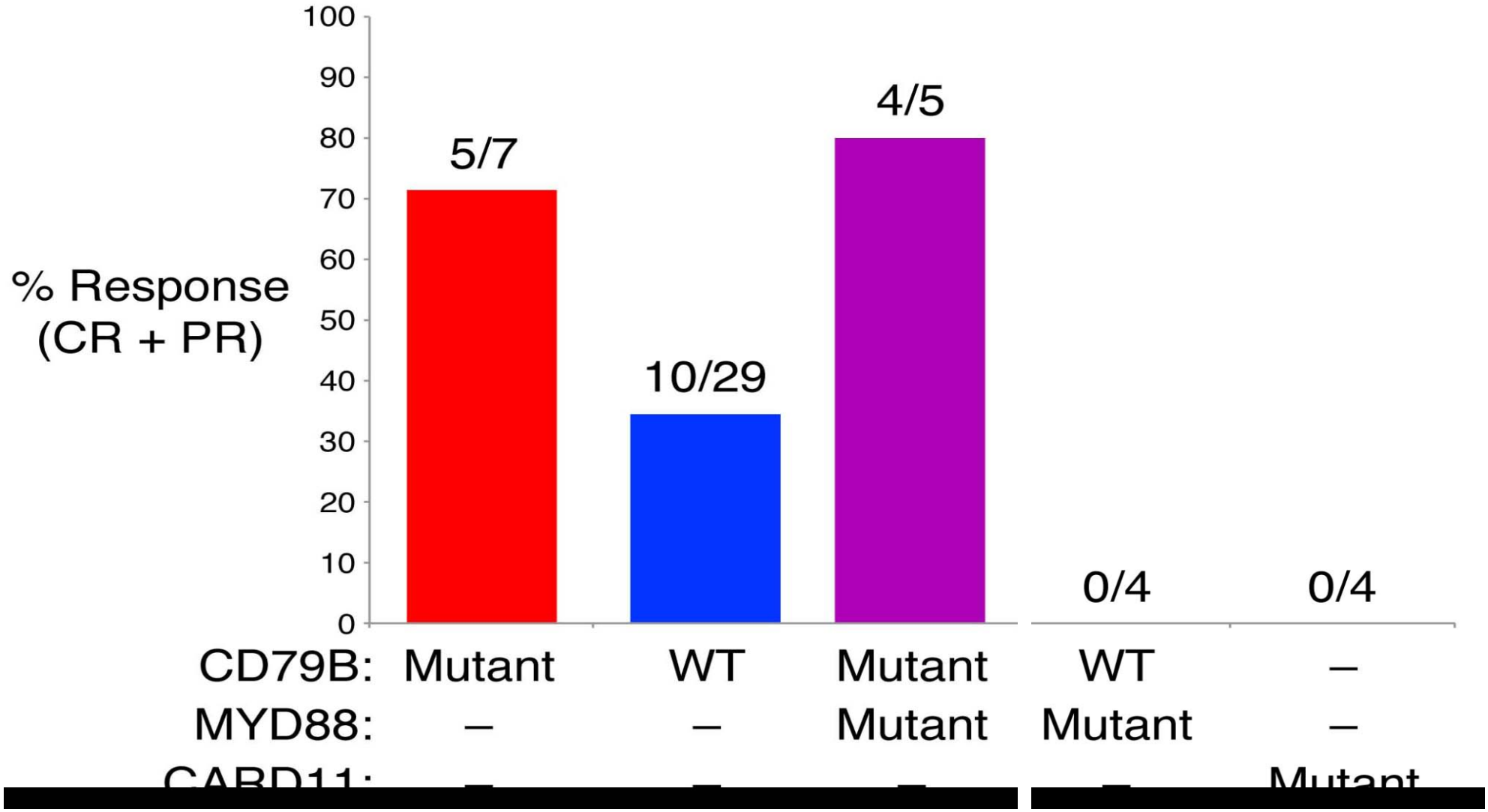


Phase I/II clinical trial of Ibrutinib in relapsed/refractory DLBCL



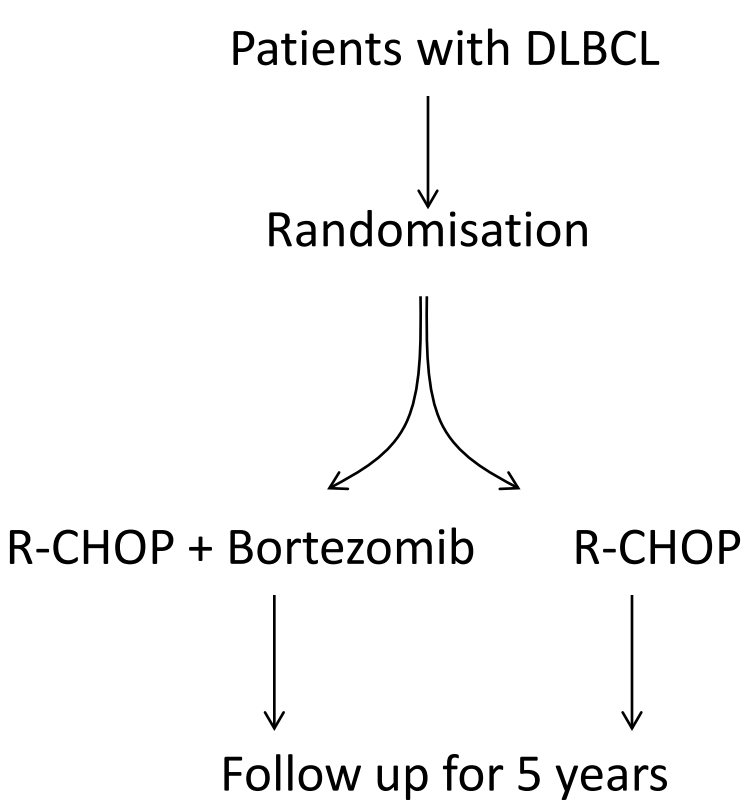
Wilson et al Nat Med 2015

Impact of CD79B, MYD88 and CARD11 mutation on Ibrutinib treatment response in ABC-DLBCL



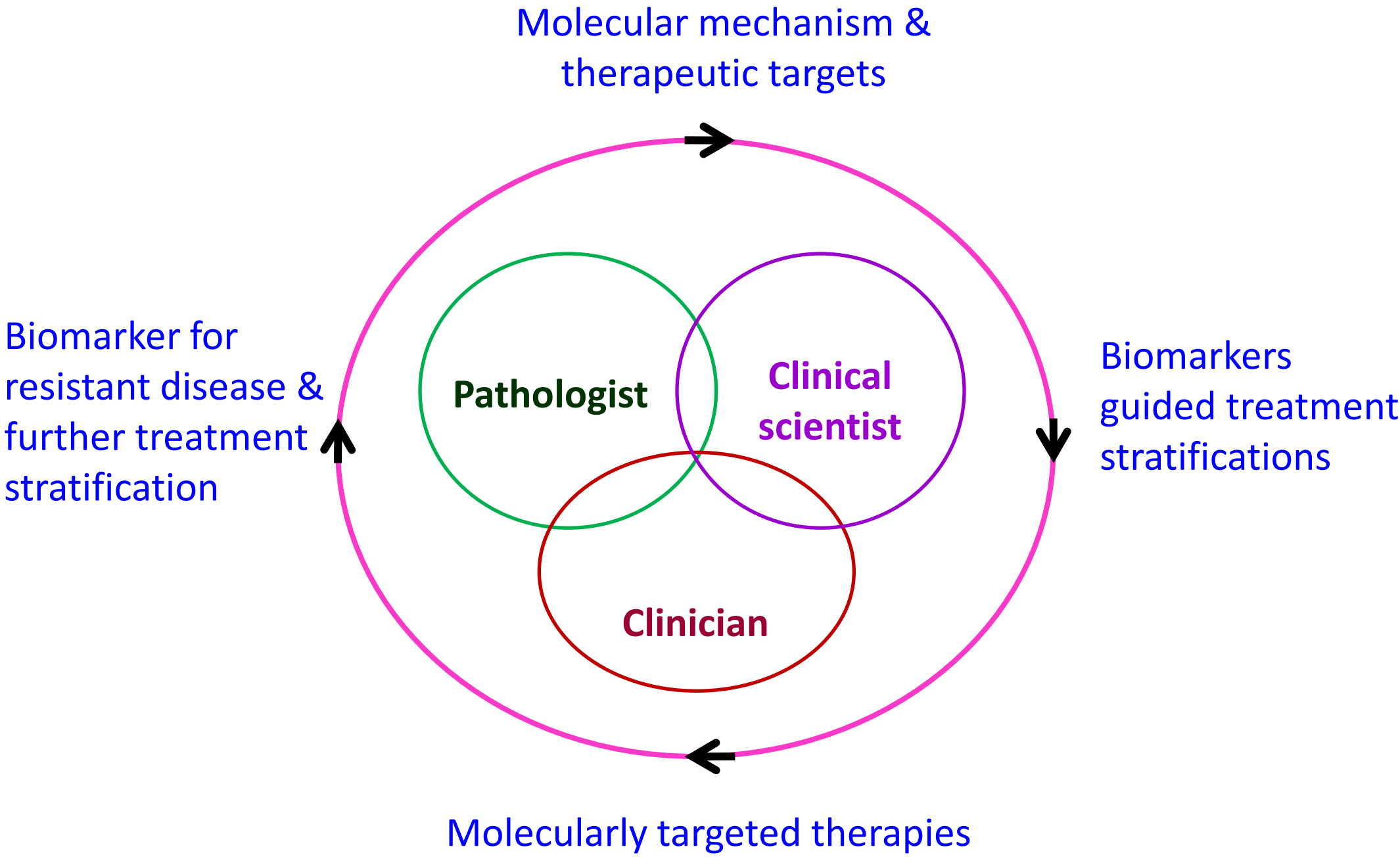
Courtesy of Louis Staudt

REMoDL-B phase-3 trial



- ABC/GCB molecular subtyping
- Mutation profiling
  - NF-kB pathway
  - B-cell differentiation
  - Cell cycle & apoptosis
  - Epigenetic programme
- NF-kB target gene signature

- Does the addition of bortezomib to R-CHOP improve progression free survival?
- Does the molecular phenotype (either ABC or GCB) determine the benefit from the addition of bortezomib?


Molecular mechanism &
therapeutic targets
Biomarker for
resistant disease &
further treatment
stratification
Pathologist
Clinical
scientist
Clinician
Biomarkers
guided treatment
stratifications
Molecularly targeted therapies

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University of Cambridge

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Western General Hospital

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Gehong Dong
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Guy's, St Thomas Hospital

Michael Neat
Bridget Wilkins
Paul Fields

Bloodwise
Beating blood cancer since 1960

Pathological Society
Understanding Disease

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