IX SIMPOSIO

Grupo Cooperativo Español de Citogenética Hematológica

Avances de las técnicas citogenéticas y moleculares en el diagnóstico de las hemopatías malignas

Organiza: GCECGH

SEHH Sociedad Española de Hematología y Hemoterapia

FEHH Fundación Española de Hematología y Hemoterapia

Alteraciones (cito)genéticas en el diagnóstico de linfomas de célula B

Sílvia Beà, PhD

sbea@clinic.cat - @SilviaBea1

Secció Hematopatologia, Servei Anatomia Patològica, Hospital Clínic

Lymphoid Program-Fundació de Recerca Clínic/IDIBAPS

Universitat Barcelona

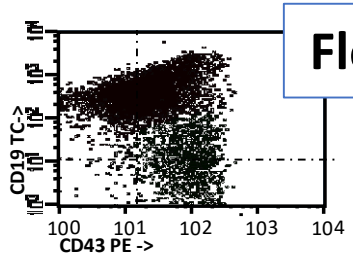
Clínic Barcelona IDIBAPS Universitat de Barcelona

www.gcecgh.org

#gcecgh

Diagnosis in B-Lymphoid Neoplasms: An integrated process

Clinical Information

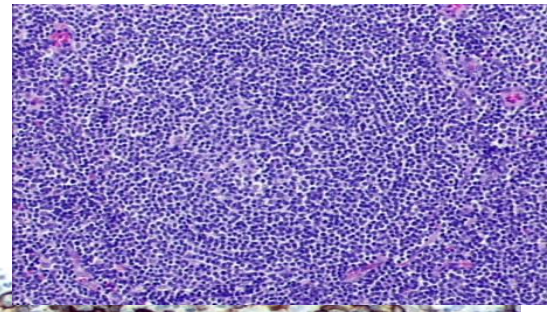


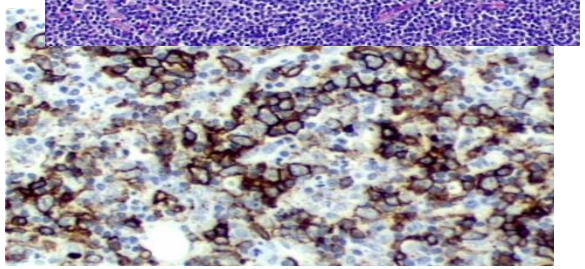
Flow Cytometry

Phenotype

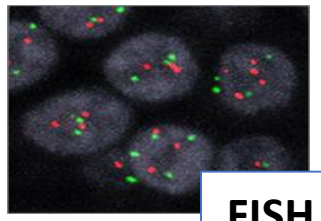
Sample

Morphology
- Architecture
- Cytology





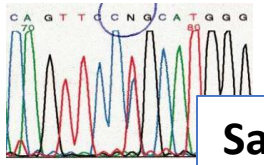
(Cyto) Genetics



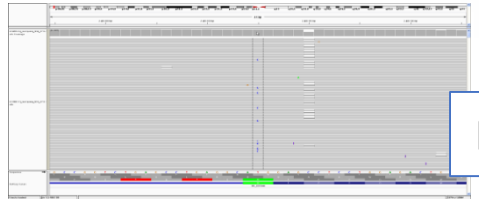
FISH

Karyotype

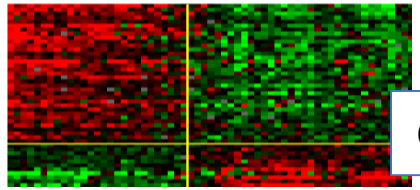
Molecular Biology



Sanger



NGS



Gene expression

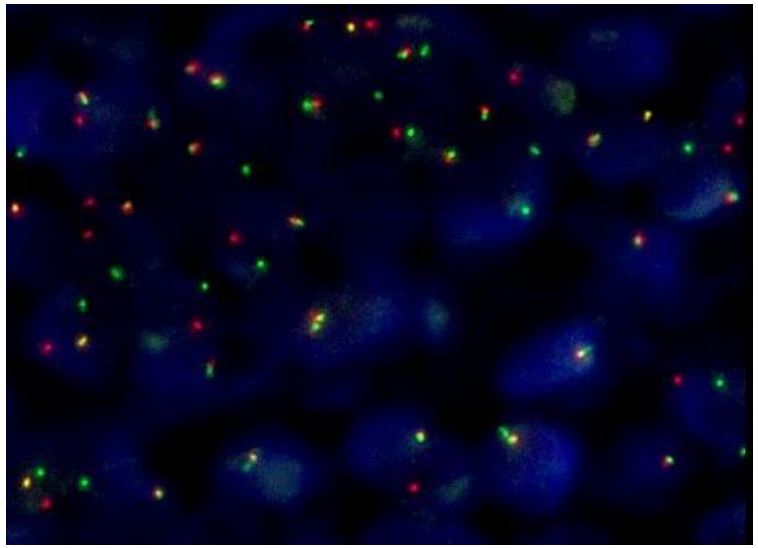
Diagnosis
- Defined Entity
- Prognosis
- Therapeutic targets

2022 WHO/ICC classifications & ICC genomic recommendations

The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. *Alaggio et al, Leukemia 2022 Jul;36(7):1720-1748*
→ *Blue Book on line beta version, provisional.*

The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee. *Campo et al, Blood. 2022 Sep 15;140(11):1229-1253*
→ *Virchows Arch 2023.*

➢ **FISH**



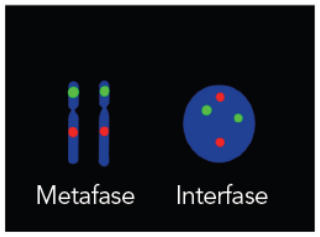
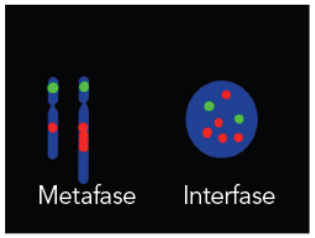
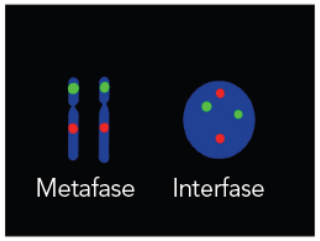
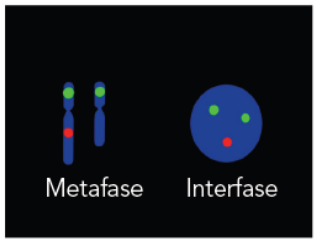
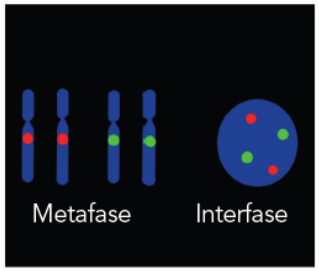
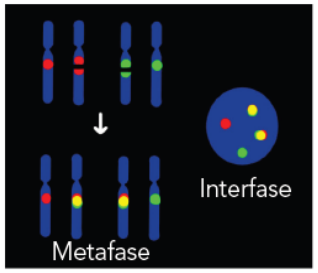
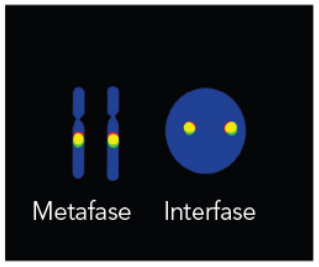
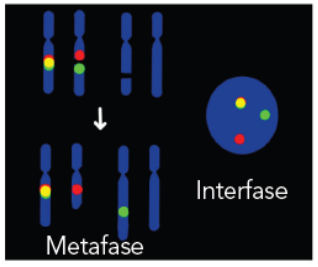
Genomic profiling for clinical decision making in lymphoid neoplasms *(Blood 2022 Nov 24;140(21):2193-2227)*

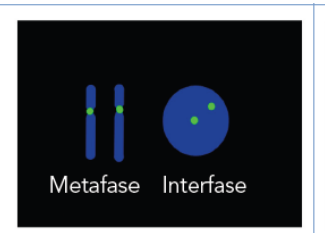
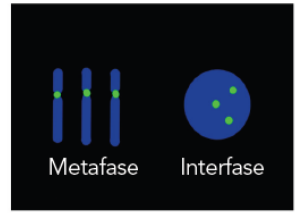
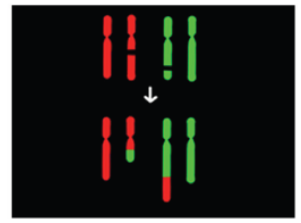
Laurence de Leval,[1,*] Ash A. Alizadeh,[2-5] P. Leif Bergsagel,[6] Elias Campo,[7] Andrew Davies,[8] Ahmet Dogan,[9] Jude Fitzgibbon,[10]

Established molecular assays and newly developed technologies complement clinical diagnoses and provide novel information important for:

- ✓ contribution to diagnosis
- ✓ refinement of entities/subtypes
- ✓ risk stratification
- ✓ therapy prediction

Type of FISH probes used in B-Lymphoid Neoplasms

		Modelo de célula normal	Modelo de célula aberrante
Sondas específicas de *locus* o sondas de secuencia única	Amplificación o ganancia de gen		
	Deleción o pérdida de gen		
	Reordenamientos con sondas de doble fusión		
	Reordenamientos con sondas de separación *(break apart)*		

Sondas centroméricas		
Sondas de pintado cromosómico Usadas únicamente en el estudio en metafases		

Our Spanish Cooperative group FISH recommendations

IX SIMPOSIO
Grupo Cooperativo Español de Citogenética Hematológica
Avances de las técnicas citogenéticas y moleculares en el diagnóstico de las hemopatías malignas

(Contar 200 núcleos/hibridación, cutoffs establecidos en cada laboratorio)

✓**Technical recommendations ONLY**

➢ **Como Grupo Cooperativo cuales son nuestras recomendaciones en relación a las sondas (y el orden) que tenemos que aplicar para el diagnóstico y pronóstico de los linfomas B?**

B-Lymphoid Neoplasms that require FISH testing

- Chronic lymphocytic leukemia → Clear Consensus Guidelines for FISH testing

- Mantle cell lymphoma
- Follicular lymphoma
- Burkitt's lymphoma

} Well-known entities with defining primary translocations - FISH -

- Diffuse Large-B-Cell Lymphoma
- High-grade B-cell lymphoma (-DH and –NOS)
- Large B-cell lymphoma with 11q aberration
- Large B-cell lymphoma with *IRF4* rearrangement

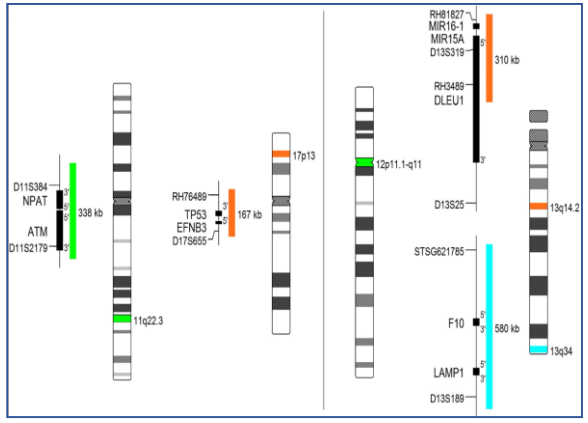
} "New" entities defined by genetic alteration - FISH -

FISH in chronic lymphocytic leukemia (CLL)

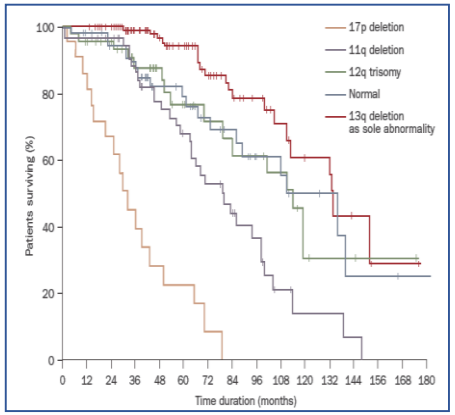
Entity	Genetic alteration: test	Diagnostic use	Clinical impact	Future assays
Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)	IGHV mutation status*: IGHV sequencing		Prognostic and predictive. IGHV gene mutational status remains stable through the disease course and only needs to be performed once	Determining BcR stereotypy and IGLV3-21^{R110} mutation status for risk stratification; tracking of resistance mutations (*BTK*, *PLCG2*, and *BCL2*; supplemental Table 3) WGS for mutations, CNAs, SVs, and complex karyotype determination MRD testing using HTS to guide therapy decisions
	del(11q), +12, del(13q), del(17p)*: FISH		Prognostic and del(17p) is predictive. FISH testing should be performed before each new course of therapy	
	TP53 mutations*: HTS		Prognostic and predictive. *TP53* sequencing should be performed before each new course of therapy unless already demonstrated	
	Detection of complex karyotype (≥5 abnormalities): cytogenetics* or SNP arrays		Prognostic	

(de Leval., Blood, 2022)

➢**FISH with XL CLL Probe Kit (XL *ATM/TP53* + XL *DLEU/LAMP*/12cen)**



(Metasystems)

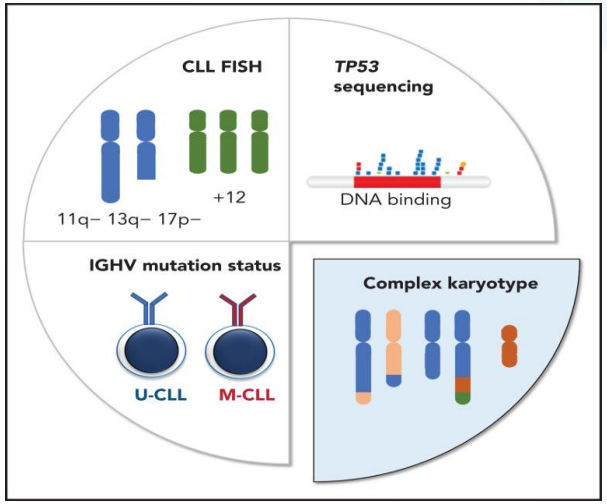


(Dohner H., NEJM, 2000)

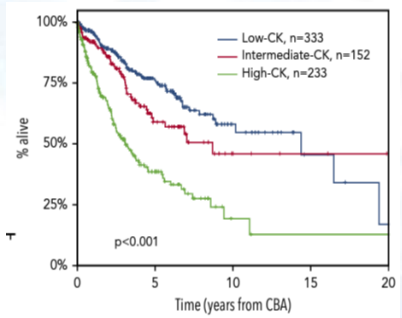
✓**International and European Guidelines**

✓**No diagnostic impact**

✓**Prognostic impact**



(Rosenquist R, The more complex, the worse outcome in CLL, Blood, 2021)



(Baliakas P, Blood 2019)

Complexity assessed by: <u>Karyotype</u>, WGS/WES, array, OGM…

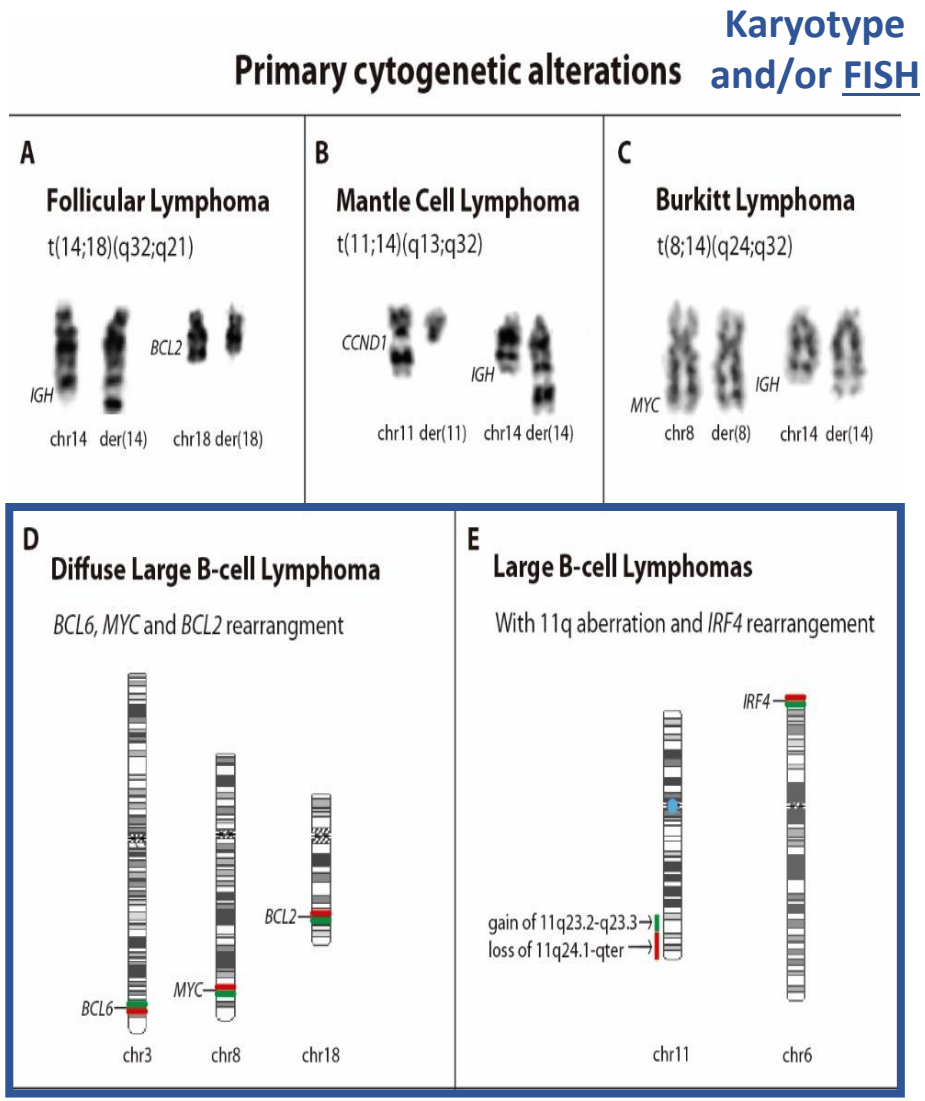
➢**ADDITIONALLY:**

FISH with <u>*IGH* break-apart probe:</u>

- **Low frequency (2%)**
- **Useful for differential diagnosis**
- **Known *IGH* partners in CLL:**
  - ***BCL2::IGH*** (good prognosis)
  - ***BCL3::IGH*** (specific subtype)
  - ***ZFP36L1::IGH*** (bad prognosis)
  - ***MYC::IGH*** (Richter T)
  - ***BCL11A::IGH…***

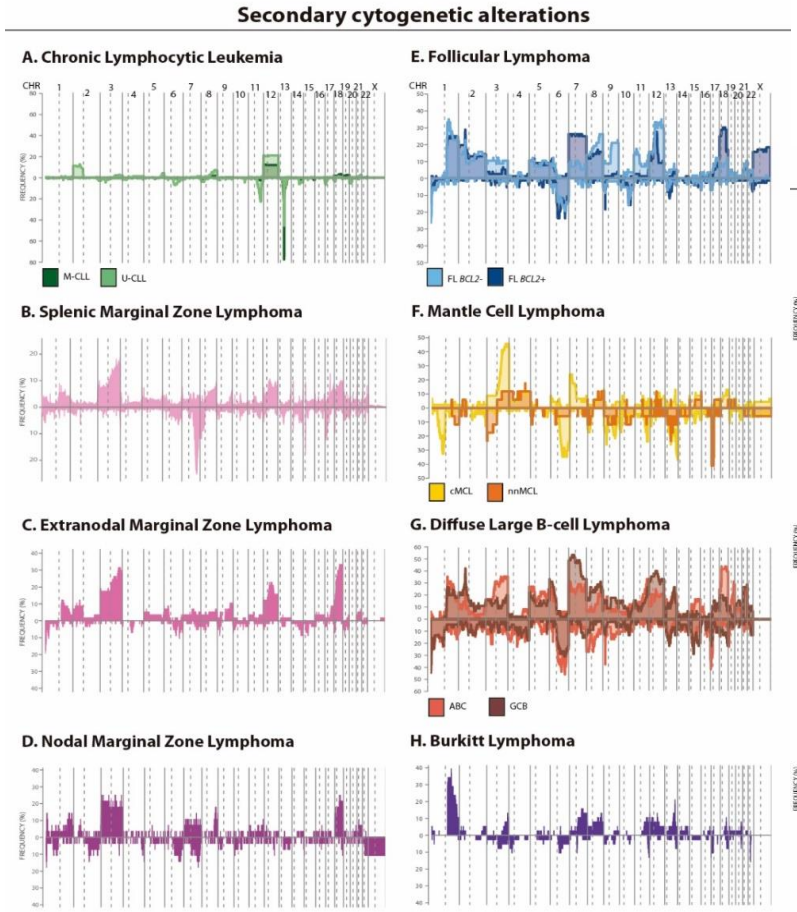
FISH in B-Lymphoid Neoplasms

Karyotype and/or FISH

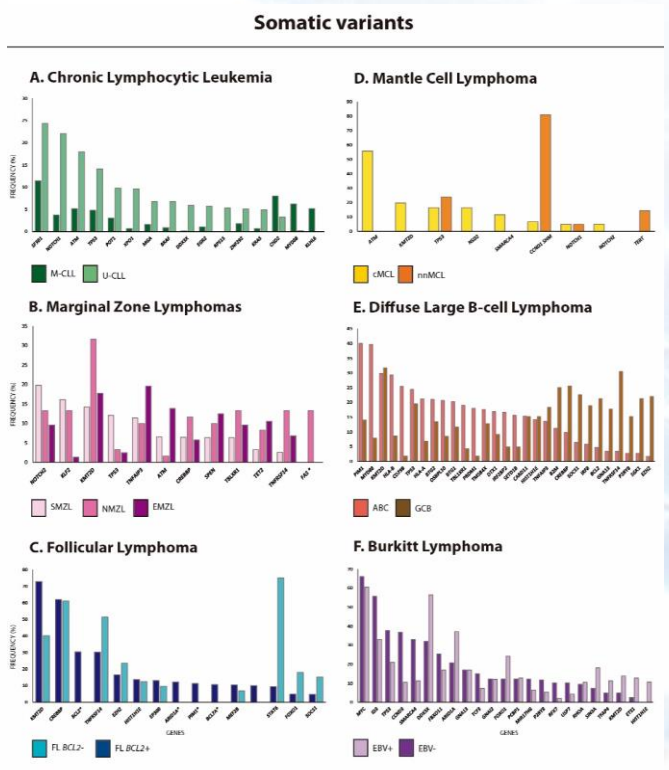


Only FISH

array, WGS/WES, OGM



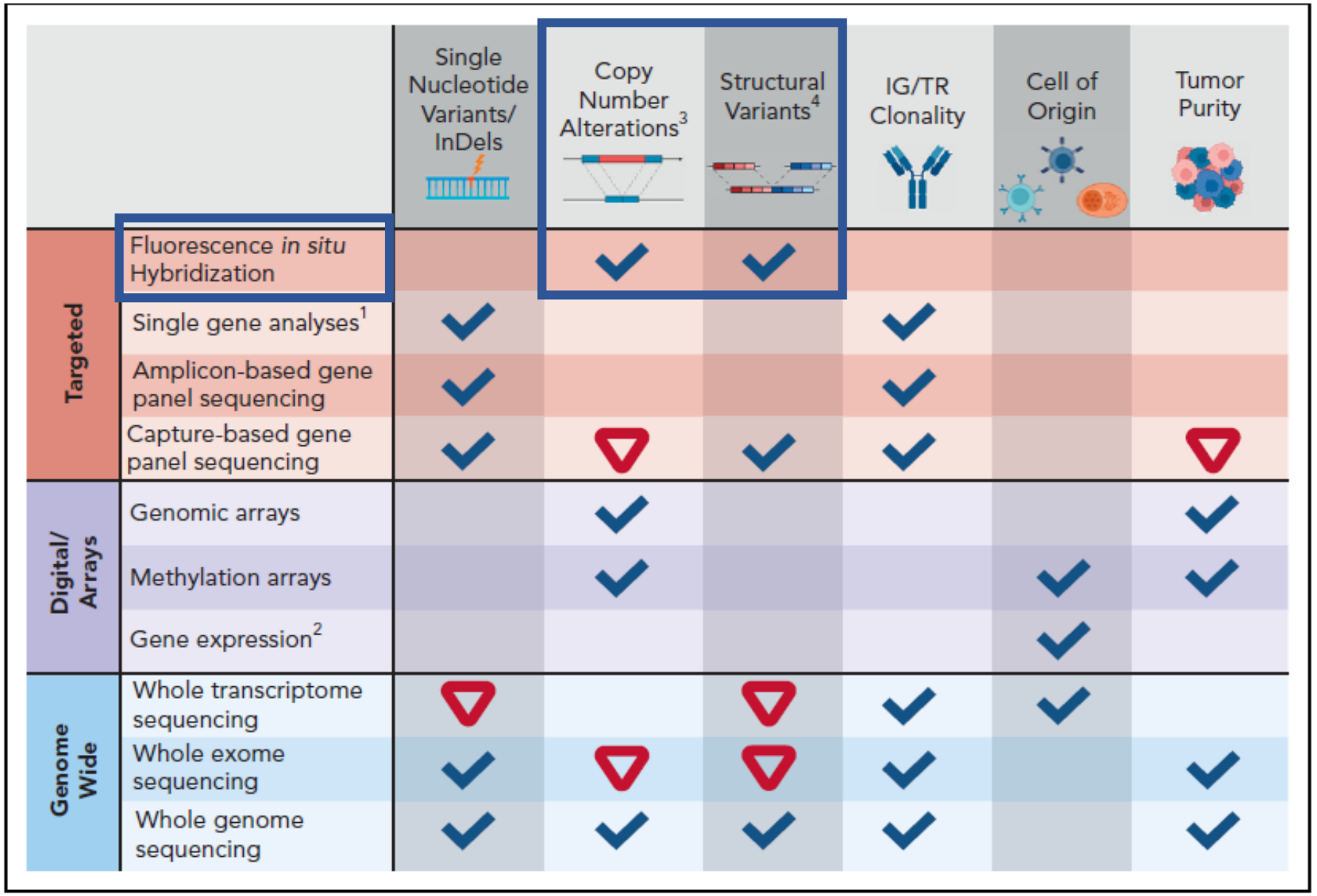
Next-generation seq. (WGS/WES/panels)



- **Additionally, the study of secondary genetic alterations (CNA and mutations) helps in:**
  - differential diagnosis of difficult cases
  - prognostic stratification

(Grau M. Cytogenomics of B-cell non-Hodgkin lymphomas: the "old" meets the "new". Best practice & research clinical haematology 2023)

Detection capacity of genomic aberrations B-Lymphoid Neoplasms with different technologies



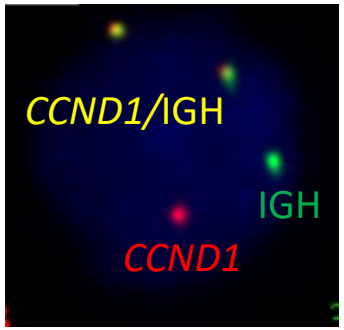
✓ Good capacity to identify a certain abnormality

▽ Limited or insufficient detection capacity

(De Leval et al Blood 2022)

Mantle cell lymphoma (MCL)

t(11;14)(q13;q32)
CCND1::IGH



Virtually all MCL have *CCND1::IGH* as primary alteration*:*

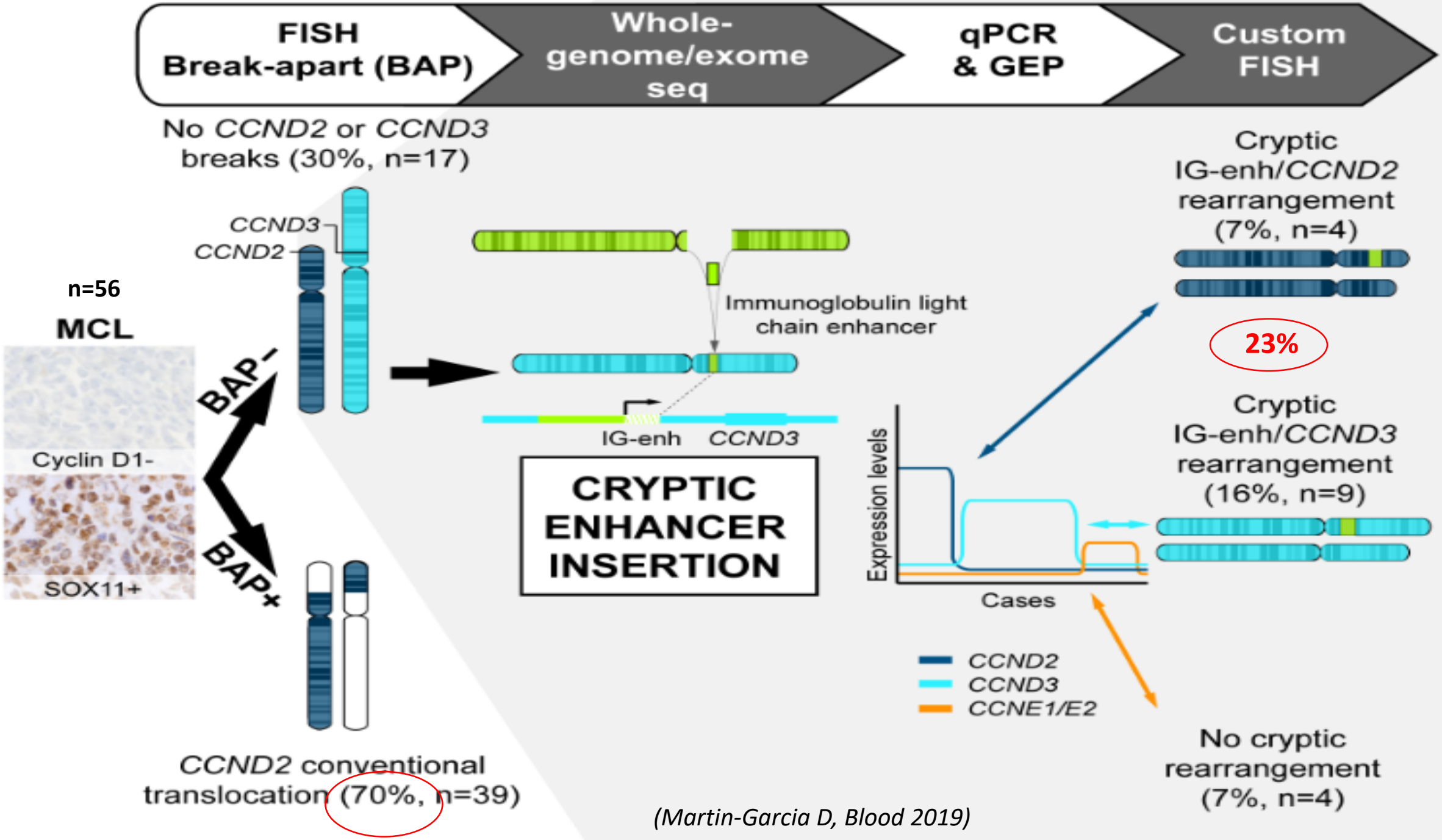
- Few variant translocations with *IGK::CCND1* or *CCND1::IGL*
- Few cryptic *CCND1r* (uncovered by WGS)
- 5-7% Cyclin D1-neg MCL *(Martin-Garcia D, Blood 2019):*
  - *CCND2r* (usually reciprocal rearrangement, few cryptic)
  - *CCND3r* (always cryptic)

- **FISH with *CCND1::IGH* and *CCND1* breakapart to avoid false-negative results**
- FISH with *CCND2* breakapart to identify most Cyclin D1-negative MCL variant

*********CCND1::IGH* may be present in non-MCL cases (and MM)

*********CCND3::IGH* reported in DLBCL, MZL and B-CLPD NOS (and MM) –ONLY 1 MCL REPORTED *(Wlodarska I, Blood 2008)*

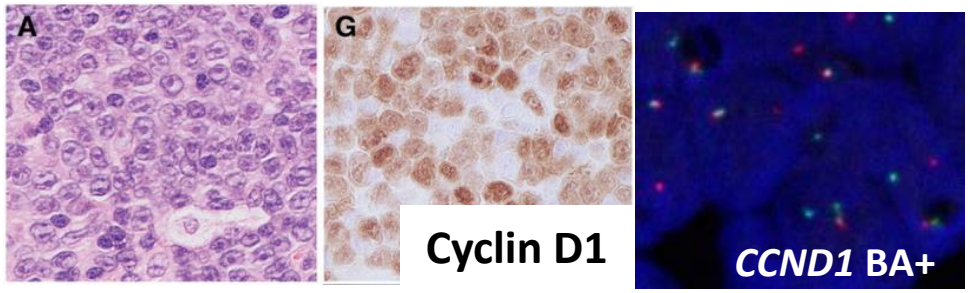
Entity	Genetic alteration: test	Diagnostic use	Clinical impact	Future assays
Mantle cell lymphoma	*CCND1* rearrangement†: FISH	Consider if CCND1 IHC is negative		MRD testing using HTS to guide treatment decisions WTS or targeted gene expression panel for proliferation and signatures of nnMCL vs cMCL
	CCND2 and *CCND3* rearrangement†: FISH	Consider in *CCND1*-R– negative tumors		
	TP53 mutation*: HTS‡	*(de Leval., Blood, 2022)*	Prognostic and guide management[111]	



(Martin-Garcia D, Blood 2019)

Cyclin D1 expression and *CCND1* rearrangement as a secondary event

High Grade B-Cell Lymphoma



- Large B cell morphology
- CD5 and SOX11-neg, express cyclin D1
- Usually *CCND1* rearrangement negative but… unusual cases *CCND1* rearranged
- Associated with multiple other translocations (*BCL6, BCL2, MYC*)
- Unusual mutations in MCL (eg *KRAS* and *TNFRSF14)*

(Hsiao Histopathology. 2012 61:685-93; Cheng J Hemasphere. 2021; 5: e505; Schliemann I Leuk Lymphoma. 2016;57(11):2672-6)

Chronic lymphocytic leukemia

LEUKEMIA & LYMPHOMA, 2016
VOL. 57, NO. 11, 2672–2676
http://dx.doi.org/10.3109/10428194.2016.1153085
Taylor & Francis
Taylor & Francis Group

LETTER TO THE EDITOR

The t(11;14)(q13;q32)/CCND1-IGH translocation is a recurrent secondary genetic aberration in relapsed chronic lymphocytic leukemia

Igor Schliemann[a*], Ilske Oschlies[b*], Inga Nagel[d], Eva Maria Murga Penas[d], Reiner Siebert[d] and Birgitta Sander[a,c]

[a]Pathology/Cytology, F46 Karolinska University Hospital Huddinge, Stockholm, SE, Sweden; [b]Department of Pathology, Hematopathology Section and Lymph Node Registry, Christian-Albrechts University, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany; [c]Department of Laboratory Medicine, Division of Pathology, F46 Karolinska Institutet, Stockholm, SE, Sweden; [d]Institute of Human Genetics, Christian-Albrechts University Kiel, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

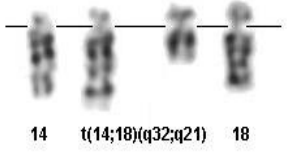
ARTICLE HISTORY Received 31 July 2015; Revised 15 January 2016; Accepted 29 January 2016

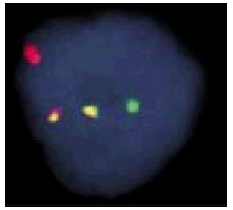
- t(11;14) in 3 CLL cases, but as secondary event
- Requires demonstration of same disease (not composite CLL+MCL), monoclonal peak, flow cytometry, FISH CLL alterations
- Integrated diagnosis

Follicular lymphoma (FL)

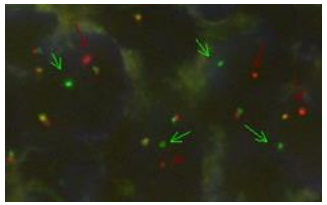
Virtually all have *IGH::BCL2* as primary alteration*:*

- **t(14;18)(q32;q21)/*IGH::BCL2* in 80-85%**
- variant t(2;18)(p12;q21)/*IGK::BCL2*
  and t(18;22)(q21;q11)/*BCL2::IGL*





IGH::BCL2



FFPE: *BCL2* BA

➢ **FISH with *IGH::BCL2* and *BCL2* breakapart to avoid false-negative**

Entity	Genetic alteration: test	Diagnostic use	Clinical impact
Follicular lymphoma (FL)	*BCL2* rearrangement†: FISH (or cytogenetics)	Consider if BCL2 IHC is negative. Further workup of *BCL2*-R–negative FL shown in scenario 1B in Table 3	
	EZH2 mutation†: HTS		*EZH2* mutation is predictive of response to EZH2 inhibition.[81] Tazemetostat is approved by the FDA for use in patients with *EZH2*-mutated FL (detected by an FDA-approved test) who have received at least 2 prior lines of systemic therapy (and all adult patients, including with wt *EZH2* with relapsed/refractory disease and no other satisfactory alternative treatment options)

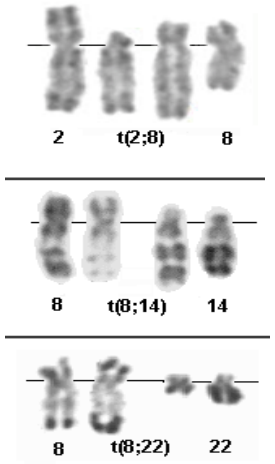
(de Leval., Blood, 2022)

*********BCL2* translocations are also present in 30% DLBCL and few CLL

FL THAT ARE NEGATIVE FOR *BCL2* REARRANGEMENT: DIFFERENT SITUATIONS

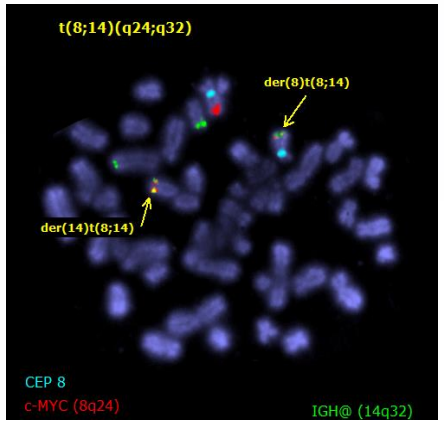
- ***BCL2-r negative*** BUT Bcl2+ protein expression, in 30%, CD10-, need LMO2 or GC markers, no clinical impact
- **FL grade 3B**, only 11-20% have *BCL2* rearrangement → recomended to perform *BCL6* and *IRF4* FISH
- **Pediatric type FL** (*MAP2K1, IRF8, TNFRS14)*
- **Testicular FL**
- ***BCL2-r negative, CD23+ follicle center lymphoma* (provisional ICC entity),** inguinal region, localized
  CD10, BCL2, BCL6, CD23 positive; frequent loss 1p36 and ***STAT6*** mutation

Burkitt lymphoma (BL)



Virtually all BL have *MYCr* as primary alteration*:*

- **t(8;14)(q24;q32)/*MYC::IGH* in 80%**
- variants t(2;8)(p12;q24)/*IGK::MYC* and t(8;22)(q24;q11)/*MYC::IGL*



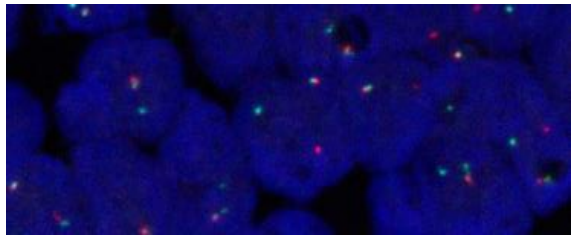
- *IG::MYC* is very specific of BL but not exclusive (can be found in DLBCL, HGBCL, MCL (pleo), B-PLL, CLL->RT... usually acquired as secondary alteration, and poor survival)
- BL have relatively simple karyotypes, low genomic complexity (frequent 1q+), useful for dif. diagnosis
- BL mutational profile is highly specific, useful for dif. diagnosis

- **FISH with *MYC* breakapart and *MYC::IGH* to avoid false-negative**
- FISH with *BCL2* and *BCL6* (only if *MYCr*) to exclude HGBCL-DH

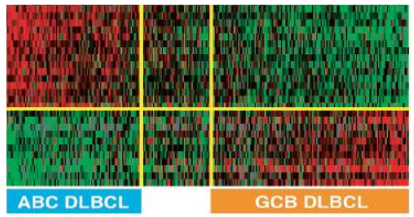
Entity	Genetic alteration: test	Diagnostic use
Burkitt lymphoma	*MYC*, *BCL2*, and/or *BCL6* rearrangement (latter two can be performed concurrently or only if *MYC* rearrangement is detected): FISH*	Required to exclude HGBCL-DH-*BCL2* and HGBCL-DH-*BCL6*

(de Leval., Blood, 2022)

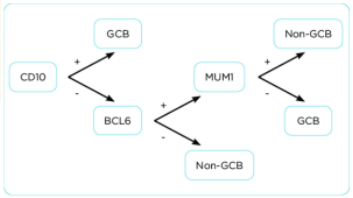
Diffuse large B-cell lymphoma (DLBCL)

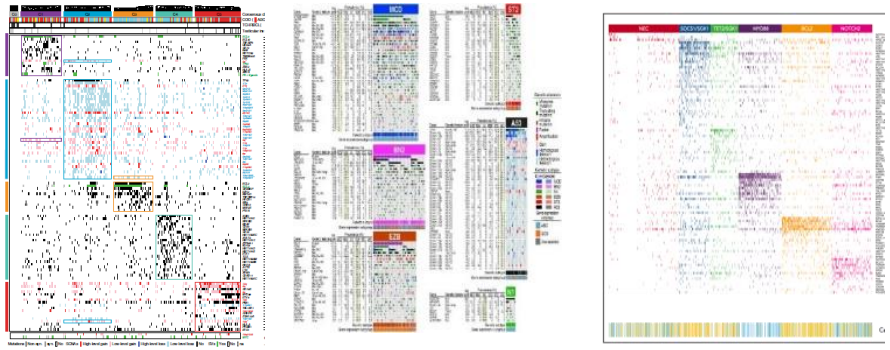


FISH



COO





Genetic subgroups

(Chapuy B et al Nat Med 2018; Wright GW et al Cancer Cell 2020; Lacy SE et al Blood 2020)

- **Heterogeous group, no completely specific chromosomal aberration**
- ***MYC, BCL2* and *BCL6* translocations should be performed to identify HGBCL (usually by FISH)**
- **Cell-of-origin in DLBCL,NOS should be maintained since it reflects a basic biological distinction. GEP is recommended but IHC acceptable**
- **Recognize the limitation of this binary COO classification to capture DLBCL complexity**
- **Genetic subgroups capture biological complexity but are still not ready for clinical use**
- **Expectation of transitioning to a molecular genetic classification in the near future**

DLBCL genetic workup: *BCL2, BCL6, MYC*

- **FISH with *MYC, BCL2 and BCL6* breakapart probes, all tests at once** (mainly FFPE material)
- FISH with *MYC* (1st step) and FISH with *BCL2* and *BCL6* (only if *MYCr*) to exclude HGBCL-DH (2nd step)

- Should we also use *MYC::IGH* and *IGH::BCL2* to avoid false-negative results?

***Diagnostic and prognostic impact

Entity	Genetic alteration: test	Diagnostic use	Clinical impact	Future assays
Diffuse large B-cell lymphoma, NOS Germinal center B-cell subtype Activated B-cell subtype	*MYC*, *BCL2*, and/or *BCL6* rearrangement (latter two can be performed concurrently or only if *MYC* rearrangement is detected): FISH*	Required to exclude HGBCL-DH-*BCL2* and HGBCL-DH-*BCL6*	See "High-grade B-cell lymphoma"	Genetic subtype assignment (eg, LymphGen[187]) by panel, exome or WGS and *BCL2* and *BCL6* rearrangement detection and WTS or targeted gene expression panels (DHITsig[29]/MHG signature[199]) HTS-based ctDNA testing[465] for response-adapted management
	COO determination: GEP or widely used IHC surrogates*	Required to assign DLBCL, NOS gene expression subtypes	Prognostic for outcomes following R-CHOP (GEP)[466]; predictive of response to treatment at relapse[177]	

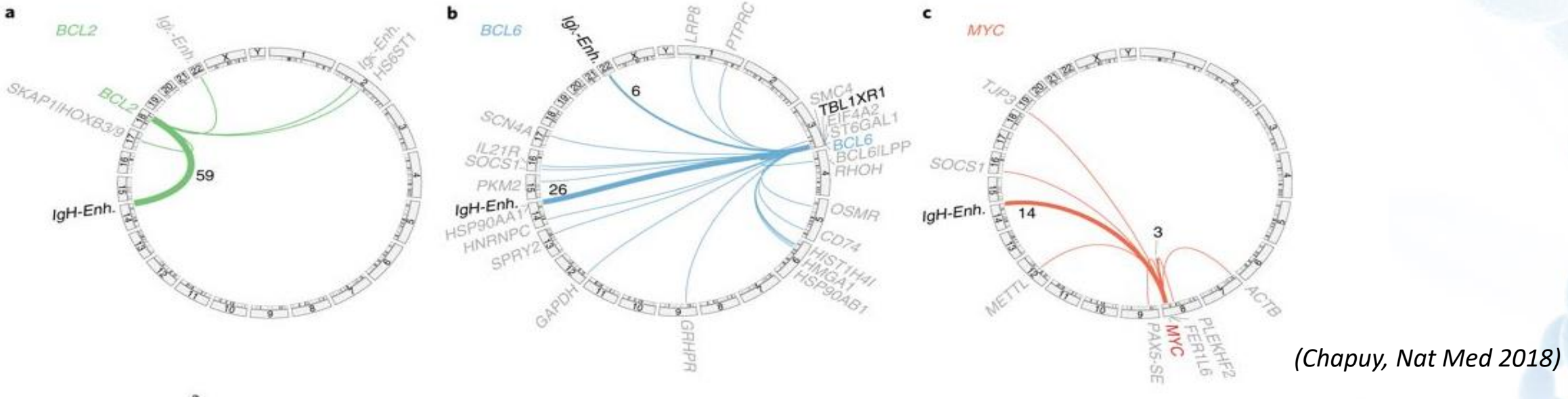
(de Leval., Blood, 2022)

DLBCL genetic workup: *BCL2, BCL6, MYC*

***BCL2*: 20-30%**
***IGH* mostly**
GCB type

***BCL6*: 20-40%**
Frequently non-*IGH*
ABC type

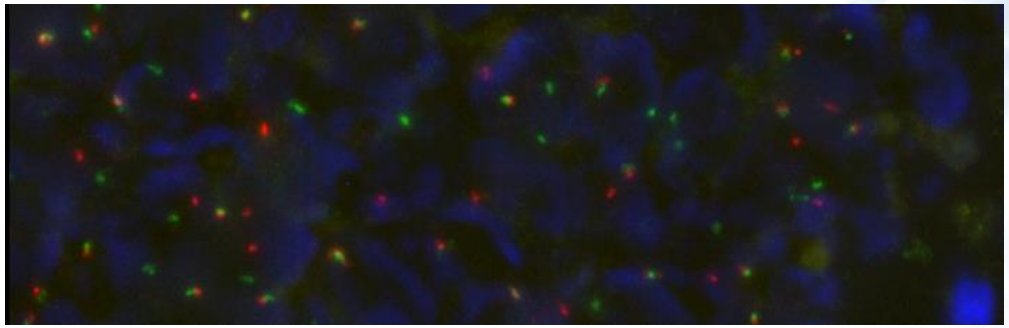
***MYC*: 10-20 %**
***IGH* in 60%**



(Chapuy, Nat Med 2018)

- **Do we need to screen by FISH *BCL2, BCL6, MYC* all DLBCL?**

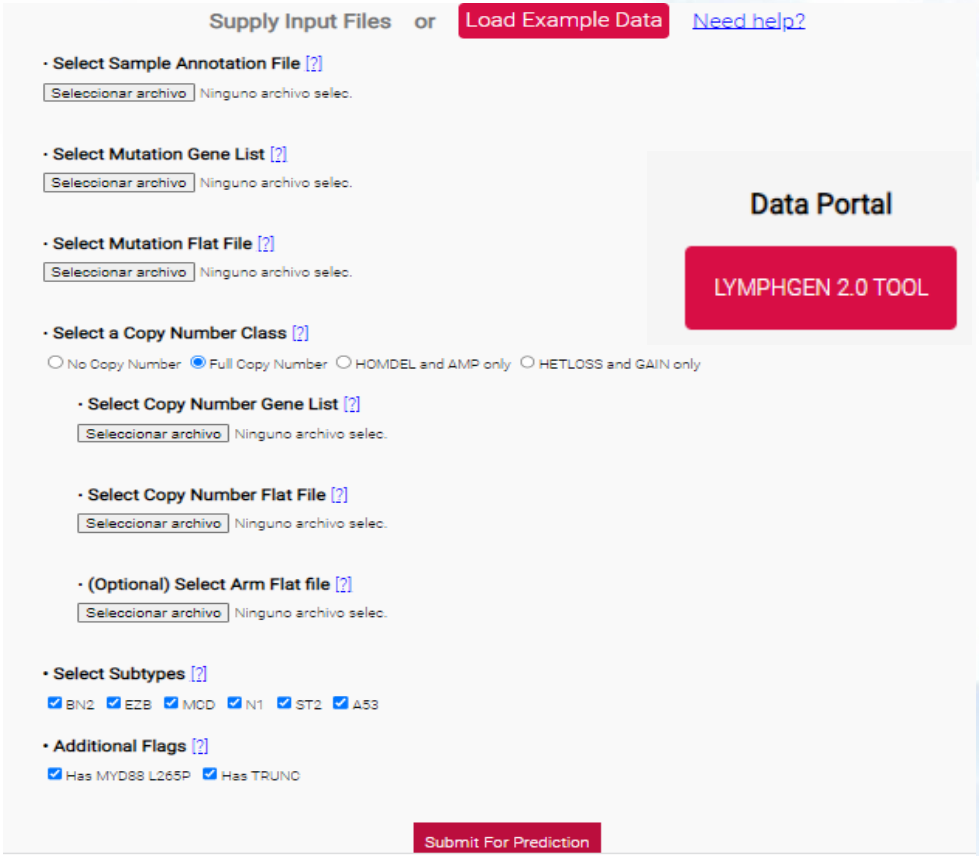
  My suggestions:
  - specially if Ki67 is high
  - all CGB subtype?
  - All high Myc protein expression?



DLBCL molecular/genetic subgroup determination

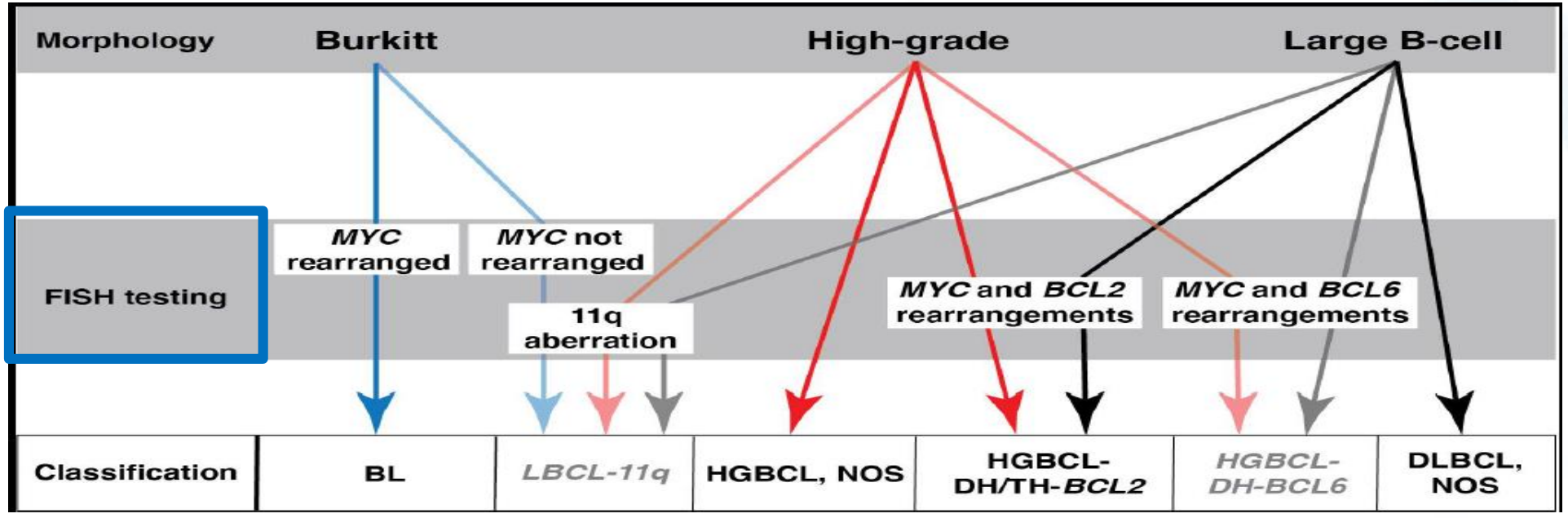
Wright 2020	Chapuy 2018	Lacy 2020	Hallmark drivers	%
MCD	C5	MYD88	***MYD88/CD79B***	14-21
BN2	C1	NOTCH2	***tBCL6/ NOTH2***	16-19
EZB-MYC-	C3	BCL2	***EZH2 tBCL2***	13-18
EZB-MYC+			***EZH2/MYCt***	
A53	C2		***TP53 Aneuploidy***	7-21
ST2	C4	SOCS1/TET /SGK1	***SOCS1/TET/ SGK1***	5-17
N1		NEC	***NOTCH1***	3
UNCLASS				37

https://llmpp.nih.gov/lymphgen/index.php



To classify a DLBCL into a molecular subtype using the lymphgen tool we need mutations, but ALSO TRANSLOCATIONS and CNA!!! (using only an NGS panel is not enought)

Diagnostic Approach for High-grade B-cell lymphomas



Entity	Genetic alteration: test	Diagnostic use	Clinical impact	Future assays
High-grade B-cell lymphomas (HGBCL) HGBCL with *MYC* and *BCL2* rearrangement (HGBCL-DH-*BCL2*) *HGBCL with MYC and BCL6 rearrangement (HGBCL-DH-BCL6)* HGBCL, NOS	*MYC*, *BCL2*, and/or *BCL6* rearrangement (latter two can be performed concurrently or only if *MYC* rearrangement is detected): FISH*	Required for the diagnosis of HGBCL-DH-*BCL2* and HGBCL-DH-*BCL6*	Prognostic and predictive: HGBCL-DH-*BCL2* has poor prognosis with R-CHOP and likely benefits from treatment intensification[467]	Rearrangement detection and *MYC* partner determination by HTS HTS analysis of HGBCL, NOS tumors to assign these tumors to definitive disease categories

(de Leval., Blood, 2022)

FISH Approach for High-grade B-cell lymphomas

DIAGNOSTIC CRITERIA:

- **FISH using breakapart probes *MYC, BCL2,* and *BCL6***
- also *MYC::IGH, BCL2::IGH, IGH*?????
- is FISH enough? or gene expression needed?

HGBCL *MYCr* and *BCL2r double-hit*

- FISH breakapart probes recommended but may miss up to 20% cases (cryptic alterations)
- *MYC* with IG partner in 50%, poor outcome? inconclusive results
- Do not consider GAINS/AMPLIFICATIONS
- COO: Germinal center origin
- Expression signature of centroblast in the GC dark zone
- Mutational profile similar to aggressive FL and GCB-DLBCL (*BCL2, **MYC**, KMT2D, CREBPP, TNFRS14, EZH2, TP53*)

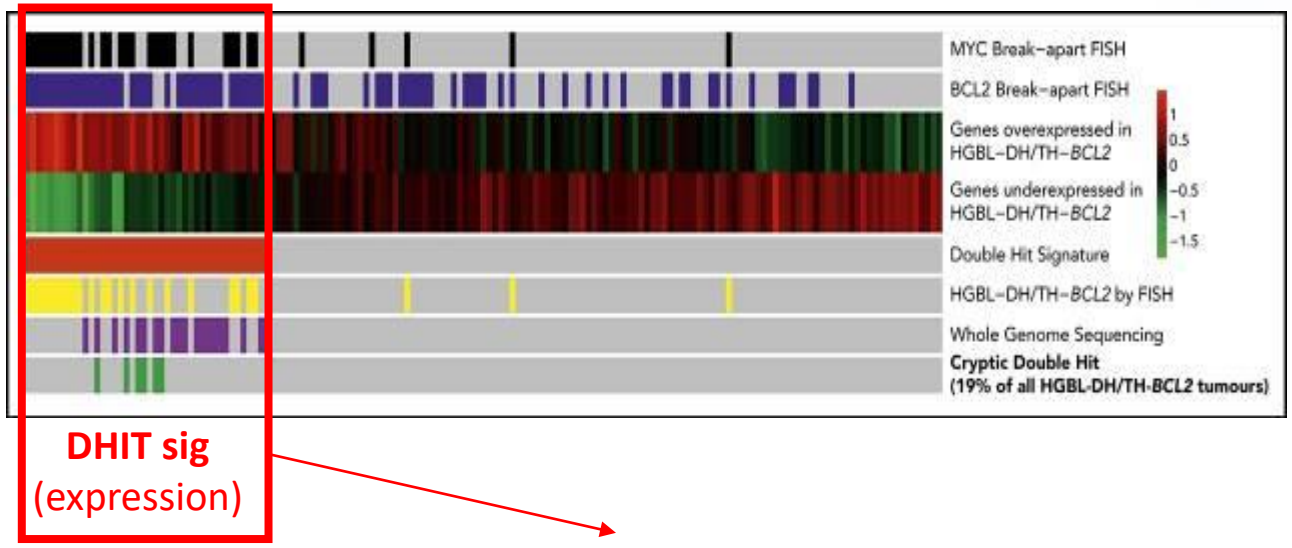
HGBCL *MYCr* and *BCL6r double-hit*

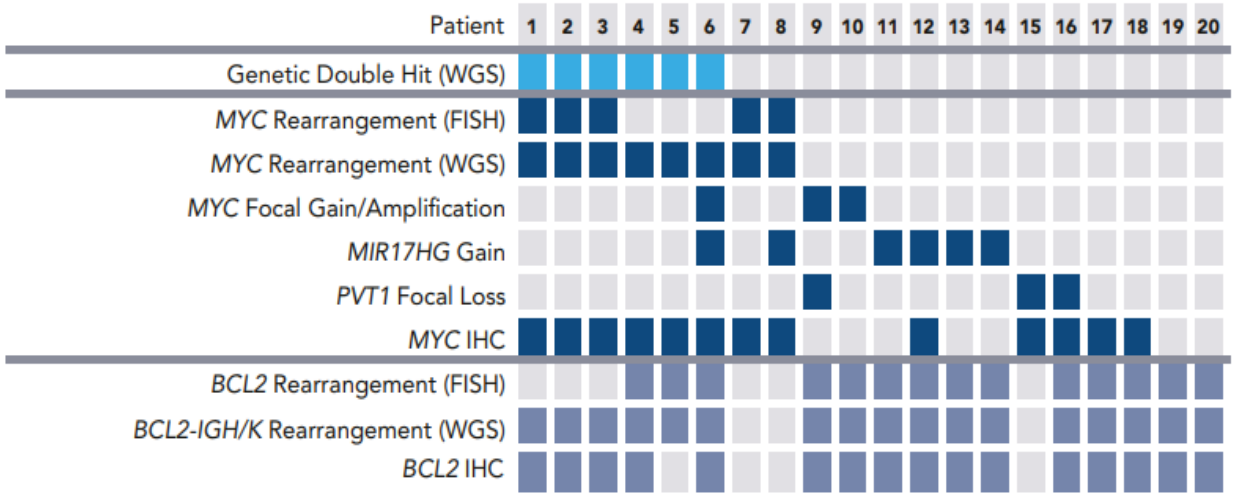
- Less frequent
- Heterogeneous in COO (ABC, GCB) and mutational profile (less FL–type, NOTCH2)
- 30% “pseudo-double” hit (*BCL6::MYC*)
- Should be considered an individual entity??

High-grade B-cell lymphoma, NOS

- No double rearrangement
- *MYC* in 50%, *MYC::IGH* much more freq.
- *BCL2* and *BCL6* unfrequent

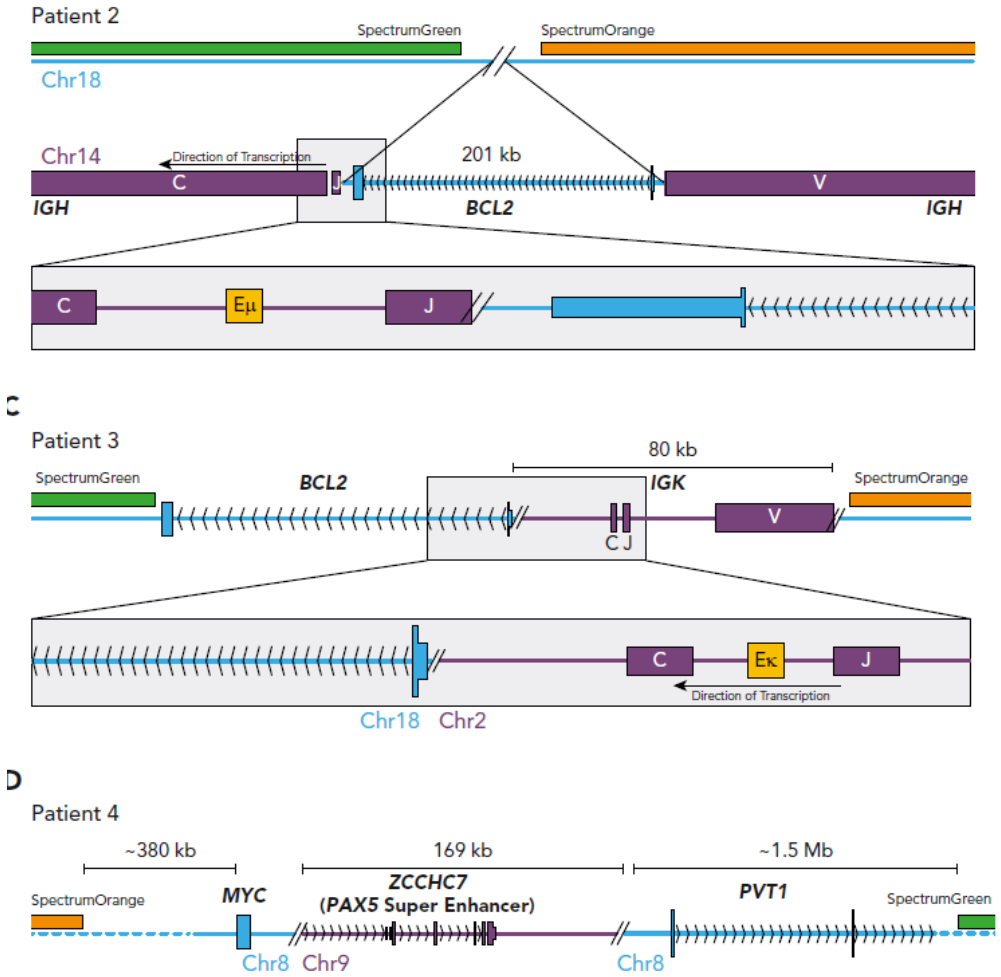
The DHITsig identifies DH-DLBCL with genetic events cryptic to FISH breakapart probes





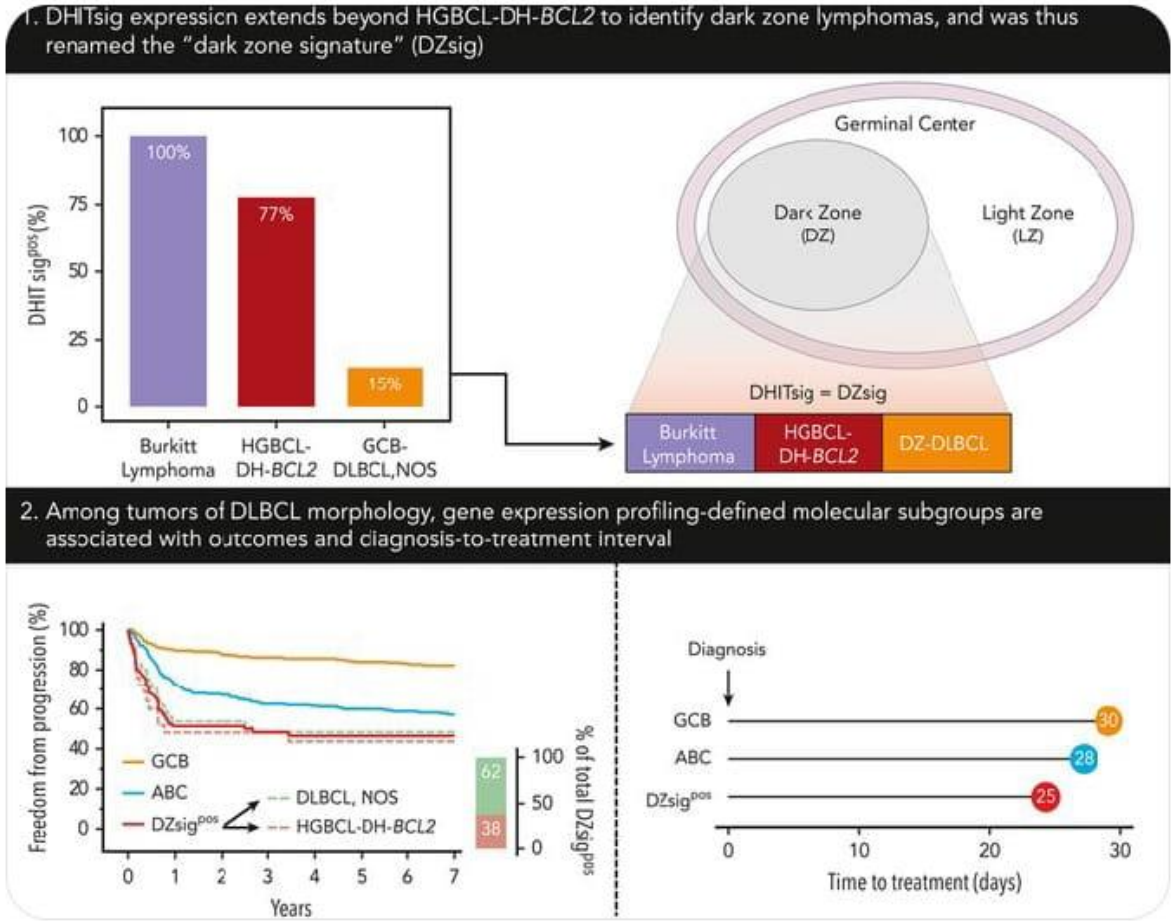
(Hilton LK, Blood 2019)

WGS of 20 DHITsig GCB-DLBCL apparently lacking *MYC* and/or *BCL2* rearrangements: 6 tumors with *MYC* or *BCL2* rearrangements cryptic to breakapart FISH probes



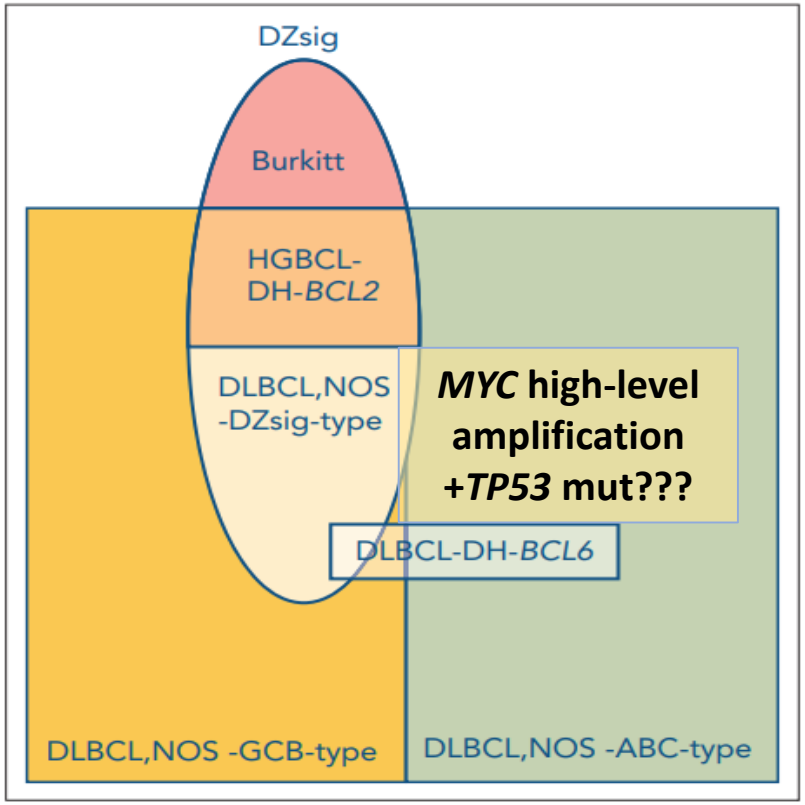
Dark zone signature (DZsig) in DLBCL

- DHITsig expression extends beyond HGBCL-DH-*BCL2* to identify dark zone lymphomas (renamed the "DZsig)
- DZsig refines COO classification by identifying patients within GCB-DLBCL with inferior OS and shorter time to treatment



(Alduaij W, Blood 2023)

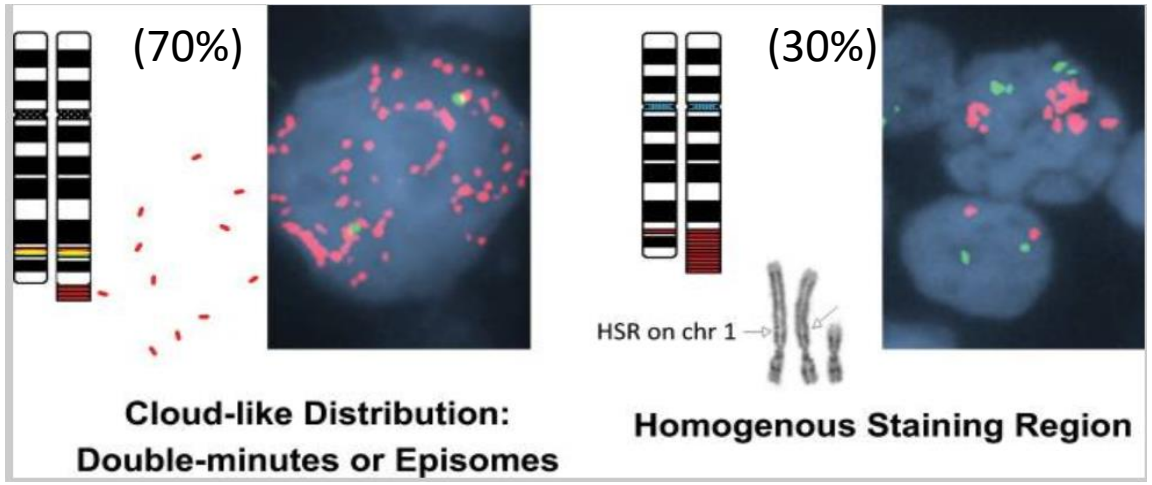
DNA or RNA? Classification of B-cell lymphomas

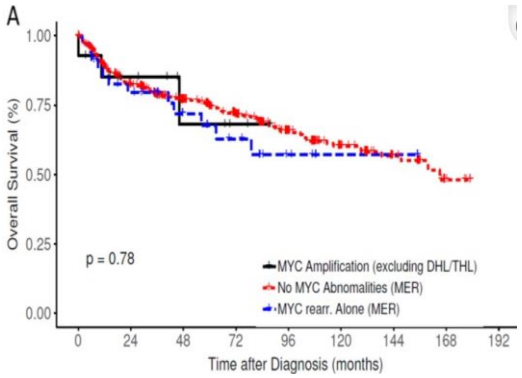


(Ylstra B, Blood 2023)

High level *MYC* amplification in B-cell lymphomas: a marker of aggressive disease?

Uncountable FISH signals:
2 main patterns of *MYC* amplification



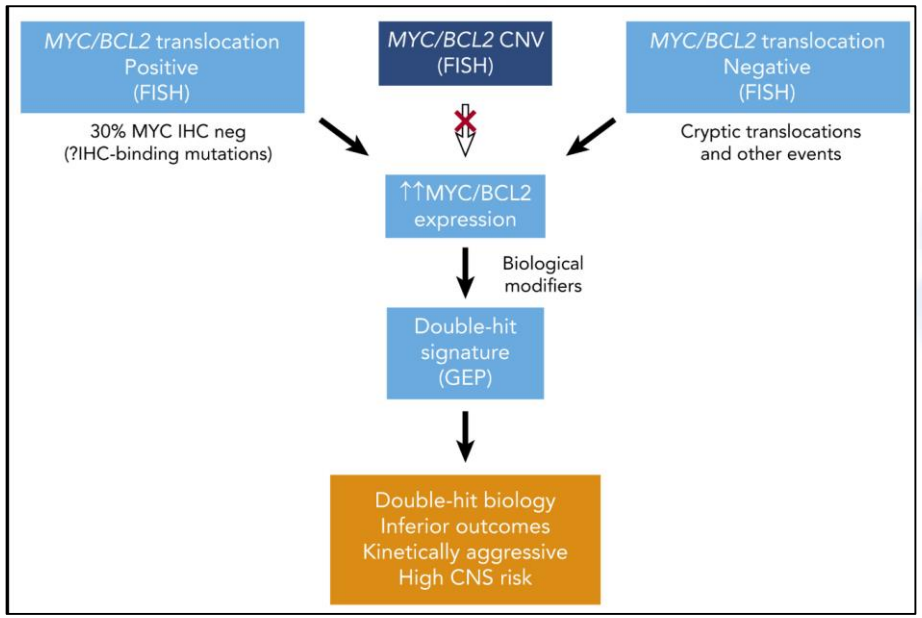


- *MYCamp* (44/9715; 0,45%)
- 12/42 (29%) were DH
- *MYCamp* did not have prognostic significance in DLBCL in this cohort *(Pophali PA, Blood Cancer J. 2020)*

…controversy

-*MYCamp* (4/385; 1%)
-*MYC* with >7 copies and *MYCamp* poorest prognosis
(Schieppati F, Haematologica 2020)

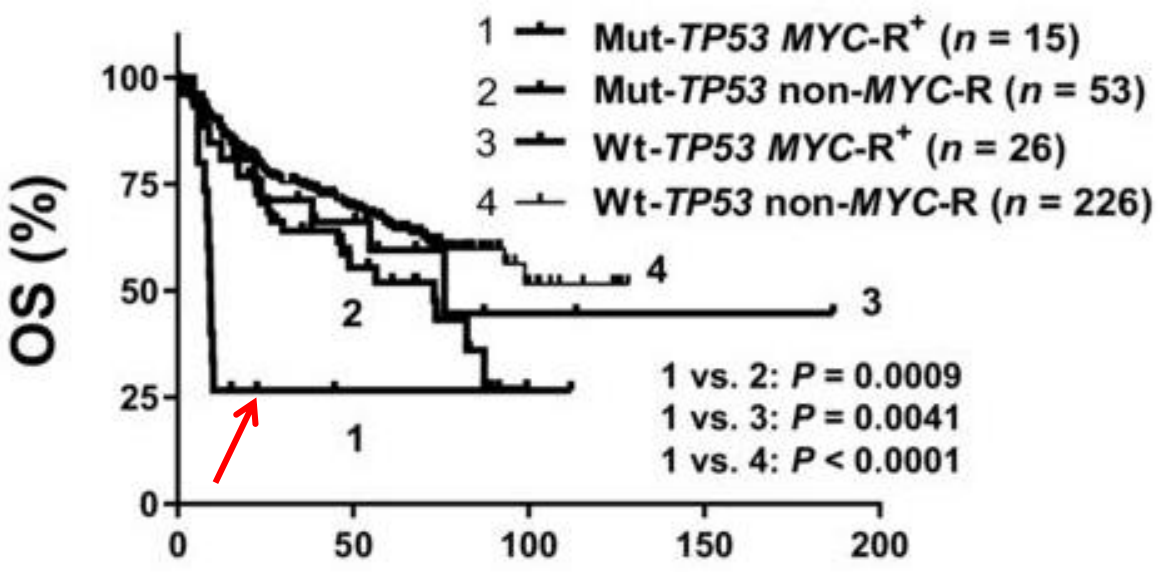
-*MYC* gains do not lead to high Myc protein
-*MYC* and *BCL2* CNV (gains/amp) are not DH-TH
(Collinge B, Blood 2021)



High level *MYC* amplification in B-cell lymphomas: a marker of aggressive disease?

DLBCL with *MYCr* and *TP53* mutation: WORST PROGNOSIS

(N=320 DLBCL)



(Manman deng et al. Mol cancer res 2021)

- **If *MYCr* & *TP53* mut DLBCL have poor OS…**
  - **do DLBCL with *MYC* amp & *TP53* mut have worse prognostic???**
  - **could be the DLBCL-NOS DZsig+ type???**

Still no data!

Large B-cell lymphoma with 11q aberration
Large B-cell lymphoma with *IRF4* rearrangement

Entity	Genetic alteration: test	Diagnostic use	Clinical impact	Future assays
				Detection of CNAs and SVs using HTS
Large B-cell lymphoma with 11q aberration	11q aberration: SNP array or FISH	Required for diagnosis of *LBCL-11q*		
Large B-cell lymphoma with *IRF4* rearrangement	*IRF4* rearrangement: FISH *CARD11*, *IRF4* mutations†: HTS	FISH required for diagnosis of LBCL-*IRF4* rearrangement Useful in certain circumstances for diagnosis; see also scenario 3A in Table 3.		

(de Leval., Blood, 2022)

➢ **2 new entities defined by a specific primary alteration, mainly detected by FISH**

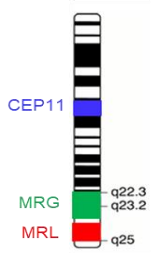
Large B-cell lymphoma with 11q aberration (ICC, new entity)
High-grade lymphoma with 11q aberration (WHO, provisional)

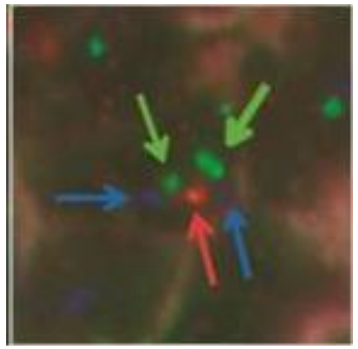
Burkitt-like lymhoma with 11q aberration (previous name)

- Low frequency
- Children and young adults
- Predominantly nodal
- Morphology from Burkitt-like (starry sky) to large cell
- Favorable prognosis with current treatment

11q-pattern karyotype

11q-pattern FISH



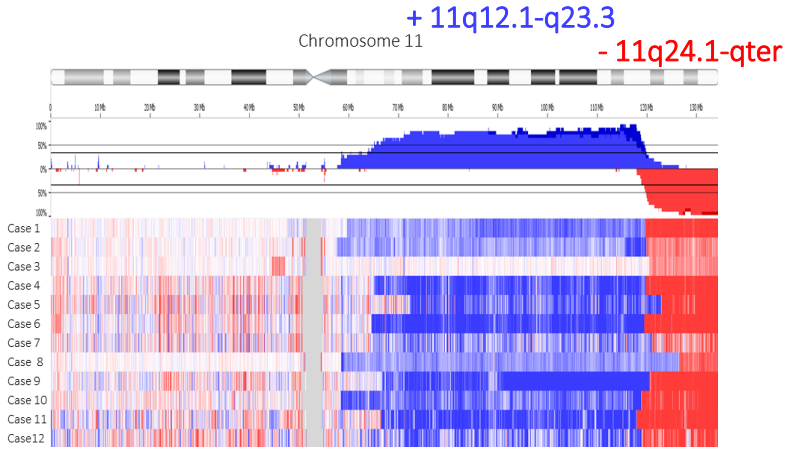


DIAGNOSTIC CRITERIA:

- **Presence of 11q22-q24 gain /11q24-qter loss (FISH, array, karyotype, OGM, WGS…)**
- Absence of *MYC, BCL6*, and *BCL2* rearrangements (FISH)

*** 11q alterations can also be found in other cases, need of integrated diagnosis

11q-pattern Copy number array

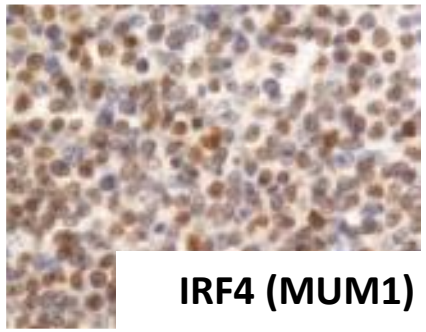


ADDITIONALLY:

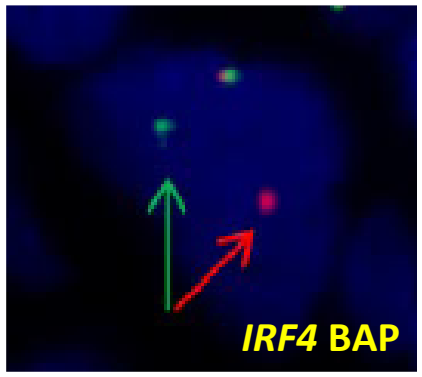
- NGS, mutational profile:
  - closer to DLBCL (frequent *BTG2, GNA13, CREBBP)*
  - different from BL (absence of *ID3, TCF3)*

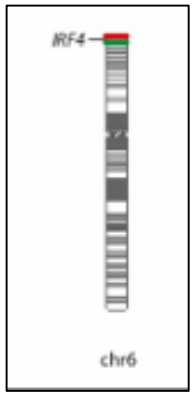
(Salaverria I et al Blood 2014; 123: 1187–1198; Gonzalez-Farre B et al Haematologica 2019; Wagener R et al Blood 2019; Horn H et al Am J Surg Pathol 2021;45:356-364*)*

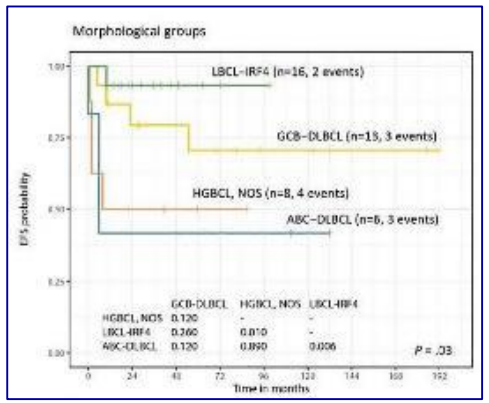
Large B-cell lymphoma with *IRF4* rearrangement



IRF4 (MUM1)







NEW ENTITY ICC/WHO

- Low frequency
- Pediatric and young adult population
- Germinal center phenotype (CD10/BCL6)
- BCL2 expression (but no *BCL2r*)
- Strong IRF4 expression and *IRF4* translocation (**mainly *IRF4::IGH,*** also *IGK, IGL*)
- Cryptic (telomeric) translocation, not detected by karyotype
- Excellent prognosis

DIAGNOSTIC CRITERIA:

- **FISH with *IRF4* break apart probe must be performed (**freq. false – and +)
- **Cases negative for *IRF4r* must have *IGH* break apart pattern**
- Absence of *BCL6* and *BCL2* rearrangements (FISH)

******IRF4*** translocations may be present in other LBCL

ADDITIONALLY:

- *IRF4* mutations as "surrogate marker" of translocation (1 or more mutations in exons 1-2, aSHM)

(Ramis-Zaldivar et al, Blood 2020)

FISH is a simple and accessible single cell technology that helps in difficult diagnosis

Clinical: Burkitt with high LDH → Lymph node: BL? Starry sky pattern, BM/PB minimal infiltration, by flow cytometry MCL phenotype (pleo?)

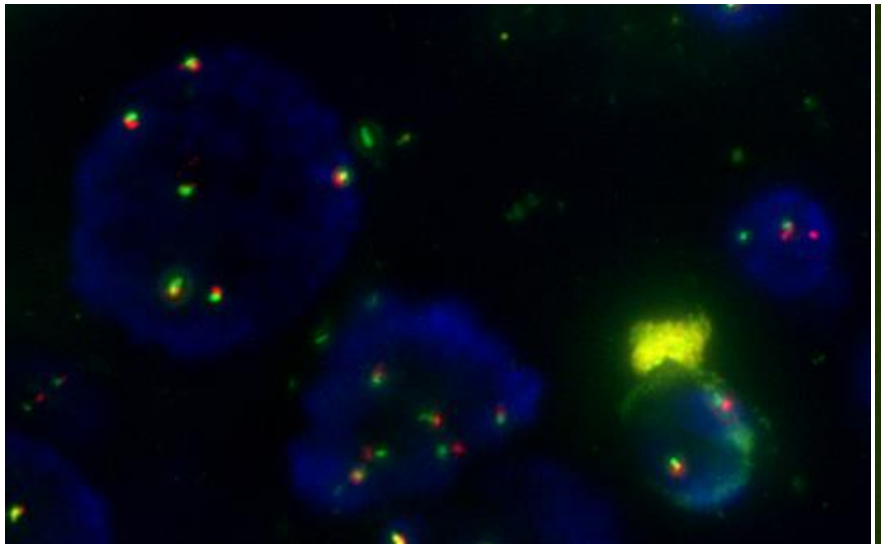
Large B-cell (CD20+, CD79a+)

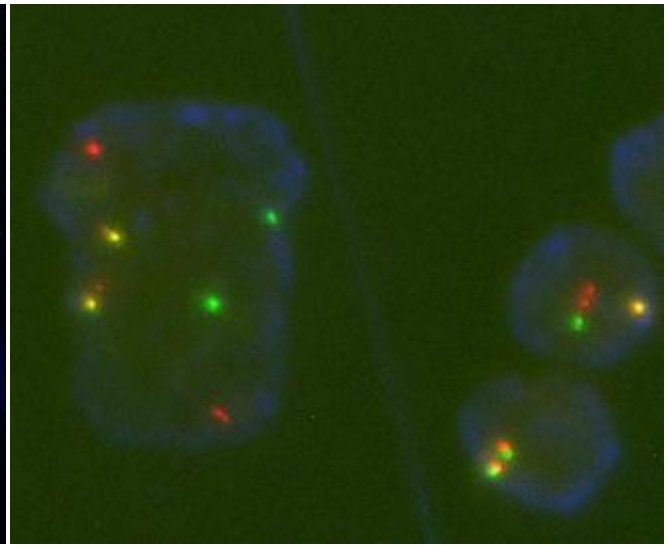
CD10+, BCL6+, p53+++
Bcl2-, cyclin D1-, Sox11-
Myc+++, Ki67 100%

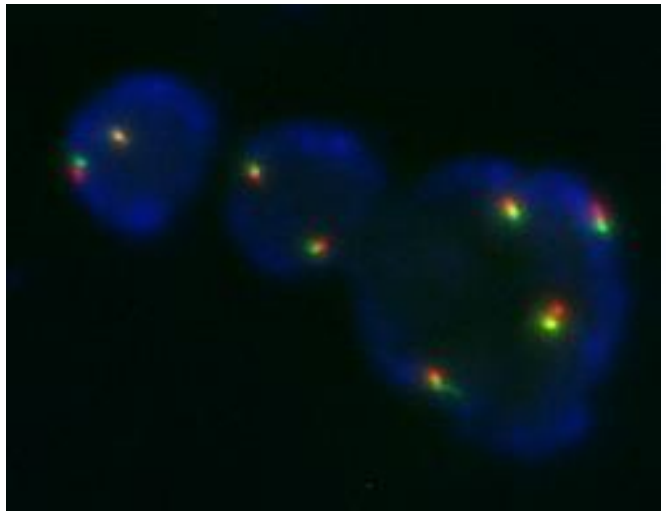
+

small B-cell (CD20+, CD79a+)

CD10-, BCL6-, p53-
BCL2+, cyclin D1+, Sox11+
Myc-, Ki67 low







***CCND1* BA:**
- 6 copies in large cells
- rearranged in some small cells

***MYC* BA:**
- 4 copies in large cells, 2 rearr
- normal in small cells

***BCL6* BA:**
- 4 copies in large cells
- normal in small cells

***BCL2* BA:**
- normal

DIAGNOSTIC: conventional MANTLE CELL LYMPHOMA + DIFFUSE LARGE B-CELL LYMPHOMA–NOS GCB (****MYC*r + *TP53*mut)**

Indication of genetic testing in small B-cell lymphomas

Category	Disease	Marker	Clinical relevance
Diagnostic	Follicular lymphoma	• *BCL2* rearrangement (FISH)	Diagnostic
	Mantle cell lymphoma	• *CCND1/D2/(D3)* rearrangement (FISH)	Diagnostic
	Hairy cell leukemia	• *BRAF* V600E	Diagnostic
	Lymphoplasmacytic lymphoma	• *MYD88* L265	Diagnostic
	Nodal marginal zone lymphoma	• +3, +8, *KLF2, NOTCH2, PTPRD*	Support the diagnosis
	Splenic marginal zone lymphoma	• del (7q), +3, +18, *KLF2, NOTCH2*	Support the diagnosis
	CD23+*BCL2*-R neg Follicle center lymphoma	• *STAT6/SOCS1*	Diagnostic
Prognostic	Chronic lymphocytic lymphoma	• *TP53*, *IGHV* mutation status • del (11q), +12, del (13q), del (17p) • Complex Karyotype (>5 alt.)	Prognostic relevant
	Mantle cell lymphoma	• *TP53* (*also del17p?)	Prognostic relevant

(Quintanilla-Martinez L, personal view)

- Which FISH probes do you apply for B-Lymphoid Neoplasms diagnosis?
- All at once? In which sequential order?
- Do you cover all entities included in the updated WHO22 and ICC22?
- Do you use other additional/alternative technologies to detect translocations? which ones?
- Are you interested in elaborating FISH useful guidelines for B-NHL?

FISH probes for B-Lymphoid Neoplasms: QUESTIONNAIRE TO AUDIENCE

		FISH (minimum)	N hyb.		FISH (extended)
Chronic lymphocytic leukemia					
		ATM/TP53			*IGH*
		DLEU/LAMP/12			*MYC (accel. & RT)*
					BCL3
					Other:
No recibimos / No estudiamos					
Follicular lymphoma					
		IGH::BCL2			*BCL6*
		or BCL2			*Other:*
No recibimos / No estudiamos					
Mantle cell lymphoma					
		IGH::CCND1			*TP53*
		or CCND1			*MYC (blastoid)*
					CCND2 (D1-neg)
					CCND3 (D1-neg)
					CCND2::IGKenh(D1-neg)
					CCND2::IGLenh(D1-neg)
					CCND3::IGKenh(D1-neg)
					CCND3::IGLenh(D1-neg)
					Other:
No recibimos / No estudiamos					
Burkitt's lymphoma					
		IGH::MYC			*Other:*
		*or **MYC***			*Other:*
		BCL2			*Other:*
		BCL6			*Other:*
No recibimos / No estudiamos					

DLBCL & High grade B-cell lymphoma (DH and –NOS)					
		IGH::MYC			*TP53*
		MYC			*IGH*
		BCL6			*IRF4 (some cases)*
		BCL2			*11q*
		IGH::BCL2			*PD-L1/L2*
					Other:
No recibimos / No estudiamos					
Large B-cell lymphoma with 11q aberration					
		11q			*Other:*
		MYC			*Other:*
		BCL6			*Other:*
		BCL2			*Other:*
No recibimos / No estudiamos					
		FISH (minimum)	**N hyb.**		**FISH (extended)**
Large B-cell lymphoma with IRF4 rearrangement					
		IRF4			*Other:*
		IGH			
No recibimos / No estudiamos					

FISH probes for B-Lymphoid Neoplasms: QUESTIONNAIRE: filled form Hospital Clinic

Nombre: Sílvia Beà
Departamento/Centro: Hematopato, AP, Hospital Clínic Barcelona
Muestras de linfoma B en cultivo (SI/NO): sí
Muestras de linfoma B en parafina (SI/NO): sí
Interesado en elaboración guías consenso para sondas linfoma? Email:

		FISH (minimum)	N hyb.		FISH (extended)	N hyb.	TOTAL hyb.
Chronic lymphocytic leukemia			**2**			(1-2)	(3-4)
	x	*ATM/TP53*		x	*IGH*		
	x	*DLEU/LAMP/12*		x	*MYC (accel. & RT)*		
					BCL3		
					Other:		
No recibimos / No estudiamos							
Follicular lymphoma			(1-2)			(0-1)	(1-3)
	x	***IGH::BCL2***		x	*BCL6*		
	x	*or BCL2*			*Other:*		
No recibimos / No estudiamos							
Mantle cell lymphoma			(1-2)				(1-5)
	x	***IGH::CCND1***		x	*TP53*		
	x	*or CCND1*		x	*MYC (blastoid)*		
				x	*CCND2 (D1-neg)*		
					CCND3 (D1-neg)		
					CCND2::IGKenh(D1-neg)		
					CCND2::IGLenh(D1-neg)		
					CCND3::IGKenh(D1-neg)		
					CCND3::IGLenh(D1-neg)		
					Other:		
No recibimos / No estudiamos							
Burkitt's lymphoma			(1-4)				(1-4)
	x	*IGH::MYC*			*Other:*		
	x	*or **MYC***			*Other:*		
	x	*BCL2*			*Other:*		
	x	*BCL6*			*Other:*		
No recibimos / No estudiamos							

DLBCL & High grade B-cell lymphoma (DH and –NOS)			(3-5)			1	(3-6)
		IGH::MYC			*TP53*		
	x	***MYC***			*IGH*		
	x	***BCL6***		x	*IRF4 (some cases)*		
	x	***BCL2***			*11q*		
		IGH::BCL2			*PD-L1/L2*		
					Other:		
No recibimos / No estudiamos							
Large B-cell lymphoma with 11q aberration			0			0	0
		11q			*Other:*		
		MYC			*Other:*		
		BCL6			*Other:*		
		BCL2			*Other:*		
No recibimos / No estudiamos							
Large B-cell lymphoma with IRF4 rearrangement			(1-2)				(1-2)
	x	*IRF4*			*Other:*		
		IGH					
No recibimos / No estudiamos							
Marginal zone lymphoma			(1-3)			0	(1-3)
	x	7q32 (x SMZL)			*IGH (IGH::MALT1 and IGH::BCL10 in EMZL)*		
	x	*BCL6* (x SMZL)			*Other:*		
	x	*MALT (x EMZL) (BIRC3::MALT1 & IGH::MALT1)*			*Other:*		
No recibimos / No estudiamos							
Multiple myeloma			(3-6)			1	(4-7)
	x	*TP53*		x	*MYC (if IGH+)*		
	x	*1p/1q*			*Other:*		
	x	*IGH (if +:)*			*Other:*		
	(x)	*t(11;14)*			*Other:*		
	(x)	*t(4;14)*					
	(x)	*t(14;16)*					
No recibimos / No estudiamos							

TAKE HOME MESSAGES

- **FISH is a very useful technique for the analysis of B-NHL**
- **FISH has high specificity and high resolution**
- **FISH is simple (no instrumentation needed) and rapid (results in 3-12h)**
- **FISH is easy to analyze and available in every lab**
- **FISH is part of the integrated diagnostic approach for B-NHL**
- **FISH results have diagnostic and prognostic impact in B-NHL**
- **Will WGS, GEP, OGM, NGS (SV) replace FISH in diagnostic?**
- **As a cooperative Group should we elaborate guidelines for FISH testing and interpretation in B-NHL? Please, fill the questionnaire…**

ACKNOWLEDGEMENTS

Clínic Barcelona

ANATOMIA PATOLÒGICA 50 anys Mirant per tu

IDIBAPS Institut D'Investigacions Biomèdiques August Pi i Sunyer

AGAUR Agència de Gestió d'Ajuts Universitaris i de Recerca

ciberonc Centro de Investigación Biomédica en Red Cáncer

ISC Instituto de Salud Carlos III

La Marató 3

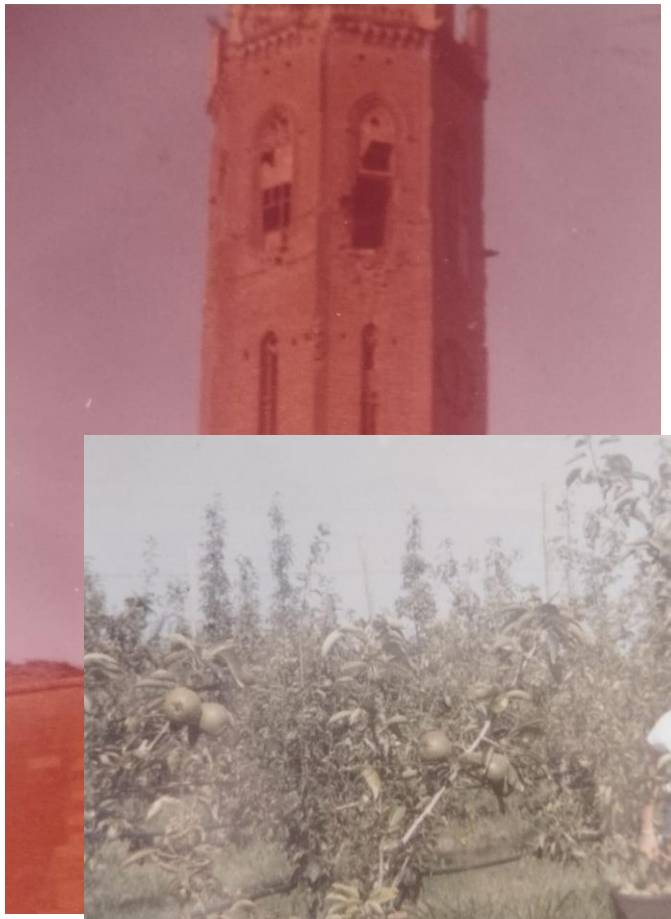
LEUKEMIA & LYMPHOMA SOCIETY®

aecc Contra el Cáncer

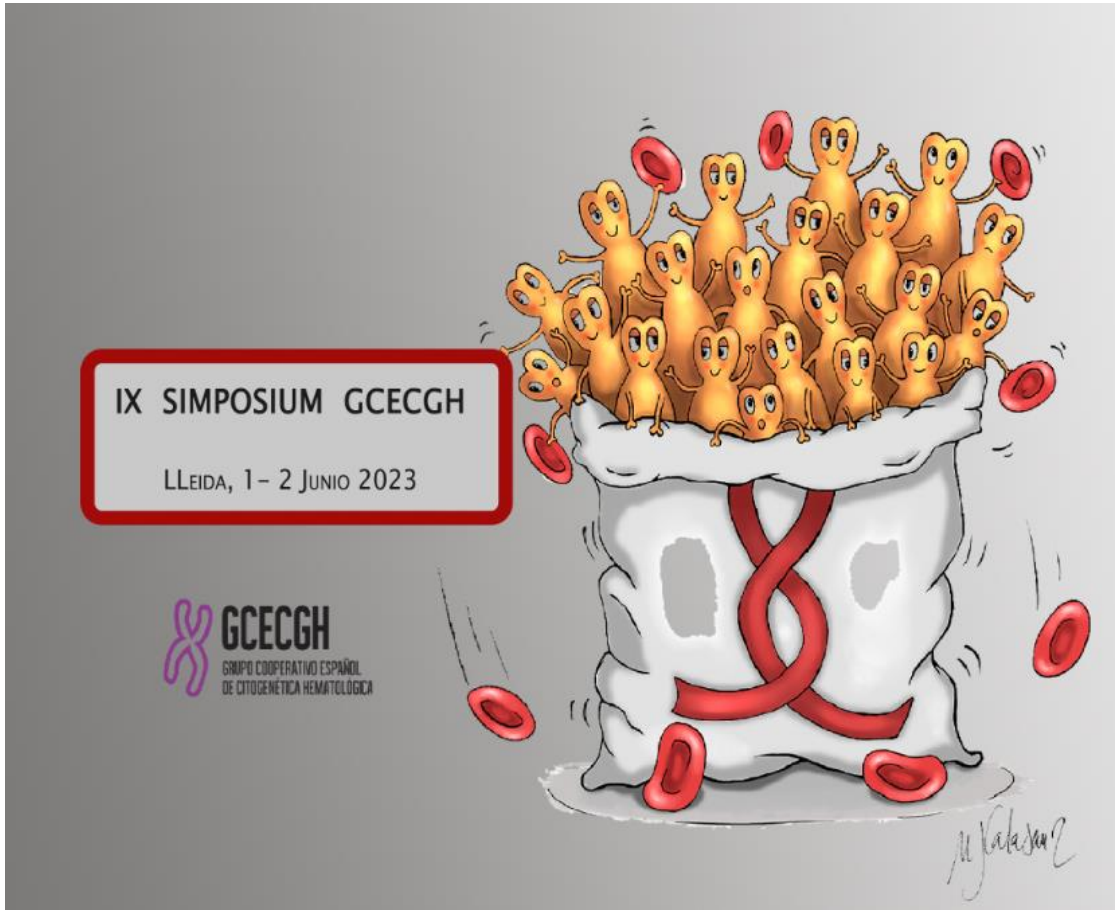
Unió Europea
Fons europeu de desenvolupament regional
Una manera de fer Europa

MY ORIGINS

IX SIMPOSIO
Grupo Cooperativo Español de Citogenética Hematológica
Avances de las técnicas citogenéticas y moleculares en el diagnóstico de las hemopatías malignas



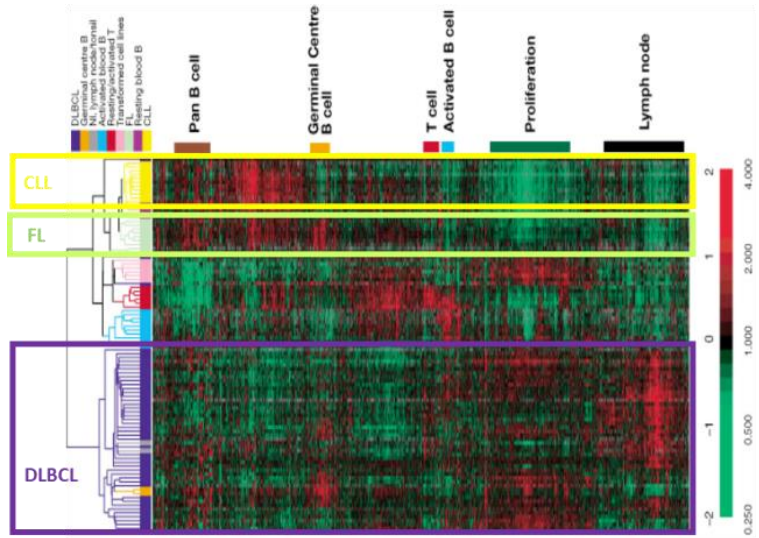
Moltes gràcies per l'atenció!!!!!!! i… visiteu lo Castell de Lleida



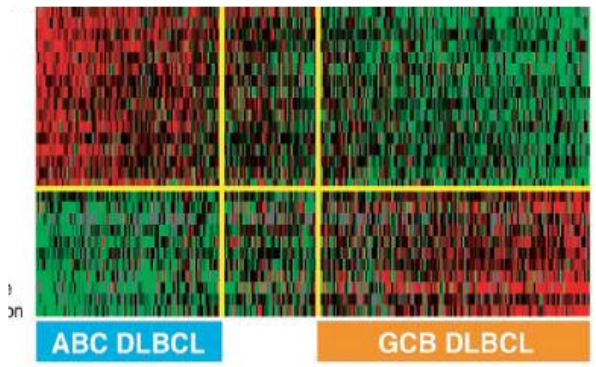
Y gracias a María José Calasanz por los diseños de libretas y tazas cromosómicos

Main contribution of gene expression techniques in B-NHL

Expression microarrays (hybridization)



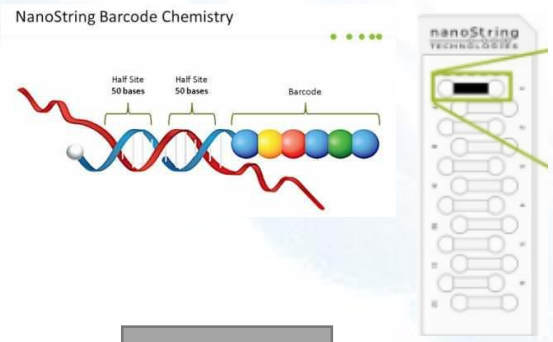
(Alizadeh A et al. Nature 2000)

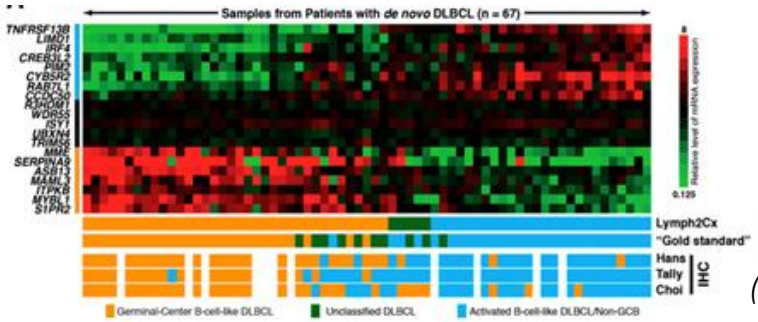


DLBCL COO

(Rosenwald A et al N Engl J Med 2001)

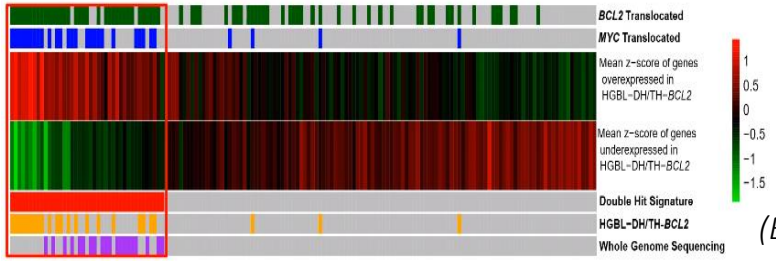
Nanostring (RNA digital quantification)





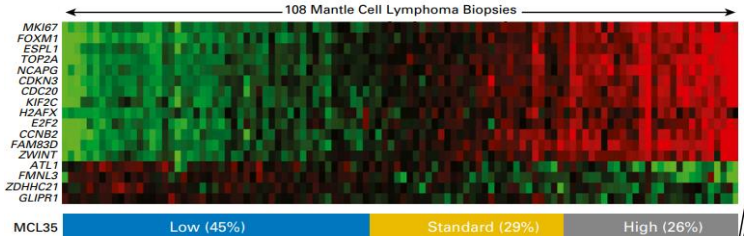
DLBCL COO

(Scott DW, et al. Blood. 2014)



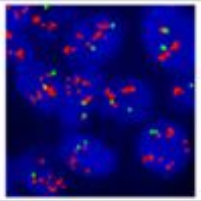
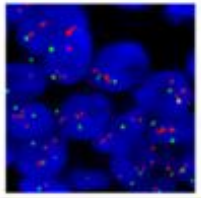
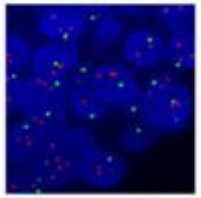
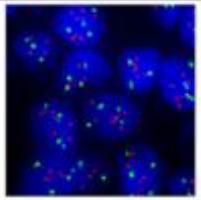
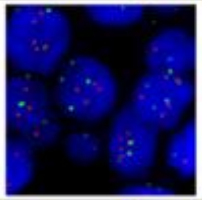
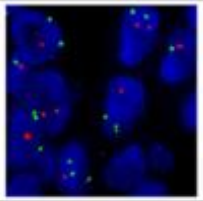
DLBCL/ HGBCL Double-hit

(Ennishi D et al J Clin Oncol 2018)



MCL proliferation

(Scott DW , JCO 2017)

Group	Examples	HER2/CEP17	HER2	2013 guidelines	2018 guidelines
1a		≥ 2.0	≥ 6.0	positive	positive
1b		≥ 2.0	≥ 4.0 and < 6.0	positive	positive
2		≥ 2.0	< 4.0	positive	negative if IHC is 0-2+; positive if IHC is 3+.
3		< 2.0	≥ 6.0	positive	negative if IHC is 0 or 1+; positive if IHC is 2+ or 3+.
4		< 2.0	≥ 4.0 and < 6.0	equivocal	negative if IHC is 0-2+; positive if IHC is 3+.
5		< 2.0	< 4.0	negative	negative

The differences of clinicopathologic characteristics among subgroups of reclassified HER2 fluorescence in situ hybridization (FISH) according to the ASCO/CAP 2018 breast cancer HER2 testing guidelines. Yang L, J Clin Pathol 2018.