Follicular lymphoma

Wolfram Klapper
Hematopathology Section, Kiel, Germany

Disclosures Wolfram Klapper

- I have received travel expenses, honoraria and research grants from Roche, Celgene Novartis, Janssen and Takeda.

- Travel expenses only cover the real costs of traveling.

- All honoraria and grants have been assigned to an account of the University Hospital Schleswig-Holstein and used for research and training purposes.

Follicular Lymphoma

Pathology and Grading → molecular biology and novel findings

Follicular Lymphoma

Pathology and Grading → molecular biology and novel findings

molecular biology and novel findings → Pathology and Grading

Follicular Lymphoma

Pathology and Grading → molecular biology and novel findings

What information do hematologist-oncologists ask for when managing FL? → molecular biology and novel findings → Pathology and Grading

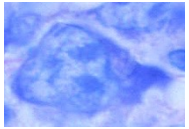
Follicular Lymphoma

WHAT INFORMATION DO HEMATOLOGIST-ONCOLOGISTS ASK FOR WHEN MANAGING FL?

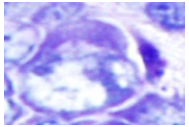
What information do hematologist-oncologists ask for when managing FL?

Diagnosis

Cytology



centroblast



centroblast

indistinguishable

follikular lymphoma

germinal center

insufficient to distinguish FL and hyperplasia

(if not at least combined with flow and/or molecular testing)

Molecular testing

	FL	DLBCL (GCB)*
IGH::BCL2	85-90%	28%
KMT2A	50-70%	40%
CREBBP	50-70%	30%
EZH2	20-40%	22%

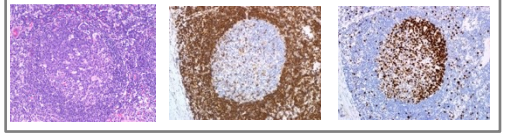
insufficient to diststinguish FL and DLBCL

(and other indolent lymphomas in IGH::BCL2 negative FL)

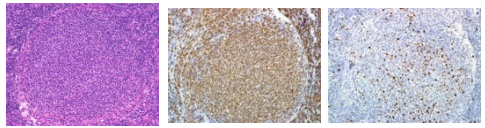
*Schmitz et al NEJM 2018

Pathology

hyperplasia



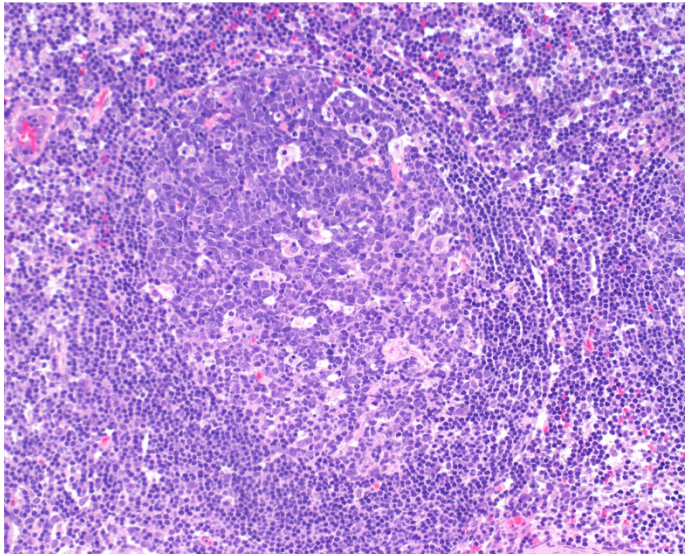
follicular lymphoma



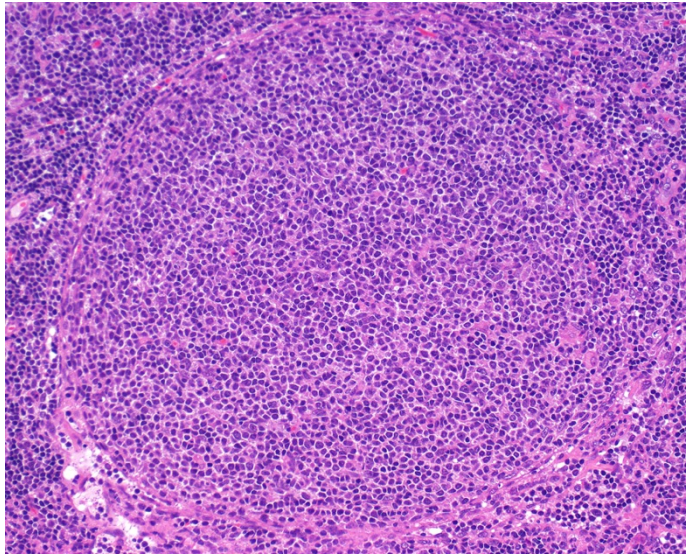
Potentially able to meet all diagnostic needs

Why is pathology strong in diagnosing FL?

physiologic



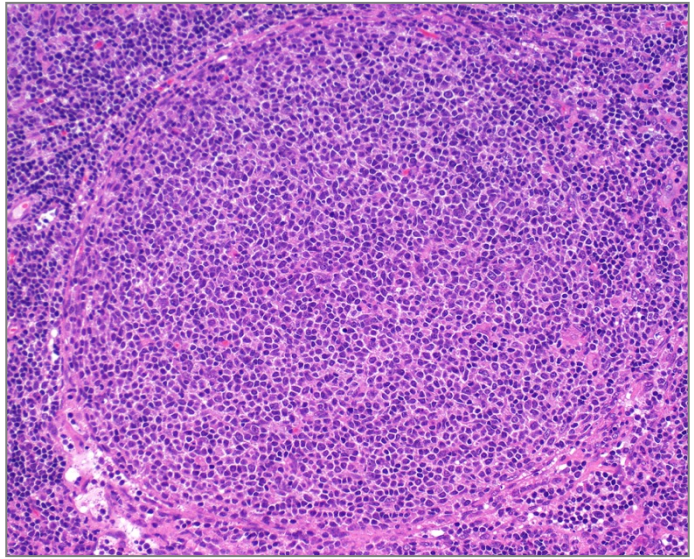
neoplastic



Pathologist perspective

- reduced centroblast content
- absence of dark/light zone
- no starry sky macrophages
- ill defined outer borders

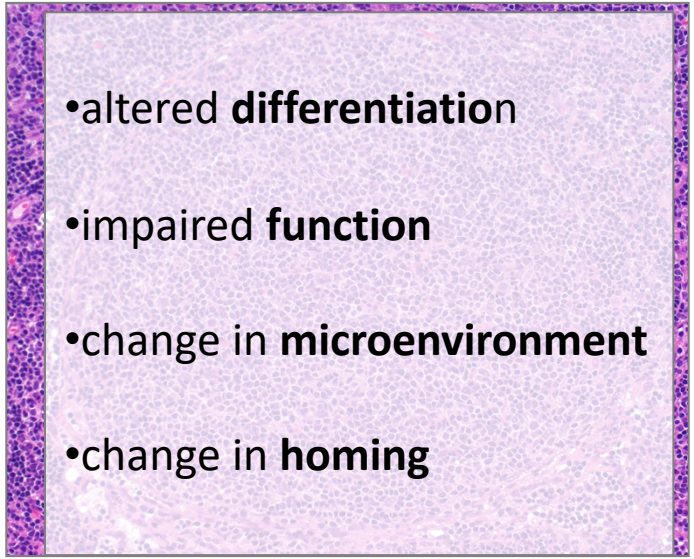
neoplastic



Pathologist perspective

- reduced centroblast content
- absence of dark/light zone
- no starry sky macrophages
- ill defined outer borders

neoplastic

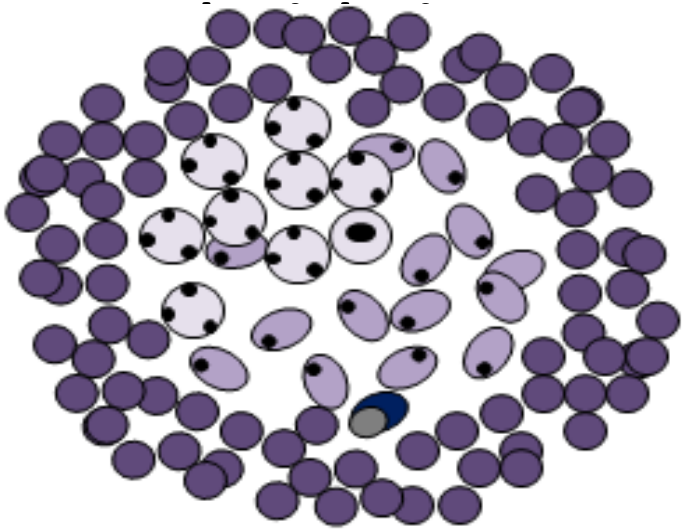


- altered **differentiation**
- impaired **function**
- change in **microenvironment**
- change in **homing**

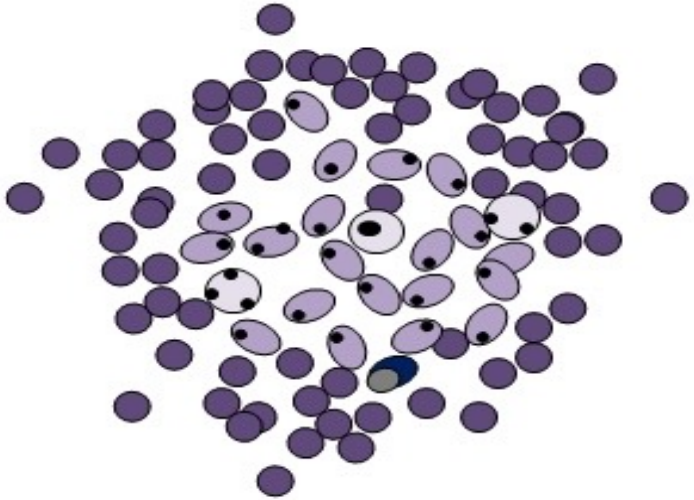
Why is pathology strong in diagnosing FL?

Pathology reflects spatial biology

physiologic



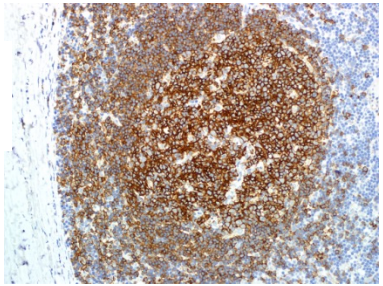
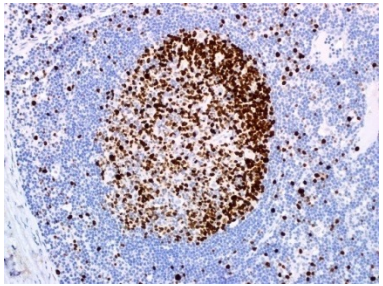
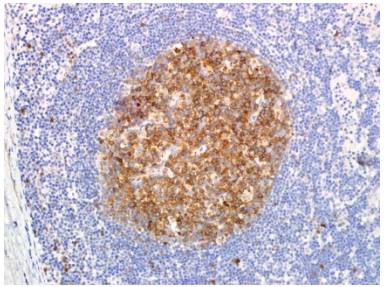
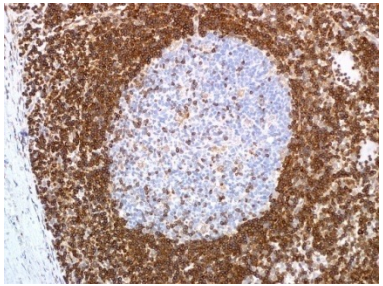
neoplastic



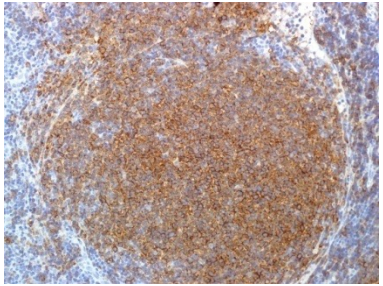
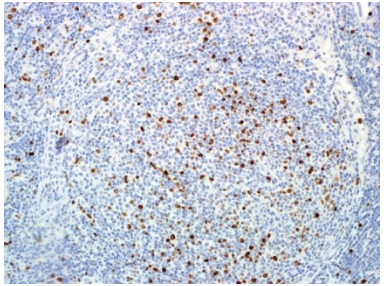
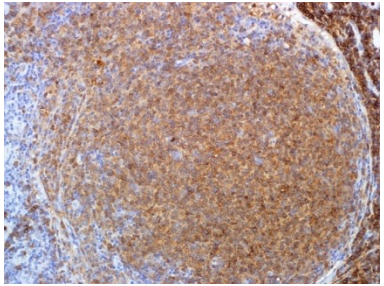
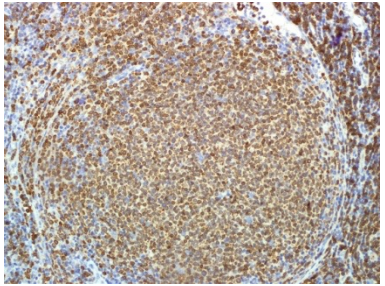
With a little help by IHC

CD20 | Ki67 | CD10 | BCL2

reactive follicle

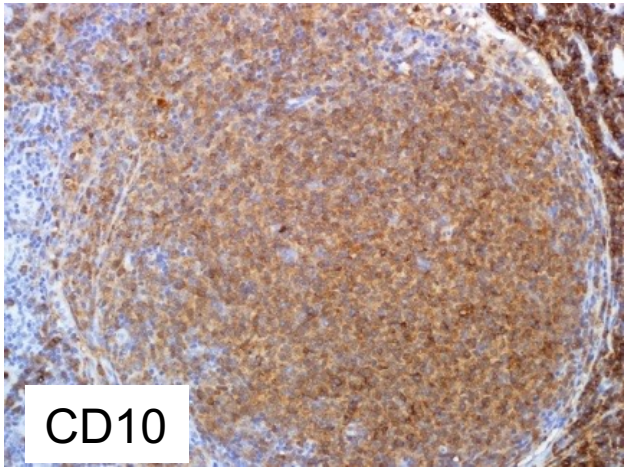





Follicular lymphoma

What information do hematologist-oncologists ask for when managing FL?

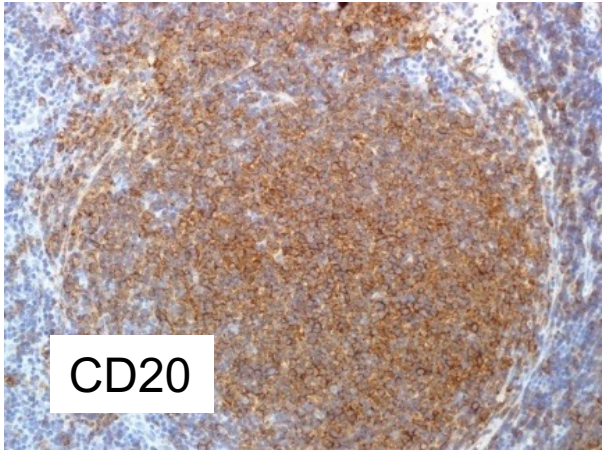
Therapeutic targets



Spatial biology:
CD10+ lymphatic cells outside of a GC
– never benign!

What information do hematologist-oncologists ask for when managing FL?

Therapeutic targets



- Targets: CD20, CD19 etc.
- reliably and easy to assess by immunohistochemistry, flow cytometry
- virtually always positive at the time of first diagnosis

What information do hematologist-oncologists ask for when managing FL?

Transformation

clinical*

- rise in the lactate dehydrogenase level
- a rapid localized nodal growth
- new extranodal sites of disease
- the presence of B symptoms or hypercalcemia

histologic

- histologic change from „low-grade/indolent“ to „high-grade/aggressive“

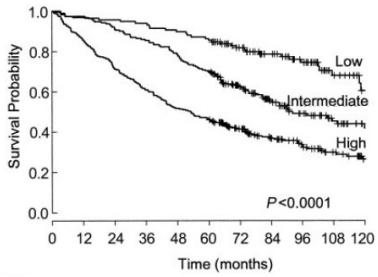
*Al-Tourah AJ et al J Clin Oncol 26:5165-5169, 2008

What information do hematologist-oncologists ask for when managing FL?

Prognosis

FLIPI

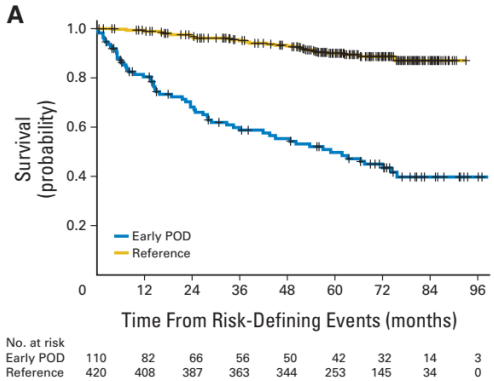
- age (> 60 years)
- Ann Arbor stage (III-IV),
- hemoglobin < 120 g/L
- number of nodal areas >4
- LDH above normal



Solae-Céligny 2004

POD24

- progression of disease <2 years after diagnosis



Casulo 2015

10-30% of patients die of lymphoma within 10 years (adv. stages, >60y).

Sarkozy 2019, Dinnessen 2022

50% of deaths are associated with transformation

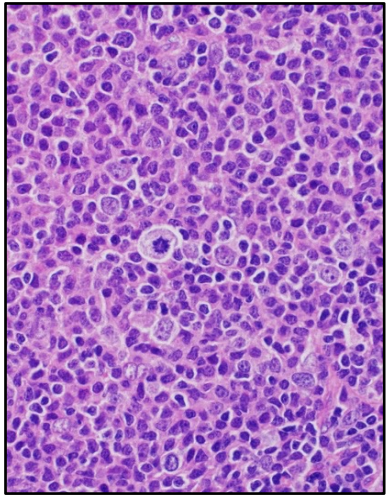
Sarkozy 2019

What information do hematologist-oncologists ask for when managing FL?

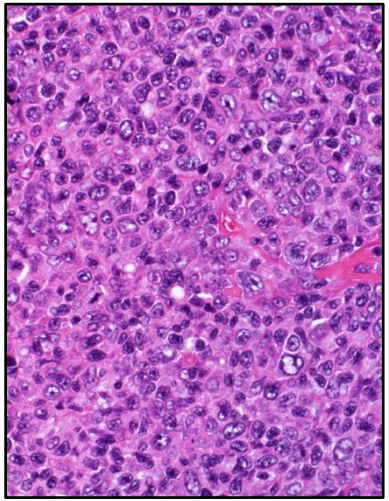
Prognosis

•Grade 1	0-5 centroblasts per HPF*
•Grade 2	6-15 centroblasts per HPF*
•Grade 3	>15 centroblasts per HPF*
–*3a*	*- centrocytes detectable*
–*3b*	*- no centrocytes, only centroblasts*

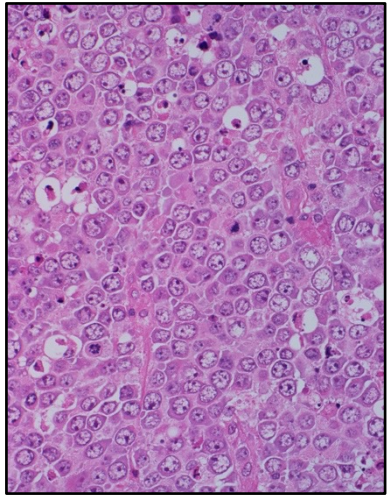
Grade 1/2



Grade 3A



Grade 3B



* 0.159 mm^2

Ask yourself

- Do you do grading when signing our FL?
- Do you count (not estimate) blasts?
- Do you use an HPF?
- Do you know how many blast are the cut-off for you personal microscopy workplace?

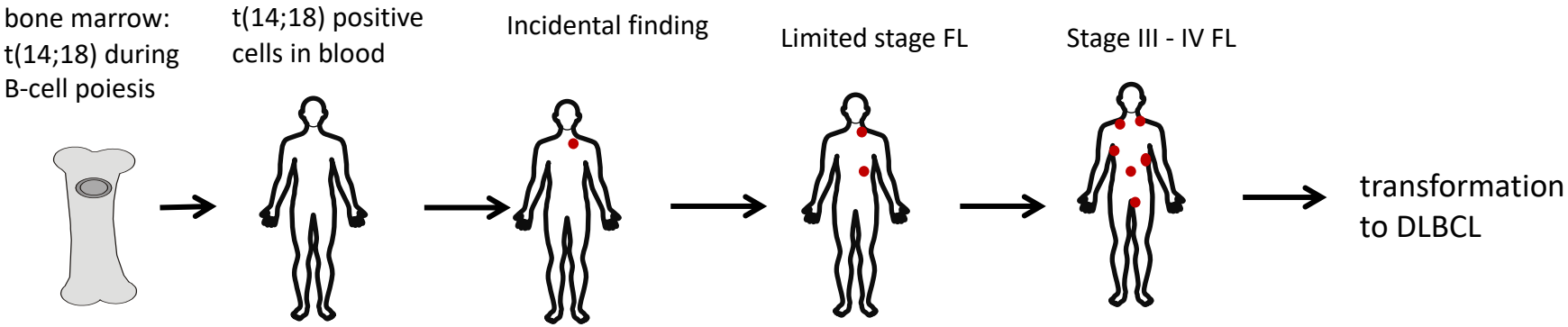
Follicular Lymphoma

MOLECULAR BIOLOGY AND NOVEL FINDINGS

Molecular biology and novel findings

Slow, continuously progressing pathogenesis

clinical scenario

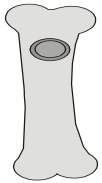


Molecular biology and novel findings

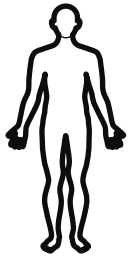
Slow, continuously progressing pathogenesis

clinical scenario

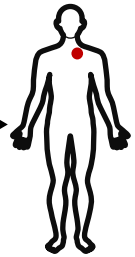
bone marrow: t(14;18) during B-cell poiesis



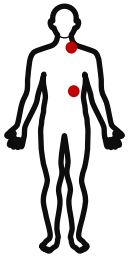
t(14;18) positive cells in blood



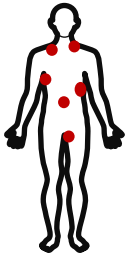
Incidental finding



Limited stage FL

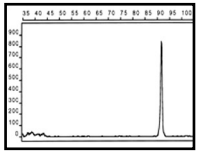


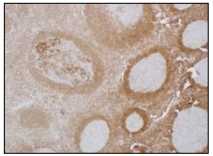
Stage III - IV FL



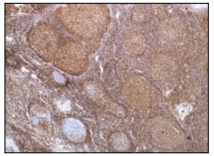
transformation to DLBCL

pathology

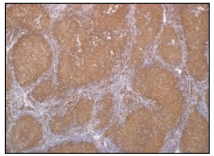




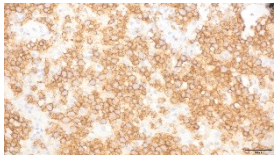
in situ foll. Neoplasia



partial infiltration



fully developed FL

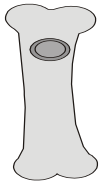


Molecular biology and novel findings

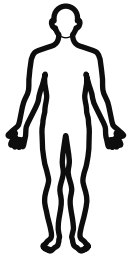
Slow, continuously progressing pathogenesis

clinical scenario

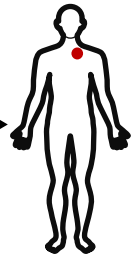
bone marrow: t(14;18) during B-cell poiesis



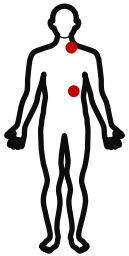
t(14;18) positive cells in blood



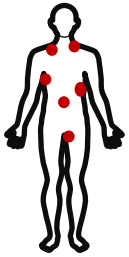
Incidental finding



Limited stage FL

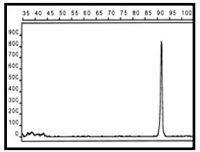


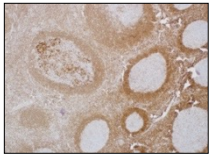
Stage III - IV FL



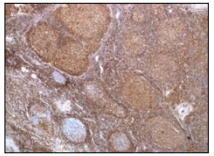
transformation to DLBCL

pathology

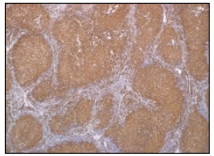




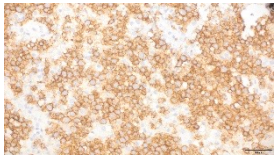
in situ foll. Neoplasia



partial infiltration



fully developed FL



molecular genetics

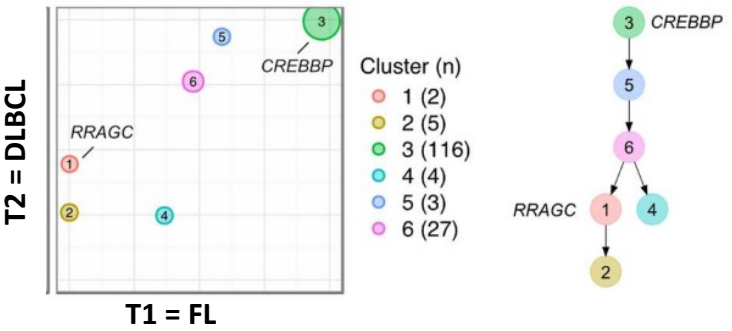
BCL2
CREBBP

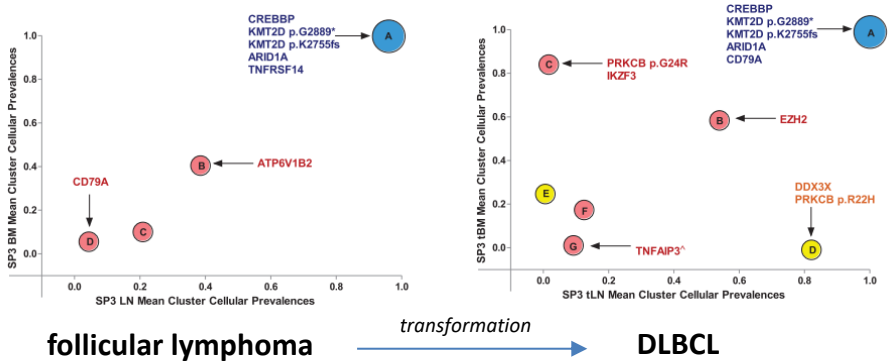
TP53, MYC, NF-KB etc

KMT2D, EZH2 etc

Molecular biology and novel findings

Sudden shift in biology and clinical behavious: Transformation





follicular lymphoma *transformation* → **DLBCL**

Evolution at transformation

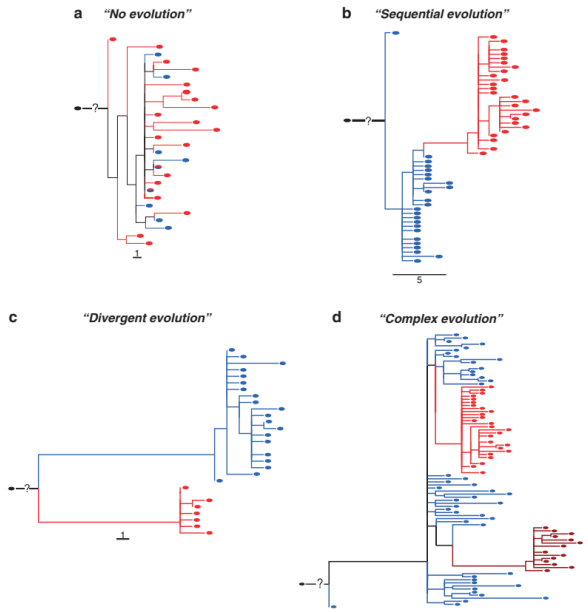
Kridel et al Plos Med 2016

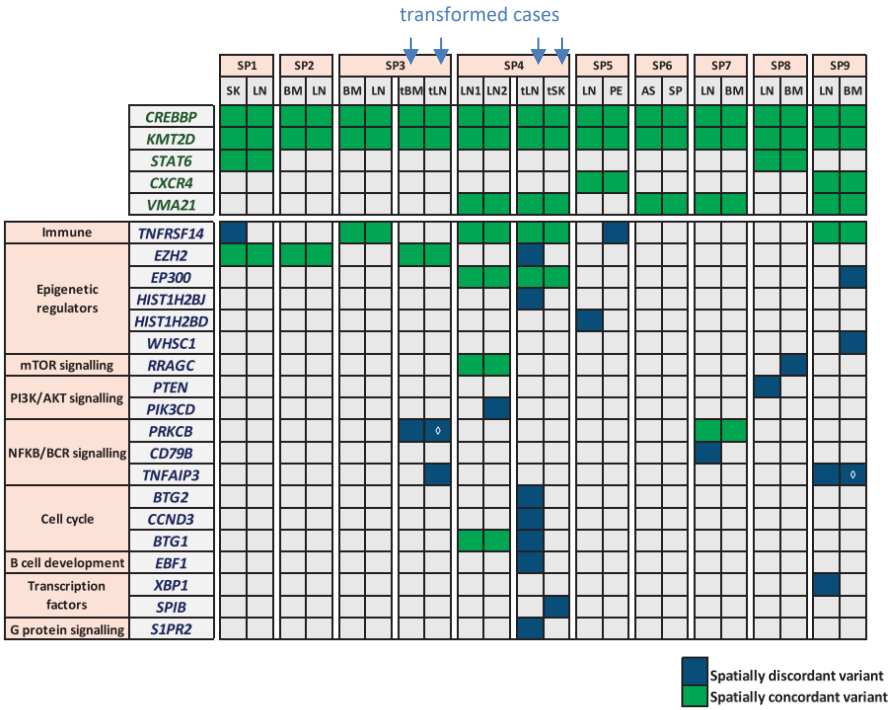
Devolution at transformation

Araf et al Leukemia 2018

Molecular biology and novel findings

intratumoral heterogeneity and divergent evolution





Sequential hererogeneity (IGHV)

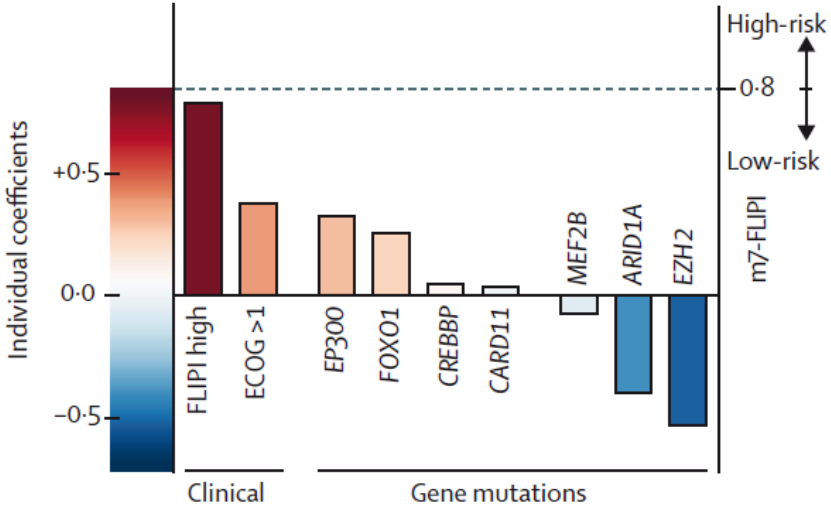
Löffler et al Leukemia 2014

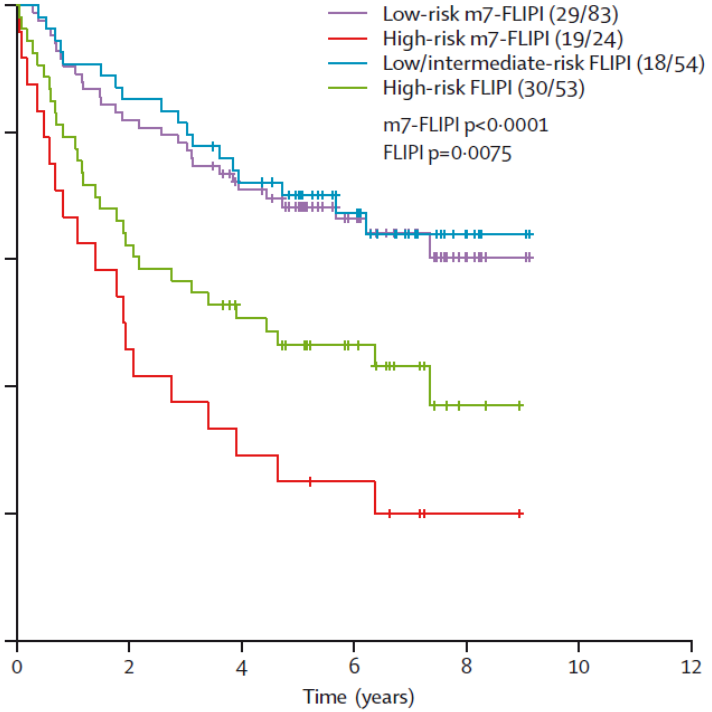
Spatial heterogeneity (som. mutations)

Araf et al Leukemia 2018

Molecular biology and novel findings

Predicting outcome by omics technology



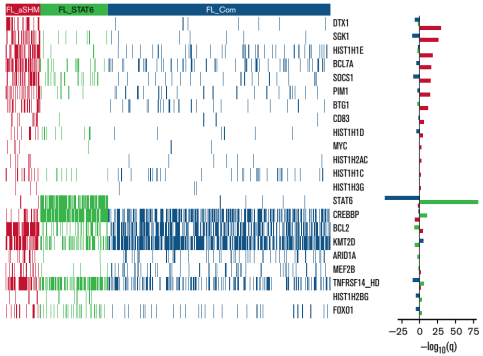


Pastore et al. Lancet 2015

Molecular biology and novel findings

Predicting outcome by omics technology

- 548 patients
- population based
- 293 gene panel NGS



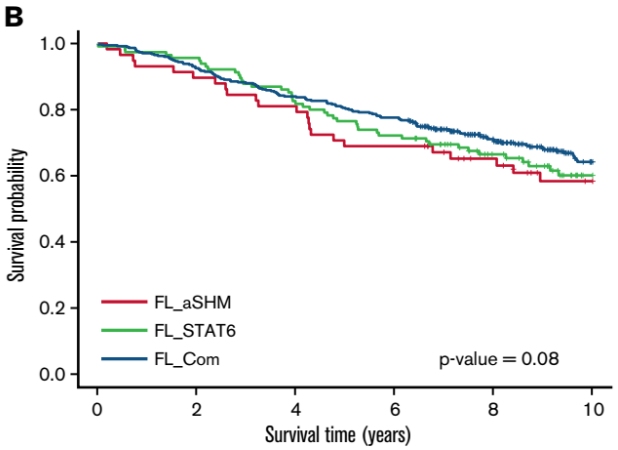


Image for all patients (same result with w&W or initially treated)

„ Our approach identifies 3 molecular subclusters of FL linked to differences in underlying mechanistic pathways. “

“... overall tumor genetics provided limited information to predict individual patient risk.“

Crouch et al Blood Adv 2021

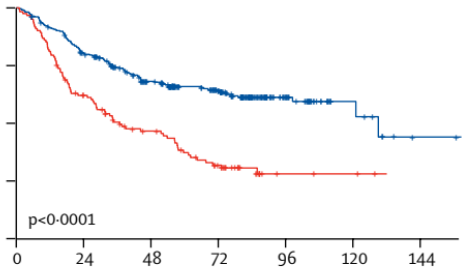
Predicting outcome by omics technology

A gene-expression profiling score for prediction of outcome in patients with follicular lymphoma: a retrospective training and validation analysis in three international cohorts

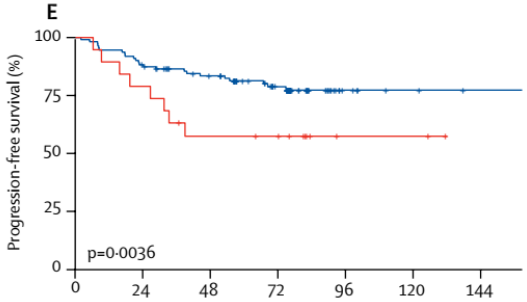
Sarah Huet, Bruno Tesson*, Jean-Philippe Jais, Andrew L Feldman, Laura Magnano, Emilie Thomas, Alexandra Traverse-Glehen, Benoit Albaud, Marjorie Carrère, Luc Xerri, Stephen M Ansell, Lucile Baseggio, Cécile Reyes, Karin Tarte, Sandrine Boyault, Corinne Haioun, Brian K Link, Pierre Feugier, Armando Lopez-Guillermo, Hervé Tilly, Pauline Brice, Sandrine Hayette, Fabrice Jardin, Fritz Offner, Pierre Sujobert, David Gentien, Alain Viari, Elias Campo, James R Cerhan, Gilles Salles*

www.thelancet.com/oncology **Vol 19 April 2018**

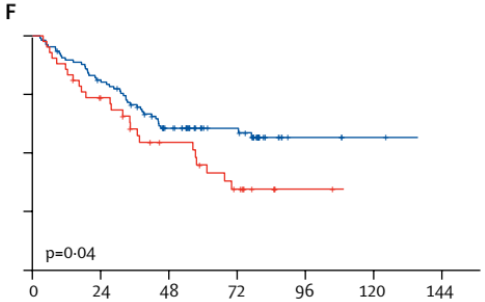
23 gene expression score



low risk FLIPI



intermediate risk FLIPI

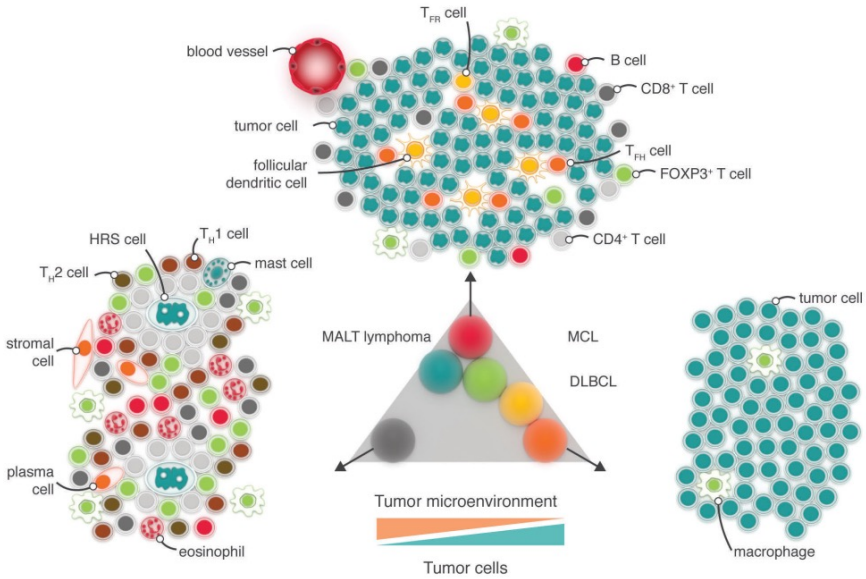


high risk FLIPI

Molecular biology and novel findings

Interaction with immunesystem

Re-education
follicular lymphoma

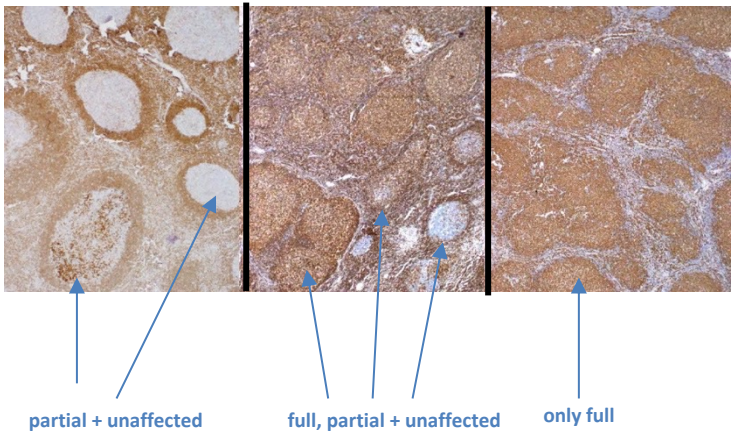


Recruitment
classic Hodgkin

Replacement
Burkitt

Fowler et al. Haematologica 2016

BCL2 to illustrate infiltration of germinal centers



Klapper Sem Diagn Pathol 2011

What can we expect from a pathologist?

Who visually inspects H&E stained slides to assess clinical behaviour?

Follicular lymphoma biology	Pathology
Slow, continuously progressing pathogenesis	performed at variable often undefined stages of pathogenesis
Interaction with immunesystem	not considering host factors (not even microenvironment)
intratumoral heterogeneity and divergent evolution	assesses only a small tumor fraction which is not selected by biological factors
complex biology	simple, non-quantitative method

Is grading a reliable test?

joint review at multi-headed microscope (calibration) → independent review on single microscopes → discussion of dicrepant cases at multi-headed microscope

	Consensus by independent review	
Final diagnosis	**all**	**pure**
FL1/2	34/62 (55%)	20/32 (63%)
FL3A	15/39 (38%)	2/9 (22%)
FL3B	4/10 (40%)	0/2 (0%)
FL3U	0/6 (0%)	n.a.

Grading of Follicular Lymphoma suffers from poor interobserver reproducibility.

Koch et al. Ann Oncol 2016

What can we expect from a pathologist?

Yes

Diagnosis and clinico-pathological subtypes with special features

- *in situ FL*
- *duodenal type*
- *pediatric type*
- *follicular large cell lymphoma (FL3B)*
- *etc*

No

prognostic information

Follicular Lymphoma

PATHOLOGY AND GRADING

History of grading FL

HEMATOLOGICAL ONCOLOGY, VOL. 1, 187–192 (1983)

SHORT COMMUNICATION

CRITERIA FOR THE CYTOLOGIC SUBCLASSIFICATION OF FOLLICULAR LYMPHOMAS: A PROPOSED ALTERNATIVE METHOD

RISA B. MANN*‡ AND COSTAN W. BERARD†

From The Department of Pathology of the The Johns Hopkins Hospital, Baltimore, Maryland and The Division of Pathology of The St. Jude Children's Research Hospital†, Memphis, Tennessee, U.S.A.*

KEY WORDS Follicular lymphomas Lymphomas Classification Diagnosis

In this brief report we would like to propose a simple alternative method for classifying follicular lymphomas which hopefully may lead to more uniformity in the diagnostic categorization of these tumours. This method has been used by one of us (CWB) for over 15 years both in clinicopathologic studies and in the routine daily teaching of fellows and residents. It is easy to apply, reproducible, and appears to divide the follicular lymphomas into three distinct groups with differing clinical survivals (Anderson *et al.*, 1977, 1982a, b; Glick *et al.*, 1981; National Cancer Institute, 1982). The cytologic subclassification is based on the relative proportions of small cleaved lymphocytes and large transformed lymphoid cells within the neoplastic nodules. Because rough estimates or actual percentages of large cells are difficult to use and reproduce, we have chosen to actually count the number of large cells observed within a field of the neoplastic nodule at high dry magnification (10× ocular, 40× objective). At least twenty fields are counted and an average is taken. To qualify as FM a case should have between 5 and 15 large neoplastic cells per high power field in the majority of the microscopic fields (Figure 1). The large cells are two to five times the size

- **Proposal to count blasts in high-power field (size not specified)**
- **No outcome data**

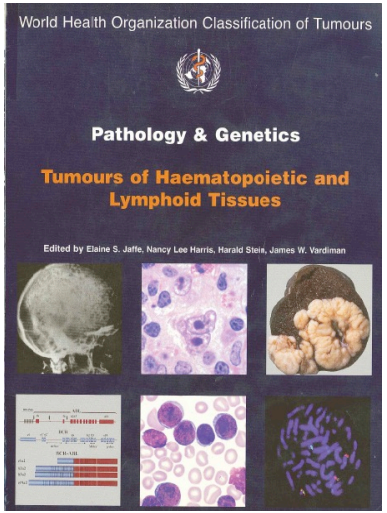
History of grading FL

1974 Kiel-classification → 1994 REAL classfication

no grading

recommendation to grade but no method specified

WHO 2000

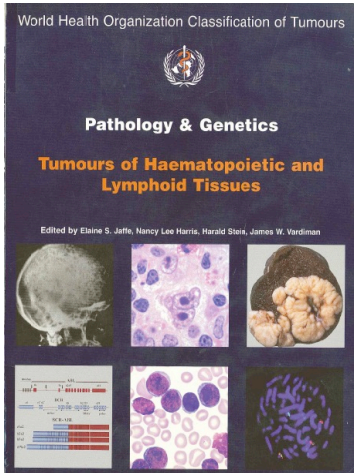


Grade 1
Grade 2
Grade 3A
Grade 3B

What evidence for grading in 2000?

- lacking scientific standards -

WHO 2000



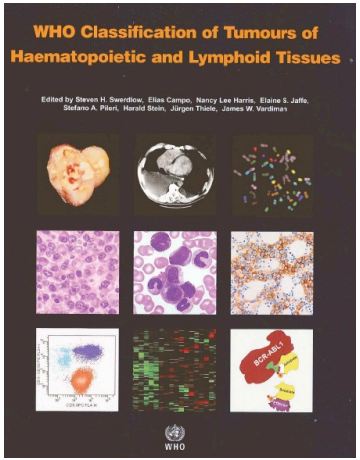
„Follicular lymphoma is graded by the proportion of centroblasts. Histological grading can predict clinical outcome, but the optimal method and the clinical significance are still debated. We recommend a 3-grade system (Grade 1-3) based on the absolute number of centroblasts in ten high-power microscopic field (hpf) (811, 870, 935)“

citation	publication	method specified?	outcome data?	prospective trial?
810	Mann et al. Haematol. Oncol 1983	not exactly	no	no
870	Metter, Nathwani et al J Clin Oncol 1985	yes	yes	yes (CHOP, n=105)
935	Nathwani, Metter et al Blood 1986	yes	yes	yes (CHOP, n=105)

identical data

What evidence for grading in 2008?

WHO 2008



„There is **insufficient evidence** at this time to recommend an alternative method or **to eliminate** grading altogether.“

- reversal of burden of proof -

Anderson et al (J Clin Oncol 1993) Nebrasca group
Working formulation (Cancer 1982)
Comment by Sandra Horning
Galagher et al (J Clin Oncol 1986) Andrew Lister Group
2x Glick et al (Blood 1981, Cancer 1982)
Jones et al (Cancer 1973)
Kantarjian et al (J Clin Oncol 1984)
Longo et al (Ann Intern Med 1984)
Martin et al (Blood 1995) Nebrasca group
Rappaport (Cancer 1956)

Why get rid of FL grading?

- Implemented for prognostication but never sufficiently proven to be prognostic.
- Poor inter-observer consensus
- Misleading nomenclature: not reflecting biology of FL and not aligned with grading of other tumors

Follicular Lymphoma – Grading and Subtyping

	before (Kiel, REAL etc)	WHO 2000	WHO 2008	WHO 2017	WHO 2023
grading	no grading (Kiel) provisional (REAL)	Grade 1 Grade 2 Grade 3A Grade 3B	Grade 1 Grade 2 Grade 3A Grade 3B	Grade 1/2 Grade 3A Grade 3B	classic large cell type
subtypes	*none*	*none*	„variants“ (pediatric, intestinal, „in situ“) •PCFCL (cutaneous)	„variants“ (testicular, duodenal, „in situ“) •PCFCL (cutaneous) •Pediatric type	•In situ follicular B cell neoplasm •Paediatric-type follicular lymphoma •Duodenal-type follicular lymphoma •Gonadal follicular lymphoma

Clinico-pathological subtypes of FL

	morphology	Immuno-phenotype	genetics	Clinical features
Duodenal type	Typical FL 1/2 with large expansive follicles	CD10+/BCL2+	IGH::BCL2 mostly positive	Required for diagnosis: mostly confined to duodenal mucosa
***In situ* follicular neoplasia**	Minimal involvement of GC in otherwise reactive lymph node	CD10+/BCL2+	IGH::BCL2 mostly positive	Incidental finding or associated with other lymphoma
Pediatric type follicular lymphoma	Large expansive follicles, rich in centroblasts	CD10+/-/BCL2 mostly negative	IGH::BCL2 negative Absence of BCL6/IRF4 translocations	Nodal disease mostly in stage I (often single lymph node)

ICC – Follicular Lymphoma

"Whether patients with grade 3A have a more adverse prognosis and deserve different management than those with grades 1 to 2 remains debatable[62-64] (...)."

"Grade 3B clearly differs in its clinical behavior, and patients are usually managed similarly to those with diffuse large B-cell lymphoma (DLBCL).[65,66] "

Campo et al. Blood 2022

ICC – Follicular Lymphoma

"Whether patients with grade 3A have a more adverse prognosis and deserve different management than those with grades 1 to 2 remains debatable[62-64] (...)."

62: Koch et al. Ann Oncol 2016:

63: Ali et al. Clin Lymphoma, Myeloma & Leuk 2017:

64: Sha et al. Clin Lymphoma, Myeloma & Leuk 2019:

Campo et al. Blood 2022

ICC – Follicular Lymphoma

"Whether patients with grade 3A have a more adverse prognosis and deserve different management than those with grades 1 to 2 remains debatable[62-64] (...)."

62: Koch et al. Ann Oncol 2016:
FL3A even better than FL1/2!

63: Ali et al. Clin Lymphoma, Myeloma & Leuk 2017:
No comparision FL3A with FL1/2 included in this paper!

64: Sha et al. Clin Lymphoma, Myeloma & Leuk 2019:
No comparision FL3A with FL1/2 included in this paper!

Campo et al. Blood 2022

Establising clinical prognostic scores for Follicular lymphoma: *Grading was not prognostic!*

FLIPI

CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS

Follicular Lymphoma International Prognostic Index

Philippe Solal-Céligny, Pascal Roy, Philippe Colombat, Josephine White, Jim O. Armitage, Reyes Arranz-Saez, Wing Y. Au, Monica Bellei, Pauline Brice, Dolores Caballero, Bertrand Coiffier, Eulogio Conde-Garcia, Chantal Doyen, Massimo Federico, Richard I. Fisher, Javier F. Garcia-Conde, Cesare Guglielmi, Anton Hagenbeek, Corinne Haïoun, Michael LeBlanc, Andrew T. Lister, Armando Lopez-Guillermo, Peter McLaughlin, Noël Milpied, Pierre Morel, Nicolas Mounier, Stephen J. Proctor, Ama Rohatiner, Paul Smith, Pierre Soubeyran, Hervé Tilly, Umberto Vitolo, Pier-Luigi Zinzani, Emanuele Zucca, and Emili Montserrat

Blood (2005)

4167 patients

grading not prognostic!

POD24

VOLUME 33 · NUMBER 23 · AUGUST 10 2015

JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Early Relapse of Follicular Lymphoma After Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone Defines Patients at High Risk for Death: An Analysis From the National LymphoCare Study

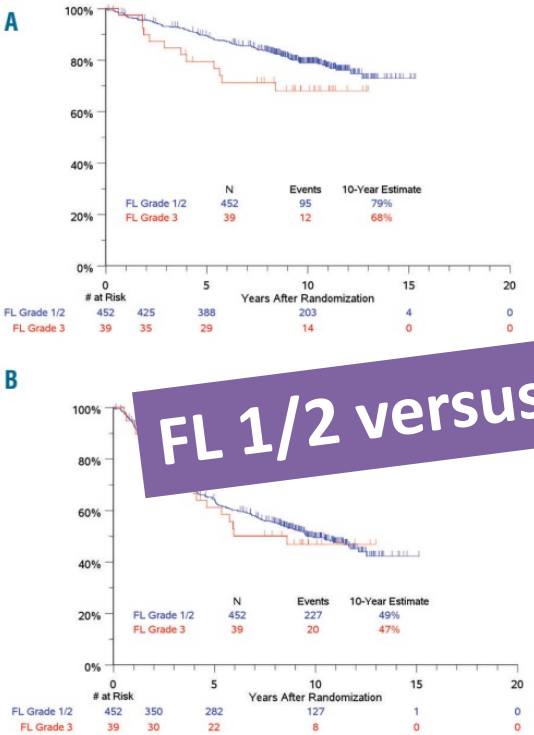
Carla Casulo, Michelle Byrtek, Keith L. Dawson, Xiaolei Zhou, Charles M. Farber, Christopher R. Flowers, John D. Hainsworth, Matthew J. Maurer, James R. Cerhan, Brian K. Link, Andrew D. Zelenetz, and Jonathan W. Friedberg

J Clin Oncol (2015)

588 + 147 patients

grading not prognostic!

Not cited paper (example): Rimza et al. Haematologica 2018

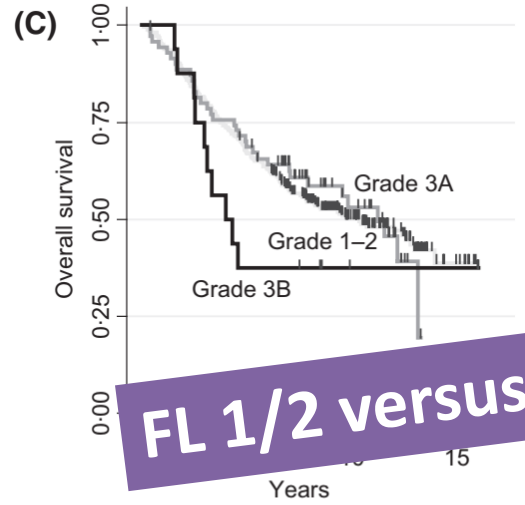


- 419 patients
- centralized review (including panel)
- prospective clinical trial

FL 1/2 versus FL3A: no prognostic difference

„We found that: (i) the impact of histological grading on patients' outcome in the rituximab plus doxorubicincontaining chemotherapy era <u>is negligible</u>; and (ii) there is a tendency to "over-grade" FL. "

Not cited paper (example): Wahlin et al. Br J Haematol 2011



No. of patients at risk

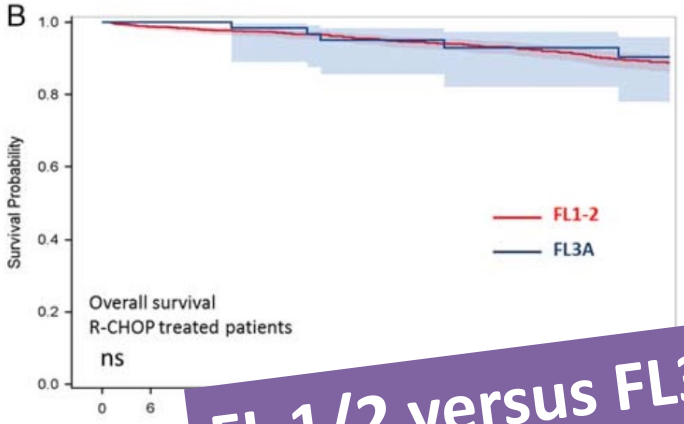
Grade 1–2	279	186	81	8
Grade 3A	70	49	20	0
Grade 3B	16	7	3	1

- >500 patients
- centralized review
- Swedisch cancer re

FL 1/2 versus FL3A: no prognostic difference

„Patients with grade 3A may safely be recruited to indolent lymphoma trials"

Not cited paper (example): Laurent et al. Am J Surg Pathol 2021

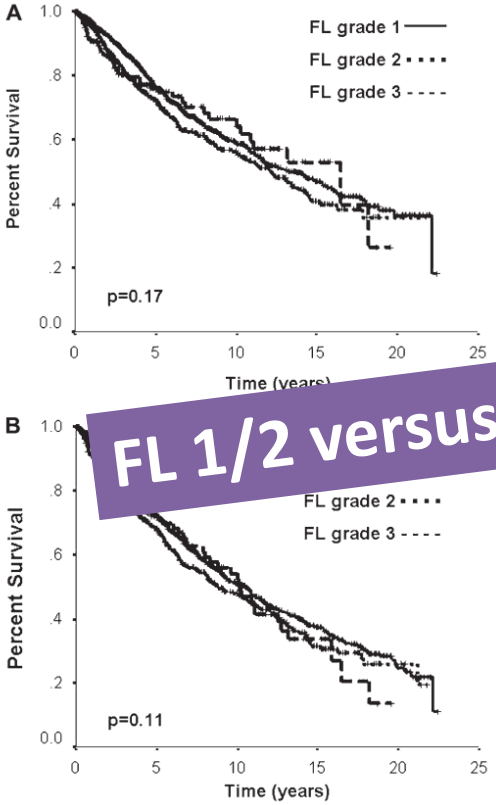


- >900 patients
- centralized review
- prospective clinical

FL 1/2 versus FL3A: no prognostic difference

„(...)our data corroborate in a large series of randomized FL patients that FL3A and FL1-2 patients share a similar prognosis."

Not cited paper (example): Shustik et al. Ann Oncol 2010



- >1000 patients
- centralized review
- BCCA registry

FL 1/2 versus FL3A: no prognostic difference

„Our findings indicate a survival pattern in FL3A/FL3B similar to that of FL grades 1–2 (...)"

ICC – Follicular Lymphoma

"Whether patients with grade 3A have a more adverse prognosis and deserve different management than those with grades 1 to 2 **remains debatable**[62-64] (...)."

Campo et al. Blood 2022

ICC – Follicular Lymphoma

"Whether patients with grade 3A have a more adverse prognosis and deserve different management than those with grades 1 to 2 remains debatable[62-64] ***and needs to be re-evaluated, given evolving non-cytotoxic therapeutic approaches.***"

Campo et al. Blood 2022

ICC – Follicular Lymphoma

"Whether patients with grade 3A have a more adverse prognosis and deserve different management than those with grades 1 to 2 remains debatable[62-64] ***and needs to be re-evaluated, given evolving non-cytotoxic therapeutic approaches.***"

Campo et al. Blood 2022

ICC – Follicular Lymphoma

"Grade 3B clearly differs in its ***clinical behavior***, and patients are usually managed similarly to those with diffuse large B-cell lymphoma (DLBCL) *65,66*"

Campo et al. Blood 2022

ICC – Follicular Lymphoma

"Grade 3B clearly differs in its ***clinical behavior***, and patients are usually managed similarly to those with diffuse large B-cell lymphoma (DLBCL) *65,66*"

65: Barraclough et al. Br J Haematol 2021:
This is a review. No original data included!

66: Koch et al Haematologica 2021:
***No comparison with other grades included*!**

Campo et al. Blood 2022

The clinical course of FL3A does not really „differ“ from FL3B

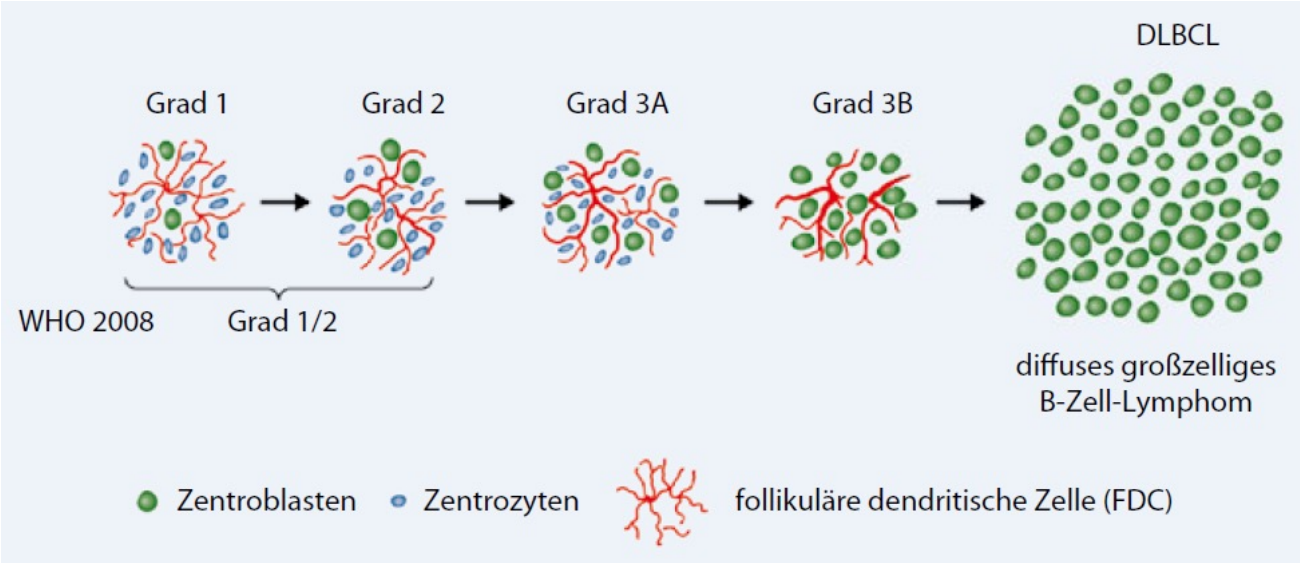
study	n	therapy	survival difference FL3A versus FL3B
Hsi et al. Arch Pathol Lab Med 2004	FL3A: 35 FL3B: 9	variable, no R	**No**
Shustik et al. Ann Oncol 2010	FL3A: 139 FL3B:22	variable, some with R	**No**
Wahlin et al. Br J Haematol 2011	FL3A: 94 FL3B: 23	variable, some with R	**yes (FL3B more relapses but no late relapses)**
Koch et al Ann Oncol 2016	FL3A: 39 FL3B: 10	R-CHOP	**No**
Barraclough et al. Haematological 2022	FL3A: 302 FL3B: 157	R-CHOP or O-CHOP	**No**

FL3A and FL3B are distinct but ...

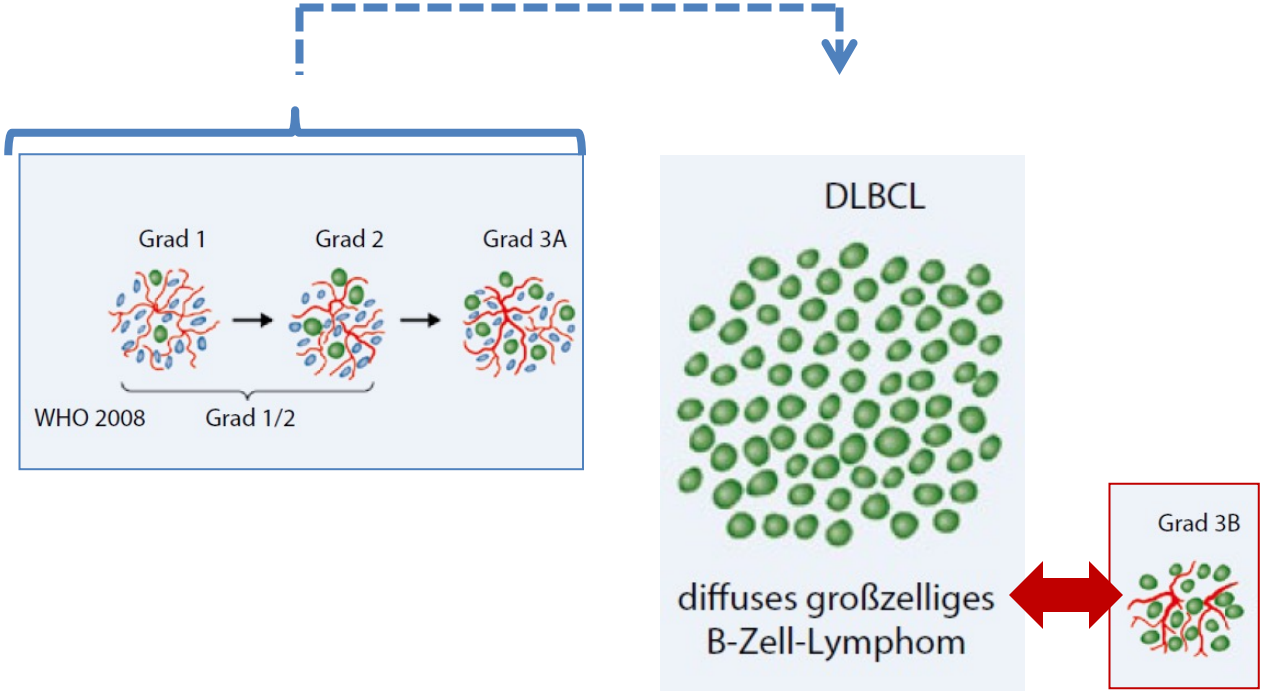
- ... not in their clinical course in most published studies and under R-chemo

- ... instead their differences are their *molecular-pathogenetic relation* of **FL3A to FL1/2** and **FL3B to DLBCL**

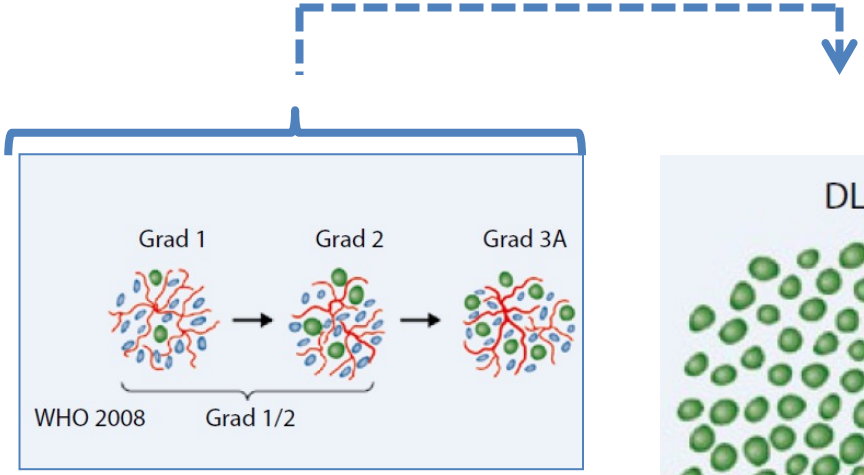
(see Horn et al. Haematologica 2011, Koch et al. Ann Oncol 2016)

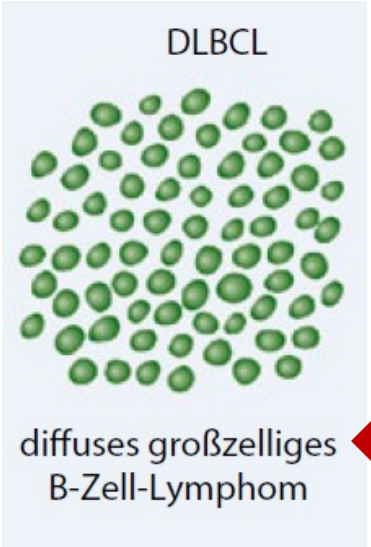


Onkologe 2011

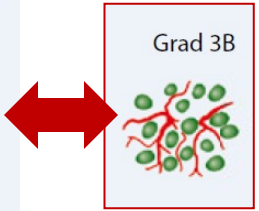

Grad 1
Grad 2
Grad 3A
WHO 2008
Grad 1/2
DLBCL
diffuses großzelliges
B-Zell-Lymphom
Grad 3B

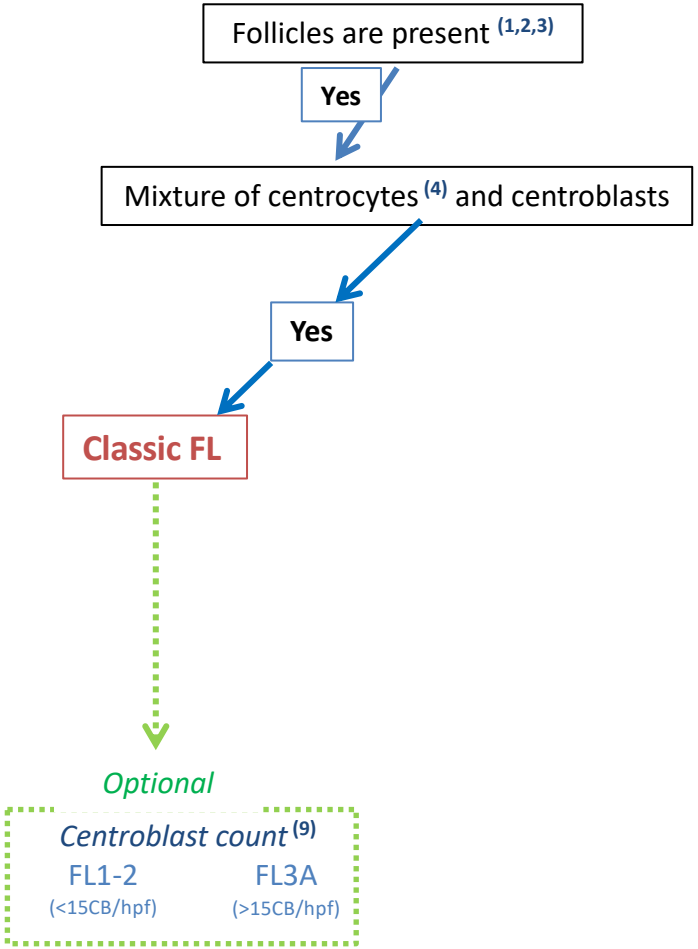
classic FL

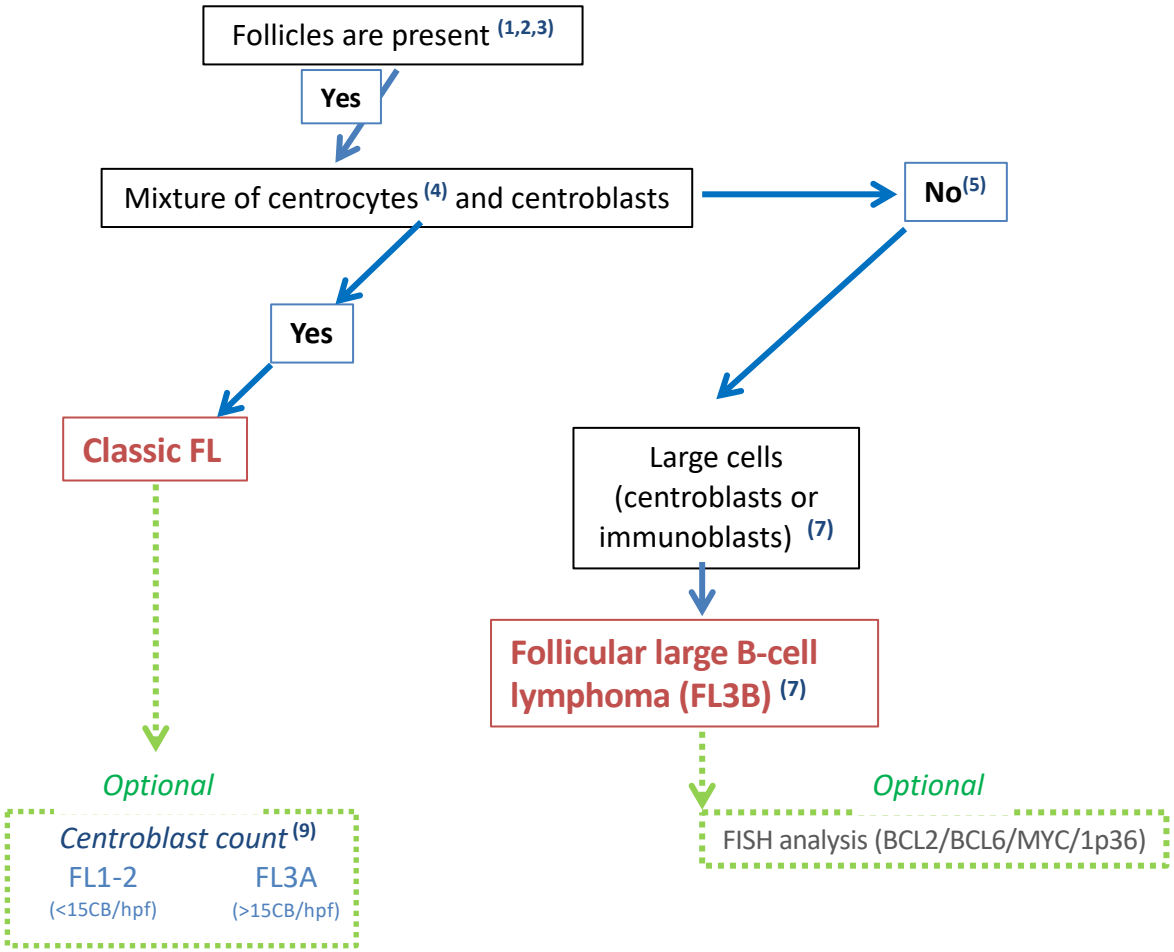


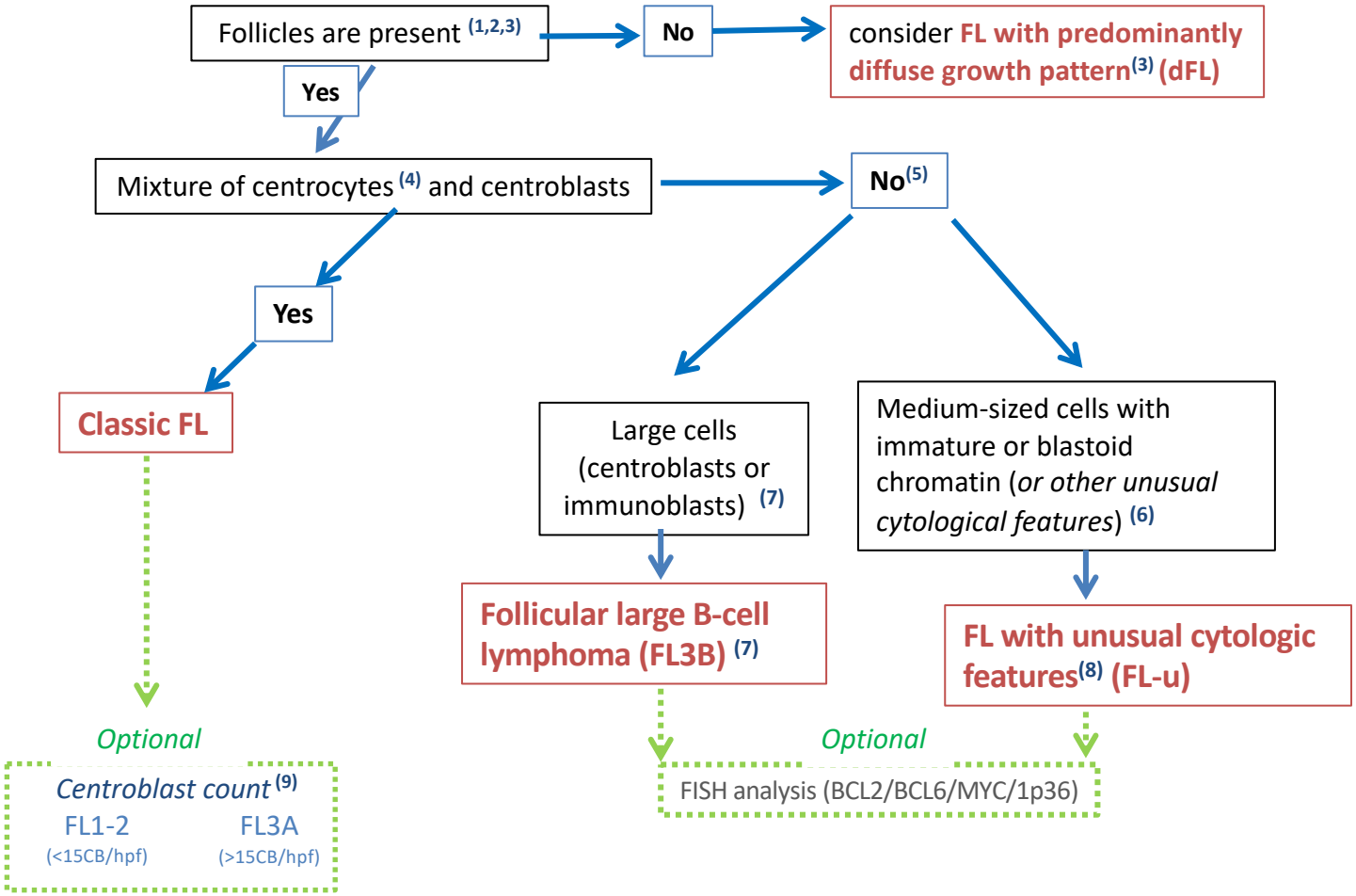


Follicular large cell lymphoma




Follicles are present (1,2,3)
Yes
Mixture of centrocytes (4) and centroblasts
Yes
Classic FL
Optional
Centroblast count (9)
FL1-2
(<15CB/hpf)
FL3A
(>15CB/hpf)


Follicles are present (1,2,3)
Yes
Mixture of centrocytes (4) and centroblasts
No(5)
Yes
Classic FL
Large cells (centroblasts or immunoblasts) (7)
Follicular large B-cell lymphoma (FL3B) (7)
Optional
Centroblast count (9)
FL1-2
(<15CB/hpf)
FL3A
(>15CB/hpf)
Optional
FISH analysis (BCL2/BCL6/MYC/1p36)

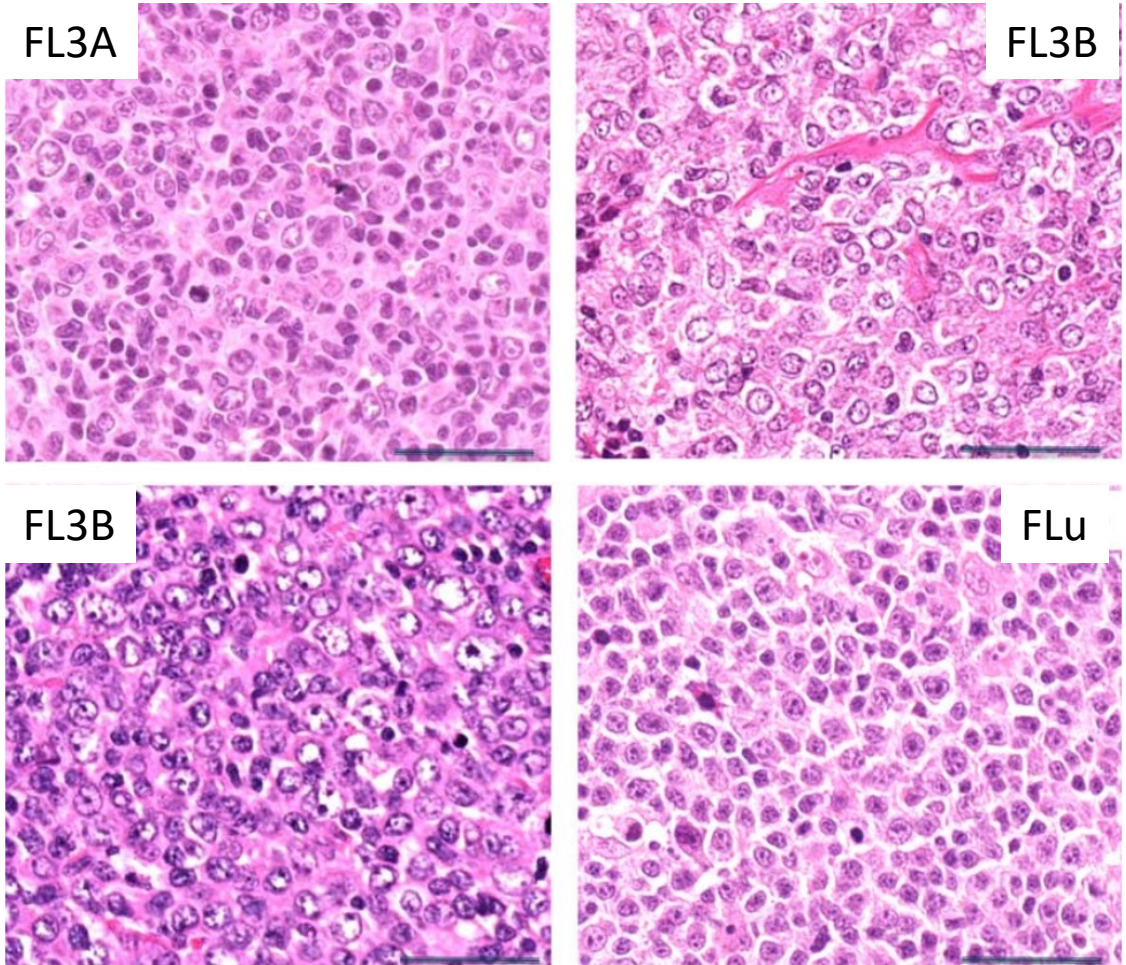

Follicles are present (1,2,3)
No
consider FL with predominantly diffuse growth pattern(3) (dFL)
Yes
Mixture of centrocytes (4) and centroblasts
No(5)
Yes
Classic FL
Large cells (centroblasts or immunoblasts) (7)
Medium-sized cells with immature or blastoid chromatin (or other unusual cytological features) (6)
Follicular large B-cell lymphoma (FL3B) (7)
FL with unusual cytologic features(8) (FL-u)
Optional
Centroblast count (9)
FL1-2
(<15CB/hpf)
FL3A
(>15CB/hpf)
Optional
FISH analysis (BCL2/BCL6/MYC/1p36)

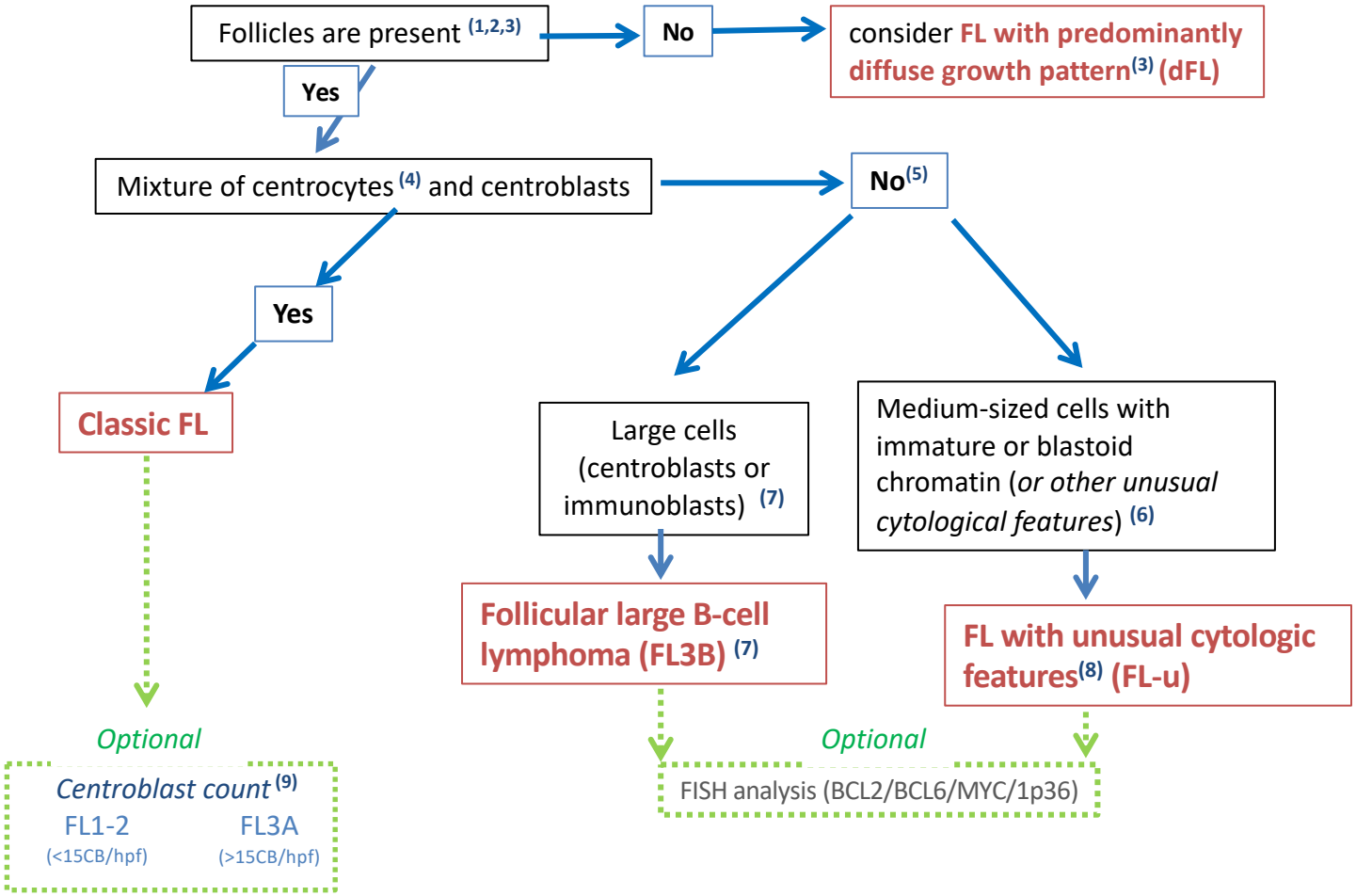
FL-u (unconventional, ungraded)

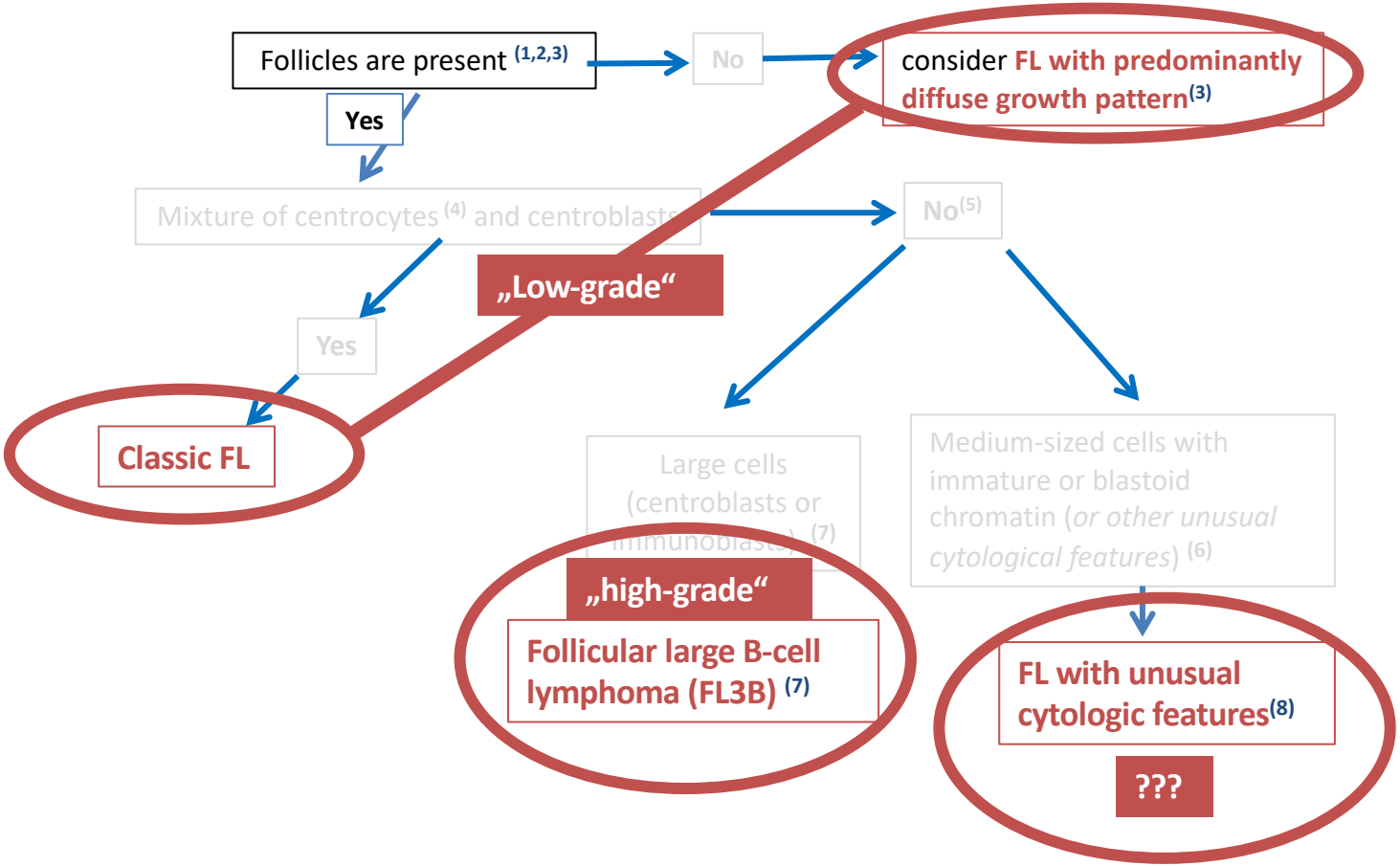
- cytology ambigious between centrocytes and centroblasts
- often „blastoid“ apearing and highly proliferative
- clinical course might be more aggressive (to be confirmed)

Horn et al. 2011, Laurent et al 2021

FL-u (unconventional, ungraded)




Follicles are present (1,2,3)
No
consider FL with predominantly diffuse growth pattern(3) (dFL)
Yes
Mixture of centrocytes (4) and centroblasts
No(5)
Yes
Classic FL
Large cells (centroblasts or immunoblasts) (7)
Medium-sized cells with immature or blastoid chromatin (or other unusual cytological features) (6)
Follicular large B-cell lymphoma (FL3B) (7)
FL with unusual cytologic features(8) (FL-u)
Optional
Centroblast count (9)
FL1-2
(<15CB/hpf)
FL3A
(>15CB/hpf)
Optional
FISH analysis (BCL2/BCL6/MYC/1p36)


Follicles are present (1,2,3)
No
consider FL with predominantly diffuse growth pattern(3)
Yes
Mixture of centrocytes (4) and centroblasts
No(5)
„Low-grade“
Yes
Classic FL
Large cells (centroblasts or immunoblasts) (7)
„high-grade“
Follicular large B-cell lymphoma (FL3B) (7)
Medium-sized cells with immature or blastoid chromatin (or other unusual cytological features) (6)
FL with unusual cytologic features(8)
???

Vielen Dank für Die Aufmerksamkeit!