Pathology and Molecular Genetics of Follicular Lymphoma

Lymphoma Forum
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Topics

• New data on the pathology and molecular pathogenesis of follicular lymphoma
• Report from the lymphoma workshop of the EAHP/SH held in Istanbul 2014
• A new tool for prognostication: the m7FLIPI
Follicular Lymphoma – The Cornerstones

The t(14;18) is found in 85% of FL grades 1/2 and 60% of 3A

BCL-2
The t(14;18) is found in reactive lymphoid proliferations and peripheral blood of healthy individuals and its frequency increases over time.


Courtesy of Bertrand Nadel
Follicular Lymphomas in all its Sites and Shapes
Variants and Subtypes

- FL grades 1&2, nodal
- FL grade 3A
- FL grade 3B

- BCL-2 expression negative FL (10%) of FL 1, 2, 3A (30%?)
  - in 30%-50% of cases due to somatic mutation at the BCL-2 Ab binding site in spite of t(14;18)
  - t(14;18) negative FL

- Predominantly diffuse follicular lymphomas

- Pediatric-type follicular lymphomas

- Primary extranodal follicular lymphomas
  - Primary cutaneous follicle centre lymphoma
  - Primary duodenal FL
  - Other extranodal follicular lymphomas
    - Ocular adnexae, breast, testis, thyroid gland

*With particular biological and/or clinical characteristics*
FL3B

- >150 blasts in 10 HPF
- Very rare!
- Rigid follicular contours
- No obvious (typical?) centrocytes
- Sometimes starry sky macrophages
- t(14;18) rare
FL3A

- >150 blasts in 10 HPF
- Typical morphology of FL
- Smooth transition of tumor to interfollicular areas
- Follicles not sharply circumscribed
- CD10+ BCL2+
- t(14;18) chromosome translocation in 60%
FL3A do show a plateau upon CHOP therapy in retrospective analyses of the German low and high grade lymphoma study groups.
Features of nodal t(14;18)-negative FL

- atypical follicles, predominantly centrocytes
- majority grade 1/2
- GEP similar to t(14;18) positive garden variety FL, **but**

- Gene expression and miRNA profiles suggest an exit population with „late“ germinal center B-cell phenotype

Leich et al., Blood 2009; Leich et al., Blood 2011
FISH Analysis of FL Enrolled in the GLSG Studies

FISH BCL2-BAP

540 III/IV FL
117 I/II FL

IHC BCL2

+ 99%
- 14%

+ 86%
- 14%

+ 100%

+ 69%
- 31%

Leich et al. Leukemia 2015
No Difference in Survival Between t(14;18) positive and –negative FL in Stages III/IV

Leich et al. Leukemia 2015
Importance of Genetic Alterations in the Foundation and Progression of FL

New Data From NGS Efforts…
Mutations of Genes Involved in Chromatin Regulation and Linker Histones are Frequently Altered in FL (and TNFRSF14)

A New Kid on the Block in FL (and DLBCL): Modification of Epigenetic Signatures

Frequent mutations of histone or chromatin modifying genes in 17-70% FL: $\text{EZH2, CREBBP, MLL2, MEF2B, etc.}$

KMT2D (MLL2) mutation

Active chromatin configuration

Heterozygous $\text{EZH2 Y641}$ Dominant mutations

Increased $\text{H3K27me3}$ Tumorigenesis

$\text{Morin et al., Nature Genetics 2010}$
$\text{Yap et al., Blood 2011, Okosun et al. Nature Medicine 2014}$
$\text{Ortega-Molina et al. Nature Medicine 2015}$
$\text{Zhang et al. Nature Medicine 2015}$
Clonal and Subclonal Mutations in FL as Markers of Early vs. Late Events
Different Patterns of Genetic Evolution in FL

Ancestral CPC clone

Okosun et al. Nature Genetics 2014
Redefining the spectrum of small B cell lymphomas in the light of current technology:
Session 5 - Follicular Lymphoma

Luc Xerri
German Ott
Issues addressed in 44 workshop cases

- In Situ Follicular neoplasia/follicular lymphoma \textit{in situ} and FL precursors
- FL with unusual immunophenotypes
- FL with localized extranodal presentation
- Predominantly diffuse variants of FL
- FL with blastoid features
- FL with monocytoid features and/or marginal zone differentiation (MZD)
- FL with unusual genetic features
- Transformation of FL
In Situ Follicular Neoplasia (ISFN)

H&E 200x

BCL2 200x

Ki67 200x
The Hallmarks of In Situ Follicular Neoplasia (ISFN)

Usually a fortuitous finding: 2% in consecutive analyses (Henopp et al., Histopathology 2011)

In 15-20% in the context of established FL or another B-NHL (MCL, CLL, MZL) (Jegalian et al., Blood 2011, Montes-Moreno et al., Histopathology 2010; Pillai et al., Haematologica 2013)

Risk of progression to FL ~6-10% (Jegalian et al., Blood 2011, Pillai et al. Haematologica 2013)

Clinical consequences:
- staging work up to rule out concurrent lymphoma
- if no lymphoma, conservative management
- monitoring B cells with t(14;18) in peripheral blood?

Genetic features:
t(14;18)+, paucity of genetic alterations compared to overt FL (Mamessier et al., Haematologica 2014; Schmidt et al., Leukemia 2014), e.g. EZH2 mutations
t(14;18) positive FL-like B cells and ISFN

Individuals with a frequency of >1 translocation-positive cell in $10^4$ leukocytes (approximately 1 in 500 B cells) have a 23-fold increased risk of developing follicular lymphoma over a period of 15 years (Roulland et al. JCO 2014)

These rare lesions have already accumulated several genetic alterations underway to follicular lymphoma and obviously may have little to do with the circulating t(14;18) cells that are present in so many normal adults (Mamessier et al., Haematologica 2014; Schmidt et al., Leukemia 2014)
t(14;18) Positive FL-like B Cells and ISFN

Nonproliferating t(14;18)+ centrocytes accumulate in the light zone of normal germinal centers

- t(14;18)
- Memory t(14;18)+ B cells in lymph nodes & blood
- Follicular lymphoma in situ
- Follicular lymphoma

Importance of GC re-entries by t(14;18) rearranged FL-like B cells

*Sungalee et al. JCI 2014*
Differential Diagnosis

- In Situ Follicular Neoplasia (ISFN) Update WHO 2016
- Partial involvement by FL (PFL)
ISFN and PFL
FL in situ / In situ Follicular Neoplasia (ISFN)

Preserved lymph node architecture, open sinuses, intact paracortex. Strong expression of CD10 and BCL2 in slightly atypical follicles. BCL2 positive B cells carry BCL2 translocation.

FL partial involvement:

Architecture partially preserved (reactive follicles!), affected follicles expanded (crowded), attenuated mantle zones, displacement of normal elements
Primary extranodal follicular lymphoma

**Testis (children and adults):**
Rare disease, grade 3 +/- DLBCL as a rule (Lones et al., J Pediatr Hematol Oncol 2012, Bacon et al., Am J Surg Pathol 2007)
Usually no BCL2 expression and no t(14;18)

**Overlap with FL of Pediatric type?**

**Ovary:**
one group usually grade 3, no BCL2 expression, no t(14;18)
one group grade 1/2 with BCL2 expression and t(14;18)
(Ozsan et al., Am J Surg Pathol 2011)

**Thyroid:**
one group mostly grade 1/2, mostly CD10-positive, t(14;18)+
one group mostly grade 3, CD10-negative, no t(14;18)
(Bacon et al. Am J Surg Pathol 2009)
Follicular Lymphoma of the Duodenum

- CD10
- BCL-2
- Ki67
Follicular Lymphoma of the Duodenum

Genetic data

PCR: t(14;18)+ in 12/18
FISH: t(14;18)+ in 7/8
Karyotyping: 4/4 t(14;18) as sole aberration

Vienna data: Courtesy of Dr. Andreas Chott
Bcl2 Negative FL Cases (Using Dako Clone)

• 10-15% of FL 1/2 reported to be BCL2 negative

2 groups of Bcl2 Clone-124 negative FL

- Half of cases: also negative IHC using Bcl2 clones E17 and SP66
  • No t(14;18), no Bcl2 mutation,
- Other half of cases: Bcl2 IHC+ using clones E17 and SP66
  • t(14;18)+, missense Bcl2 mutation

Xerri et al. Virchows Arch 2015

Burkhart et al, Hematol Oncol 2014
Adam et al, Hum Pathol 2013
Hoeller et al, Hum Pathol 2012
ISFN and FL BCL2 pseudonegative in the same lymph node (LYWS 0127)

Xerri et al. Virchows Arch 2015
FL, pediatric type

Extended serpiginous GC
No polarization
BCL2 negative
Ki67 high
t(14;18) negative
Must be clonal!
FL with IRF4/MUM1

- Higher level of expression associated with BCL2 negative FL 1/2 and FL grade 3
- Some pediatric/young adult cases with IRF4 translocation (Salaverria et al. Blood 2011, Quintanilla-Martinez et al. Virchows Arch 2015)
  - LYWS 44: MUM1+ FL3B and DLBCL of the tonsil
- Low/moderate expression in FL 1/2
  - LYWS 63: MUM1+ FL1/2 with GC markers
  - LYWS 237: FL not gradable; low PI
LYWS0272 - T. Molina –
5 year old girl – tonsillectomy specimen

Quintanilla-Martinez et al. Virchows Arch 2015
50% of FL in the tonsil of children and young adults harbor translocations involving IRF4/MUM1

- Often with diffuse component

Quintanilla-Martinez et al. Virchows Arch 2015
A Big Topic: Follicular Lymphoma with Uncommon Morphologies (and Immunophenotypes)
How to deal with “blastoid” FL?

Diffuse blastoid variant of t(14;18)-negative FL:
CD10+, BCL6+, BCL2+
No t(14;18), +18, others
Structural abnormalities of BCL6
High Ki-67 (>80%)

Chiu et al., Mod Pathol 2009
Panel Dx: FL, not gradable, with blastoidoid features
**FL with Unusual Morphology (and Phenotype)**

- FL with small cells and strong Ki67 positivity
  - LYWS 193: High PI and mitotic count associated with small centrocyte-like cells
  - LYWS 246

Xerri et al. Virchows Arch 2015

Panel Diagnosis: FL 1/2 with high PI
Transformed FL

of DLBCL type

of BCLU type

„Double Hit“

Rare: Lymphoblastic Type (TdT positive) Geyer et al. 2015

Xerri et al. Virchows Arch 2015
Rare Transformation Type: Histiocytic/dendritic cell neoplasms

- Most common lymphoma types:
  - CLL
  - Follicular lymphoma
  - Lymphoblastic neoplasm

- Most common histiocytic/dendritic cell neoplasms:
  - Histiocytic sarcoma
  - Dendritic cell tumors, including Langerhans cell histiocytosis

Clonally related! t(14;18) positive
Follicular lymphoma transformation into histiocytic sarcoma: indications for a common neoplastic progenitor.

Case 71: A CD163 positive histiocytic sarcoma arising after several FL relapses and clonally identical to the primary FL.
Mutated B-cell or multipotent progenitor cell → B-cell lymphoma → Histiocytic neoplasm

Transdifferentiation? New line of differentiation? Dedifferentiation?

Courtesy of J. Chan and St. Dirnhofer
Prognostication
Analysis of Somatic Mutations in a GLSG FL Cohort

Pastore et al. Lancet Oncol 2015
Clinicogenetic Risk Model: “m7-FLIPI“

Pastore et al. Lancet Oncol 2015
FLIPI and m7-FLIPI

Pastore et al. Lancet Oncol 2015
FLIPI and m7-FLIPI

**GLSG training cohort**
- FLIPI: 77 high risk, 34 non-high risk
- m7-FLIPI: 43 high risk, 108 non-high risk

**BCCA validation cohort**
- FLIPI: 53 high risk, 29 non-high risk
- m7-FLIPI: 24 high risk, 83 non-high risk

*Pastore et al. Lancet Oncol 2015*
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All participants of the Workshop who – again – taught us how fascinating Hematopathology can be!
Call for Abstracts

**Lymphoma & Bone Marrow Symposia**

The Symposia will be devoted to “What causes aggressive behavior”, with topics on:
- Genetic changes
- Micro-environment interactions
- Deregulation of signalling pathways

Abstracts on these subjects are welcome to be submitted. In a separate session on “Recent Advances”, new and ground-breaking developments in lymphoma research will be discussed.

**Important Dates**

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<tr>
<th>Event</th>
<th>Date</th>
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<tbody>
<tr>
<td>Submission starts</td>
<td>December 1, 2015</td>
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<tr>
<td>Submission deadline</td>
<td>April 15, 2016</td>
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<td>Notification of acceptance</td>
<td>June 3, 2016</td>
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