Molecular genetics of follicular lymphoma

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I have the following financial relationships to disclose:

- Advisory board for: BeiGene

- Grant/Research support from: BeiGene, Gilead Sciences

- AND -

- I will discuss the following off label use and/or investigational use in my presentation: Tazemetostat
Follicular Lymphoma (FL) – clinical challenges

What is ‘high risk’ FL?

- Relapsing, remitting
- High risk FL: early progression, transformed FL
- Lymphoma: leading cause of death
  - ‘Incurable’: QoL

Spanish-Lymphoma Oncology Registry
Newly diagnosed FL

Provencio et al, Plos One 2017

Cause of Death in Follicular Lymphoma in the First Decade of the Rituximab Era: A Pooled Analysis of French and US Cohorts

Sarkozy et al, JCO 2019

Pooled French and US cohorts = 1654 patients

CAUSES OF DEATH

- Lymphoma progression 27%
- Transformation 30%
- Treatment related 17%
- Other malignancy 13%
- Other Causes 13%

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Barts patients: Contrasting clinical courses

Patient 1

Relapsing-remitting

Early progression

Patient 2
Model of FL tumorigenesis and evolution

85-90% of FL have the t(14;18)

First genetic hit: t(14;18) BCL2 over-expression

Normal immature B cell

‘primed’ malignant cell

Peripheral blood

Additional (epi) genetic hits

FLLC: follicular lymphoma like cells (Roulland et al, JCO 2014; Sungalee et al, JCI 2014)

ISFN: in-situ follicular neoplasia (Cong et al, Blood 2002)
Technologies: From DNA to Genome

- **CYTOGENETICS**
- **SANGER SEQUENCING**
- **FISH**
- **SNP/CGH arrays**
- **NGS**

- Single cell, AI, ML
- Multiple genes
  - Translocations
  - Copy number

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Dissecting FL genetics in the NGS era

Interpatient

Intrapatient

Longitudinal

Spatial

XX years
Epigenetic deregulation appears fundamental
(90% of FL cases have mutations)

Genetic catalogue of FL tumours – not t(14;18)-centric

GENES FREQUENTLY MUTATED

BIOLOGICAL PATHWAYS

Epigenetic modifiers >90%
NFkB signalling >15%
JAK-STAT Signalling 20%
mTOR signalling >30%

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An epigenetic addiction – why?

- **KMT2D**
  - 60-80%
  - LOSS OF FUNCTION
  - KMT2D
  - BCL6–SMRT–HDAC3

- **CREBBP**
  - 60-70%
  - LOSS OF FUNCTION
  - CREBBP
  - EP300

- **EZH2**
  - 20-25%
  - GAIN OF FUNCTION
  - EZH2

**Lymphoma onset in combination with BCL2**

↓MHC class I and II – immune evasion

↑ GC formation; proliferation

Impairs terminal differentiation of B cells

Adapted from Huet et al, *Nat Rev Cancer* 2018


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Genetic alterations in metabolic pathways – a new player?

RRAGC is recurrently mutated in FL

Okosun et al, Nature Genetics 2016

Also: Ying et al, Clin Cancer Res 2016

N = 142 FL patients
mTORC1 - a critical pathway for follicular lymphoma

RRAGC and v-ATPase genes mutated in about a third of FL patients
Okosun et al, Nature Genetics 2016

Activate mTORC1 signalling

Sestrin1 lost in about 20% of FL patients
Oricchio et al, Sci Transl Med 2017

Enhanced B cell activation
Decreased T cell help
Ortega-Molina et al, Nature Metabolism 2019

Role for mTOR inhibitors?
Targeting metabolic programs?

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Genetics driving a tumour-supportive microenvironment

>30% of FL
Inactivating mutations
Activates BCR signalling
Recruits T follicular helper cells

TNFRSF14 (HVEM)

~5-6% of FL
40% protein overexpression
Promotes CD4 recruitment & restricts CD8 infiltration
Supports B-Tfh communication

Boice et al, Cell 2016

Dheilly et al, Cancer Cell 2020
Bararia et al, Cell Reports 2020

CTSS (Cathepsin S)
Distinguishing features of other FL variants

In situ follicular neoplasia (ISFN)

- BCL2+ B cells in GC
- Pre-malignant event
- **Genetic events = classical FL**

### t(14;18)-negative FL

- Late GC phenotype/NF-kB
- Majority express BCL2
- **Genetic events = classical FL**

### Duodenal-type FL

- BCL2/BCL6 rearrangements
- **KMT2D** mutations <25%
- **Chronic inflammation signature**

**ISFN:** Schmidt et al, *Leukemia* 2014; Mammesier et al, *Haematologica* 2014

**t(14;18)-neg FL:** Leich et al, 2011, 2015; Zamo et al, *Leukemia* 2018


**Duodenal FL:** Hellmuth et al, *Blood* 2018

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Temporal heterogeneity and the evidence for a reservoir population - ‘CPC’

Progression occurs via divergent evolution

Reservoir population (CPC)

Okosun et al, Nature Genetics 2014

Also: Green et al, PNAS 2015
Pasqualucci et al, Cell Rep 2014
Kridel et al, Plos Medicine 2016
CREBBP and KMT2D mutations are early events in FL

Early initiating – epigenetic modifiers

Okosun et al, Nature Genetics 2014

Kridel et al, Plos Medicine 2016
No simple genetic explanation for progression and transformation

Transformation clone undetectable at diagnoses

Cancer repopulating reservoir (CPC)

FL diagnosis

Transformation

FL clones

tFL clones

Adapted from Kridel et al, Plos Med 2016

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Spatial heterogeneity: Different targets at different sites

Araf et al, Leukemia 2018

EZH2 in LN but NOT in BM
Increased spatial heterogeneity with progression

Araf et al, Leukemia 2018
Evidence for a reservoir population: **donor-derived FL**

**Donor-derived FL after stem cell transplantation**

**Case 1**
- D → FL
  - 3 yrs
- R → FL
  - 11 yrs


**Case 2**
- D → FL
  - 9 yrs
- R → FL
  - 10 yrs

Weigert et al, *Cancer Discovery* 2012

**Overt FL from a pre-existing precursor**
- Identical t(14;18)
- Shared IGH V(D)J rearrangements
- Shared mutations
Why are we not curing FL?

- Early initiating events
- Dominant clone
- Minor clone
- ‘Progressor’ or late events

Cancer repopulating reservoir (CPC)

- Co-operating mutations
- Must be drug resistant
- Must have renewal capacity or ‘stemness’

FL

t(14;18)
Will any of this knowledge be useful for patients?
How to utilise the molecular information?

Tailoring treatment
- Lenalidomide
- Obinutuzumab
- Ibrutinib
- R-CHOP
- Autograft
- Tazemetostat
- Car-T
- Bispecifics antibodies
- Checkpoint inhibitors
- PI3K inhibitors
- R-Benda

Progression/Survival
- Prognostic biomarker
- Predictive biomarker

Real-time monitoring
- Dynamic biomarker
- ctDNA
- MRD

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Gene mutations can predict response: EZH2 inhibition

<table>
<thead>
<tr>
<th>Best Response</th>
<th>FL EZH2 MT (n=45)</th>
<th>FL EZH2 WT (n=54)</th>
</tr>
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<tbody>
<tr>
<td><strong>Objective Response Rate (CR + PR)</strong></td>
<td>20 (71%)</td>
<td>18 (33%)</td>
</tr>
<tr>
<td>Complete Response (CR)</td>
<td>4 (9%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Partial Response (PR)</td>
<td>31 (69%)</td>
<td>15 (28%)</td>
</tr>
<tr>
<td>Stable Disease</td>
<td>10 (22%)</td>
<td>16 (30%)</td>
</tr>
<tr>
<td>SD study drug ongoing</td>
<td>6 (25.7%)</td>
<td>1 (5.6%)</td>
</tr>
<tr>
<td>Progressive Disease</td>
<td>0</td>
<td>17 (31%)</td>
</tr>
<tr>
<td>Duration of response, median months</td>
<td>8.3 months</td>
<td>14.7 months</td>
</tr>
<tr>
<td>Progression Free Survival, median months</td>
<td>13.8 months</td>
<td>5.6 months</td>
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Tazemetostat, an oral EZH2 inhibitor

- Phase 2 multi-centre study in relapsed/refractory FL
- Stratified by EZH2 mutation status

Morschhauser et al, ASH 2019

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Incorporating tumour genotype into prognostic tools: m7-FLIPI

- Pre-treatment risk model: Integrating 7 genes + PS + FLIPI score

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<thead>
<tr>
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<th>5 yr FFS</th>
<th>5 yr OS</th>
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<tbody>
<tr>
<td>High risk m7-FLIPI</td>
<td>38.29%</td>
<td>65.25%</td>
</tr>
<tr>
<td>Low risk m7-FLIPI</td>
<td>77.21%</td>
<td>89.98%</td>
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Challenges in exploring lymphoma biology: ‘Tissue is the Issue’

<table>
<thead>
<tr>
<th>Fresh Frozen (FF)</th>
<th>Formalin-fixed (FFPE)</th>
<th>Core Biopsy</th>
</tr>
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<tbody>
<tr>
<td>GOLD-STANDARD</td>
<td>QUALITY</td>
<td>QUANTITY</td>
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Is liquid biopsy the alternative?

Cell free DNA (cfDNA) – circulating tumour DNA (ctDNA)

**Advantages:**
- Quick
- Minimally invasive
- Easily obtained
FL mutations can be tracked in liquid biopsies

Different clones at different sites
Role for liquid biopsy
Araf et al, Leukemia 2018; Unpublished

Can ctDNA serve as dynamic surveillance tools?

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Are we just at the tip of the iceberg?
Beyond 2020 – what are the translational priorities

Clinical heterogeneity ↔ biological heterogeneity

Better therapies (subset of patients)
Biomarkers of response, resistance and prognosis

Targeting the CPC
But......more knowledge needed
Conclusions: Breaking the bottlenecks to progress in FL

Epigenetic deregulation is a key genetic feature of FL

FL is **NOT** a single disease

![Diagnosis](diagnosis.png) → ![Relapse](relapse.png)  
Spatial and temporal **heterogeneity**

A single biopsy will not capture this heterogeneity

Is this the **Achilles’ heel** of FL?

Maximise clinical trials

Can we improve **prognostic and predictive markers** and move to biology-based therapy?

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