Molecular Genetics of Small Mature B-cell Lymphomas

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Genetic Drivers in Lymphomagenesis

Clonal Population

Primary Genetic Alterations

Overt Lymphoid Neoplasia

Secondary Genetic Alterations

Progression/Transformation

Microenvironment

Genetic alterations
The mutational profile is a dynamic process
Impact of NGS in Small B-cell Neoplasms

- Disease specific profiles
- Understanding evolution of the disease
- Prognostic groups and risk stratification
- Orient management strategies
Driver mutated Genes and CNA in CLL
ICGC & Danna-Farber (DFCI)

Driver genes (n=80)

ICGC

DFCI

36

23

21

Driver mutated Genes in CLL genomes (ICGC vs Danna Farber (DFCI))

Population based vs Clinical trial

Median age DFCI cohort 10 yr younger than ICGC

DFCI/Broad (n=123) Only pretreatment cases considered
CLL8 (n=278): Clinical trial (Landau et al. Nature 2015)
Mutated Pathways in CLL
Genomic Heterogeneity and Driverless in CLL

Puente et al Nature 2015

452 CLL & 54 MBL (150 whole genomes)
13.631 mut in coding genes: 27 per tumor
951 CNA (2 per tumor)
59 driver genes
NOTCH1 Mutations in CLL


Stilgenbauer S et al. Blood 2014;123:3247-3254
TLR/MYD88 mutated pathway in CLL
A specific subgroup of patients?

NFκB pathway
MAPK pathways
Inflammatory cytokines and chemokines

Clinical relevance of individual mutated genes in CLL

**NOTCH1**

Puente et al. 2011, Nature

Young et al. 2017, Leukemia

**EGR2**

Quesada et al. 2011, Nat Genetics

**SF3B1**

Herling et al. 2016, Blood

**RPS15**

**NFKBIE**

Ljungström et al. 2015, Blood

Mansouiri et al. 2015, JEM
Co-occurrence of driver alterations in CLL

Nadeu F et al  Leukemia 2017  (In press)
Prognostic impact of number of mutated drivers and subclonal alterations in CLL

Number of drivers

Clonal/subclonal heterogeneity

Small TP53 variant subclone mixed with TP53 wt clones

Removal of TP53 wt clones and selection of the TP53 variant subclone

Expansion of the TP53 variant clone

Rosi D et al Blood 2013
Small mutated subclones are identified in virtually all genes

- 406 untreated patients
- 28 CLL driver genes
- Mean coverage 1500x NGS
- Sensitive pipeline 0.1% allelic frequency

Nadeu F et al Leukemia 2017 (In press)
Clinical Impact of clonal and subclonal mutations in CLL

Nadeu F et al Blood 2016; Leukemia 2017 (In press)
Ibrutinib-resistant mutations may be detected before clinical relapse

Detection of mutated subclones may precede treatment
Median 8-9 months (3-15 months)

Arnason & Brown Curr Oncol Report 2017
Somatic Mutations in Lymphoplasmacytic Lymphoma

MYD88 L265P
- 95% WM/LPL
- 29% DLBCL-ABC
- 6% MZL
- 3% CLL

CXCR4
- 25-35% WM/LPL
- Associated with MYD88
- More active disease
- Less lymphadenopathy
- More resistant disease to new drugs

BTK

Useful information in the differential diagnosis of LPL
Need to be interpreted in the global context of the disease

Patients treated with Ibrutinib
Mutations before clinical progression

When should *MYD88* L265P mutations be studied in small B-cell neoplasms?

- Differential diagnosis of LPL strongly considered but findings not conclusive
- Differential diagnosis between LPL and IgM PCM
- If LPL is not in the differential diagnosis *MYD88* mutations may not be useful

*Swerdlow SH et al Virchows Arch. 2016;468(3):259-75*
Somatic Mutations in HCL and HCLv

Hairy Cell Leukemia

- 50% HCLv
- 50% HCL IgH V4-34
- 50% Pediatric Type FL

Vemurafenib Resistance

- NF1/NF2 downregulation
- KRAS mutation

MAP2K1

- 50% HCLv
- 50% HCL IgH V4-34
- 50% Pediatric Type FL

CCND3, U2AF1

7q deletion

Biological effects of somatic mutations in HCL and HCLv.

References:
Follicular lymphoma Mutational Landscape


Krysiak K et al Blood 2017; 129: 473-483
Early and Late Mutations in Follicular Lymphoma

Early driver mutations in chromatin regulator genes (CREBBP, EZH2 and KMT2D (MLL2)),

Gained at transformation: EBF1 and regulators of NF-κB signaling (MYD88 and TNFAIP3)

Clinical Impact of FL Mutations

**EZH2 inhibitor** (Tazemetostat) in Refractory/Relapsed FL

- OR: 63% in patients with EZH2 mutations ($N = 8$)
- 28% in with wild type EZH2 ($N = 46$)

*Pastore et al Lancet Oncol. 2015 Sep;16(9):1011-1012*

*Morschhauser F et al Hematol Oncol 2017; 35, S2: 24–25*
NOTCH1/2 Mutations in FL

7/112 FL cases (6.3%)

- 5 NOTCH1
- 2 NOTCH2
- NOTCH3/4 (4%)

Female
Splenic/extranodal involvement
Negative for t(14;18)
DLBCL component

Karube K et al J Pathol 2014; 234:423-30
Krysiak K et al Blood 2017; 129: 473-483
# Pediatric Type Follicular Lymphoma

<table>
<thead>
<tr>
<th>Genes</th>
<th>PTFL (n=71) (%)</th>
<th>t(14;18)-neg FL (%)</th>
<th>t(14;18)-pos FL (%)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>TNFRSF14</td>
<td>33-51</td>
<td>36</td>
<td>18-46</td>
<td>Ns</td>
</tr>
<tr>
<td>KMT2D</td>
<td>14-16</td>
<td>36</td>
<td>67-82</td>
<td>Ns</td>
</tr>
<tr>
<td>CREBBP</td>
<td>3*</td>
<td>45</td>
<td>33-64</td>
<td>0.001</td>
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<tr>
<td>FOXO1</td>
<td>5</td>
<td>27</td>
<td>-</td>
<td>Ns</td>
</tr>
<tr>
<td>GNA13</td>
<td>11</td>
<td>0</td>
<td>-</td>
<td>Ns</td>
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<tr>
<td>EZH2</td>
<td>3*</td>
<td>18</td>
<td>7-20</td>
<td>0.0049</td>
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* >18yr

<table>
<thead>
<tr>
<th>Genes</th>
<th>PTFL</th>
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<tbody>
<tr>
<td>MAP2K1</td>
<td>38-49%</td>
</tr>
<tr>
<td>MAPK1</td>
<td>10%</td>
</tr>
<tr>
<td>IRF8</td>
<td>15-50%</td>
</tr>
</tbody>
</table>

Mutational Profile of Nodal and Splenic Marginal Zone Lymphoma

Targeted Pathways

- KLF2, NOTCH2, activation (40-25%)
- NFkB activation (36-51%)
- BCR signaling (SMZL 8%)
- PTPRD (NMZL 20%)

Clinical Impact of Somatic Mutations in SMZL

Parry et al Clin Cancer Res 2015

Campos-Martin Y et al Haematologica 2017
Molecular Pathogenesis and Clinical Subtypes of MCL
Mantle cell lymphoma
**CCND1-negative variant**

<table>
<thead>
<tr>
<th>Rearrangements</th>
<th>No. (%)</th>
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</thead>
<tbody>
<tr>
<td><strong>CCND2</strong></td>
<td>22 (55%)</td>
</tr>
<tr>
<td><strong>CCND3</strong></td>
<td>0</td>
</tr>
<tr>
<td><em>No Cyclin D gene translocation</em></td>
<td>18 (45%)</td>
</tr>
</tbody>
</table>

Mozos et al Haematologica 2009; Salaverria et al Blood 2013
Cryptic IG-CCND1/2/3 rearrangements in MCL

Bea S et al unpublished
Recurrent gene mutations in MCL

Bea et al. & unpubl (n=170)
Greiner et al (n=92)
Zhang et al (n=56)
Rahal et al (n=165)
Kridel et al (n=108)
Meissner et al (n=102)
Rossi et al (n=151)
Wu et al (n=13)
Somatic Mutations in MCL

MCL primary tumors
(n=29)

- **SOX11 +**
- **SOX11 -**
- **IGHV-mut**
- **IGHV-unmut**

**SOX11 +**
- ATM (55%)
- Chromatin (10%)
  - NOTCH1/2 (5%)
  - TP53 (23%)
  - BIRC3 (7%)
  - High CNA

**SOX11 -**
- TLR2 (29%)
  - NOTCH1/2 (5%)
  - TP53 (23%)
  - BIRC3 (14%)
  - Low CNA

Bea S et al PNAS 2013
Prognostic impact of 17p TP53 aberrations in MCL

SOX11-negative MCL

Royo C et al, Leukemia 2012

SOX11 +

Eskelund CW et al Blood 2017
Clinical Relevance of Mutational Profiles in Lymphoid Neoplasms

- Diagnostic criteria to refine entities
- Identification of subsets of patients
- Prognostic and predictive significance
- Monitoring disease evolution: Dynamic evolution of mutational landscape
- Targets for therapy decisions:
  - Selection of patients
  - Actionable mutations
# Recurrently Mutated Pathways in Small B-cell Neoplasms

<table>
<thead>
<tr>
<th>Pathway</th>
<th>U-CLL</th>
<th>M-CLL</th>
<th>cMCL</th>
<th>nmMCL</th>
<th>FL</th>
<th>PTFL</th>
<th>LPL</th>
<th>MZL</th>
<th>HCL</th>
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<tbody>
<tr>
<td>DNA-damage</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
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<tr>
<td>SF3B1</td>
<td>+</td>
<td>+/-</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOTCH1/2</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td></td>
<td></td>
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<tr>
<td>Chromatin Remodeling</td>
<td></td>
<td></td>
<td>+</td>
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<tr>
<td>BCR-signaling</td>
<td></td>
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<td></td>
<td>+</td>
<td></td>
<td></td>
<td></td>
<td>+/-</td>
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<tr>
<td>NFkB</td>
<td>+</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>+/-</td>
<td></td>
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<tr>
<td>TLR/MYD88</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td></td>
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<tr>
<td>MAPK</td>
<td>+/-</td>
<td>+/-</td>
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</tbody>
</table>

+ indicates that the pathway is recurrently mutated in the specified type of B-cell neoplasm.