The use of microarrays in the diagnostic work-up of CLL

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Prognostic markers in CLL

Clinical markers
- *Clinical stage* (Rai / Binet staging systems)
- *Lymphocyte doubling time* (LDT)
- *Serum markers*

Protein and RNA expression markers
- *ZAP70 and CD38 expression levels*
- *Expression of miRNAs*

Genetic markers
- *IGHV gene mutation status*
- *Chromosomal aberrations*
- *Gene mutations*
Hierarchical model  Döhner et al 2000

**GOOD** ⇔ del 13q > normal > tris 12 > del 11q > del 17p ⇔ **POOR**
Different techniques
Karyotyping
FISH: Recurrent chromosomal aberrations in CLL
(diagnosis/presentation)

<table>
<thead>
<tr>
<th>Aberration</th>
<th>Genes involved</th>
<th>Frequency</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>del(11)(q22-23)</td>
<td><strong>ATM, MLL, FDX, BIRC3</strong></td>
<td>15-20%</td>
<td>intermediate/poor</td>
</tr>
<tr>
<td>trisomy 12</td>
<td>?</td>
<td>10-20%</td>
<td>Intermediate/good</td>
</tr>
<tr>
<td>del(13)(q14)</td>
<td><strong>RB1, DLEU2, miR-15A, miR-16-1</strong></td>
<td>40-50%</td>
<td>good</td>
</tr>
<tr>
<td>del(17)(p13)</td>
<td><strong>TP53</strong></td>
<td>5-7%</td>
<td>poor</td>
</tr>
<tr>
<td>normal FISH</td>
<td></td>
<td>10-20%</td>
<td>intermediate</td>
</tr>
</tbody>
</table>

Döhner et al (2000)  
Zenz et al (2011)
Microarray

Advantages:
- whole genome analysed
- small deletions and duplications detected
- CN-LOH
- Additional (recurrent) chromosome aberrations
- no dividing cells needed

Disadvantages
- no detection of balanced structural abnormalities
Microarray analysis

- Recurrent abnormalities
- 13q14 deletion type I or II
- Other abnormalities ≥ 5Mb
- CN-LOH ≥ 10Mb
- Clinical relevance (literature)
- Guidelines Schoumans et al
  (GENES, CHROMOSOMES & CANCER 2016, 55:480-491)

- Disclaimer
  Resolution microarray 7.4 kB
  Sensitivity 10-15%
Whole Genome
- Loss 11q22-23 (ATM)
- Loss 13q14-region
- Gain 2p25-p14

Agilent 180K oligo Platform
13 Kb median spacing
Small deletions and duplications: del 13q: 0.9 Mb

Agilent 400K oligo Platform
7.4 Kb median spacing
Small deletions and duplications: del 17p: 6,4 kb

Agilent 400K oligo Platform
7,4 Kb median spacing
CLL case  Loss 13q14 - region

Agilent 180K oligo
Platform
13 Kb median spacing
Type I en Type II deletions 13q14-region

Type II deletions (large)
- 20% of CLL cases
- Elevated genomic complexity
- Contribute to CLL disease evolution (genomic destabilization)
  ➢ shorter OS? / TTT?

Type I deletions (small)
- 30% of CLL cases
- Good prognosis

Conclusion
The clinical course of CLL is accelerated in patients with large (type II) 13q14 deletions that (also) span the RB1 gene.
Copy Neutral Loss of Heterozygosity:  
17p, no deletion, mutation P53 present
Additional (recurrent) chromosome aberrations by microarray analysis in CLL

- Gain 2p  (poor)
- Deletion 6q  (intermediate)
- Gain 8q  (poor)

**Progressive disease**

**More advanced stage CLL**

... So far, not really independent prognostic (clinical) value ...

- Other abnormalities
- **Complex array profile**!
Complex karyotype and OS

A. OS according to FISH for all patients
B. OS according to the presence or absence of a complex metaphase karyotype

Thompsom et al 2015
# Overview of cohort

<table>
<thead>
<tr>
<th>N=2423</th>
<th>N, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1504, 75%</td>
</tr>
<tr>
<td>Female</td>
<td>707, 25%</td>
</tr>
<tr>
<td>Median age diagnosis</td>
<td></td>
</tr>
<tr>
<td>&lt;55</td>
<td>379/1595, 24%</td>
</tr>
<tr>
<td>&gt;70</td>
<td>286/1595, 24%</td>
</tr>
<tr>
<td>MBL/SLL</td>
<td></td>
</tr>
<tr>
<td>Binet A</td>
<td>17/1449, 1%</td>
</tr>
<tr>
<td>Binet B</td>
<td>837/1449, 58%</td>
</tr>
<tr>
<td>Binet C</td>
<td>410/1449, 28%</td>
</tr>
<tr>
<td>Binet C</td>
<td>185/1449, 13%</td>
</tr>
<tr>
<td>M-CLL</td>
<td>544/1090, 50%</td>
</tr>
<tr>
<td>TP53abs</td>
<td>167/1359, 12%</td>
</tr>
<tr>
<td>del(11q)(22.3)</td>
<td>406/2386, 17%</td>
</tr>
<tr>
<td>trisomy 12</td>
<td>316/2386, 13%</td>
</tr>
<tr>
<td>del(13q)(14)</td>
<td>1235/2385, 52%</td>
</tr>
</tbody>
</table>

2423

CGH/SNP array results

[Link to ERIC: European Research Initiative on CLL]
Effects recurrent aberrations on survival

C: Survival Functions
- del(11q)
  - not present
  - present
  
P<0.001

D: Survival Functions
- trisomy 12
  
P=0.81

E: Survival Functions
- del(13q)
  
P<0.05

F: Survival Functions
- del(17p)
  
P<0.001
Effects IGHV and p53 mutation status

TP53abs=del(17p)(13.1)
and/or TP53mut
Overview of chromosomal aberrations

GC ≥3abs
24%

non-GC
76%
Overview of chromosomal aberrations

GC=genomic complexity (≥3 chromosomal aberrations)
Dissecting GC

Survival Functions

- non-GC
- GC=3
- GC=4
- GC≥5

P<0.001

Cum Survival vs. Follow up (years)
**TP53mut**

- **Survival Functions**
  - non-GC
  - GC ≥ 3

**TP53wt**

- **Survival Functions**
  - non-GC
  - GC ≥ 3

**P-values**

- **TP53mut**
  - GC ≥ 3: P < 0.01
  - GC ≥ 4: P < 0.01
  - GC ≥ 5: P < 0.001

- **TP53wt**
  - GC ≥ 3: P < 0.05
  - GC ≥ 4: P < 0.01
  - GC ≥ 5: P < 0.001
Multivariable analysis 'low' vs 'high' genomic complexity

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<tr>
<th>N=1570</th>
<th>HR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1.17</td>
<td>0.943-1.452</td>
<td>0.16</td>
</tr>
<tr>
<td>&gt;70</td>
<td>2.633</td>
<td>2.075-3.341</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BinetB/C</td>
<td>1.756</td>
<td>1.424-2.165</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>U-CLL</td>
<td>3.242</td>
<td>2.567-4.095</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TP53abs</td>
<td>1.848</td>
<td>1.351-2.528</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>del(11q)</td>
<td>1.067</td>
<td>0.829-1.375</td>
<td>0.61</td>
</tr>
<tr>
<td>trisomy12</td>
<td>0.871</td>
<td>0.630-1.206</td>
<td>0.41</td>
</tr>
<tr>
<td>del(13q)</td>
<td>1.005</td>
<td>0.812-1.243</td>
<td>0.97</td>
</tr>
<tr>
<td>GC≥3</td>
<td>1.019</td>
<td>0.775-1.341</td>
<td>0.89</td>
</tr>
</tbody>
</table>

≥5 aberrations detected by arrays linked to high-risk disease independent of clinical stage, IGHV and TP53 status

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<tr>
<td>Male</td>
<td>1.165</td>
<td>0.939-1.446</td>
<td>0.17</td>
</tr>
<tr>
<td>&gt;70</td>
<td>2.644</td>
<td>2.085-3.354</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BinetB/C</td>
<td>1.723</td>
<td>1.398-2.123</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>U-CLL</td>
<td>3.219</td>
<td>2.550-4.064</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TP53abs</td>
<td>1.624</td>
<td>1.207-2.184</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>del(11q)</td>
<td>1.008</td>
<td>0.788-1.288</td>
<td>0.95</td>
</tr>
<tr>
<td>trisomy12</td>
<td>0.899</td>
<td>0.650-1.242</td>
<td>0.52</td>
</tr>
<tr>
<td>del(13q)</td>
<td>0.978</td>
<td>0.793-1.206</td>
<td>0.83</td>
</tr>
<tr>
<td>GC≥5</td>
<td>1.672</td>
<td>1.177-2.377</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

GC ≥3 chromosomal aberrations

GC ≥5 chromosomal aberrations
Conclusions

1. Microarray in the diagnostic work-up of CLL

✓ Microarray can pick up the recurrent aberrations found in CLL
✓ Genomic complexity can also be defined by microarray

2. Effect genomic complexity (GC) in CLL

✓ Genomic complexity defined by array is a prognostic factor in CLL
✓ The presence of ≥5abs rather than >3abs is associated with the worst clinical outcome
✓ Genomic complexity is associated with worse clinical outcome even amongst cases harboring TP53abs
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Array procedure

DNA labeling
- patient DNA with Cy3
- reference DNA with Cy5

1:1 pooled hybridization on microarray slide

180,000 oligos
Array procedure  \( \log_2 \) ratios

Ratio patient / reference
1 / 2 = 0.5 \( \rightarrow \) deletion (loss)

Ratio patient / reference
2 / 2 = 1 \( \rightarrow \) normal

Ratio patient / reference
3 / 2 = 1.5 \( \rightarrow \) duplication (gain)

Normal \( \log_2 (2/2) = \log_2 (1) = 0 \)

Loss \( \log_2 (1/2) = \log_2 (0.5) = -1 \)

Gain \( \log_2 (3/2) = \log_2 (1.5) = 0.58 \)