Complex karyotype in CLL

Arnon Kater, Panagiotis Baliakas, Alexander Leeksma MD
On behalf of the ERIC “complex karyotype project”
Introduction

Accepted diagnostic workup CLL prior to treatment

- FISH 13q, tris 12, 11q and 17p; TP53 mutation according to ERIC guidelines

- Since 2006 data individual groups of predictive value of complex karyotype (CK), never integrated in guidelines

- New interest in CK in era of novel agents (Thompson, Cancer 2015)
Questions

• How to define CK?
• Predictive value of CK?
• Differences in methodology?
• Prospective analysis?

→ ERIC wide registry
→ Group of dedicated researches
→ First meeting at MLL Munich fall 2016

→ Grant Janssen
Overview

• Short summary results complex karyotype (CK) classical cytogenetics study (on behalf of Panagiotis Baliakas/Kostas Stamatopoulos)

• Preliminary results CK CGH array study
## Overview of the cohort

<table>
<thead>
<tr>
<th>N=3580</th>
<th>N, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2252, 63%</td>
</tr>
<tr>
<td>Female</td>
<td>1328, 37%</td>
</tr>
<tr>
<td>Median age diagnosis</td>
<td>65.6 years</td>
</tr>
<tr>
<td>&lt;55</td>
<td>698/3560, 20%</td>
</tr>
<tr>
<td>&gt;70</td>
<td>1184/3560, 33%</td>
</tr>
<tr>
<td>MBL</td>
<td>258/2863, 9%</td>
</tr>
<tr>
<td>Binet A</td>
<td>2098/2893, 73%</td>
</tr>
<tr>
<td>Binet B</td>
<td>357/2893, 12%</td>
</tr>
<tr>
<td>Binet C</td>
<td>258/2893, 9%</td>
</tr>
<tr>
<td>M-CLL</td>
<td>1222/2051, 60%</td>
</tr>
<tr>
<td>TP53abs</td>
<td>299/3308, 9%</td>
</tr>
<tr>
<td>del(11q)</td>
<td>377/3256, 12%</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>507/3260, 16%</td>
</tr>
<tr>
<td>del(13q)</td>
<td>1769/3271, 54%</td>
</tr>
<tr>
<td>Treated (median FU: 4.6 years)</td>
<td>1413/3393, 42%</td>
</tr>
</tbody>
</table>

3656

3580 ≥10 metaphases
Method and timing of testing

- CPG/IL2: n=379, 11%
- TPA: n=1846, 52%
- TPA, CPG+IL2: n=1355, 37%

Tested within 6 months from diagnosis
N=2684/3549, 76%

Tested within 1 year from diagnosis
N=2814/3549, 79%

Tested before treatment (treated cases)
N=1238/1406, n=88%
Overview of chromosomal aberrations

- 3580
  - 3179 (89%) Non-CK
  - 401 (11%) CK
  - 2032 (64%) Normal/del(13q)
  - 741 (23%) 1 aberration [non-del(13q)]
  - 406 (13%) 2 aberrations

No difference between cell stimulation protocols
## CK (≥3 aberrations) vs non CK

<table>
<thead>
<tr>
<th></th>
<th>CK</th>
<th>Non CK</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td>275/401, 69%</td>
<td>1977/3179, 63%</td>
<td>0.012</td>
</tr>
<tr>
<td><strong>&lt;55 years</strong></td>
<td>81/397, 20%</td>
<td>617/3163, 20%</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>&gt;70 years</strong></td>
<td>144/397, 36%</td>
<td>1040/3163, 33%</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>MBL</strong></td>
<td>13/295, 4%</td>
<td>245/2568, 10%</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Binet A</strong></td>
<td>186/295, 63%</td>
<td>1912/2568, 74%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Binet B</strong></td>
<td>66/295, 22%</td>
<td>291/2568, 11%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Binet C</strong></td>
<td>30/295, 10%</td>
<td>120/2568, 5%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>M-CLL</strong></td>
<td>98/254, 38%</td>
<td>1124/1797, 63%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>TP53abs</strong></td>
<td>117/381, 31%</td>
<td>182/2927, 6%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>del(11q)</strong></td>
<td>106/369¹, 29%</td>
<td>271/2887, 9%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Trisomy 12</strong></td>
<td>98/368², 27%</td>
<td>409/2892, 14%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>del(13q)</strong></td>
<td>237/391³, 60%</td>
<td>1532/2900, 53%</td>
<td>0.003</td>
</tr>
</tbody>
</table>

1: 14/105, 13% TP53abs, 2: 17/97 TP53abs, 39/97: +12, +19, 3: 71/235 (30%) negative for TP53abs, del(11q), +12
Selected cases karyotyped before treatment

p=0.0001
CK and TP53mut

Include condition: v34=1

Multivariate analysis
- CK
- Age
- Mutation
- TP53
3abs vs 4abs vs ≥5abs

% Alive vs Time

- CK, 3 abs, n=195
- CK, 4abs, n=80
- CK, 5abs, n=99

p=0.00001
Are all CK equal?
Preliminary conclusions
Classical cytogenetics
(retrospective analysis)

• Different mitogens homogeneous results

• CK independent risk factor but:
  • different subgroups can be found (e.g. +12,+19)
  • exact definition of CK to be determined

Submitted to IWCLL on behalf of ERIC
Overview

• short summary results complex karyotype (CK) classical cytogenetics study *(on behalf of Panagiotis Baliakas/Kostas Stamatopoulous)*

• preliminary results CK CGH array study
Preliminary results CK CGH array study (first analysis)

• ERIC survey: 25 centers will provide data (different sizes)

• Very preliminary results of first 9 centers

• Methods:
  
  – For del13q, del11q, del17p: no cut-off

  – All other aberrations: cut-off of >5MB (Schoumans diagnostic guidelines)

  1. effect complex karyotype (CK) on survival

  2. effect different aberrations on survival
# Overview of the CGH array cohort

<table>
<thead>
<tr>
<th></th>
<th>N=1322</th>
<th>N, %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td>892</td>
<td>67,5%</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>427</td>
<td>32,3%</td>
</tr>
<tr>
<td><strong>Median age diagnosis</strong></td>
<td>63 years</td>
<td>63 years</td>
</tr>
<tr>
<td>&lt;55</td>
<td>146</td>
<td>22%</td>
</tr>
<tr>
<td>&gt;70</td>
<td>168</td>
<td>25%</td>
</tr>
<tr>
<td><strong>MBL</strong></td>
<td>13</td>
<td>2%</td>
</tr>
<tr>
<td>Binet A</td>
<td>432</td>
<td>73%</td>
</tr>
<tr>
<td>Binet B</td>
<td>102</td>
<td>17%</td>
</tr>
<tr>
<td>Binet C</td>
<td>47</td>
<td>8%</td>
</tr>
<tr>
<td><strong>M-CLL</strong></td>
<td>311</td>
<td>56%</td>
</tr>
<tr>
<td><strong>TP53abs</strong></td>
<td>55</td>
<td>9%</td>
</tr>
<tr>
<td>del(11q)</td>
<td>182</td>
<td>14%</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>174</td>
<td>13%</td>
</tr>
<tr>
<td>del(13q)</td>
<td>584</td>
<td>45%</td>
</tr>
</tbody>
</table>
Overview of chromosomal aberrations

- 1322
  - 1148 (87%) Non-CK
  - 174 (13%) CK
  - 405 (31%) Normal
  - 552 (42%) 1 aberration
  - 191 (14%) 2 aberrations
Overview effect recurrent aberrations

**del13q**
- % Alive
- Time (years)
- not present vs present
- p<0.0001

**tris12**
- % Alive
- Time (years)
- not present vs present
- NS

**del11q**
- % Alive
- Time (years)
- not present vs present
- p<0.01

**del17p**
- % Alive
- Time (years)
- not present vs present
- p<0.0001
Overview effect other aberrations

trisomy18

trisomy19

dup2p

del6q

dup8q

% Alive

Time (years)

p<0.05

p<0.0001

NS

p<0.05

not present

present

not present

present

not present

present

not present

present

not present

present

not present

present
Dissecting CK

- non-CK: 87%
- CK 3abs: 6%
- CK 4abs: 3%
- CK≥ 5: 4%
Dissecting CK

% Alive

Time

non-CK
CK

p<0.0001
Dissecting CK

CK subdivided

- blue: non-CK
- orange: 3abs
- red: 4abs
- black: 5 or >5abs

non-CK vs CK 5abs p<0.0001
3abs vs 4abs NS
4abs vs 5abs NS
Effect *TP53* mutation

![Graph showing the effect of TP53 mutation on survival over time. The graph compares two groups: non-CK (blue line) and CK (red line). The x-axis represents time in years, ranging from 0 to 20, and the y-axis represents the percentage of survival, ranging from 0 to 100. The graph shows a significant difference between the two groups, with the non-CK group having a higher percentage of survival than the CK group. The p-value is p<0.01, indicating a statistically significant difference.]

*Note: This description is based on interpreting the visual data presented in the graph.*
Preliminary conclusions

Array-based

(work in progress)

• Complex karyotype is a prognostic factor in CLL
• The presence of $\geq 5$ abs rather than $>3$ abs is associated with the worst clinical outcome
• Genomic complexity is associated with worse clinical outcome even amongst cases harboring $TP53$ mut
Acknowledgements

ERIC CK working group

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Blanca Espinet, Barcelona, Spain
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Sarka Pospisilova, Brno, Czech Republic
Karla Pevlova, Brno, Czech Republic
Kaja Malinka, Brno, Czech Republic
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Hidde Posthuma, Amsterdam, the Netherlands
Clemens Mellink, Amsterdam, the Netherlands
Arnon Kater, Amsterdam, the Netherlands

(If you are not on board and want to be part of this large initiative to address the impact of genomic complexity in CLL please send an email to a.c.leeksma@amc.uva.nl)
CK and IGHV status (classical cytogenetics)

Include condition: v24=1

- M-CLL/CK, n=96
- M-CLL/non CK, n=1118

p=0.054
CK and IGHV status (classical cytogenetics)
CK and IGHV status (CGH array)
Dissecting CK (CGH array)

IGHV UM

% Alive

Time

non-CK

CK

NS

 european research initiative on CLL
CK and IGHV status (classical cytogenetics)

Include condition: v24=0

- U-CLL/CK, n=142
- U-CLL/nonCK, n=605

p=0.11

% Untreated vs. Time
Are all TP53mut equal?
Are all TP53mut equal?
The Process of Array CGH

1. Patient and control DNA labeled with fluorescent dyes are applied to the microarray.

2. Patient and control DNA are hybridized to the microarray.

3. The fluorescent signals are measured by the microarray scanner.

4. The data is then analyzed by computer software which generates a plot.

DNA DOSAGE

DNA GAIN DNA LOSS NO CHANGE
Figure 3

Agilent gDNA labeling kit

DNA-Cy3

DNA-Cy5

Agilent CGH Microarrays

X: log2 Ratios

gain

loss

CGH Analytics software
Dissecting CK

CK (13q not incl)

% Alive

non-CK
CK

p<0.0001
Effect of IGHV

% Alive

Time

p<0.01

IGHVmut

IGHV UM
Effect TP53 mutation

TP53mut

% Alive

Time

p<0.0001

not present

present
Effect TP53 mutation

TP53

% Alive

CK TP53wt

CK TP53mut

p<0.0001

Time (years)

p<0.0001

ERIC
european research initiative on CLL
# CGH array vs FISH recurrent aberrations

<table>
<thead>
<tr>
<th>N=256</th>
<th>N, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>177, 69%</td>
</tr>
<tr>
<td>Female</td>
<td>79, 31%</td>
</tr>
<tr>
<td>Median age diagnosis</td>
<td>65 years</td>
</tr>
<tr>
<td>&lt;55</td>
<td>43/248, 17%</td>
</tr>
<tr>
<td>&gt;70</td>
<td>86/248, 35%</td>
</tr>
<tr>
<td>MBL</td>
<td>13/209, 6%</td>
</tr>
<tr>
<td>Binet A</td>
<td>146/209, 70%</td>
</tr>
<tr>
<td>Binet B</td>
<td>32/209, 15%</td>
</tr>
<tr>
<td>Binet C</td>
<td>18/209, 9%</td>
</tr>
<tr>
<td>M-CLL</td>
<td>47/119, 39%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>aberration</th>
<th>CGH array</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>13qmono</td>
<td>110</td>
<td>122</td>
</tr>
<tr>
<td>13qbi</td>
<td>29</td>
<td>21</td>
</tr>
<tr>
<td>trisomy12</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>del11q</td>
<td>52</td>
<td>56</td>
</tr>
<tr>
<td>del17p</td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>244</td>
<td>259</td>
</tr>
</tbody>
</table>