Hyperdiploid acute myeloid leukaemia (AML) represents a distinct molecular and clinical entity

Matt D Taylor (mattdavt@gmail.com)

UKCCCG Presentation
Outline of Presentation

- What is AML?
- AML with complex karyotype
- The role of TP53 in AML
- AML with high hyperdiploidy (49-65 chromosomes)
- Aims, study design and techniques
- Mutational and copy number profile of AML with high hyperdiploidy
- Effect on patient outcome
- What could this mean for management of patients with AML with high hyperdiploidy?
- Suggestions for future work
- Conclusions
- Acknowledgements
- References
What is AML?

Heterogeneous clonal disorder of immature myeloid precursor cells

Bone marrow failure
• Anaemia
• Thrombocytopenia
• Neutropenia
Infiltration of extramedullary tissues

Most common form of adult acute leukaemia

Cytogenetic risk classification

<table>
<thead>
<tr>
<th>Risk group</th>
<th>10-year overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favourable</td>
<td>55-81%</td>
</tr>
<tr>
<td>Intermediate</td>
<td>32-38%</td>
</tr>
<tr>
<td>Adverse</td>
<td>3-21%</td>
</tr>
</tbody>
</table>

Grimwade, D., et al. (Blood 2010)
AML with complex karyotype (CK-AML)

- AML with at least 3 chromosomal abnormalities in the absence of prognostically favourable chromosomal abnormalities
- 10-15% of adult AML, with frequency increasing with age
- Characterised by losses of 5q, 7q and 17p

### Adverse cytogenetic risk group*

<table>
<thead>
<tr>
<th>Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>abn(3q) - excluding t(3;5)(q25;q34)</td>
</tr>
<tr>
<td>-5 or abn(5q)</td>
</tr>
<tr>
<td>-7 or abn(7q)</td>
</tr>
<tr>
<td>t(6;9)(p23;q34)</td>
</tr>
<tr>
<td>t(6;11)(q27;q23)</td>
</tr>
<tr>
<td>any t(11q23) - excluding t(9;11)(p21~22;q23) and t(11;19)(q23;p13)</td>
</tr>
<tr>
<td>t(9;22)(q34;q11)</td>
</tr>
<tr>
<td>-17 or abn(17p)</td>
</tr>
</tbody>
</table>

### Complex karyotype

*Vardiman, J.W., et al (Blood 2009)*
*Döhner, H., et al. (Blood 2010)*
*Grimwade, D., et al. (Blood 2010)*
TP53

Encodes the transcription factor p53, which forms a tetramer and interacts with target genes via a central DNA-binding domain (exons 4-8)

Role of mutant p53 in cancer
• Reduced function
• Pro-oncogenic properties

Mutations in TP53 are rare in AML (5-15%)

In CK-AML, rates of TP53 mutation and/or deletion are 50-75% (≥90% of mutations involve exons 5-8)
AML with high hyperdiploidy (49-65 chromosomes)

<table>
<thead>
<tr>
<th>HH-AML SUBGROUPS</th>
<th>DEFINITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numerical (NUM)</td>
<td>Numerical high hyperdiploidy only</td>
</tr>
<tr>
<td>Structural (STR)</td>
<td>High hyperdiploidy with additional intermediate-risk or unknown-risk chromosomal abnormalities</td>
</tr>
<tr>
<td>Adverse cytogenetic risk (ACR)</td>
<td>High hyperdiploidy with additional adverse-risk chromosomal abnormalities</td>
</tr>
</tbody>
</table>

Chilton, L. et al. (Leukemia, 2014)
Aim

- To investigate the mutational and copy number profile of AML with high hyperdiploidy, with particular focus on TP53 and any associated patterns of chromosomal gain or loss
Study Design

**Samples**
- DNA
  - 55 high hyperdiploid AML
  - 24 CK-AML controls
- Fixed cells
  - 15 high hyperdiploid AML

**Preparation**
- PCR and agarose gel electrophoresis
- DHPLC
- Sanger sequencing
- Mutation screening

**Screening**
- Copy number analysis
- MLPA
- FISH 17p13.1

**Validation**
Denaturing high-performance liquid chromatography (DHPLC)

- Adapted reverse-phase ion-pair chromatography.
- Size-dependent separation of dsDNA is lost at partially-denaturing temperatures.
- Heteroduplexes (Synthetic base-pair mismatch hybrids) will denature more readily than homoduplexes and thus will have different retention behaviour.
**TP53 mutation screening**

![Bar chart showing number of TP53-mutant patient samples per subgroup](chart.png)

<table>
<thead>
<tr>
<th></th>
<th>Mutant</th>
<th>Wild-type</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUM</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>STR</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>ACR</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>CK-AML</td>
<td>10</td>
<td>13</td>
</tr>
</tbody>
</table>

NUM/STR vs ACR: $p<0.01$
Study Design

- **Samples**
  - DNA: 55 high hyperdiploid AML, 24 CK-AML controls
  - Fixed cells: 15 high hyperdiploid AML

- **Preparation**
  - PCR and agarose gel electrophoresis
  - DHPLC

- **Screening**
  - Sanger sequencing

- **Validation**
  - Copy number analysis
  - MLPA
  - FISH 17p13.1
Multiplex ligation-dependent probe amplification (MLPA)

- PCR-based quantification method using two adjacent probes for each target site - successful amplification is dependent on ligation of the two probes
- Experimental X060-AML-MDS MLPA probemix from MRC Holland. Targets various commonly affected genes and regions including TP53(17p), NPM1(5q), EZH2(7q), KMT2A(11q) and RUNX1(21q)
- Fragments separated by capillary electrophoresis and results analysed using Coffalyser.net software
No cryptic deletions of TP53 in NUM or STR patients were identified by MLPA.

No differences in numbers or patterns of chromosomal losses between ACR and CK-AML (except AEBP2 in CK-AML).

Overall survival (ACR group, intensive therapy)

- TP53 altered
- TP53 unaltered

p<0.001

Mutation screening  Copy number analysis

Copy number and patient outcome
What does this mean for patients with AML with high hyperdiploidy?

- Potential reclassification of NUM and STR patients to intermediate-risk could alter therapeutic options (e.g. haematopoietic stem cell transplantation)
- Recognition that $TP53$ status has an important prognostic impact in AML
- Access to any $TP53$-targeted treatments that may be developed in the future
Future work

- Alternative methods of *TP53* inactivation
  - p53 regulatory molecules (e.g. MDM2)
  - Upstream and/or downstream signalling pathways
    - Interactions with target genes
    - Interactions with target proteins
    - Interactions with miRNA networks

- *TP53* expression profile
- Whole exome sequencing
Conclusions

- In AML, high hyperdiploidy without individual adverse-risk chromosomal abnormalities (NUM/STR) represents a separate entity to CK-AML and could be reclassified into the intermediate cytogenetic risk group.

- TP53 mutations and deletions play a significant role in high hyperdiploid AML with individual adverse-risk chromosomal abnormalities (ACR), and are associated with an particularly poor prognosis.
Acknowledgements

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- MRC Holland
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  - Suvi Savola
- Wolfson Foundation
References

- Bowen, D.T., et al. (2005) *RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years*.
References

Thank you for listening

- Any questions?