A novel algorithm to identify, characterize and define the prognostic impact of complex catastrophic events in Multiple Myeloma

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Conflict of interest

The authors declare no conflict of interests
Complex Catastrophic Events (CCEs)

→ neoplastic cells are characterized by **genomic instability**, which might cause the **rapid evolution** of the tumour

→ **chromoanagenesis** = complex structural rearrangements leading to the formation of new aberrant chromosomes

**ONE STEP EVENTS (chromothripsis)** are complex chromosomal rearrangements, which happen in a single temporal moment, in contrast to events that acquire mutations gradually over time.

**STEPWISE EVENTS (chromoanasyntesis & cromoplexy)** that are the consequence of multiple, small and sequential genomic events, occurring during subsequent cell cycles.

chromoanagenesis incidence = 2-3% in all tumours

P.J Stephens et al.; 2011 Jan , Cell
M.N.H. Luijten et al; 2018; Sep; Mutat. Res
CCEs in Multiple Myeloma

STEP WISE event

CHROMOTRIPSIS

PROGRESSIVE rearrangements model

Experimental Plan

Results

Conclusion

>3 CN state

LOH in 2N region

<3 CN state

HETEROZIGOSITY in 2N region
Aim & experimental plan

1. 488 MM samples
   1. CD138+ enrichment
   2. DNA extraction
   3. SNP Array (Affymetrix SNP Array 6.0 e Cytoscan HD)

2. Genomic profiling:
   - ChAS v3.3-Affymetrix
   - Rawcopy tool
   - Personalized R scripts

3. CCEs characterization:
   - Set-up of an original algorithm to detect and characterize CCEs

CLINICAL CORRELATION

1. To detect CCEs in MM, with a focus on Chromotriipsis, by using an original and reliable bio-informatic algorithm
2. To characterize the genetic and genomic context of Chromotriipsis
3. To correlate the presence of Chromotriipsis with patient prognosis
C.C.E. detector 3.0

- count of the CN changes as compared to the diploid region (2N)
- if the total number of CN changes is >3, it continues with detection and categorization of the event
- the events are categorized according to the reported guidelines
- the file output includes a list of detected events and their chromosomal position for each individual sample
# Frequency & co-segregation

### Chromosonal aberration

<table>
<thead>
<tr>
<th>Chromosomal aberration</th>
<th>Targeted gene</th>
<th>p_value</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>del chr 17p</td>
<td>17p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>del TP53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mut TP53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>del chr 1p</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>del CDKN2C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>del FAF1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amp chr 1q</td>
<td>amp CKS1B</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traslocations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(4;14)</td>
<td></td>
<td>0.002</td>
<td>3.4</td>
</tr>
<tr>
<td>t(14;20)</td>
<td></td>
<td>0.01</td>
<td>8</td>
</tr>
<tr>
<td>t(14;16)</td>
<td></td>
<td>0.04</td>
<td>3.55</td>
</tr>
</tbody>
</table>

### Position

<table>
<thead>
<tr>
<th>Position</th>
<th>p_value</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr 1p</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>chr 2q</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>chr 11q</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>chr 22q</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Chromotripsis

<table>
<thead>
<tr>
<th>Chromotripsis</th>
<th>p-value</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*chromotripsis events are mainly carried by specific chromosomes*
**Chromotripsy is predictive of clinical outcome**

**PFS**

<table>
<thead>
<tr>
<th>Event</th>
<th>p.value</th>
<th>C.I</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALL.Chromotripsy</td>
<td>0.04601 *</td>
<td>1.0065 – 2.055</td>
</tr>
<tr>
<td>del TP53 (17p13.1)</td>
<td>0.2854</td>
<td>0.4945 – 1.230</td>
</tr>
<tr>
<td>T(4;14)</td>
<td>0.00591 **</td>
<td>1.1275 – 2.041</td>
</tr>
</tbody>
</table>

**OS**

<table>
<thead>
<tr>
<th>Event</th>
<th>p.value</th>
<th>C.I</th>
</tr>
</thead>
<tbody>
<tr>
<td>CALL.Chromotripsy</td>
<td>0.0387 *</td>
<td>1.0246 – 2.490</td>
</tr>
<tr>
<td>del TP53 (17p13.1)</td>
<td>0.2766</td>
<td>0.3298 – 1.373</td>
</tr>
<tr>
<td>T(4;14)</td>
<td>0.0011 **</td>
<td>1.2897 – 2.771</td>
</tr>
</tbody>
</table>

**Results**

The impact of chromotripsy on PFS and OS is **independent** from other adverse prognostic factors.

**Multivariate analysis**

- **PFS**
  - HR 1.52, C.I. 1.07-2.15
  - p = 0.019

- **OS**
  - HR 1.68, C.I. 1.09-2.59
  - p = 0.019
PI-based therapy & chromotripsy

→ **ER stress pathway** deregulation is related to the decrease of response to PI-based therapy

<table>
<thead>
<tr>
<th>Targeted gene</th>
<th>chr position</th>
<th>p_value</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>amp XBP1</td>
<td>22q12.1</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>del XBP1</td>
<td>22q12.1</td>
<td>&gt;0.001</td>
<td>4.68</td>
</tr>
<tr>
<td>amp ATF4</td>
<td>22q13.1</td>
<td>0.03</td>
<td>6.05</td>
</tr>
<tr>
<td>del ATF4</td>
<td>22q13.1</td>
<td>0.01</td>
<td>3.01</td>
</tr>
<tr>
<td>amp/del ATF6</td>
<td>1q23.3</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>amp/del CRBN</td>
<td>3p26.2</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>amp/del DDIT3 (CHOP)</td>
<td>12q13.3</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>amp/del EIF2AK3 (PERK)</td>
<td>2p11.2</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>amp/del ERN1 (IRE1a)</td>
<td>17q23.3</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

→ **CNAs in 2 genes** of the **ER stress pathway** correlates with the presence of chromotripsy

Sinan X. et al., 2021, Cell and Molecular life Sciences
Chromothripsis & Clonal Evolution

Chromothripsis is detectable as clonal event in MGUS and SMM that will progress to multiple myeloma.

Chromothripsis is conserved over time after precursor progression and at relapse after treatment, as clonal, without any significant changes in its structure and copy number profile.

Maura F et al, 2021, Semin Cell Dev Biol
Conclusions

1. **CCEs Dectector 3.0** highlights and characterizes CCEs across the whole genome

2. CCEs frequency was 36%; chromotripsis frequency was 9%

3. chromotripsis **significantly impact** PFS and OS of newly diagnosed MM patients

4. chromotripsis events significantly correlate with CNAs in *TP53, XBP1, ATF4*

5. in the genome throughout MM course, thus suggesting their key-role in driving disease progression
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