Importance of minor TP53 mutated clones in the clinic

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The Diagnostic Lab of the IOSI analyzes TP53 mutations by Sanger sequencing:

1. The evidence that TP53 mutations associate with chemorefractoriness comes from Sanger sequencing-based studies.

2. The current evidence support small TP53 mutated subclones as prognostic.

3. Lack of harmonization for small TP53 mutated subclone identification.

4. Patients/week number does not cost/effectively support the switch to NGS.
TP53 lesions are mostly subclonal

Landau et al, Cell 2013
Detection limit of Sanger sequencing

**Scenario 3**
- Case no.: 474

**Scenario 2**
- Case no.: 390

**Scenario 1**
- Case no.: 73-2

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Mutation</th>
<th>Sanger seq</th>
<th>VAF by NGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>474</td>
<td>c.G524A p.R175H</td>
<td></td>
<td>3.30%</td>
</tr>
<tr>
<td>390</td>
<td>c.G329T p.R110L</td>
<td></td>
<td>9.11%</td>
</tr>
<tr>
<td>73-2</td>
<td>c.A488G p.Y163C</td>
<td></td>
<td>11.71%</td>
</tr>
<tr>
<td>1461</td>
<td>c.G607C p.V203L</td>
<td></td>
<td>12%</td>
</tr>
<tr>
<td>701</td>
<td>c.G743A p.R248Q</td>
<td></td>
<td>17.34%</td>
</tr>
</tbody>
</table>

Nadeu F et al Blood 2016
Backtracking of TP53 mutations clonally acquired at later stages of the disease

NOTCH 1 EX34: c.7544_7545delCT p.P2515fs*4 (heterozygous)

TP53 EX7: c.716A>C p.N239T (heterozygous)

30 months 62 months

RS precursor

RS transformation
Small TP53 mutated subclones account for ~30% of all cases harboring TP53 defects

**Sanger sequencing**
- Positive: n=35
- Negative: n=50

**Ultra deep-NGS**
- 85 TP53 mutations in 46/309 (15%) CLL

**TP53 mutations**
- N=309
- 85% (N=263)
- 3% (N=10)
- 6% (N=18)

**TP53 lesions***
- N=309
- 84% (N=259)
- 7% (N=22)
- 5% (N=15)
- 4% (N=13)

*TP53 mutations and 17p13 deletion

Rossi, Blood 2014
**Subclonal TP53 mutations have the same detrimental impact on TP53 as clonal lesions**

**Subclonal TP53 mutations are mainly missense substitutions**

- Missense: Clonal M: 195, Subclonal M: 39; Frequency: 76%, 78%; p=1
- Insdel: Clonal M: 46, Subclonal M: 2; Frequency: 18%, 4%; p=0.0408
- Nonsense: Clonal M: 257, Subclonal M: 50; Frequency: 2%, 10%; p=0.0808
- Splicing sites: Clonal M: 257, Subclonal M: 50; Frequency: 2%, 8%; p=1

**Subclonal TP53 mutations are preferentially transitions**

- Transitions: GC>AT: Clonal M: 86, Subclonal M: 19; Frequency: 41%, 40%; p=1
  AT>GC: Clonal M: 42, Subclonal M: 13; Frequency: 20%, 27%; p=1
- Transversions: GC>CG: Clonal M: 210, Subclonal M: 48; Frequency: 8%, 13%; p=1
  GC>TA: Clonal M: 29, Subclonal M: 4; Frequency: 14%, 8%; p=1
  AT>CG: Clonal M: 18, Subclonal M: 2; Frequency: 9%, 4%; p=1
  AT>TA: Clonal M: 18, Subclonal M: 4; Frequency: 9%, 8%; p=1

**Subclonal TP53 mutations affect hot spot codons within the DBD**

**Subclonal TP53 mutations negatively impact on TP53 function**

**CDKN1A promoter transcriptional activity measured in yeast functional assays from the IARC database**

Clonal mutations from Zenz et al, Leukemia 2010
Small *TP53* mutated subclones have the same unfavorable prognostic impact as clonal *TP53* defects.
Small $TP53$ mutated subclones account for ~30% of all cases harboring $TP53$ defects.
Small TP53 mutated subclones have the same unfavorable prognostic impact as clonal TP53 defects.
Small TP53 mutated subclones detected before treatment subsequently expand under the selective pressure of therapies.
TP53 subclone evolution in CLL treated within the CLL8 trial

INCREASING CCF

<table>
<thead>
<tr>
<th>Gene</th>
<th>CCF</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>17</td>
</tr>
<tr>
<td>del (17p)</td>
<td>14</td>
</tr>
<tr>
<td>IKZF3</td>
<td>4</td>
</tr>
<tr>
<td>del (18p)</td>
<td>4</td>
</tr>
</tbody>
</table>

Landau, Nature 2015
Small TP53 mutated subclones do not evolve under watch and wait

Rossi, Blood 2014
Scenarios of *TP53*-mutated subclones evolution

**UNTREATED**

**RELAPSE 1**

**RELAPSE 2-n**

**NO TREATMENT**

Expands

Slow expansion

Persistence

Disappearance

Persistence

Expansion

Malcikova, Leukemia 2015
Challenges in the identification of small *TP53* mutated subclones

**True mutations**

- Library preparation chemistry?
- Coverage?
- Bioinformatic pipeline for variant calling?
The mutational landscape of CLL

The wordcloud shows the genes that are reported as mutated in CLL by the v77 of the Catalogue of Somatic Mutations in Cancer (COSMIC). The size of the font is proportional to the mutation frequency.
Subclonal mutations impact on CLL outcome

Landau DA, Nature 2015
Small *NOTCH1*, *SF3B1* and *BIRC3* subclones occur in a significant fraction of CLL

**NOTCH1 mutations**

- Sanger sequencing positive, n=32
  - Ultra deep-NGS: 46 *NOTCH1* mutations in 43/304 (14%) CLL
  - Median allele frequency: 3.9% (range: 1.4-9%)

- Sanger sequencing negative, n=14
  - Median allele frequency: 3.9% (range: 1.4-9%)

**SF3B1 mutations**

- Sanger sequencing positive, n=18
  - Ultra deep-NGS: 43 *SF3B1* mutations in 35/304 (11%) CLL
  - Median allele frequency: 4.3% (range: 0.5-17%)

- Sanger sequencing negative, n=25
  - Median allele frequency: 4.3% (range: 0.5-17%)

**BIRC3 mutations**

- Sanger sequencing positive, n=7
  - Ultra deep-NGS: 37 *BIRC3* mutations in 26/304 (8%) CLL
  - Median allele frequency: 1.4% (range: 0.2-6%)

- Sanger sequencing negative, n=30
  - Median allele frequency: 1.4% (range: 0.2-6%)
The molecular spectrum of *NOTCH1*, *SF3B1* and *BIRC3* subclonal mutations was consistent with that of clonal mutations.

*NOTCH1*

*SF3B1*

*BIRC3*
Small mutated subclones of *NOTCH1*, *SF3B1* and *BIRC3* genes are clinically irrelevant

**NOTCH1**

- **NOTCH1** wild type
- Solely subclonal NOTCH1 M
- Clonal NOTCH1 mutation

<table>
<thead>
<tr>
<th>Events</th>
<th>Total</th>
<th>Median OS</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>93</td>
<td>261</td>
<td>8.8</td>
<td>7.7-10.0</td>
</tr>
<tr>
<td>21</td>
<td>31</td>
<td>3.1</td>
<td>2.0-4.2</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>12.2</td>
<td>-</td>
</tr>
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Rasi, Haematologica 2016

**SF3B1**

- **SF3B1** wild type
- Solely subclonal SF3B1 M
- Clonal SF3B1 mutation

<table>
<thead>
<tr>
<th>Events</th>
<th>Total</th>
<th>Median OS</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>99</td>
<td>270</td>
<td>9.0</td>
<td>7.2-10.8</td>
</tr>
<tr>
<td>11</td>
<td>16</td>
<td>2.8</td>
<td>0.6-4.2</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>6.6</td>
<td>3.2-10.0</td>
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</tbody>
</table>

**BIRC3**

- **BIRC3** wild type
- Solely subclonal BIRC3 M
- Clonal BIRC3 lesions

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<tr>
<td>95</td>
<td>268</td>
<td>9.0</td>
<td>7.5-10.5</td>
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<td>11</td>
<td>16</td>
<td>3.1</td>
<td>1.6-4.7</td>
</tr>
<tr>
<td>12</td>
<td>20</td>
<td>6.8</td>
<td>2.4-11.3</td>
</tr>
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Small NOTCH1, SF3B1 ATM and BIRC3 subclones occur in a significant fraction of CLL.
Small mutated subclones of *NOTCH1*, *SF3B1* *ATM* and *BIRC3* genes are clinically irrelevant

Nadeu F et al Blood 2016
Mixed shift in clonal representation of \textit{NOTCH1} and \textit{SF3B1} clones

\textbf{NOTCH1}

\textbf{SF3B1}

\textbf{SHIFTING CCF}

\begin{itemize}
  \item \textit{ATM} 33
  \item \textit{SF3B1} 33
  \item \textit{amp (2p)} 13
  \item \textit{DDX3X} 8
  \item \textit{POT1} 8
  \item \textit{NOTCH1} 6
  \item \textit{SAMHD1} 6
  \item \textit{KRAS} 5
  \item \textit{del (6q21)} 4
  \item \textit{MGA} 4
  \item \textit{BIRC3} 4
  \item \textit{IRF4} 4
\end{itemize}

Nadeu F et al Blood 2016
Landau, Nature 2015
Biomarkers that are inert under chemoimmunotherapy may become dangerous under new agents and vice versa.
Clinical trial samples

IOSI-EMA001

- CLL
- TN
- R/R
- TP53 M

Ibrutinib

- W0
- W2
- W24
- W48
- W72
- W96
- yearly

Patients

- Biobanked
- Pending

Timepoints
- baseline
- week 2
- week 24
- week 48
- week 72
- week 96
- yearly
Longitudinal assessment of CLL clonal architecture under ibrutinib
**TP53 clone dynamics under ibrutinib**

Changes of *TP53* clone abundance

- VAF > 1.5%
- VAF <1.5%

![Graph showing allele frequency (%) changes over time with time elapsed from ibrutinib start.](image)

Changes of tumor burden

![Graph showing hGE/ml of plasma changes over time with time elapsed from ibrutinib start.](image)
Conclusions

• Small \( TP53 \) mutated subclones occur in ~5% untreated CLL

• CLL patients harboring small \( TP53 \) mutated subclones have the same clinical outcome as patients with clonally represented \( TP53 \) lesions (prognostic)

• Chemoimmunotherapy invariably select small \( TP53 \) mutated subclones

• Small \( TP53 \) mutated subclones are inert under watch and wait

Perspectives

• Meta-analysis of ultra-deep-NGS studies to identify a cut-off (if any)

• Comparative assessment of NGS protocols (and validation by PCR)

• Small \( TP53 \) mutated subclones upon treatment with novel agents