Clonal evolution in therapy-related AML

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Mike Schmitt, MD, PhD
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Categories of AML

- **Therapy-related AML**
  - AML that arises following chemotherapy and/or radiation. Very poor prognosis.

- **Secondary AML**
  - AML that arises following known MDS or MPN

- **de novo AML**
  - All other cases of AML

Risk of therapy-related AML varies by cancer type

Adjuvant treatment of breast cancer: Risk of AML varies depending on the regimen

Without anthracycline (CMF): low risk of t-AML

With anthracycline (CEF): high risk of t-AML

Risk of t-AML in Hodgkin lymphoma varies with different regimens


High alkylator exposure and extended-field radiation: 5.5% incidence of t-AML/MDS
Low alkylator exposure and involved-field radiation: 0.3% incidence of t-AML/MDS
Risk of therapy-related AML is increasing for some cancers...expected to become more of a problem as survival improves.

Mutations in de novo AML likely come from normal mutations during aging

AML: average of 10 mutations per exome
Normal stem cells: by age 50, average of ~10 mutations per exome.

Question: What causes therapy-related AML?

Do chemotherapy/radiation cause increased mutations, and thus an accelerated rate of AML development?
Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia

Terrence N. Wong¹*, Giridharan Ramsingh²*, Andrew L. Young³*, Christopher A. Miller⁴, Waseem Touma¹, John S. Welch¹, Tamara L. Lamprecht¹, Dong Shen⁶, Jasreet Hundal⁴, Robert S. Fulton⁴, Sharon Heath¹, Jack D. Baty⁷, Jeffery M. Klco⁸, Li Ding¹,⁵, Elaine R. Mardis⁴,⁵,⁹, Peter Westervelt¹,⁵, John F. DiPersio¹,⁵, Matthew J. Walter¹,⁵, Timothy A. Graubert¹,⁵, Timothy J. Ley¹,⁵, Todd E. Druley³, Daniel C. Link¹,⁵ & Richard K. Wilson⁴,⁵,⁹

Experimental approach: sequence DNA from therapy-related AML and de novo AML

- Are there more mutations in therapy-related AML relative to de novo AML?
- Are specific genes mutated in therapy-related AML?

Mutations are **not** increased in therapy-related AML
Spectrum of mutations is not different in therapy-related AML

C→T mutations are most frequent. These arise as spontaneous errors during normal cell division (from spontaneous deamination of cytosine to uracil).
Chemotherapy-resistance mutations are frequent in t-AML (TP53, ABC)
Observations:
Mutations are not increased in t-AML.
Mutations in chemotherapy resistance genes (e.g. TP53) are common in t-AML.

Hypothesis:
TP53 mutations are present in a small population of cells prior to treatment. Chemotherapy selects for these cells, and they evolve into AML.
Predictions of this model

1. The exact TP53 mutation found at AML diagnosis can be found years earlier in a small fraction of cells, prior to chemotherapy
2. Healthy individuals have small populations of TP53-mutant cells
3. TP53 mutated cells will selectively grow following chemotherapy
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2. Healthy individuals have small populations of TP53-mutant cells

3. TP53 mutated cells will selectively grow following chemotherapy
Detection of sub-clonal mutations can be challenging, due to the high error rate of next-generation sequencing.

The authors used "unique adapter sequencing" to detect mutations down to ~0.1% of cells.
Result: the TP53 mutations are present as small populations, years before development of t-AML

Extended Data Table 3 | Previously banked tissue samples in patients with t-AML/t-MDS with clonal TP53 mutations

<table>
<thead>
<tr>
<th>Patient</th>
<th>Position (Chr 17)</th>
<th>Mutation</th>
<th>Coding change</th>
<th>Year of Banking</th>
<th>Year of Diagnosis</th>
<th>Prior Banked Tissue</th>
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<tbody>
<tr>
<td>236041</td>
<td>7,518,261</td>
<td>T to A</td>
<td>R249W</td>
<td>2007</td>
<td>2011</td>
<td>BM FFPE</td>
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<td>341666</td>
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<td>2002</td>
<td>2005</td>
<td>Pharesis</td>
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<tr>
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<td>C to T</td>
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<td>* 895681</td>
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<td>* 967645</td>
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<td>2005</td>
<td>2010</td>
<td>BM Flow</td>
</tr>
</tbody>
</table>

All patients had one or more clonal TP53 mutations in their diagnostic t-AML/t-MDS samples (530447 had biallelic mutations). Cases in which the previously banked sample had detectable TP53 mutated cells are highlighted in red. See Supplementary Table 1 for the clinical and molecular features of these cases. BM FFPE, formalin-fixed paraffin-embedded sample; BM flow, snap-frozen bone marrow leukocyte pellet.

* Sample obtained before initial chemotherapy
Predictions of this model

1. The exact TP53 mutation found at AML diagnosis can be found years earlier in a small fraction of cells, prior to chemotherapy

2. Healthy individuals have small populations of TP53-mutant cells

3. TP53 mutated cells will selectively grow following chemotherapy
19 healthy individuals (age 68-89): 9 of them had subclonal TP53 mutations

Extended Data Table 4 | Somatic TP53 mutations in 19 cancer-free individuals

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chr</th>
<th>Exon</th>
<th>Start</th>
<th>Stop</th>
<th>Ref</th>
<th>Var</th>
<th>Amino acid</th>
<th>COSMIC ID</th>
<th>Var count</th>
<th>Total read family count</th>
<th>VAF (read-family)</th>
<th>VAF (ddPCR)</th>
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<tr>
<td>34</td>
<td>17</td>
<td>7</td>
<td>7518230</td>
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<td>7518273</td>
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<td>T</td>
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<tr>
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<td>7520035</td>
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<td>0.37%</td>
<td>0.28%</td>
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<tr>
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<td>7518264</td>
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<td>C</td>
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<tr>
<td>338</td>
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<td>7</td>
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<td>A</td>
<td>R248W</td>
<td>COSM10656</td>
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<td>51001</td>
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<td>N.D.</td>
</tr>
</tbody>
</table>

Note: their assay background is ~0.05%. Some of their results are at or below this threshold. Disregarding these, 4/19 patients had subclonal TP53 mutations.
Predictions of this model

1. The exact TP53 mutation found at AML diagnosis can be found years earlier in a small fraction of cells, prior to chemotherapy
2. Healthy individuals have small populations of TP53-mutant cells
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Mouse experiment:
TP53 mutant cells selectively grow after DNA damage

Mice were transplanted with a 7:1 ratio of wild-type to TP53 +/- bone marrow, then treated with ENU or placebo.

TP53 mutant cells selectively grew after ENU treatment.
Conclusions:

• Mutations are not increased in t-AML

• t-AML can arise from selective growth of pre-existing, chemotherapy-resistant cells.

• Chemotherapy-resistant cells are present in healthy individuals.
Questions:
• Can we stratify patients at high risk of t-AML (e.g. those with pre-existing subclonal mutations in TP53) to safer treatments?
• Can we monitor for emergence of therapy-related AML by tracking the growth of TP53-mutated cells?
• Are TP53 mutations really present in ~50% of healthy individuals?
  • Genovese et al NEJM 2014: 0.03% of individuals (4/11,845)
  • Jaiswal et al NEJM 2014: 0.2% of individuals (33/17,182)
  • Wong et al Nature 2015: 47% of individuals (9/19)
• Could sub-clonal TP53 mutations be relevant to other diseases?
Might subclonal TP53 mutations have broader implications in other diseases? **Example in CLL:**

Subclonal TP53 mutations in CLL are not detected by current clinical assays. However, they are equally predictive of poor outcome, even when present in <10% of cells.

Thanks

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