GENOMICS OF ACQUIRED APLASTIC ANEMIA

1. Clones, as in clonal evolution, myeloid neoplasms
2. Clones, as in HSC derived progeny
3. (Clones, as in T cell clones)
APLASTIC ANEMIA AND MYELOID MALIGNANT DISEASE

Clonal evolution

Diagnostic confusion

Pathophysiologic similarity

Clinical phenotype

Percent evolution

Time (days)

Percent

V\beta-subfamily

trisomy 8 patient
DESIGN FOR GENOMICS OF SAA

- Targeted deep sequencing of 106 candidate mutational targets reported in myeloid malignancies in 3 cohorts from USA (NIH and Cleveland) and Japan (Kanazawa Univ) (N = 426).
- Whole exome sequencing (SureSelect v.5.0) in 57 cases.

<table>
<thead>
<tr>
<th></th>
<th>NIH</th>
<th>Kanazawa</th>
<th>Cleveland</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases enrolled</td>
<td>293</td>
<td>205</td>
<td>24</td>
<td>522</td>
</tr>
<tr>
<td>Cases excluded</td>
<td>46</td>
<td>42</td>
<td>0</td>
<td>88</td>
</tr>
<tr>
<td>Eligible cases (targeted)</td>
<td>256</td>
<td>159</td>
<td>24</td>
<td>434</td>
</tr>
<tr>
<td>With germline control</td>
<td>238</td>
<td>19</td>
<td>24</td>
<td>281</td>
</tr>
<tr>
<td>Serial samples (WES)</td>
<td>35</td>
<td>15</td>
<td>2</td>
<td>52</td>
</tr>
</tbody>
</table>

ACQUIRED MUTATIONS IN SAA (NIH COHORT)

% of patients

- PIGA
- BCOR/BCORL1
- DNMT3A
- ASXL1
- Splicing
- cohesin
- JAKs
- RUNX1
- TP53
- TET2
- SETBP1
- GNAS
- PRC2
- CSMD1
- LAMB4
- WT1
- TERF1/TERT
- ATRX
- PHF6
- ATM
- RIT1
- CDAN1
- IDH2
- CUX1
- RBBP4
- CBL
- PRPF8
- KRAS
- MPL
- NF1
- POT1
- RAP1A
- STAT3
- PEG3
- DIS3
- SH2B3
ACQUIRED MUTATIONS IN SAA (NIH COHORT)

% of patients

- **multiple**
- **missense**
- **nonsense**
- **frameshift**
- **splicing**

PIGA
BCOR/BCORL1
DNMT3A
ASXL1
LOW ALLELIC BURDEN OF ACQUIRED MUTATIONS IN SAA

Variant allele frequency

Frequency

0.000
0.025
0.050
0.075
0.100
0.125
0.150
0.175
0.200
0.225
0.250
0.275
0.300
0.325
0.350
0.375
0.400

0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
0.50

no mutations
1 mutation
2 mutations
>2 mutations
SOMATIC MUTATIONS AND PATIENT AGE

1.2 age

<table>
<thead>
<tr>
<th>Age Group</th>
<th>All mutations</th>
<th>PIGA &amp; BCOR/BCORL1</th>
<th>Non PIGA/BCOR/BCORL1 mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>1.0</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>10-19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-39</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥70</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Number of mutations

C > A
C > G
C > T
T > A
T > C

WES (N=205)

Targeted sequencing (N = 176)

DNMT3A, ASXL1 age-related
C > T transitions predominate
A CASSETTE OF ACQUIRED MUTATIONS CORRELATES WITH CLINICAL OUTCOMES

p=0.0085

p=0.027
BEHAVIOR OF MUTATED CLONES OVER TIME

ASXL1

VAF

years

Dx 0.5 1 2 3 4 5 6 7 8 9 10 11 12

0.0 0.1 0.2 0.3 0.4

CR
PR
NR
BEHAVIOR OF MUTATED CLONES OVER TIME

**DNMT3A**

**ASXL1**

**BCOR/BCORL1**

VAF (Variant Allele Frequency) over time for different clones and conditions (CR, PR, NR). The graphs show changes in VAF from diagnosis (Dx) up to 12 years.
CLONAL HEMATOPOIESIS IN SAA: >12 YEAR COURSE

NIH075 74 y.o. male

Gradual transfusion independence
PHYSIOLOGIC VS PATHOLOGIC TELOMERE LOSS

Telomere loss due to mitosis:
- normal aging; tissue regeneration

Accelerated telomere attrition from inadequate repair/maintenance:
- constitutional mutations in telomerase/shelterin genes

**Physiologic telomere loss:**
- Telomere loss due to mitosis
- Normal aging; tissue regeneration

**Pathologic telomere loss:**
- Dyskeratosis (DKC1)
- Telomere disease (TERT, TERC)
TELOMERE LENGTH AND CLONAL EVOLUTION IN SAA

A

Telomere length

<table>
<thead>
<tr>
<th>Telomere length</th>
<th>1st (shortest) quartile</th>
<th>&gt; 1st quartile</th>
</tr>
</thead>
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<tr>
<td>TL &lt; 1st quartile</td>
<td>46</td>
<td>124</td>
</tr>
<tr>
<td>TL &gt; 1st quartile</td>
<td>137</td>
<td>128</td>
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</tbody>
</table>

Follow-up, years

<table>
<thead>
<tr>
<th>Follow-up, years</th>
<th>No. at risk</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>1</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

Log rank P=0.009

B

Telomere length

<table>
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<th>Telomere length</th>
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</tr>
<tr>
<td>1</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>

Log rank P=0.002
**“GENES VS CHROMOSOMES” IN CLONAL EVOLUTION**

**“Stable AA” – responders or non-responders to immunosuppressive therapy**

\[ \approx 15\% \text{ clonal evolution to MDS/AML} \]

<table>
<thead>
<tr>
<th></th>
<th>SAA TO MDS/7-“clonal evolution” (n=13)</th>
<th>SAA CONTROLS “stable SAA” (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis of SAA</td>
<td>40±20</td>
<td>38±20</td>
</tr>
<tr>
<td>Male (%)</td>
<td>69%</td>
<td>63%</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>31%</td>
<td>100%*</td>
</tr>
<tr>
<td>PNH clone (%)</td>
<td>27%</td>
<td>40%*</td>
</tr>
<tr>
<td>Neutrophils (K/uL)</td>
<td>0.46±0.4</td>
<td>0.52±0.3</td>
</tr>
<tr>
<td>Reticulocytes (K/uL)</td>
<td>16.2±16.0</td>
<td>28.9±22.9*</td>
</tr>
<tr>
<td>Lymphocytes (K/uL)</td>
<td>1.3±0.6</td>
<td>1.6±1.0</td>
</tr>
<tr>
<td>Platelets (K/uL)</td>
<td>12±8</td>
<td>9±3</td>
</tr>
</tbody>
</table>

Dumitriu B, et al; Blood 2014; 125:706
ACCELERATED TELOMERE LOSS PRECEDES CLONAL EVOLUTION

Approximately 7x higher

Normalized telomere length

Months post-IST