AML Genomics

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Normal neutrophil maturation

**Acute Myeloid Leukemia (AML)** = block in differentiation

- AML with minimal differentiation
  - FAB M1
- Promyelocytic leukemia
  - FAB M3
- Myelomonocytic
  - FAB M5
Principle 1: AML incidence increases with age


Principle 2: AML emerges through branching evolution

Nowell, PC Science 1976.
Predictions

- Where do mutations come from?
  - Genomic instability?
  - How many mutations?
  - Distribution of mutations?

- Pre-leukemic state?
  - Leukemic evolution?

- Mechanism of relapse?
% of the Human Genome in Each Tier

- Tier 1: Genes (1.3%)
- Tier 2: Conserved (8.6%)
- Tier 3: Non-repetitive (48.7%)
- Tier 4: rest (41.4%)

Lots of mutations; are they all required?

Validated Single Nucleotide Variants

- M1: Total
- M3: Total
- M1: Tier 1
- M3: Tier 1
- M1: Recurrent
- M3: Recurrent

Drivers

Passengers
The number of AML mutations correlates with the age of patient

Pre-leukemic state

• Predictions?
What is the genomic structure of normal aging hematopoietic stem cells?

Healthy Volunteers → Sort single Lin^- CD34^+ CD38^- → Culture 2-3 weeks Pick 3 colonies

Total WBCs vs Exome Sequencing

Somatic variants in healthy-donor HSPCs

![Graph showing somatic variants per HSPC clone]
The number of AML and HSC mutations correlates with the age of patient

HSC mutation rate

2.4 – 4.0 x 10⁻⁸ variants/nt/yr

Intergenerational mutation rate:

~4 x 10⁻¹⁰ variants/nt/yr
Mutation spectrum:
7 healthy-donors’ HSCs vs 24 AML cases

C > T vs G > A mutations

The Oncologist June 1, 2004 vol. 9 no. 3 353-354
Clonal Expansion and Allelic Fractions.

Prevalence of Mosaic Hematopoiesis increases with age

Laurie et al Nature Genetics 44:642. 2012
Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis

Lambert Busque1,2,15, Jay P Patel3,15, Maria E Figueroa5, Aparna Vasanthakumar6, Sylvie Provost7, Zineb Hamilou3,9, Luigina Mollica1,3, Juan Li3, Agnes Viale3, Adriana Heguy4, Maryam Hassim8, Nicholas Socci9, Parva K Bhatt4, Mithat Gonen10, Christopher E Mason11,12, Ari Melnick12,13, Lucy A Godley6, Cameron W Brennan14,15, Omar Abdel-Wahab4,15,17 & Ross I. Levine4,12,15,17

Table 1. TET2 somatic mutations found in normal elderly individuals (n = 10)

<table>
<thead>
<tr>
<th>Nucleotide substitution</th>
<th>Amino-acid substitution</th>
<th>Chromosome</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.296_298delGCGAC</td>
<td>p.Arg94fsX12</td>
<td>4</td>
<td>106155385</td>
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<tr>
<td>c.1330delA V1180fsX12</td>
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<td>4</td>
<td>106156429</td>
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<td>c.1346delA</td>
<td>p.Lys448fsX*2</td>
<td>4</td>
<td>106156447</td>
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<tr>
<td>c.1517delC</td>
<td>p.Pro505fsX*16</td>
<td>4</td>
<td>106156646</td>
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<tr>
<td>c.1603C&gt;T V130fsX</td>
<td>p.Arg544fsX</td>
<td>4</td>
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<tr>
<td>c.3311_3319delAT</td>
<td>p.Phe1104fsX*3</td>
<td>4</td>
<td>106158411</td>
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<tr>
<td>c.3991A&gt;C</td>
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<td>4</td>
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<tr>
<td>c.5205G&gt;A</td>
<td>p.Met1742fsX*11</td>
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<tr>
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<td>p.Glu1909fsX</td>
<td>4</td>
<td>106197392</td>
</tr>
</tbody>
</table>

*The reference sequence used to annotate TET2 mutations was NM_016121.3

Figure 1. Prevalence of Somatic Mutations, According to Age.
Colored bands, in increasing lighter shades, represent the 50th, 75th, and 15th percentiles.

Leukemic Evolution

Predictions?
Subclonal architecture

- 1170 reads at TET2 locus
- 490 reads with mutation
- 42% of alleles carry mutation
- Therefore, 84% of the bone marrow cells carry the mutation.

Each point represents a unique mutation

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Subclonal architecture

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Sub-clonal architecture
Model of AML mutation acquisition

Relapse

- Predictions?
Clonal selection at relapse

- Rounding clone variants
- Subclone 1 variants
- Subclone 2 variants
- Subclone 3 variants

Relapse from this subclone

Subclones lost at relapse

Klco et al. Cancer Cell 2014

Mutations

• Predictions?
How many mutations does it take?

Order of mutation acquisition?

Drivers vs passengers ... Initiation vs progression

• Subclonal architecture by Next-gen
• Temporal changes in mutations
Cytogenetics: single cell analysis

- \( t(15;17) \) and +8
  - XY, \( t(15;17) \), +8[16]/XY, \( t(15;17)[3] \), XY[1]
  - 36 cases published with \( t(15;17) \) and +8
  - 16 of these report \( t(15;17) \) cells without +8
  - None have +8 without \( t(15;17) \)

- \( t(15;17) \), -7
- \( t(8;21) \), -X, -Y
- Inv(16), +13, +22

NRAS and FLT3 mutations in subclones
Response of subclone to chemotherapy

Subclone mutation confers sensitivity

Mutations Lost at Relapse

Percent of cases

Schnittger ASH 2012: TET2 lost in 0/17 cases

Mutations Gained at Relapse

Percent of cases

FLT3-ID (N=31)  MT (N=37)  RAS (N=29)  FLT3-KR (N=19)  CEBPA (N=28)  IDH1 (N=23)  DNMT3A (N=25)  TP53 (N=27)  TET2 (N=29)

Schnittger ASH 2012: TET2 gained in 1/423 cases.
Summary of mutations

- Founding clone/initiation mutations:
  - t(15;17), CBF, MLL, DNMT3A, TET2

- Commonly subclonal mutations:
  - +8, +22, -X, -Y, FLT3, NRAS/KRAS, WT1, KIT, CEBPA

- Uncertainty:
  - NPM1, IDH1/2
Chromosome X analysis

Residual disease detection: \textit{NPM1}

\textbf{A} Overall Survival

\begin{tabular}{|c|c|c|}
\hline
 & No. of Patients & No. of Events \\
\hline
M RD-negative & 164 & 40 \\
M RD-positive & 30 & 21 \\
\hline
\end{tabular}

\textbf{B} Relapse in All Patients

\begin{tabular}{|c|c|c|}
\hline
 & No. of Patients & No. of Events \\
\hline
M RD-negative & 164 & 50 \\
M RD-positive & 30 & 25 \\
\hline
\end{tabular}

Ivey \textit{et al.} \textit{NEJM} 374: 422, 2016

\textbf{C} Patient 33428 (9 mutations)

\begin{tabular}{|c|c|}
\hline
 & \\
\hline
Mutation & Blasts \\
\hline
\end{tabular}

\textbf{D} Patient 174516 (16 mutations)

\begin{tabular}{|c|c|}
\hline
 & \\
\hline
Mutation & Blasts \\
\hline
\end{tabular}

Klco \textit{et al.} \textit{JAMA} 314: 811 2015
Genomic Features of AML

• Cell specific mutations are acquired in each HSPC over its lifespan.
  – $3.2 \times 10^8$ variants/nt/yr
  – 1 cell division/month

• Most mutations are randomly distributed across the genome.
  – Mutations favor C>T transitions.

• Founding clones and subclones that evolve during leukemogenesis and
  leukemic relapse via branching evolution.

• $DNMT3A$ and $TET2$ may act as initiating events.
  – $FLT3$, $RAS$, $KIT$, $CEBPA$, $WT1$ are commonly progression events
  – $NPM1$ and $IDH1/2$ early, but not always initiating.

• AML is not a disease of genomic instability.

Acknowledgements

Genomics of AML PPG
  - Daniel Link
  - Timothy Graubert
  - John Dipersio
  - Geoffrey Uy
  - Amanda Cashen
  - Ravi Vij

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  - Rick Wilson
  - Elaine Mardis
  - Li Ding
  - Chris Miller
  - Dave Larsen
  - Bob Fulton

Our Patients.
Random Distribution of Mutations

Genes
1.5% Conserved 8% Non-redundant 45%

SNVs per Mbp
0.0 0.2 0.4 0.6
Tier 1 Tier 2 Tier 3

SNV position on chromosome (Mbp)
0 1 5 10 15 20 XY

Frequency
0 100 200 300 400 500 600
Number of SNVs per Mbp bin

AML24
Expected Poisson distribution
Contamination of leukemia variants in skin sample

Clonal selection during xenograft

- A single clone engrafts per mouse
- Variability in the engrafting clone