AML Genomics

John S. Welch

Department of Medicine
Siteman Cancer Center
Washington University School of Medicine

Normal neutrophil maturation

Acute Myeloid Leukemia (AML) =
block in differentiation

AML with minimal differentiation,
FAB M1

Promyelocytic leukemia,
FAB M3

Myelomonocytic,
FAB M5
Why does the AML incidence increase with age?


Does AML emerge from branching evolution?

Nowell, PC Science 1976.
Predictions

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

• 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: 41.4%
- Tier 4: rest: 48.7%

Tier 1: Genes
Tier 2: Conserved
Tier 3: Non-repetitive
Tier 4: rest
Lots of mutations; are they all required?

Random Distribution of Mutations

- Genes: 1.5%
- Conserved: 8%
- Non-redundant: 45%
The number of AML mutations correlates with the age of patient

![Graph showing correlation between number of AML mutations and age]

Pre-leukemic state

- Predictions?
Prevalence of Mosaic Hematopoiesis increases with age


Xie et al. Nature Medicine
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

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Somatic variants in healthy-donor HSPCs

The number of AML and HSC mutations correlates with the age of patient
HSC mutation rate

\[2.4 \text{ – } 4.0 \times 10^{-9} \text{ variants/nt/yr}\]

Intergenerational mutation rate:
\[\sim 4 \times 10^{-10} \text{ variants/nt/yr}\]

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

Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis

Lambert Busque1,3,16, Jay P Patel1,3,16, Maria E Fiqueroa5, Aparna Vasanthakumar5, Sylvie Provost7, Zineb Hamilou3,3, Luigina Mollica5,3, Juan Li5, Agnes Viale3, Adriana Heguy3, Moryam Hassim5, Nicholas Socci5, Parca K Bhat4, Mithat Gonen5, Christopher E Mason1,3, Ari Melnick2,3, Lucy A Godley6, Cameron W Brennak9,14, Omar Abdal-Wahhab1,5,4,7 & Ross L Levine3,11,15,17

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

<table>
<thead>
<tr>
<th>Nucleotide substitution</th>
<th>Amino-acid substitution</th>
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*The reference sequence used to annotate TET2 mutations was NM_001127208.
Leukemic Evolution

Predicitions?
Subclonal architecture

Each point represents a unique mutation

Sub-clonal 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.
Model of AML mutation acquisition

Relapse

- Predictions?
Clonal selection at relapse

Klco et al. Cancer Cell 2014
Clonal selection during xenograft

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

Mutations

- Predictions?
Heterogeneity

Comprehensive analysis of genetic alterations and their prognostic impacts in adult acute myeloid leukemia patients

Significantly Mutated Genes
200 cases

How many mutations does it take?

TCGA NEJM 2013
Which mutations initiate M1-AML?

Drivers vs passengers ... Initiation vs progression

• Single cell level

• Comparison with M3 AML

• 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
Cooperating Events

NRAS and FLT3 mutations in subclones

AML2

NRAS

AML3

FLT3

SMC1A

NPM1

IDH1

PML-RARα

FLT3

TTN

EPB41L5

WT1

PHF6

DIS3

ETV6

ZNF687

MUC5B

GDPD4

ABCA10

CACNA1B

DNAH9

GPR123

NPM1

DNMT3A

IDH1

TEKT2
Response of subclone to chemotherapy

Schnittger ASH 2012: TET2 gained in 1/423 cases.
Response of subclone to chemotherapy

Subclone mutation confers sensitivity

Mutations Lost at Relapse

Schnittger ASH 2012: TET2 lost in 0/17 cases

NPM1
DNMT3A
FLT3-ITD
FLT3-TKD
NRAS
IDH1
IDH2
MLL-PTD
TP53
ASXL1

Stability  Acquired at Relapse

91%  na
97%  5%
75%  41%
9%  0%
36%  13%
75%  9%
92%  0%
a  6%
a  2%
a  4%

Kroenke et al Blood 2013
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

Genomic Features of AML

• Cell specific mutations are acquired in each HSPC over its lifespan.
  – \(3.2 \times 10^9\) 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.
Why is AML an age-associated disease?

Chromosome X analysis
Contamination of leukemia variants in skin sample

Skin contamination in M4/M5 AML
Comparison of tumor burden calculated by clinical and sequence methods

![Graph showing comparison of tumor burden calculated by clinical and sequence methods.](image)

## Acknowledgements

Genomics of AML PPG
- Tim Ley
- Peter Westervelt
- Sharon Heath
- Tami Lamprecht

Washington University Genome Institute
- Rick Wilson
- Elaine Mardis
- Li Ding
- Chris Miller
- Dave Larsen
- Bob Fulton

Our Patients.

Daniel Link
- Timothy Graubert
- John Dipersio
- Geoffrey Uy
- Amanda Cashen
- Ravi Vij