AML Genomes: lessons learned

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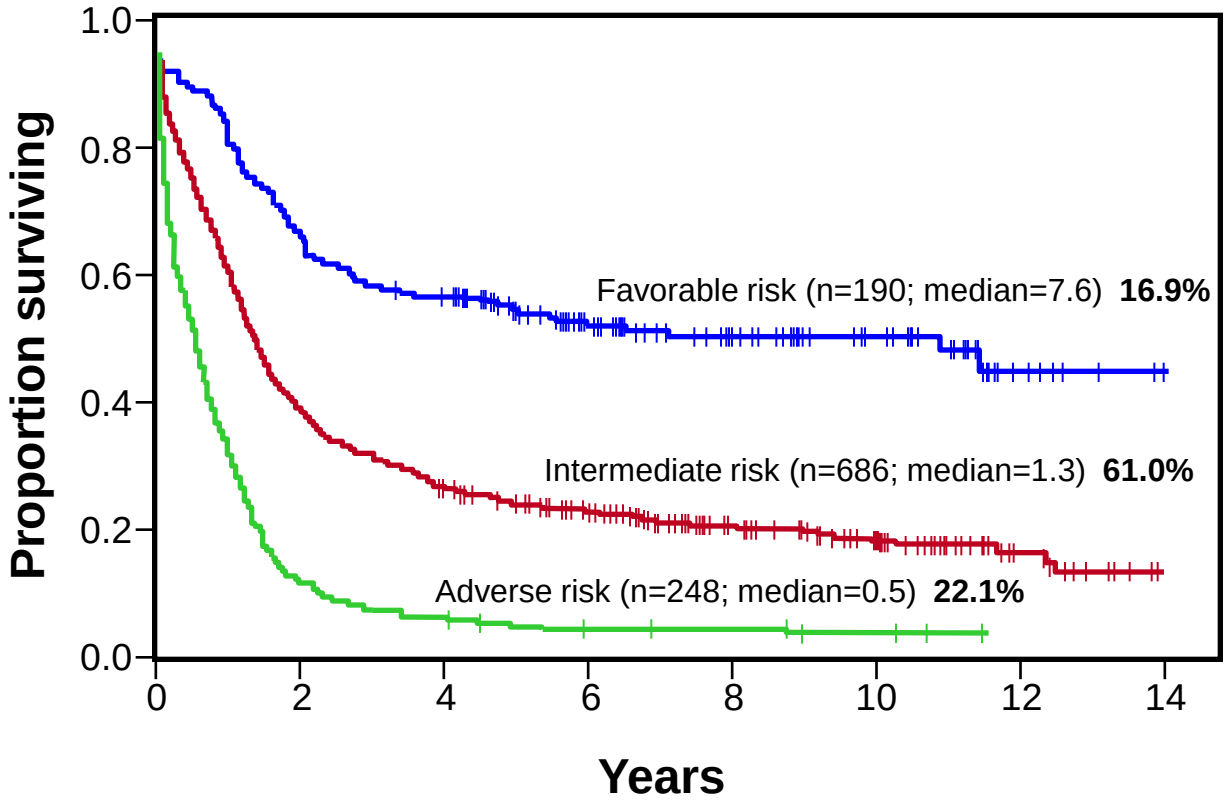
Acute Myeloid Leukemia and genomics

- Very little is known about the key initiating mutations for most patients (except for canonical translocations)
- Tumor tissue is easy to access repeatedly, and most samples are relatively free of contaminating normal cells
- Many genomes are diploid
- Low resolution genomic screening (cytogenetics) is already a paradigm for disease classification and treatment decisions

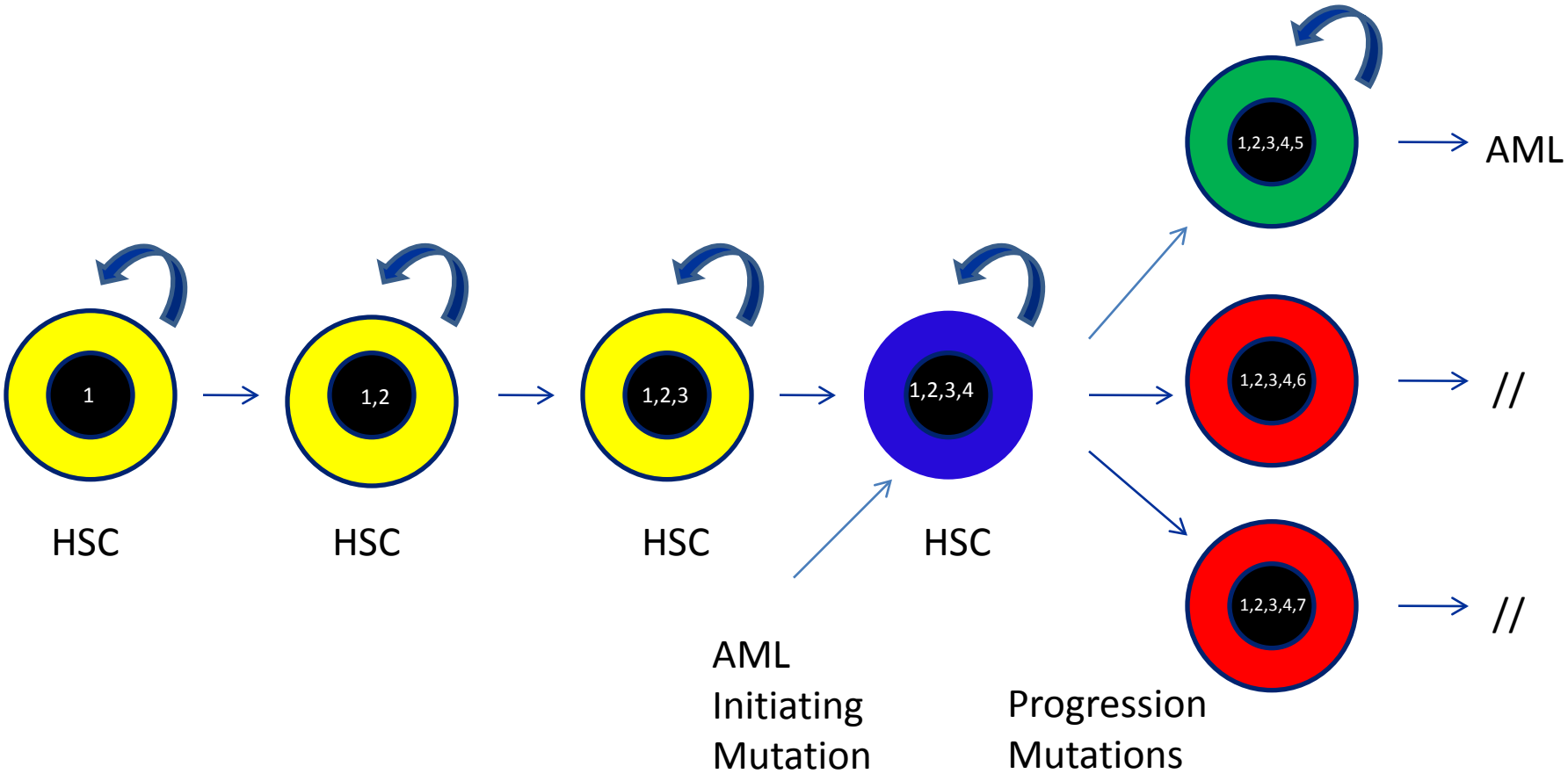
Cytogenetics (low resolution genomics) and survival in AML



Byrd JC et al., Blood. 2002 Dec 15;100(13):4325-36.

The founding conundrum

- Hundreds of mutations per AML genome
- All mutations are found in all tumor cells
- Suggests that all mutations may have arisen simultaneously
- Clonal evolution: hundreds of relevant mutations per genome?
- Both seem impossible


1
1,2
1,2,3
1,2,3,4
1,2,3,4,5
1,2,3,4,6
1,2,3,4,7
AML
//
//
HSC
HSC
HSC
HSC
AML
Initiating
Mutation
Progression
Mutations

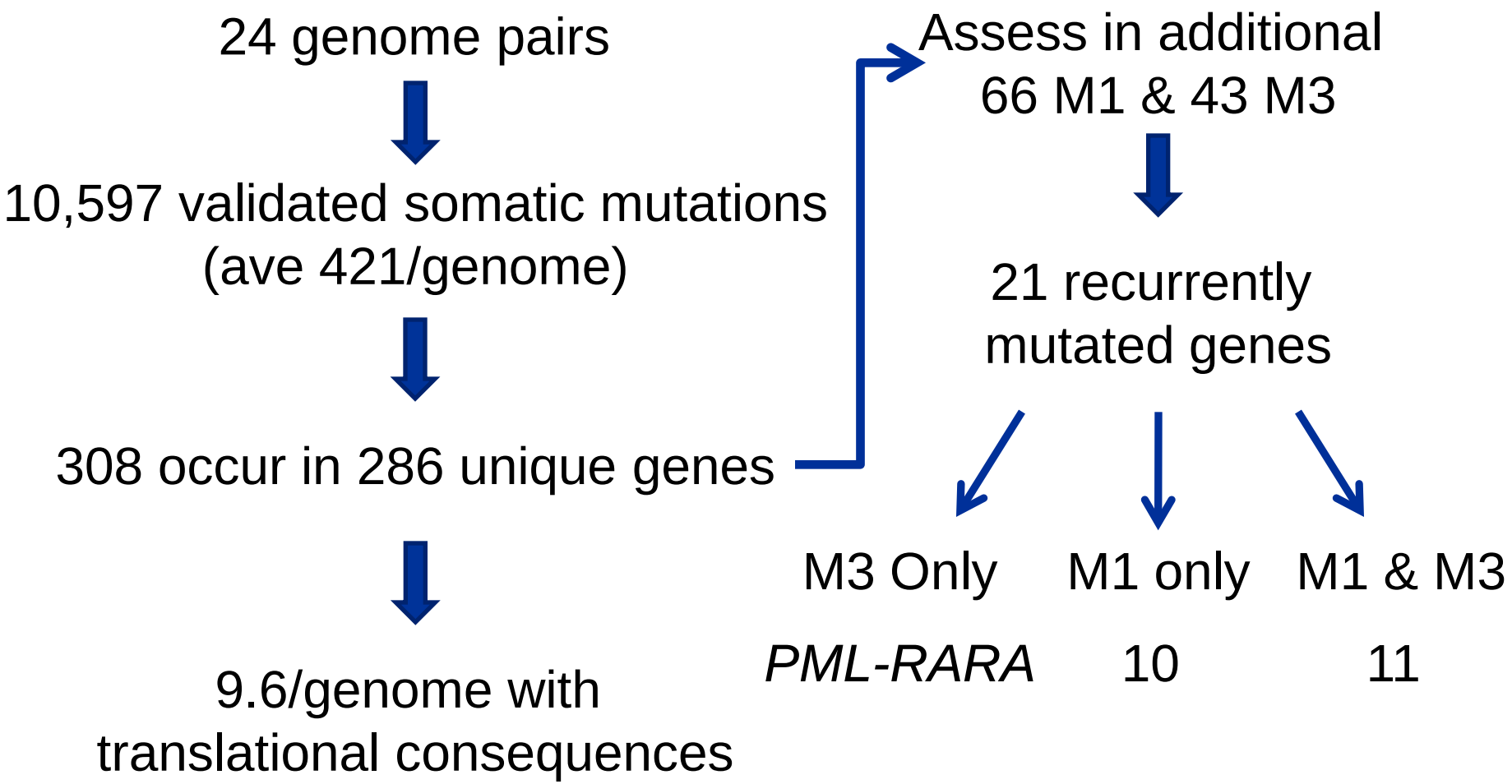
A central question :

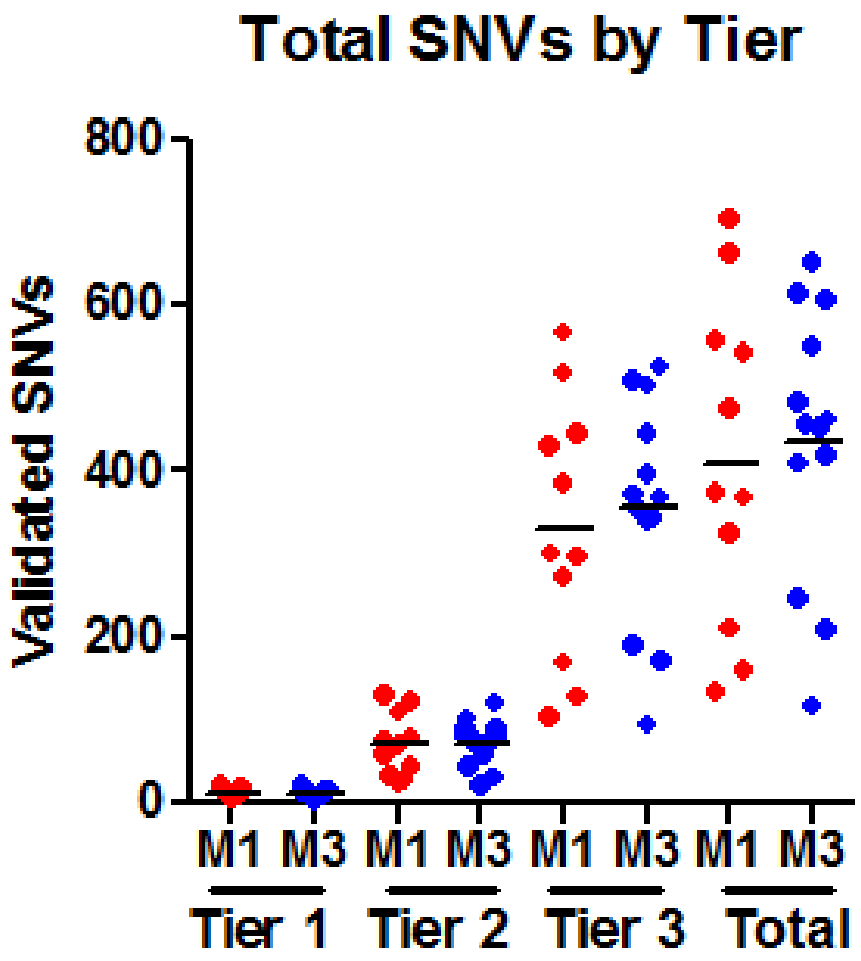
- How many mutations does it take to cause AML?
- Compare the mutational burden in M3 AML (initiated by *PML-RARA*) vs. M1 AML with normal karyotype (NK)

- Predictions:
  - Total mutations per genome will be the same (since most antedate the initiating event)
  - Most mutations will be random and irrelevant
  - M1 will have novel mutations never seen in M3 **(*initiation*)**
  - M1 and M3 genomes will share some mutations **(*progression*)**
  - How many recurring mutations per genome?

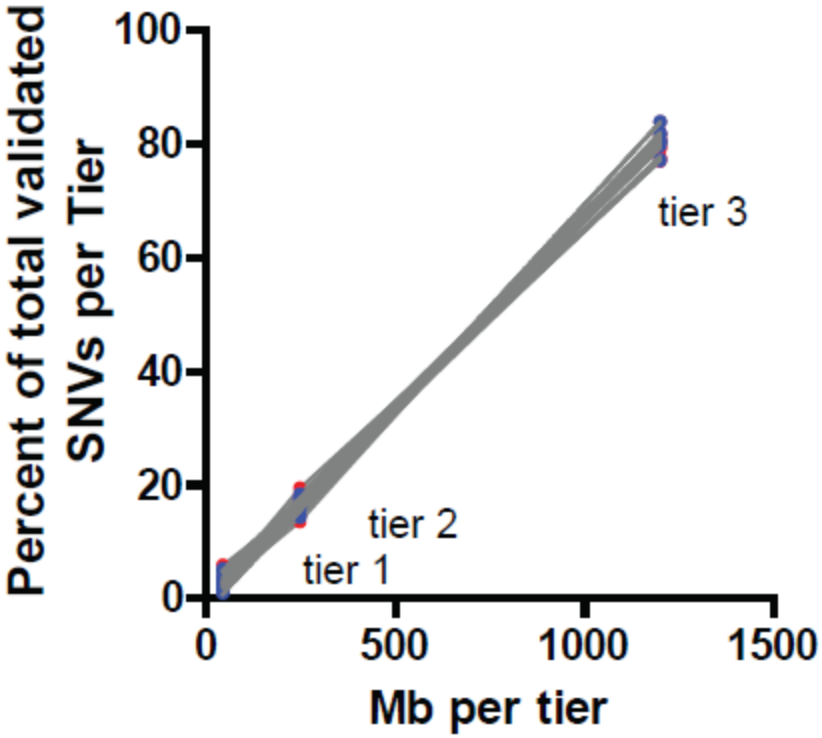
John Welch, Dave Larson, Chris Miller, Li Ding

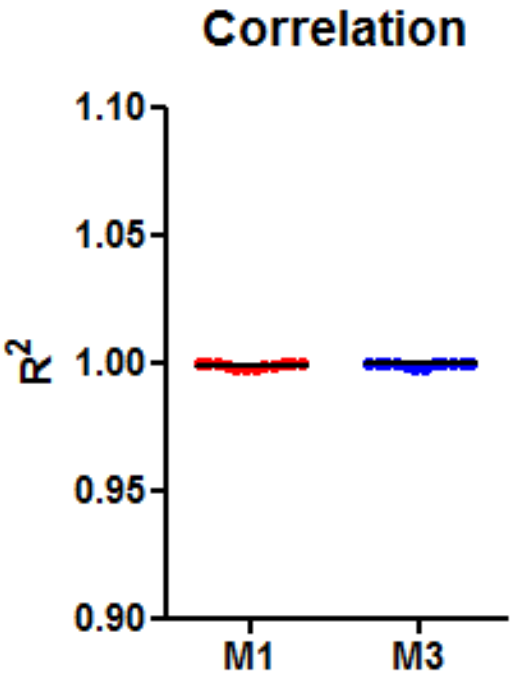
Mutation distributions



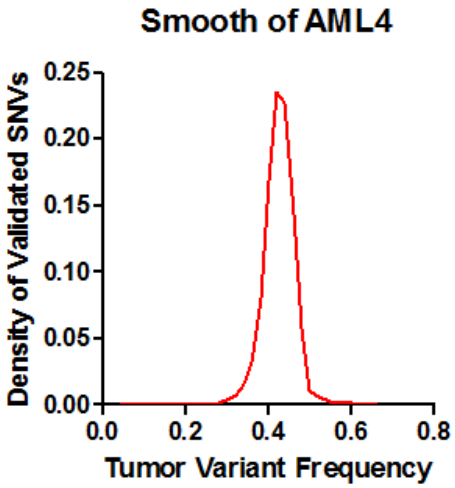

Total SNVs by Tier
Validated SNVs
800
600
400
200
0
M1
M3
M1
M3
M1
M3
M1
M3
Tier 1
Tier 2
Tier 3
Total

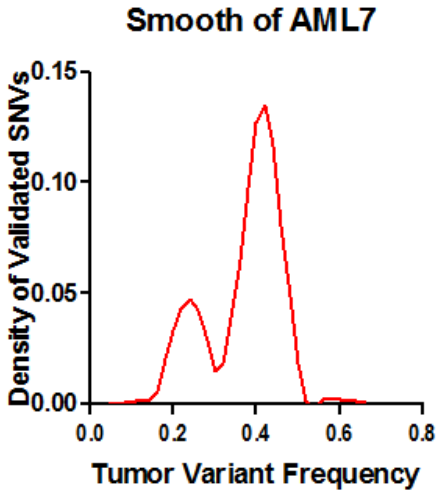
Distribution of Validated SNVs by Tier

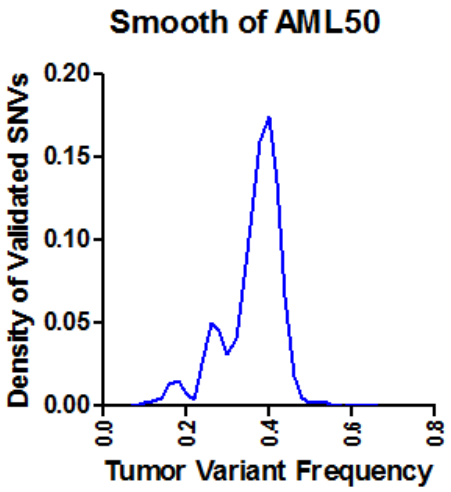


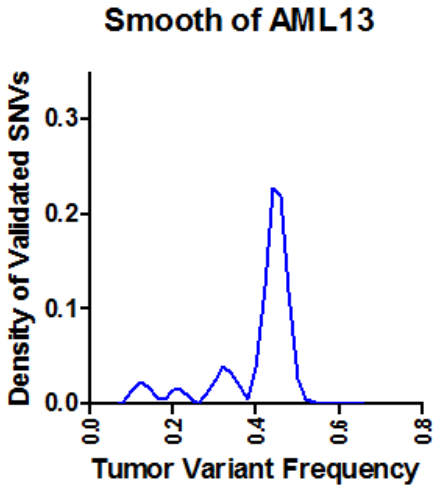


All de novo AMLs have founding clones-and some have subclones

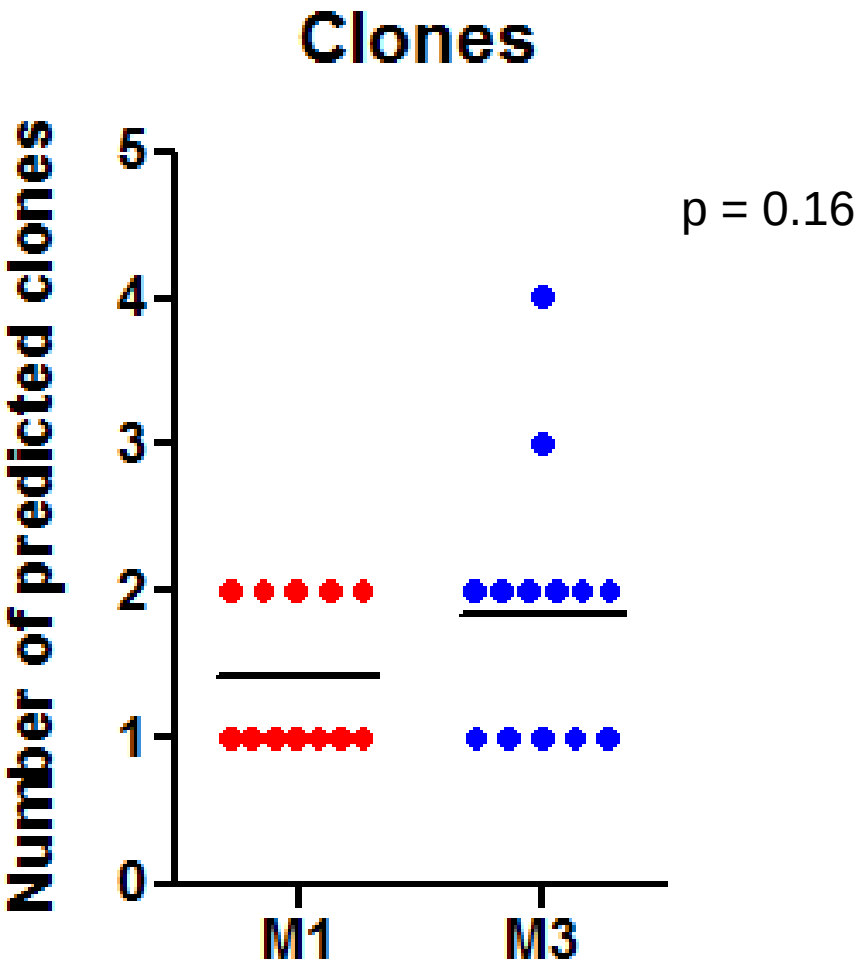




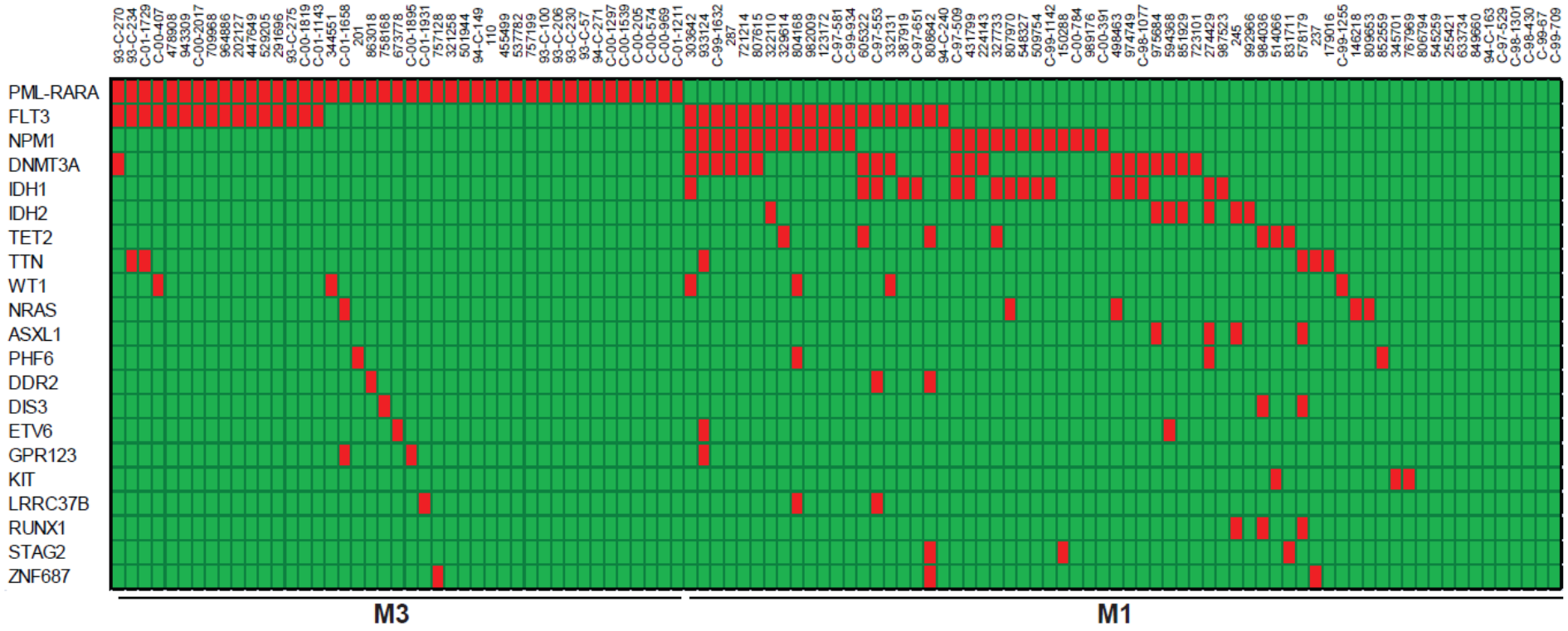




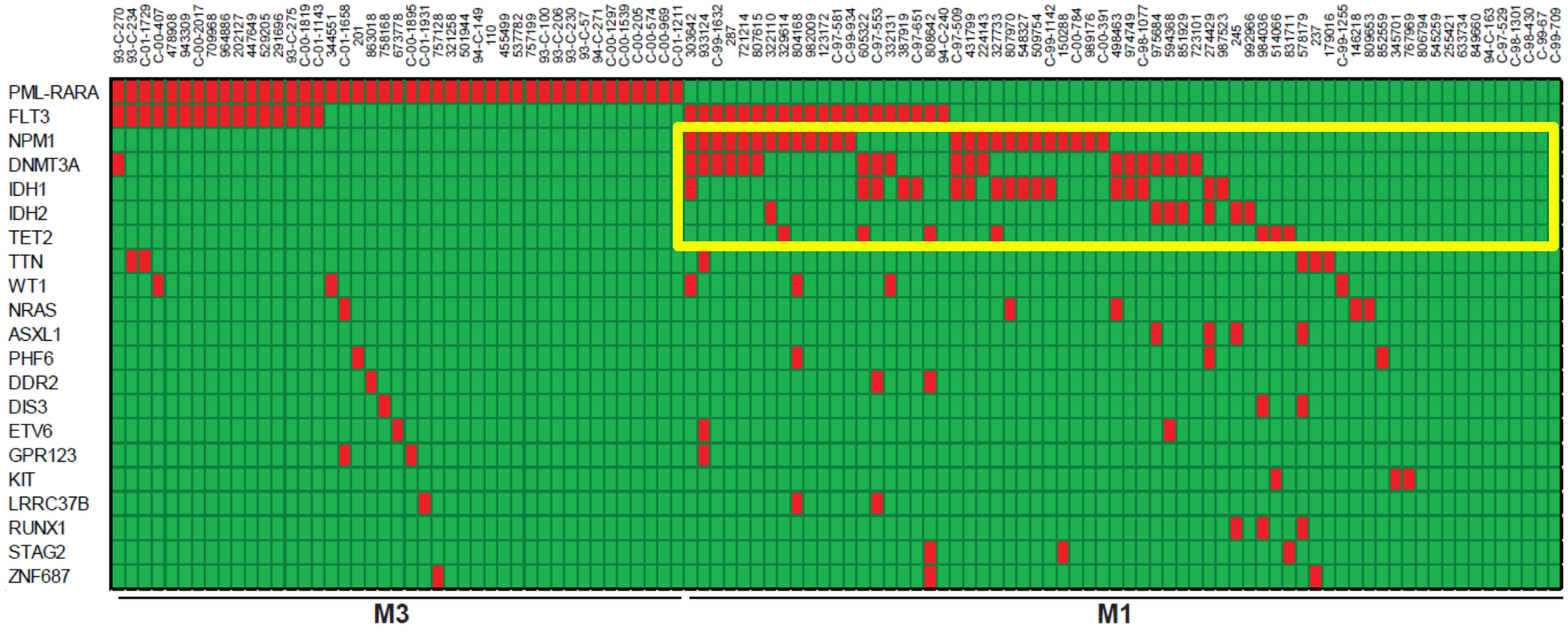
Clonality in M1 vs. M3 AML



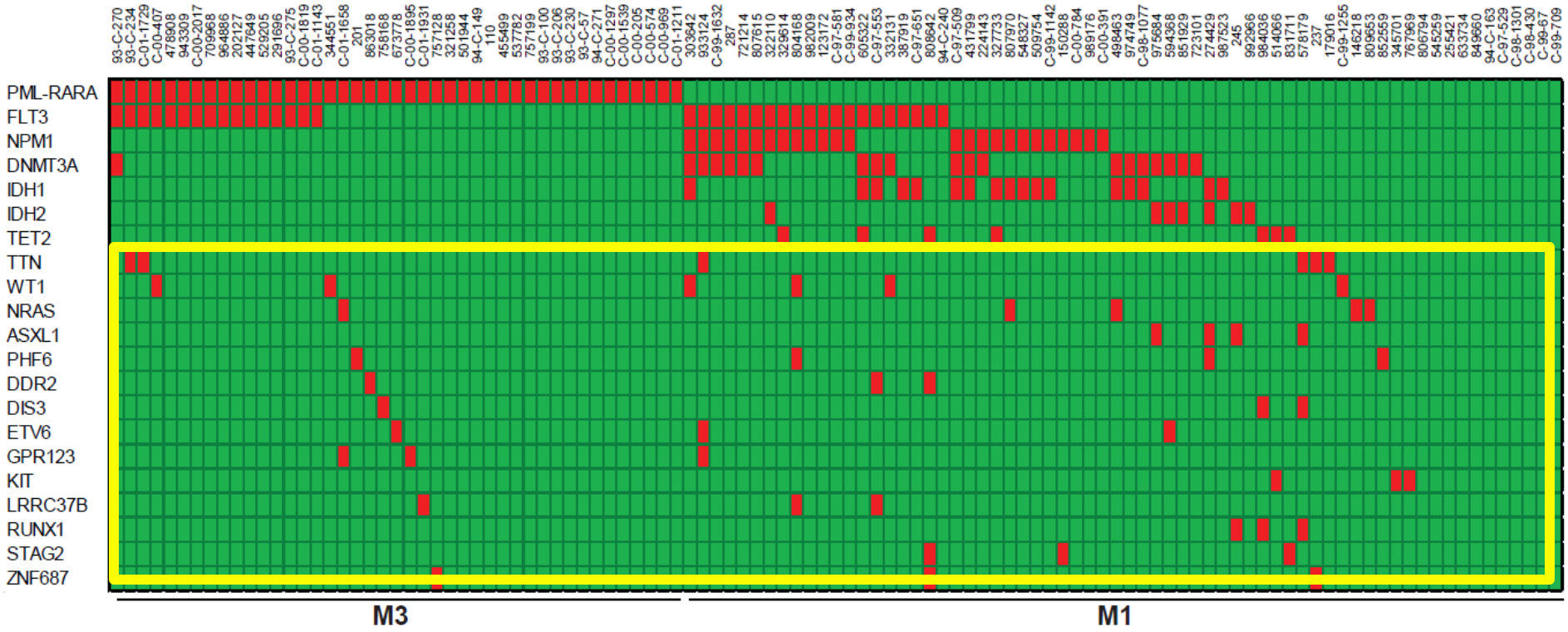
Recurrently mutated genes



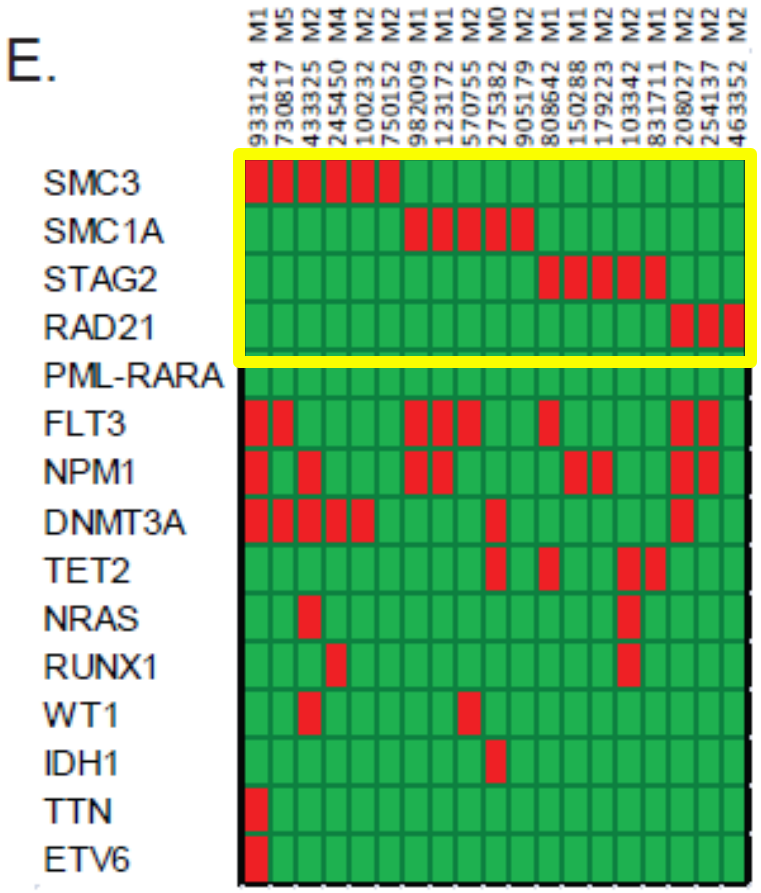
Recurrently mutated genes

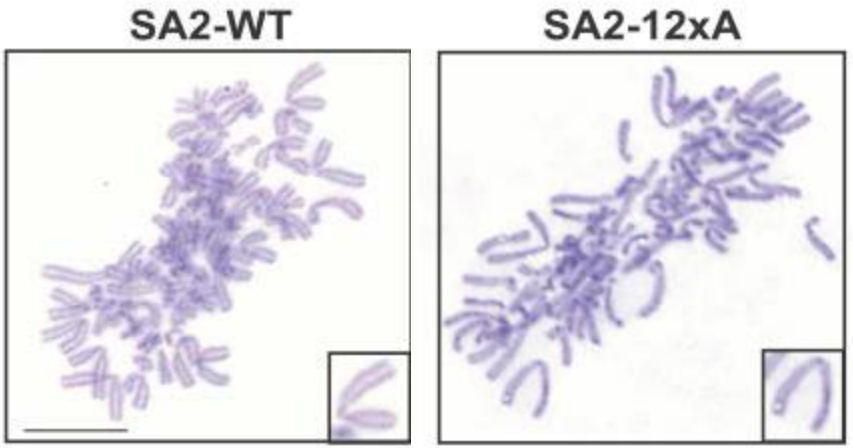


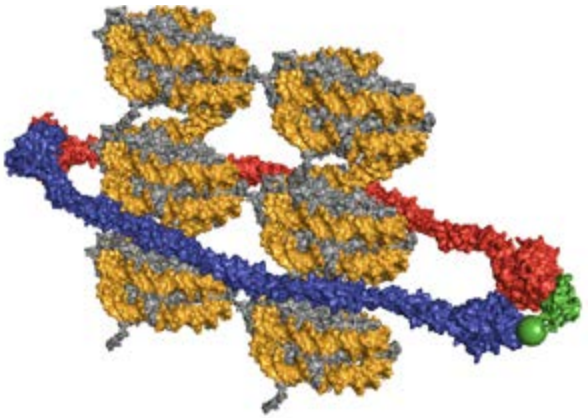
Recurrently mutated genes



All four cohesin complex genes are mutated in AML

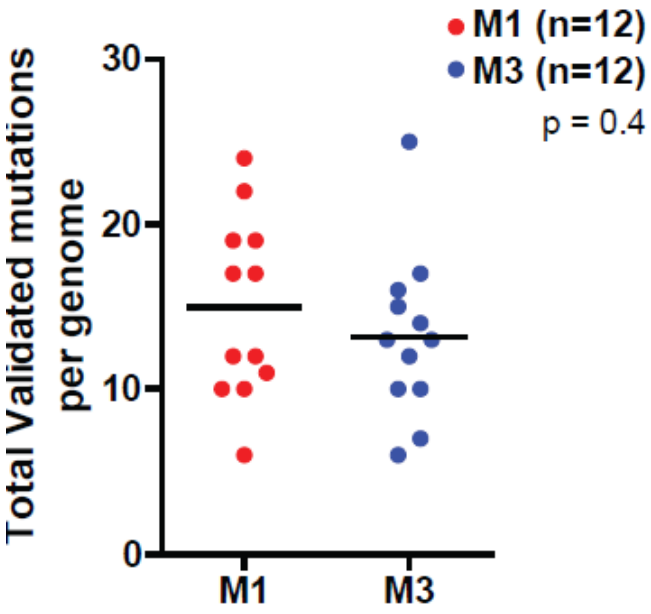




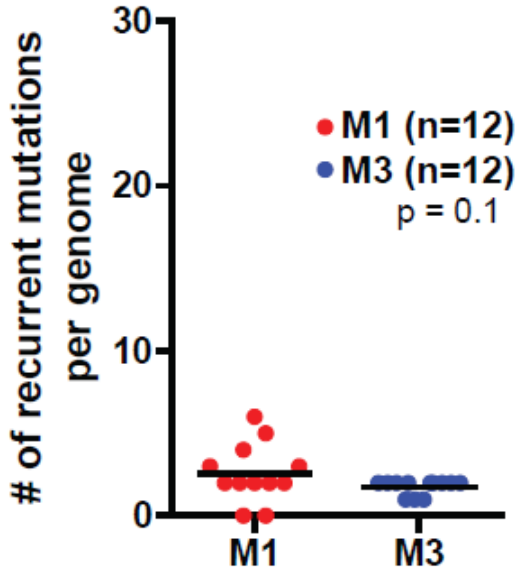


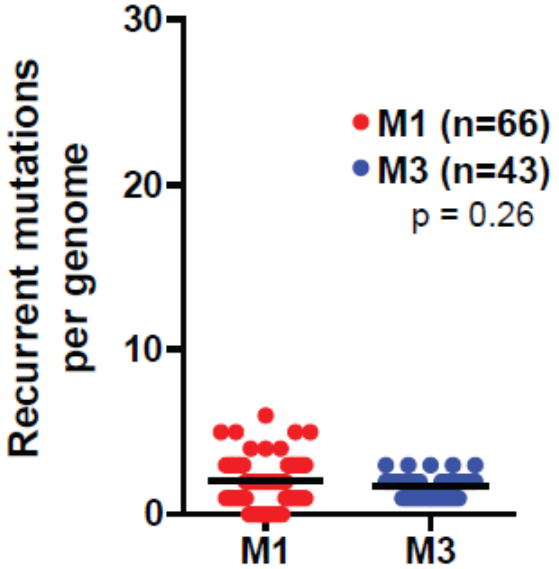
Hauf et al 2005 and Kim Nasmyth

Number of recurrently mutated genes per genome

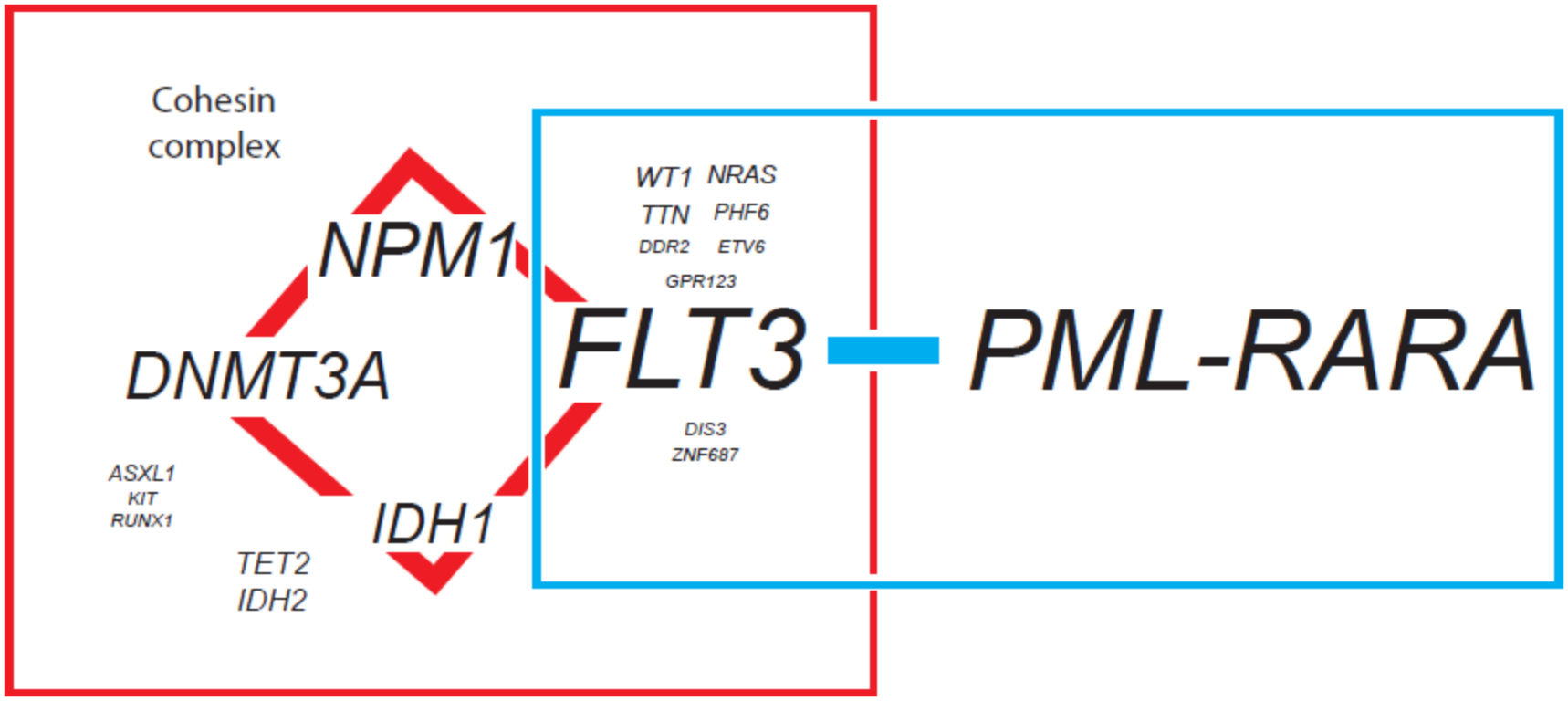


All Tier 1 mutations





Recurrent mutations with translational consequences

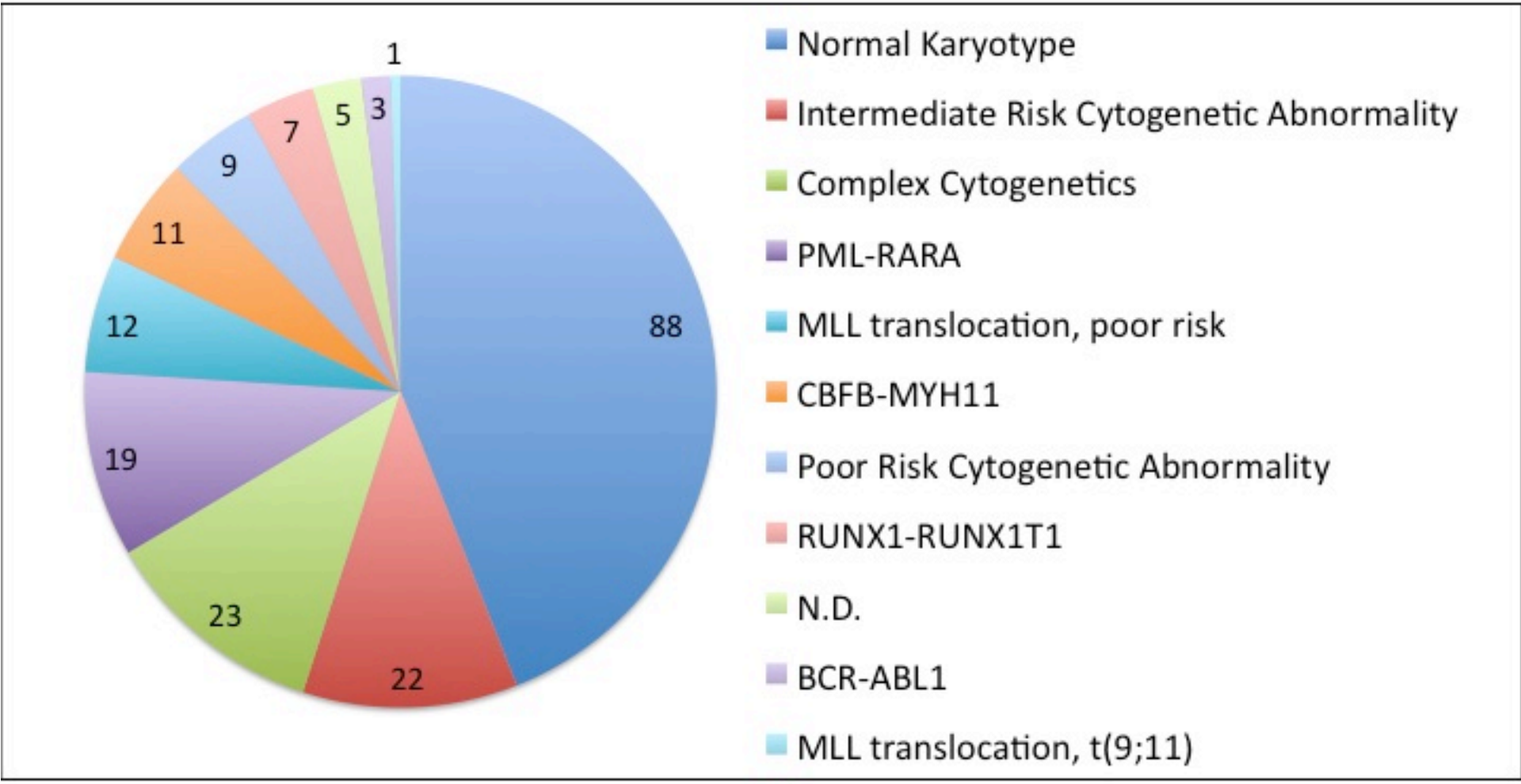

Cohesin complex
NPM1
DNMT3A
ASXL1
KIT
RUNX1
IDH1
TET2
IDH2
WT1
NRAS
TTN
PHF6
DDR2
ETV6
GPR123
FLT3
DIS3
ZNF687
PML-RARA
M1 initiating mutations
Progression mutations
M3 initiating mutation

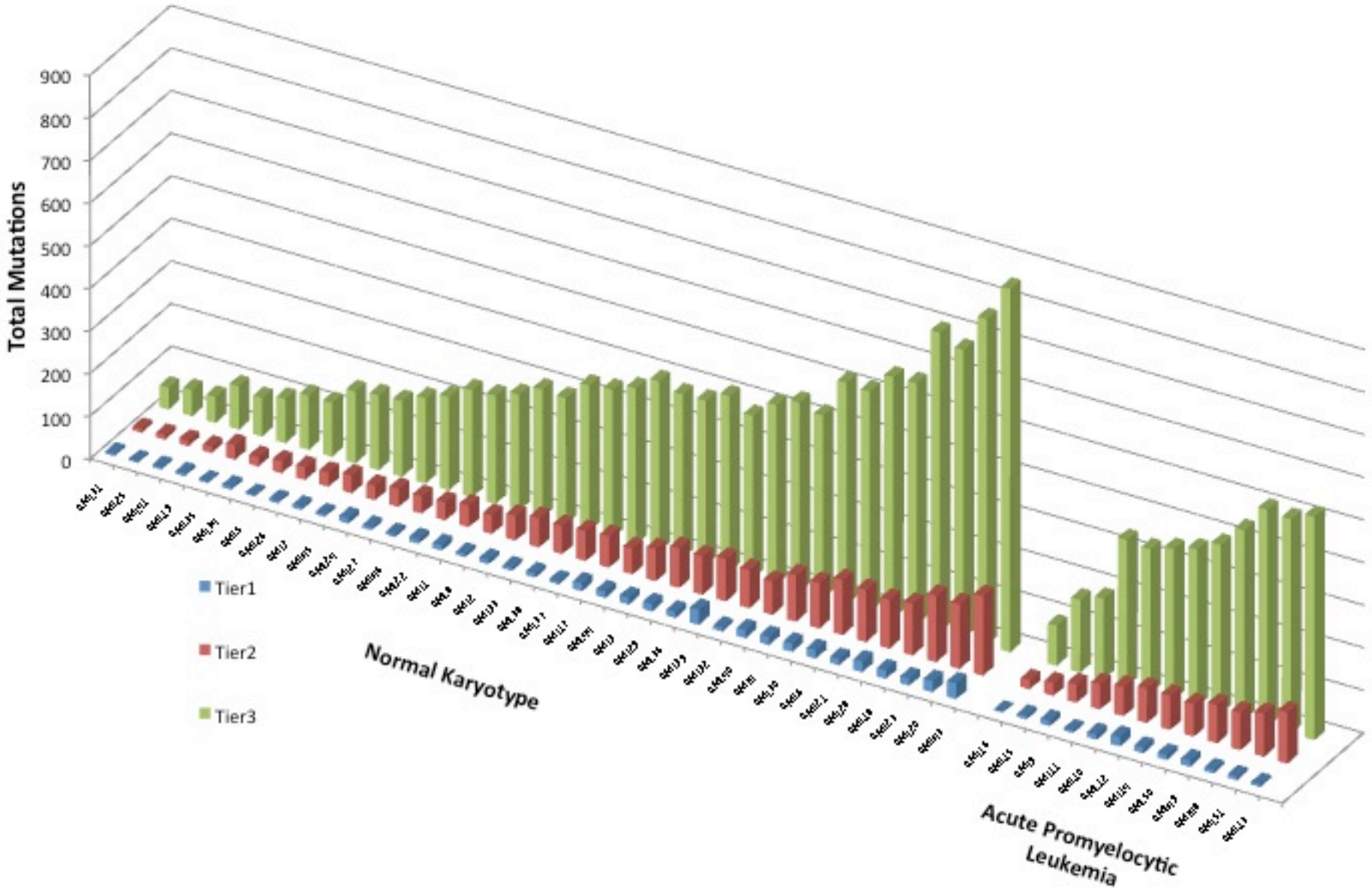
The AML 200 project

- 50 WGS, *de novo* AML tumor/normal pairs
  - 12 M1 with NK
  - 12 M3 with t(15;17)
  - 26 NK, any FAB subtype
- 150 exomes (Broad)
- 173 transcriptomes (BC)
- 192 Methylation arrays (USC)

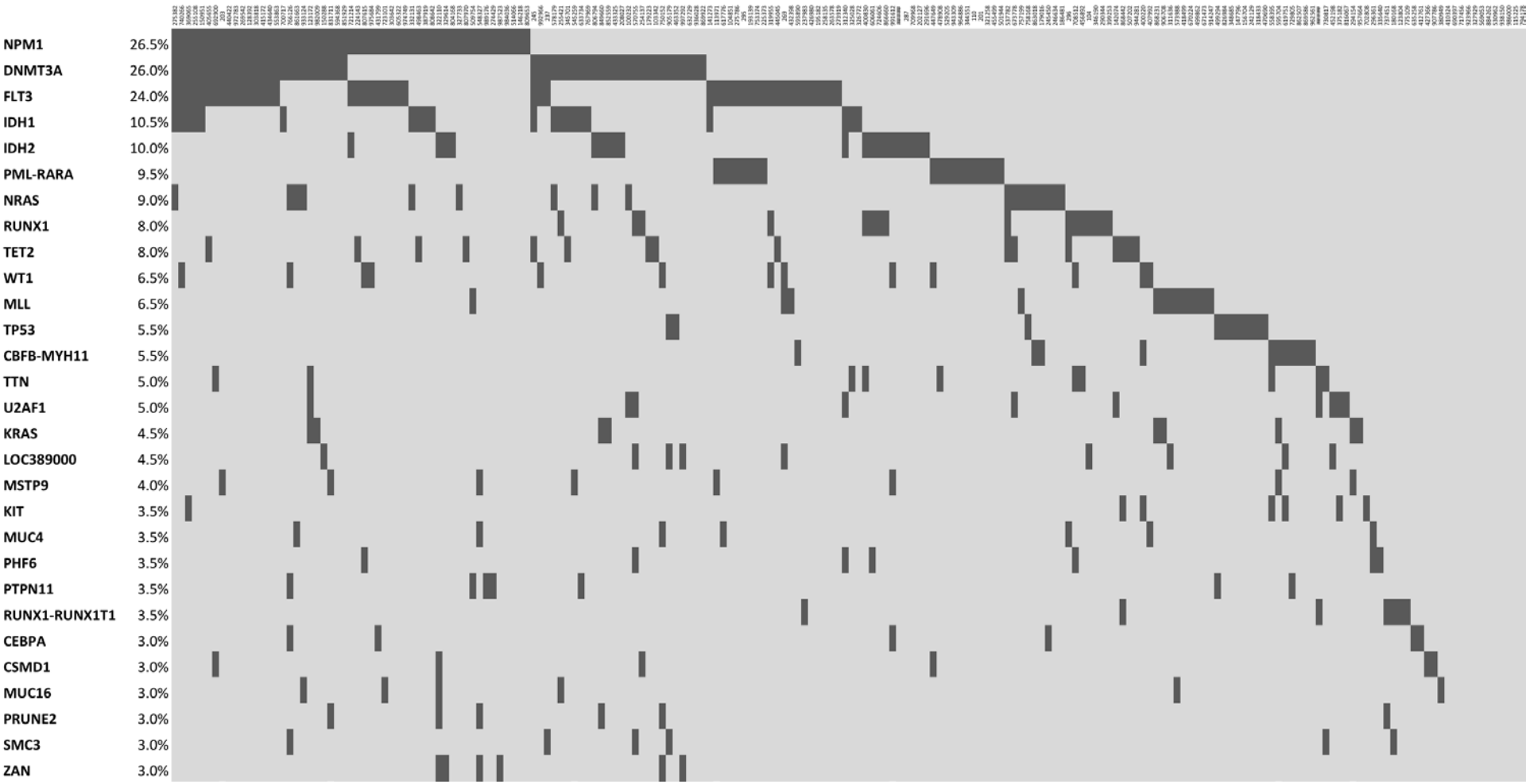
- 50 WGS with primary refractory/early relapse

AML200


88
22
23
19
12
11
9
7
5
3
1
Normal Karyotype
Intermediate Risk Cytogenetic Abnormality
Complex Cytogenetics
PML-RARA
MLL translocation, poor risk
CBFB-MYH11
Poor Risk Cytogenetic Abnormality
RUNX1-RUNX1T1
N.D.
BCR-ABL1
MLL translocation, t(9;11)

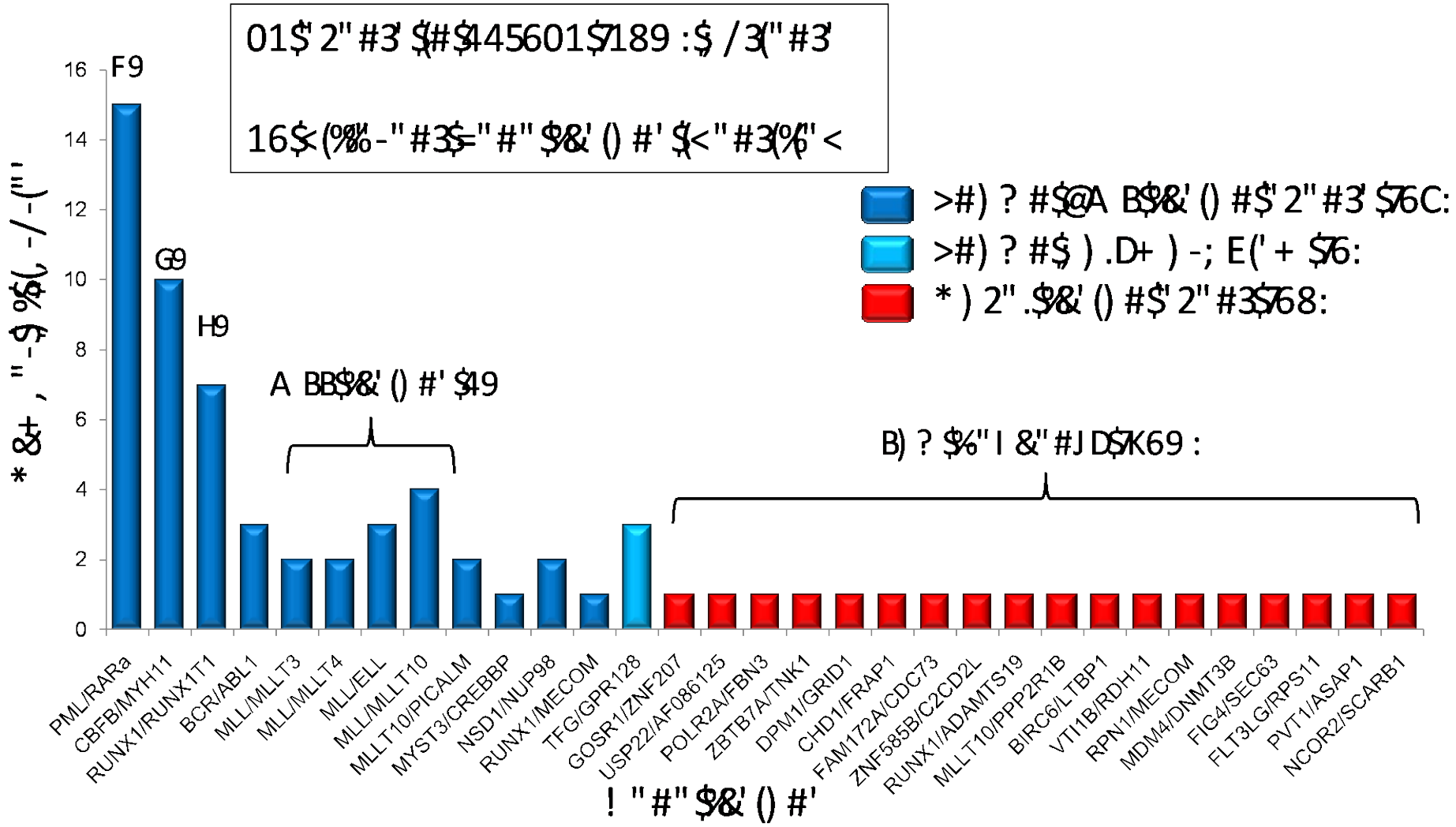

Total Mutations
900
800
700
600
500
400
300
200
100
0
Tier1
Tier2
Tier3
Normal Karyotype
Acute Promyelocytic Leukemia

Recurrent non-synonymous mutations
not all validated



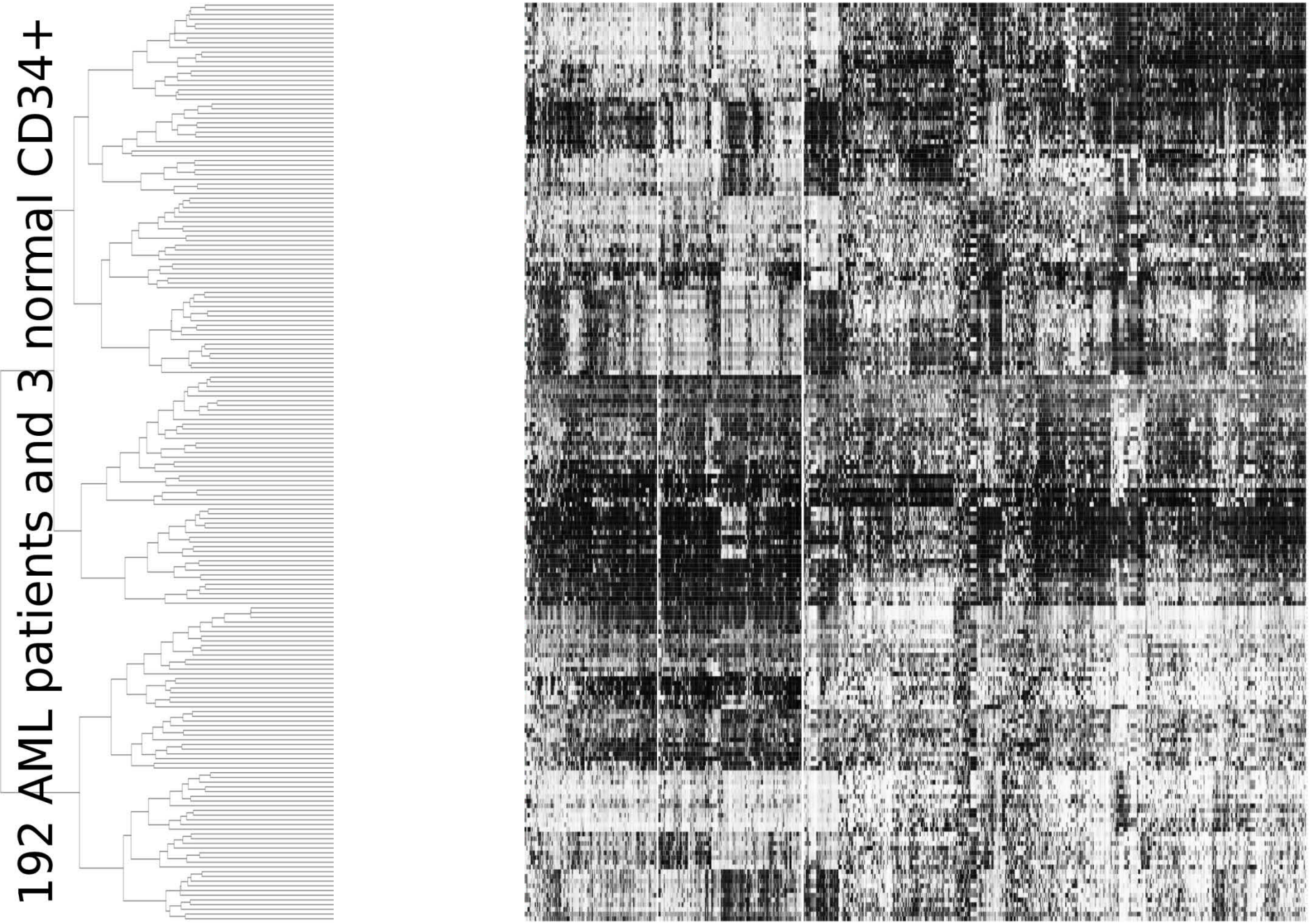
J Klco, Ben Raphael

Verified gene fusions identified from *de novo* assembly of AML transcriptomes



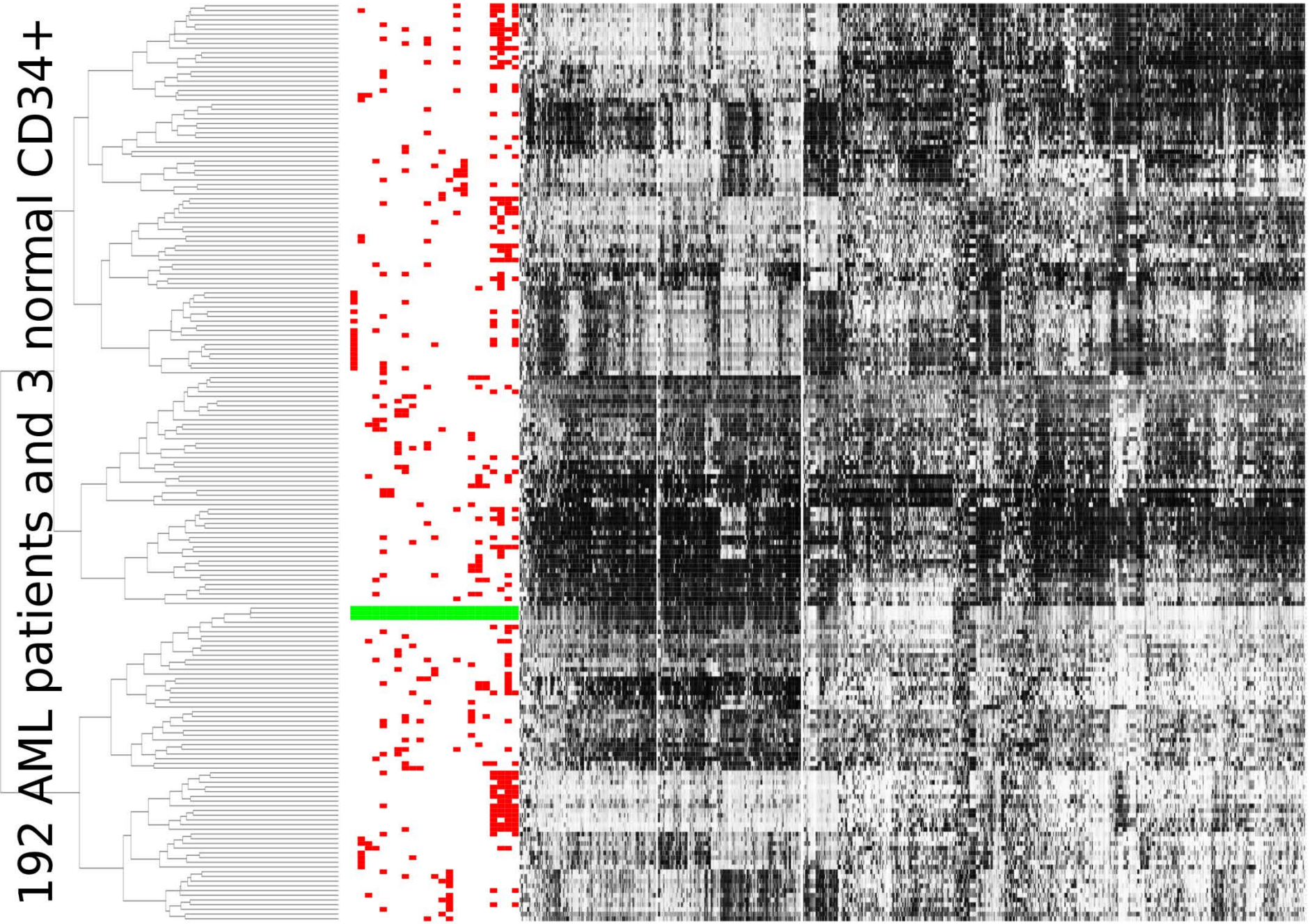
Andy Mungall, BC

450K Illumina methylation data vs Common AML mutations

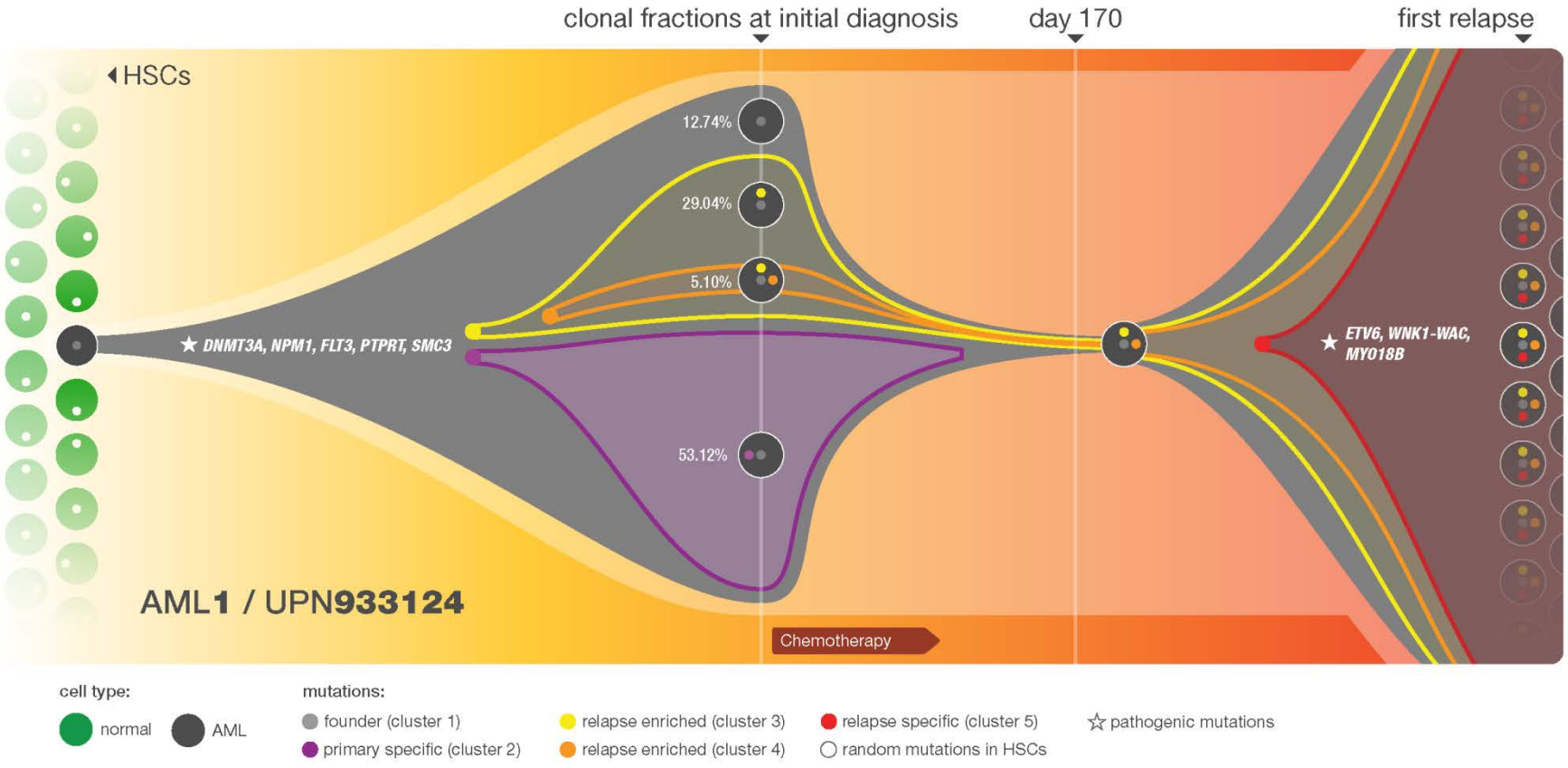


Tim Triche, Peter Laird

450K Illumina methylation data vs Common AML mutations



Tim Triche, Peter Laird


clonal fractions at initial diagnosis
day 170
first relapse
HSCs
12.74%
29.04%
5.10%
53.12%
★ DNMT3A, NPM1, FLT3, PTPRT, SMC3
★ ETV6, WNK1-WAC, MYO18B
AML1 / UPN933124
Chemotherapy
cell type:
normal
AML
mutations:
founder (cluster 1)
primary specific (cluster 2)
relapse enriched (cluster 3)
relapse enriched (cluster 4)
relapse specific (cluster 5)
random mutations in HSCs
☆ pathogenic mutations

Acknowledgements:

Our patients
TCGA
NCI
NHGRI
Alvin J. Siteman

Acknowledgements

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  - Bob Fulton
  - Lucinda Fulton
  - Sean McGrath
  - Mike McLellan
  - Dan Koboldt
  - Joelle Veizer
  - Heather Schmidt
  - And many more
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  - Jackie Payton
  - John Welch
  - Lukas Wartman
  - Jeff Klco
  - Dan Link
  - Michael Tomasson
  - Tim Graubert
  - Peter Westervelt
  - Sharon Heath
  - Shashi Kulkarni
  - Mark Watson
  - Bill Shannon
  - Rakesh Nagarajan
  - Jack Baty
  - Tamara Lamprecht
  - And many more