Somatic mutations drive specific, but reversible epigenetic heterogeneity states in leukemia

Sheng Li, PhD
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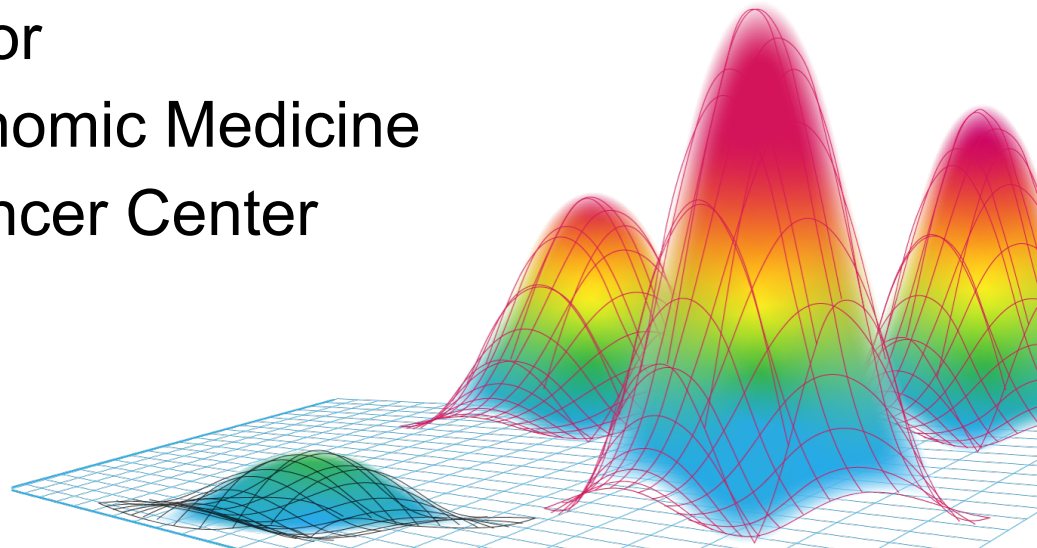
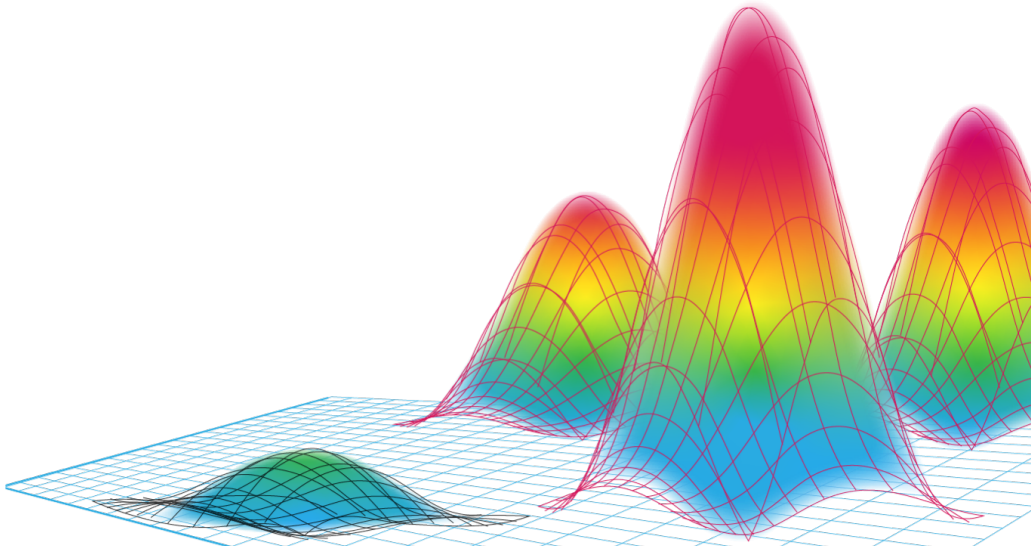


The Jackson Laboratory for Genomic Medicine

Computational epigenomics and therapeutics

- Algorithms | data mining
- Somatic mutations | epigenome heterogeneity
- Leukemia | aging
- Therapeutics | prevention

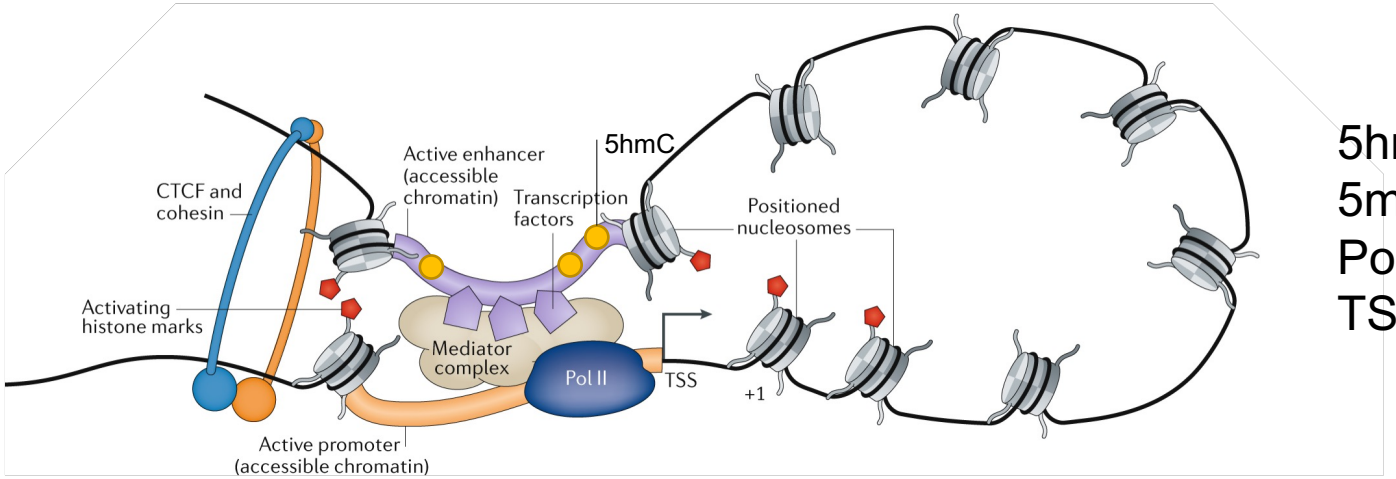


Epigenetic modifications turn genes “on” and “off” to affect expression

- Epigenetic modifications:
  - Heritable
  - Reversible
  - Major types:
    - DNA:
      - methylation (5mC)
      - hydroxymethylation (5hmC)
    - Histone:
      - Methylation
      - Acetylation
    - Non-coding RNAs

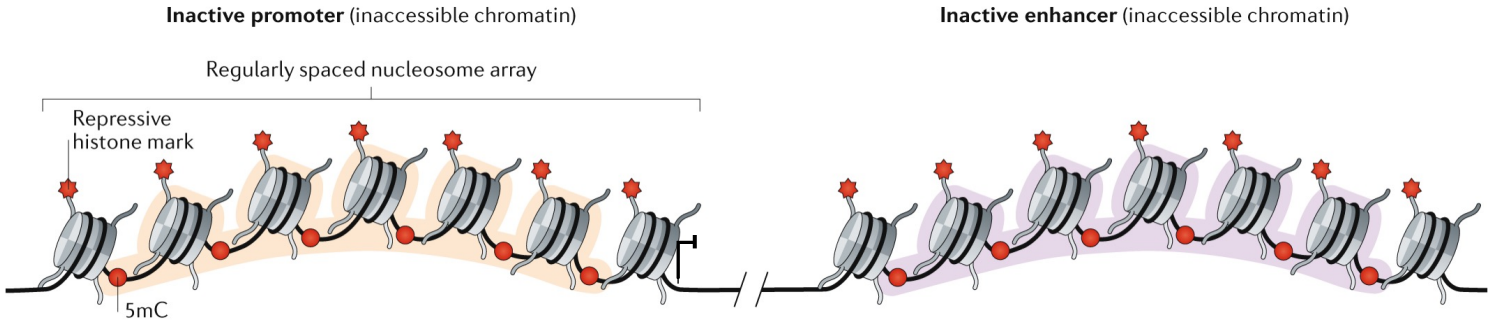
Epigenetic landscapes of transcribed and silent genes in eukaryotes

Active genes "On"



5hmC, 5-hydroxymethylation
5mC, 5-methylcytosine
Pol II, Polymerase II
TSS, transcription start site

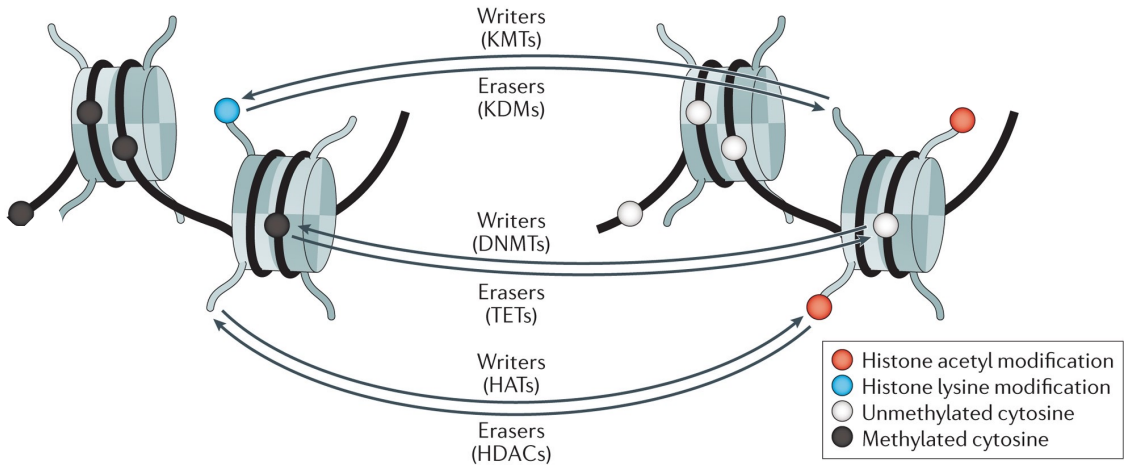
Repressed genes "Off"

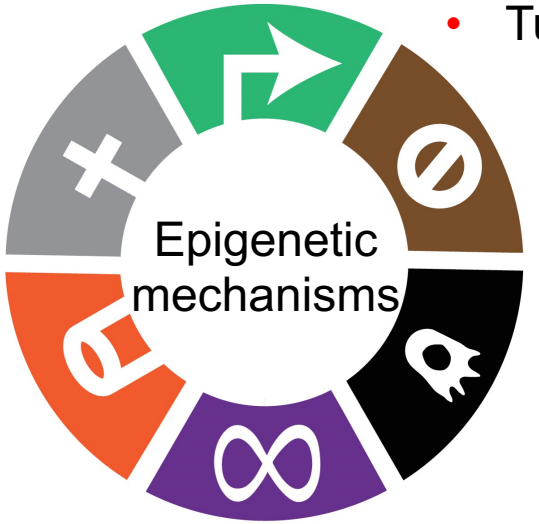


DNA and histone modifications controls the accessibility of DNA to transcription factors and other regulators.

Adapted from Carter and Zhao, Nature Reviews Genetics, 2021

More than 50% of human cancers harbor mutations in genes that encode regulators of the epigenome





- Tumor suppressor silencing
- Oncogene activation
- Cell fate transitions

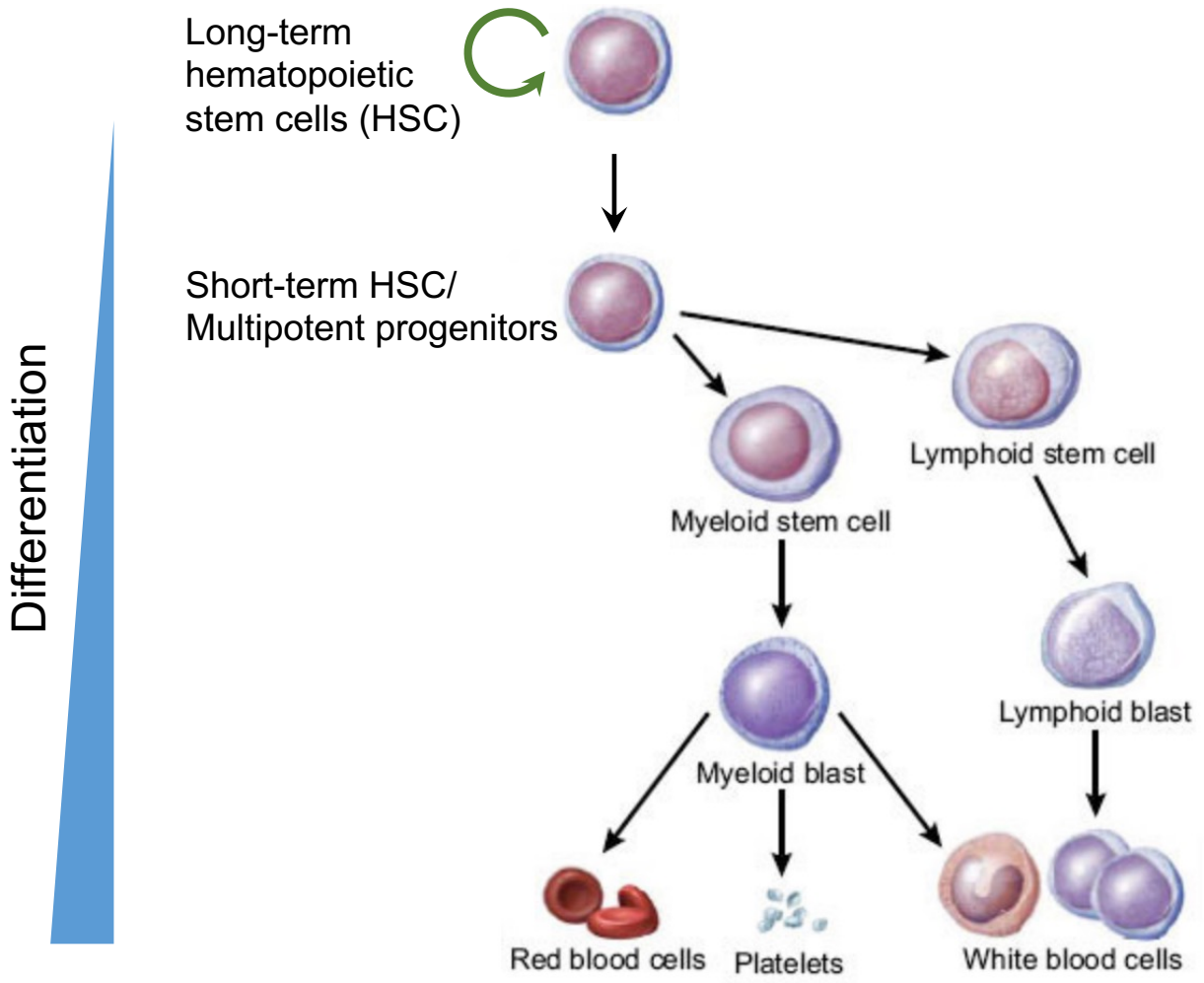
- Writers:
  - DNA methyltransferases (DNMTs)
  - Histone acetyltransferases (HATs)
  - Histone methyltransferases (HMTs)
- Erasers:
  - Ten-eleven translocation (TET) family of 5mC oxidases
  - Histone deacetylases (HDACs)
  - …

FDA-approved drugs targeting the epigenome

- 2 DNMT inhibitors
- Isocitrate dehydrogenase (IDH) 1 and 2 mutant inhibitors
  - IDH: TET regulators
- 4 HDAC inhibitors
- 1 HMT inhibitor

Jones, Issa, and Baylin, Nature Review Genetics, 2016
Adapted from Flavahan, Gaskell, and Bernstein, Science 2017
Bates, NEJM, 2020

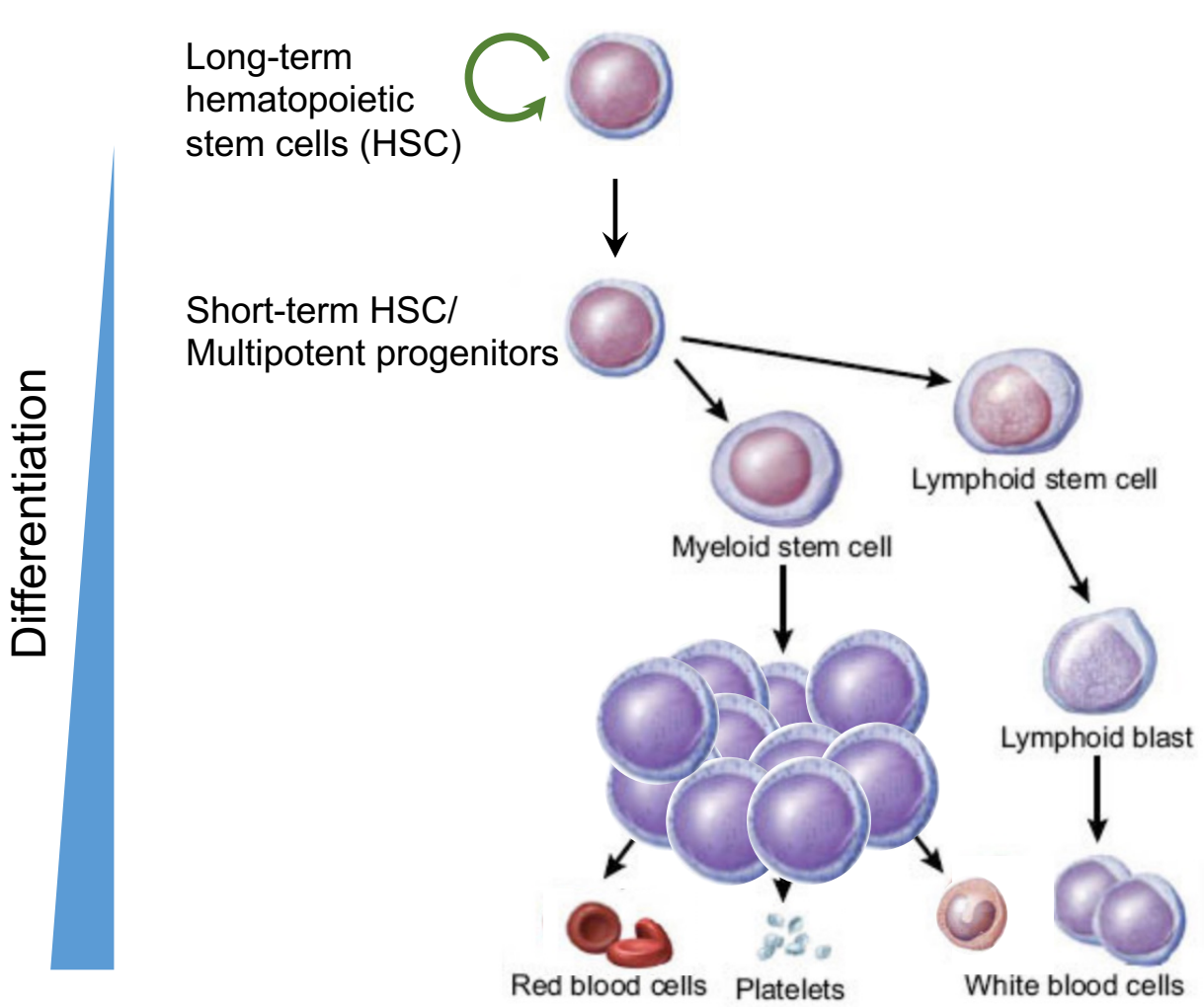
Epigenetic regulation of cell fate decisions of hematopoietic system

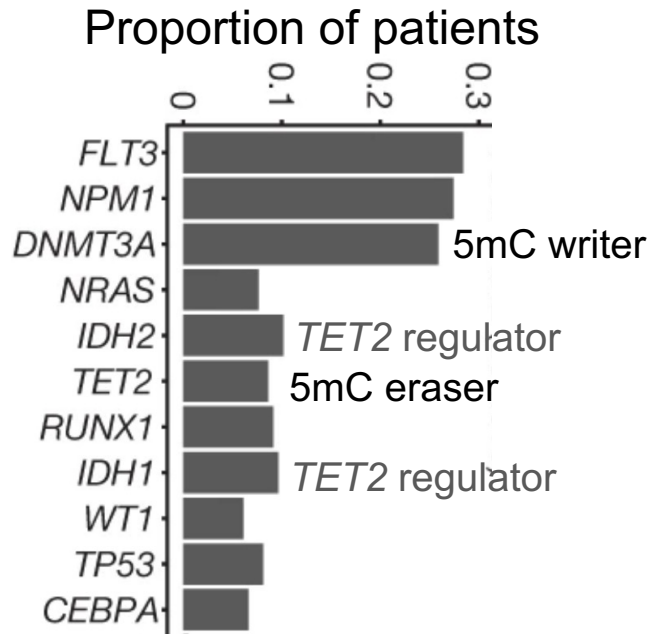


Gatekeepers of hematopoiesis:

- TET2 (5mC eraser)
- IDH1/2 (TET regulators)
- DNMT3A (5mC writer)
- …

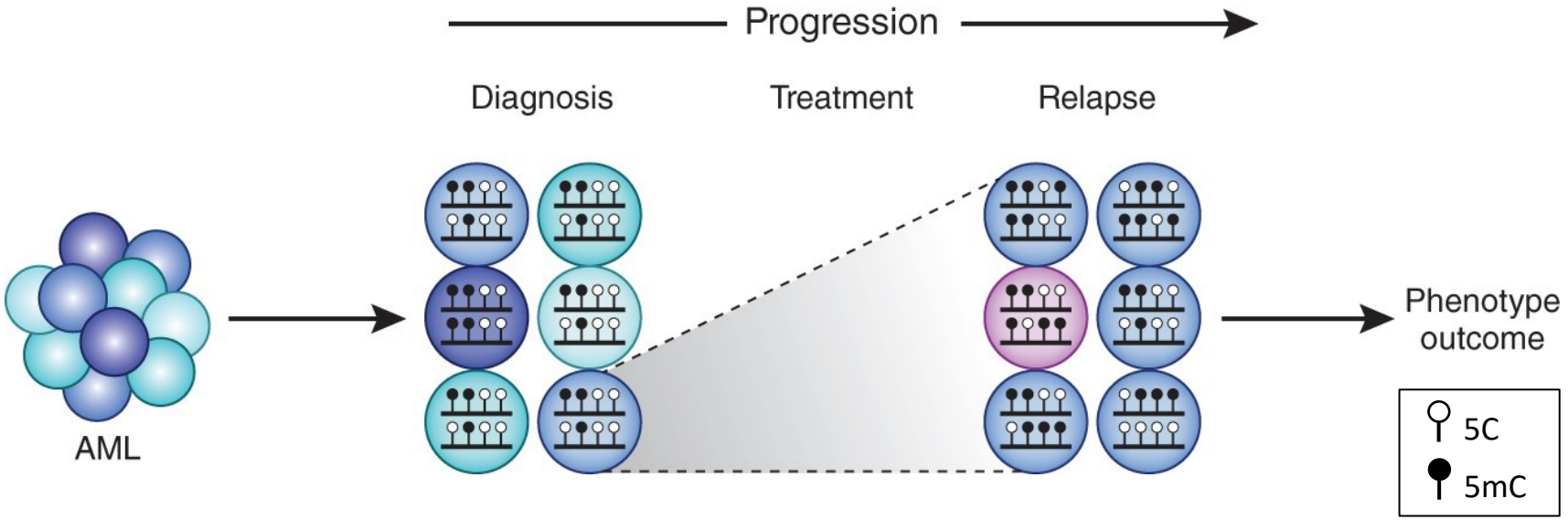
Epigenetic dysregulation: a hallmark of AML

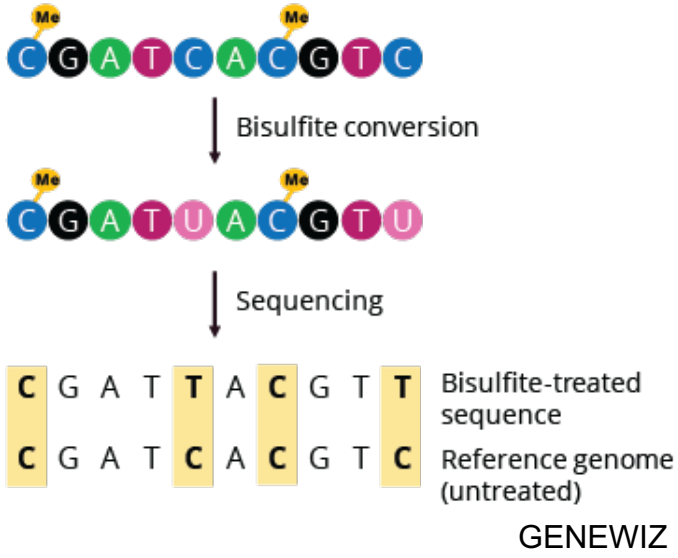




Tyner, et al., 2018. Nature

Hematopoietic clones from relapse AML manifest *selection* of malignant cells harboring specific epigenetic alleles





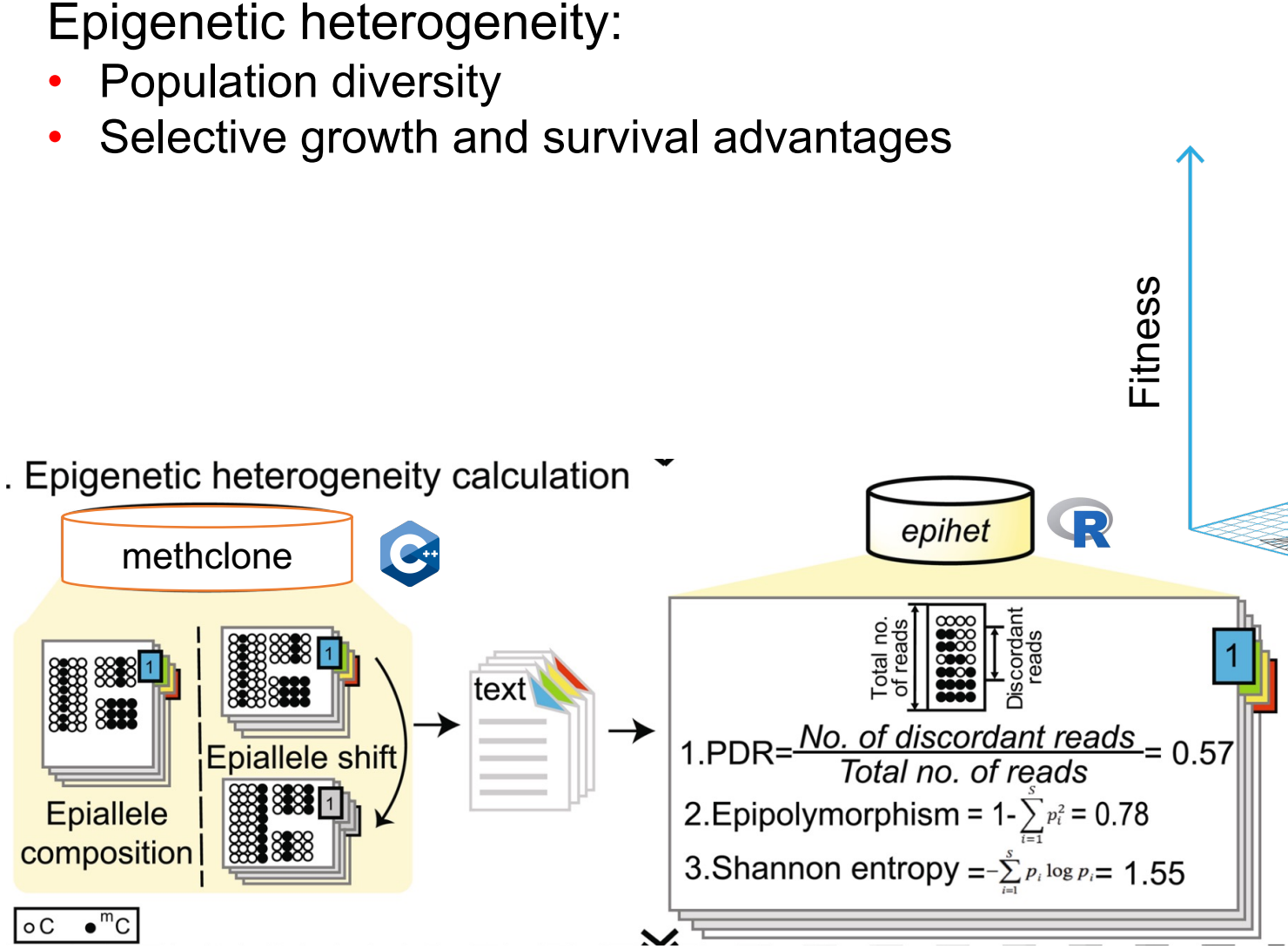
- Epialleles (epigenetic alleles) selection:
  - *Phased* DNA methylation patterns
  - Disease severity of newly diagnosed AML

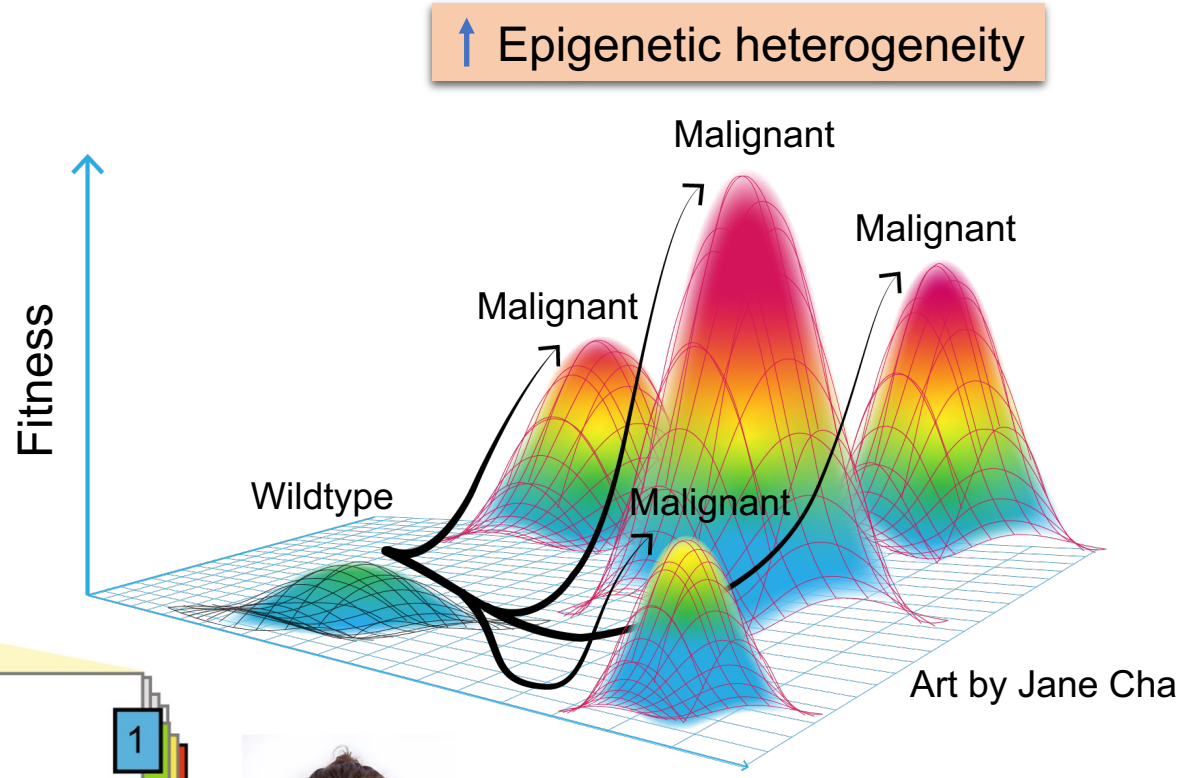
Li S et al., Genome Biology. 2014
Li S et al., Nature Medicine. 2016
Sasca & Huntly, Nature Medicine, 2016

AML epigenetic heterogeneity may provide malignant cells with an additional layer of fitness beyond genetic heterogeneity

Epigenetic heterogeneity:

- Population diversity
- Selective growth and survival advantages





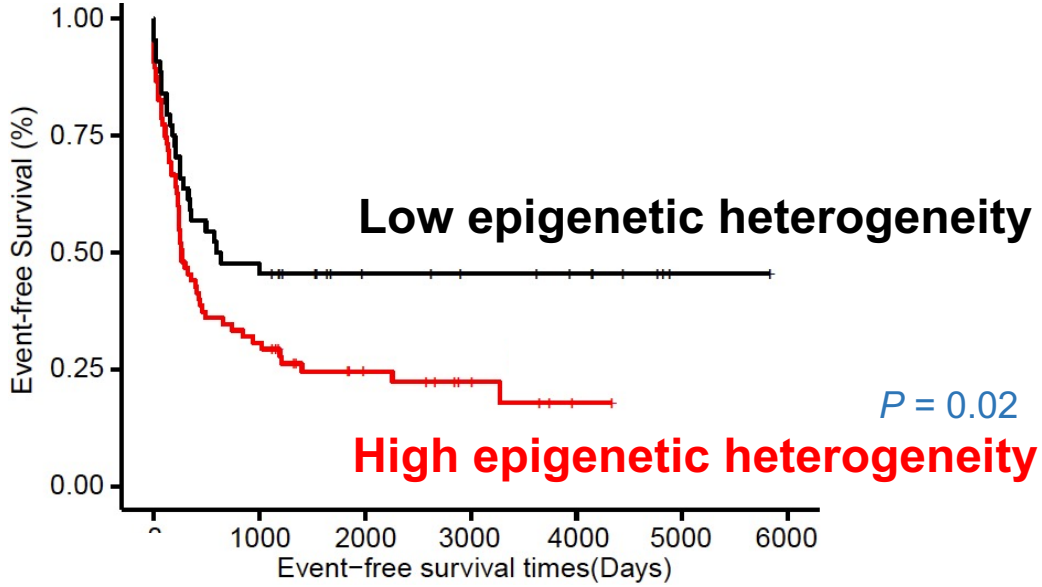
Xiaowen Chen, Ph.D.
Research Scientist

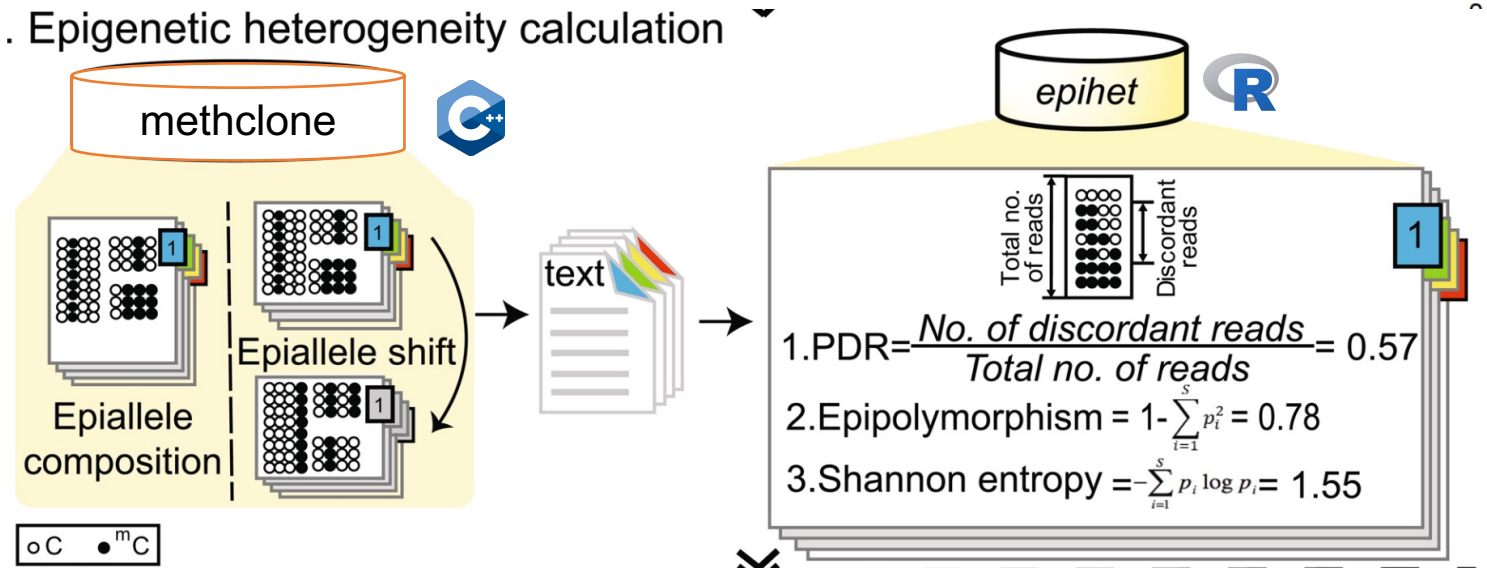
Landan, et al., Nature Genetics, 2012
Li, et al., Genome Biology, 2014
Landau, et al., Cancer Cell, 2014
Li, et al., Cancer Discovery, 2020
Chen, … Li, Scientific Reports, 2021

AML epigenetic heterogeneity may provide malignant cells with an additional layer of fitness beyond genetic heterogeneity

Epigenetic heterogeneity:

- Population diversity
- Selective growth and survival advantages
- *Unfavorable outcomes of AML*





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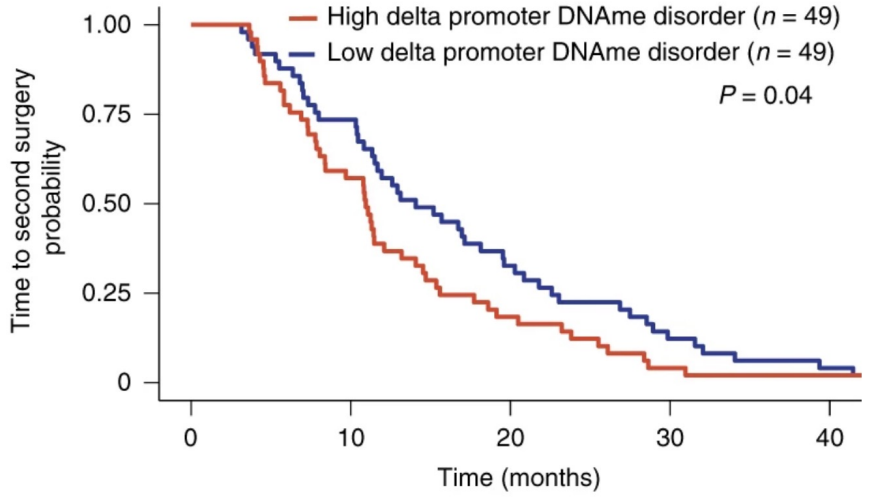
Li, et al., Cancer Discovery, 2020
Chen, … Li, Scientific Reports, 2021

Higher epigenetic heterogeneity is associated with poor clinical outcome in hematopoietic and solid tumors

- Other hematopoietic malignancies:
  - Landau, et al., *Locally disordered methylation forms the basis of intra-tumor methylome variation in **chronic lymphocytic leukemia***. Cancer Cell. 2014
  - Pan, et al., *Epigenomic evolution in **diffuse large B-cell lymphomas***. Nature Communications. 2015
  - Gaiti, et al., *Epigenetic evolution and lineage histories of **chronic lymphocytic leukaemia***. Nature. 2019
- Solid tumors:
  - Johnson, et al., *Single-cell multimodal **glioma** analyses identify epigenetic regulators of cellular plasticity and environmental stress response*. Nature Genetics. 2021



Source?

Two possible scenarios for the source of epigenetic heterogeneity

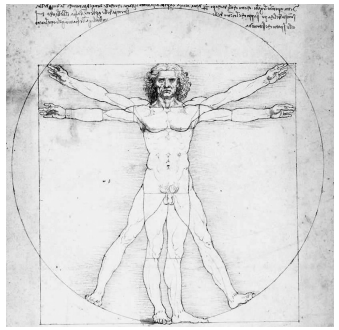
- Stochastic
  - Unrelated to somatic mutations
  - A by-product of AML disease progression

- Induced by somatic mutations
  - Disrupt regulatory states
  - Normally under strict control
  - Precede and even contribute to malignant transformation

What are the source of epigenetic heterogeneity in AML?

Hypothesis: somatic mutations affecting epigenetic regulators induce epigenetic heterogeneity

Primary AML (n =119) & Normal bone marrow (n = 14)



67 Males; 52 Females;
Median Age: 44.

Blood or bone marrow

Lymphocytes depletion

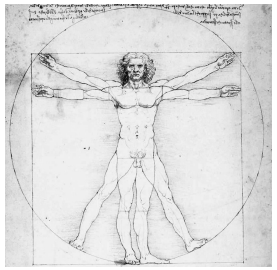
Genomic Profiling

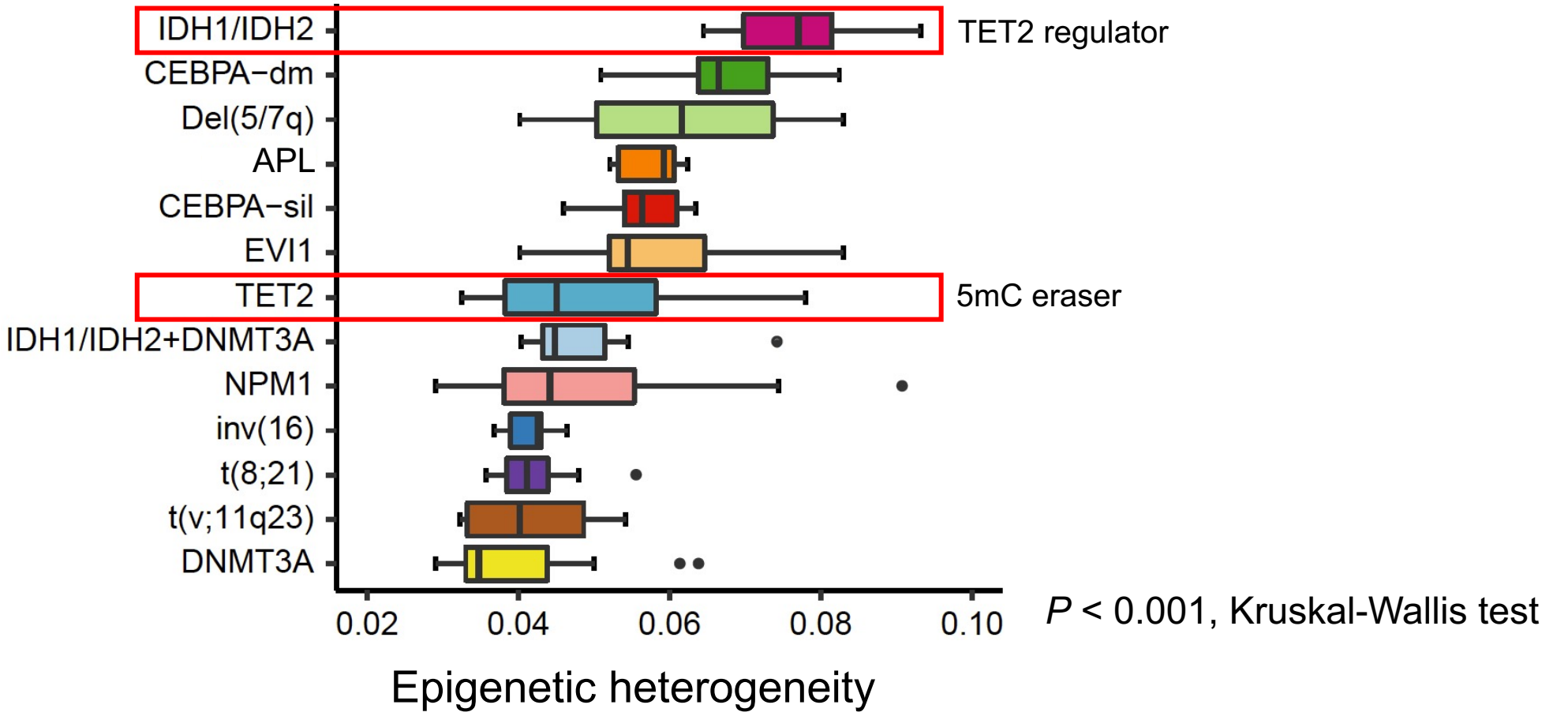
RRBS: single molecule DNA methylation profile

Targeted sequencing: genetic lesions profiles

Glass J, et al., Cancer Discovery, 2017

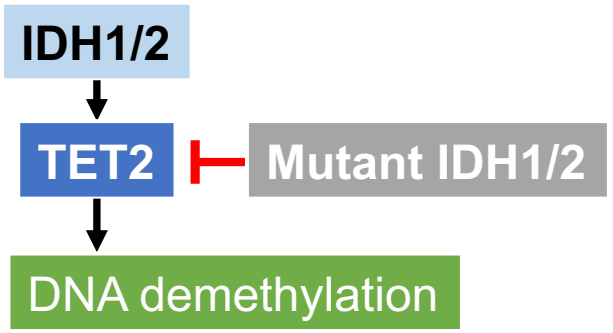
Epigenetic heterogeneity links to underlying genetic lesions

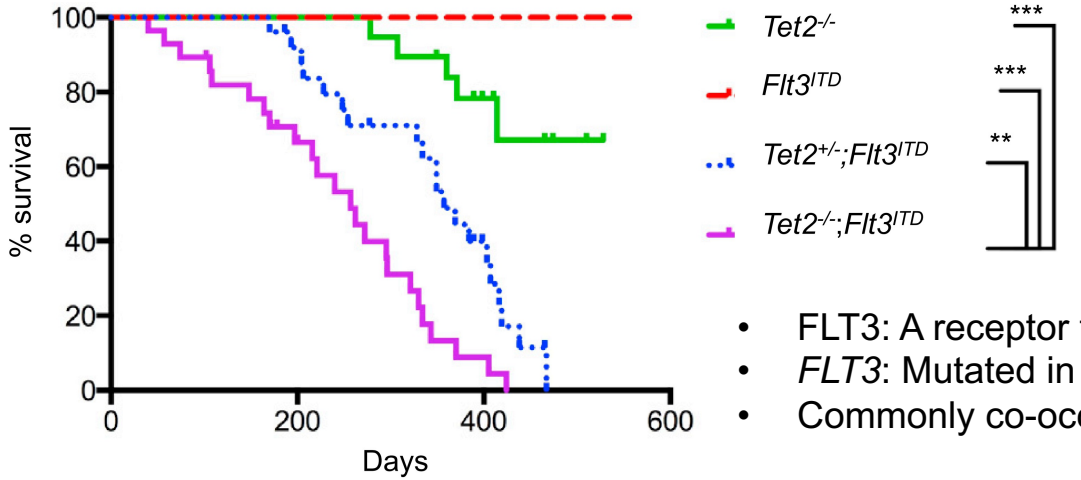




Li S*#, Chen X*, …, Melnick#, Cancer Discovery, 2020

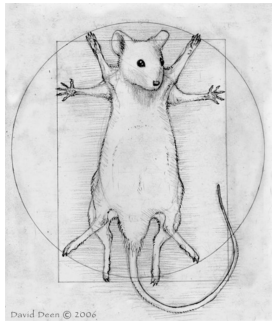
Hypothesis: somatic mutations induce epigenetic heterogeneity





- FLT3: A receptor tyrosine kinase
- *FLT3*: Mutated in 30% AML
- Commonly co-occur with *TET2* mutations

Idh2^{R140Q};Flt3^{ITD}
Tet2$^{-/-}$;Flt3^{ITD}



Healthy (non-leukemic) mice

LSK (lin$^-$Sca$^+$cKit$^+$) cells

Early form of HSCs

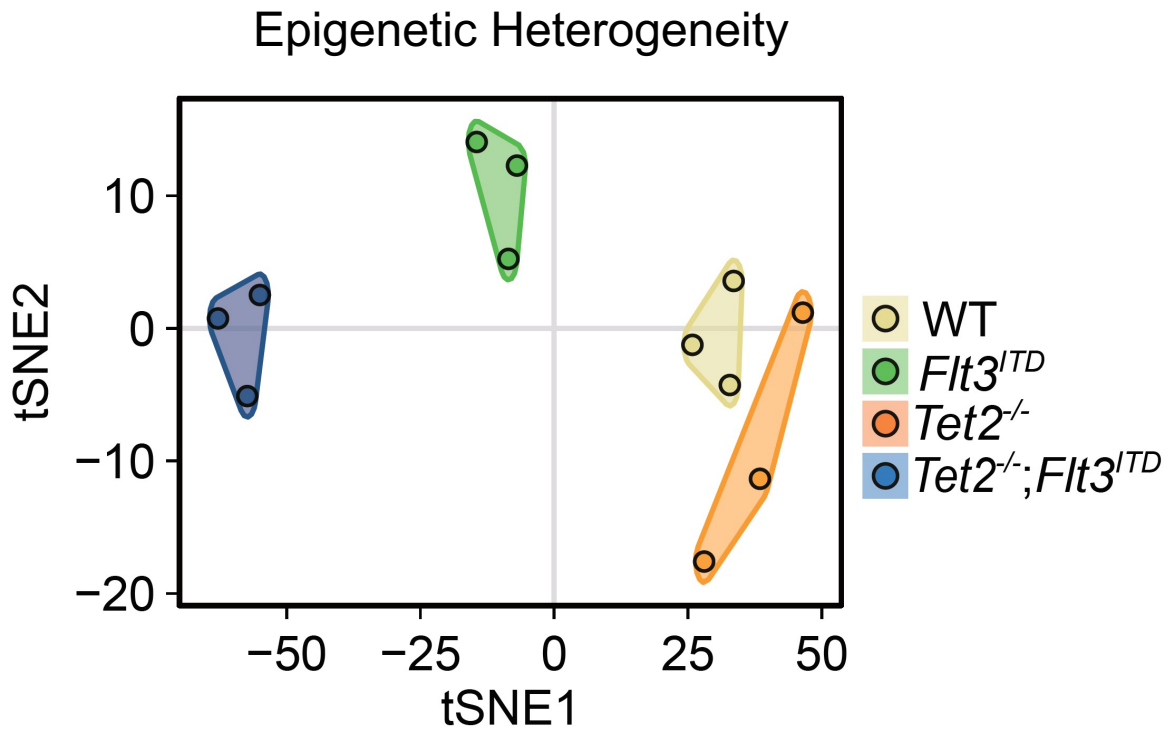
Genomic Profiling

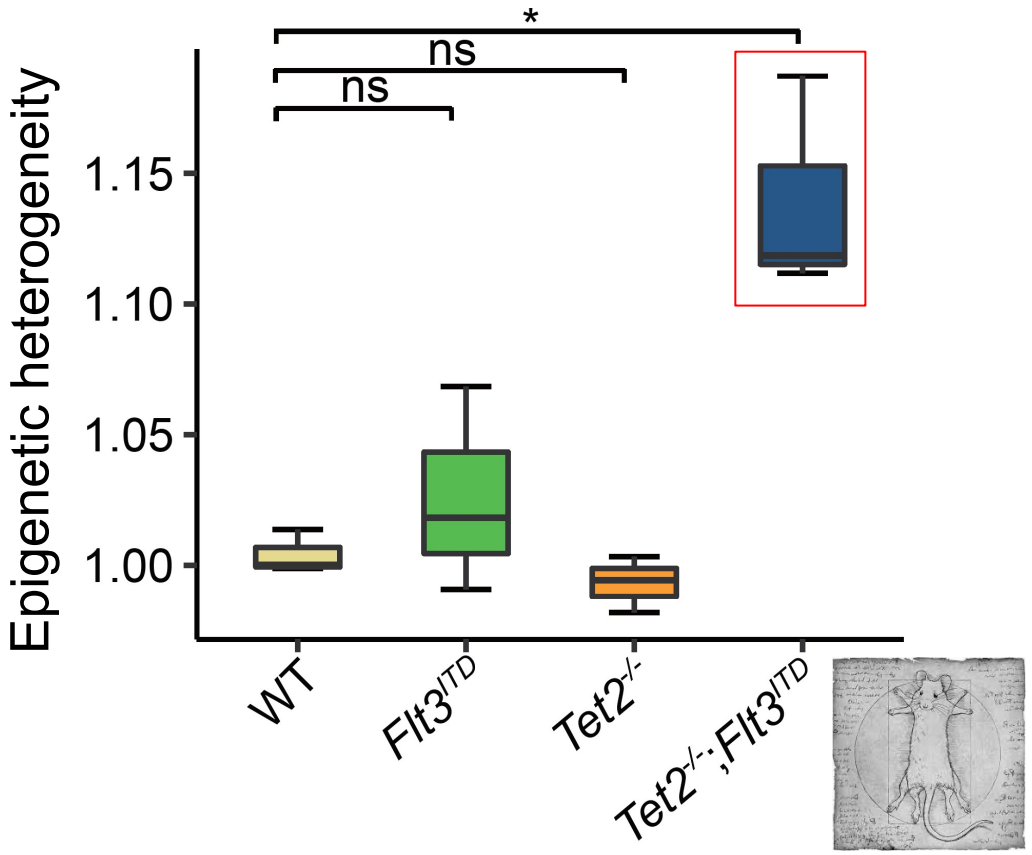
RRBS: single molecule DNA methylation profile

RNA-seq: gene expression profiles

Shih, et al., Cancer Cell, 2015

Cooperation between somatic mutations lead to enhanced epigenetic heterogeneity prior to leukemic transformation



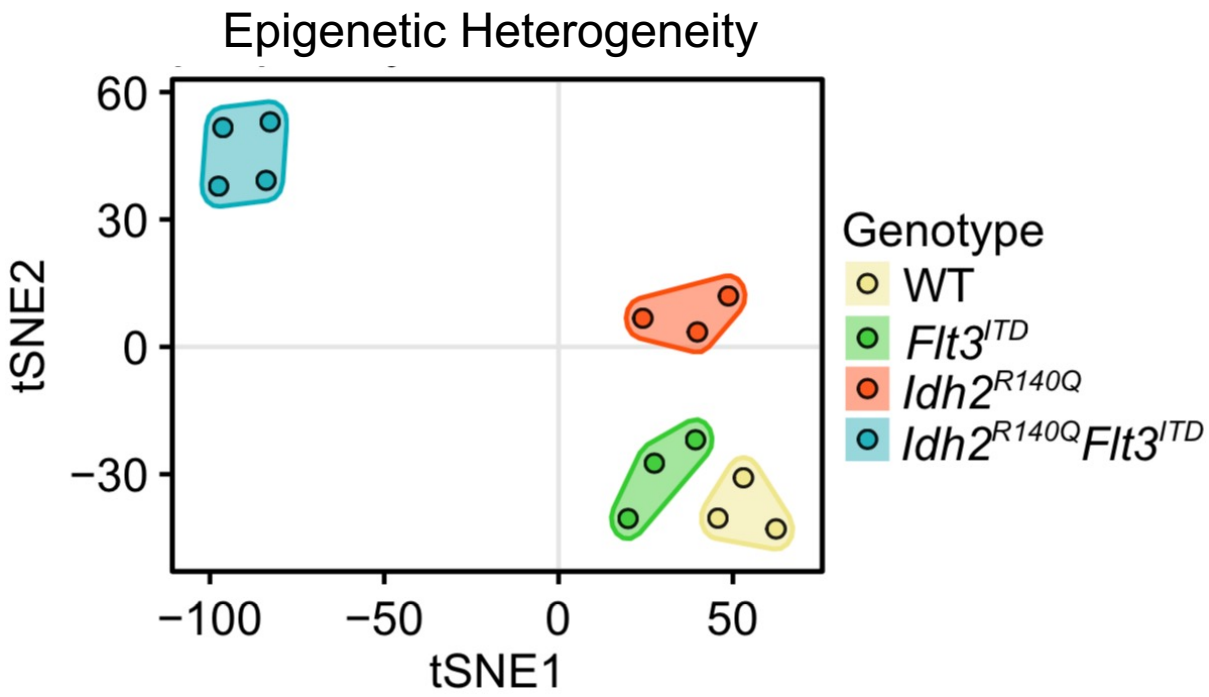


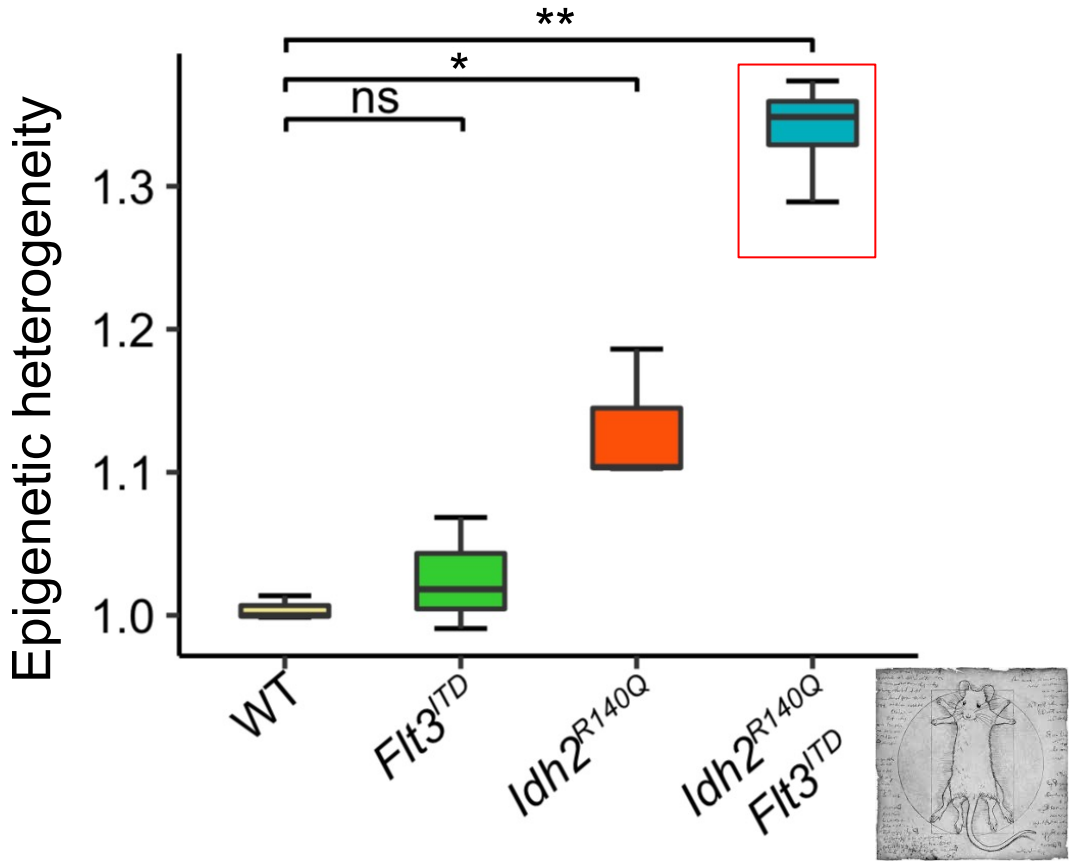
Methylomes data:

- LSK (lin⁻Sca⁺cKit⁺) cells
- ***Healthy*** (non-leukemic) mice

Li S*#, Chen X*, …, Melnick#, Cancer Discovery, 2020

Cooperation between somatic mutations lead to enhanced epigenetic heterogeneity prior to leukemic transformation



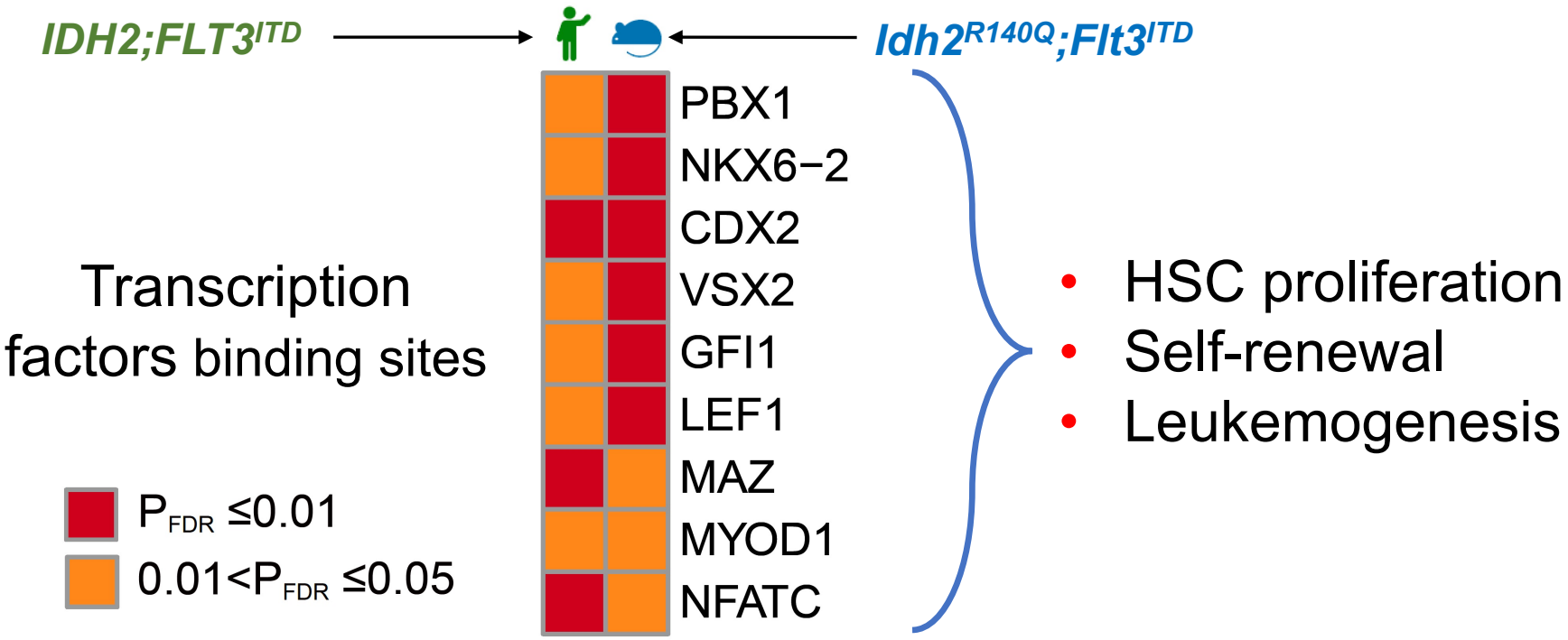


Methylomes data:

- LSK ($lin^{-}Sca^{+}cKit^{+}$) cells
- ***Healthy*** (non-leukemic) mice

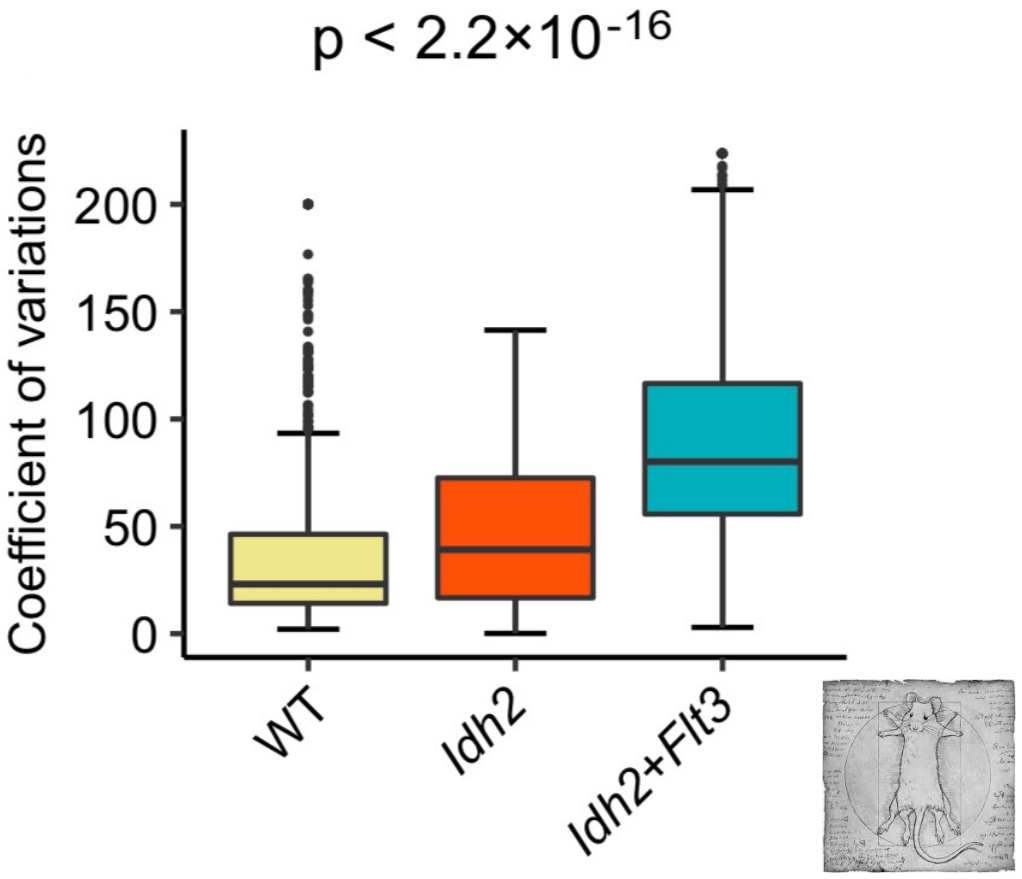
Li S*#, Chen X*, …, Melnick#, Cancer Discovery, 2020

Epigenetic heterogeneity arising in humans and mice may affect similar transcription factors relevant to AML biology



Li S*#, Chen X*, …, Melnick#, Cancer Discovery, 2020

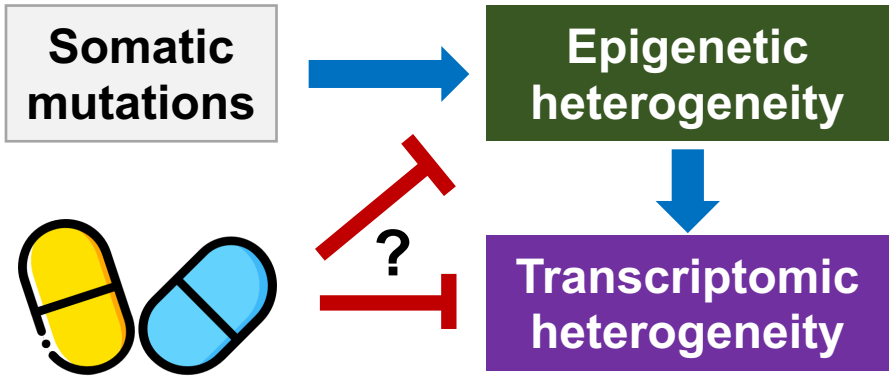
Transcriptomic heterogeneity is associated with epigenetic heterogeneity



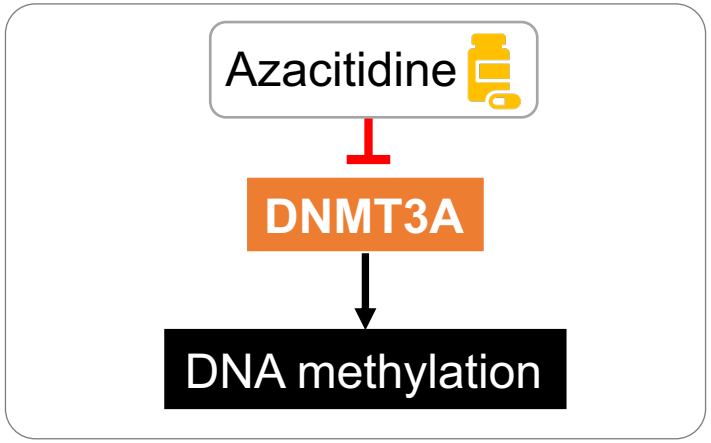
Li S*#, Chen X*, …, Melnick#, Cancer Discovery, 2020

Can epigenetic therapy reverse epigenetic and transcriptomic heterogeneity?

Clonal reduction as a therapeutic target?

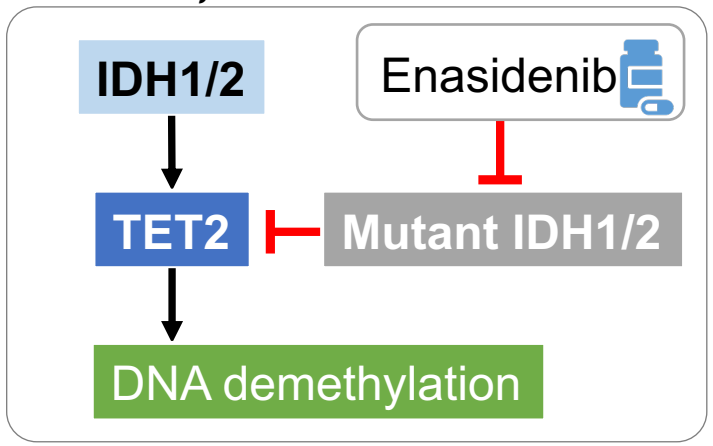


***Tet2*$^{-/-}$;*Flt3*^{ITD} AML mouse model**



- Azacitidine: global hypomethylating agent; FDA approval: 2004 (myelodysplastic syndrome); 2020 (oral, AML)

***Idh2*^{R140Q};*Flt3*^{ITD} AML mouse model**

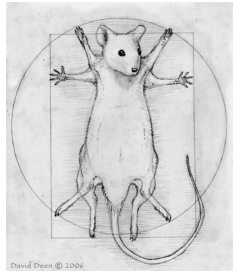


- Enasidenib: IDH2-mutant inhibitor; FDA approval: 2017; formerly AG-221

Can epigenetic therapy reverse epigenetic and transcriptomic heterogeneity?

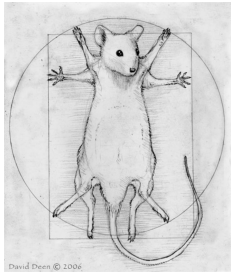
Leukemic mice

***Idh2*^{R140Q};*Flt3*^{ITD}**



Treatment:
IDH2 mutant inhibitor **Enasidenib**
FLT3 inhibitor
Vehicle

***Tet2*$^{-/-}$;*Flt3*^{ITD}**



Treatment:
Hypomethylating agent **Azacitidine**
FLT3 inhibitor
Vehicle

LSK ($lin^{-}Sca^{+}cKit^{+}$) cells

Early form of hematopoietic stem cells

Genomic Profiling

RRBS: single molecule DNA methylation profile

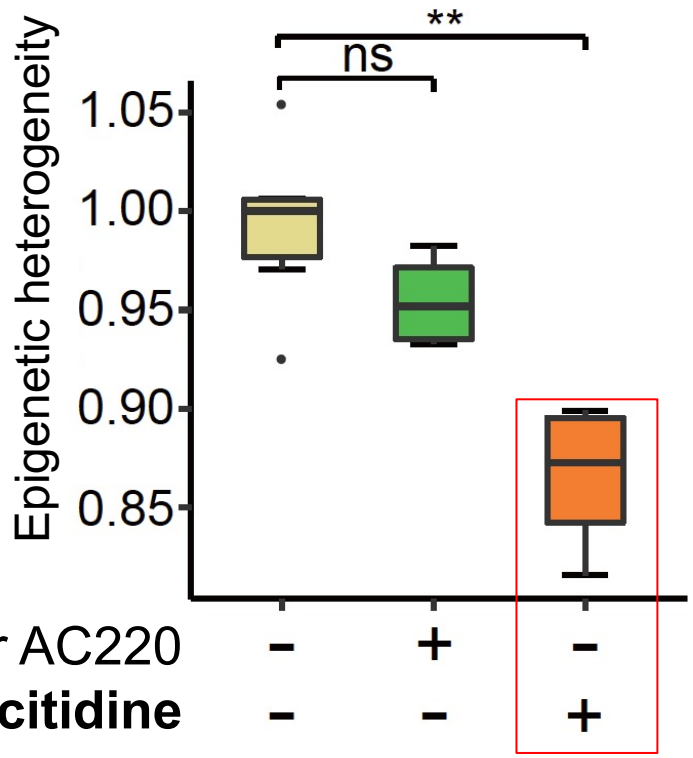
RNA-seq: gene expression profiles

Shih, et al., Cancer Discovery, 2017

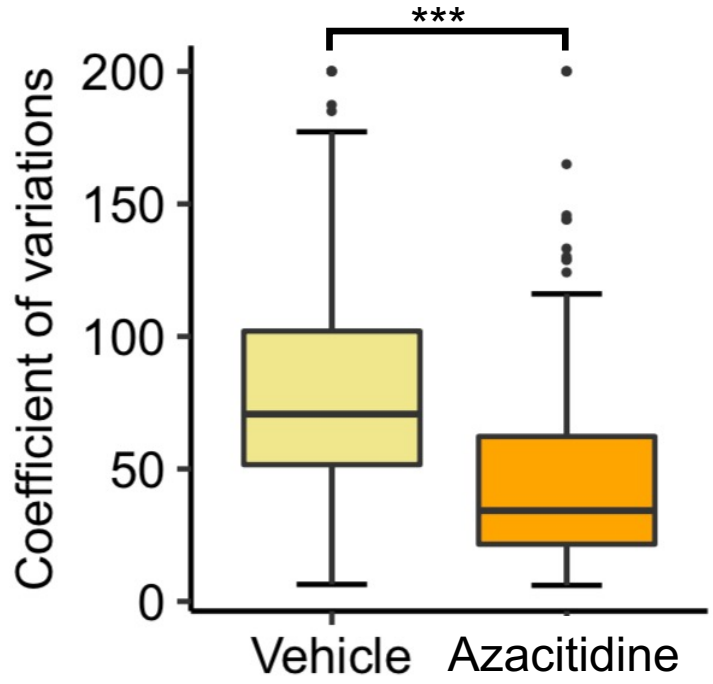
Hypomethylating agent Azacitidine alone can suppress epigenetic and transcriptomic heterogeneity

***Tet2*$^{-/-}$;*Flt3*^{ITD} mouse model**

Epigenetic Heterogeneity



Transcriptomic Heterogeneity

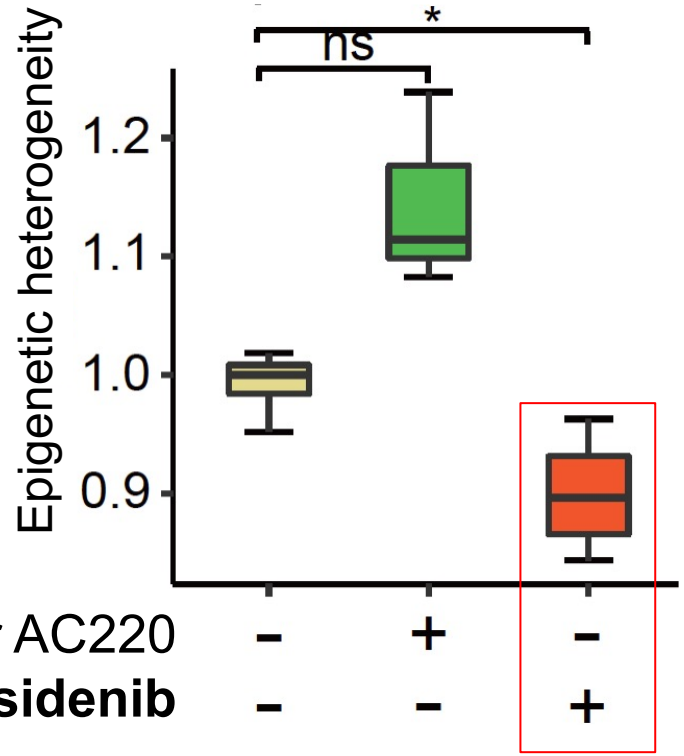


Li S*#, Chen X*, …, Melnick#, Cancer Discovery, 2020

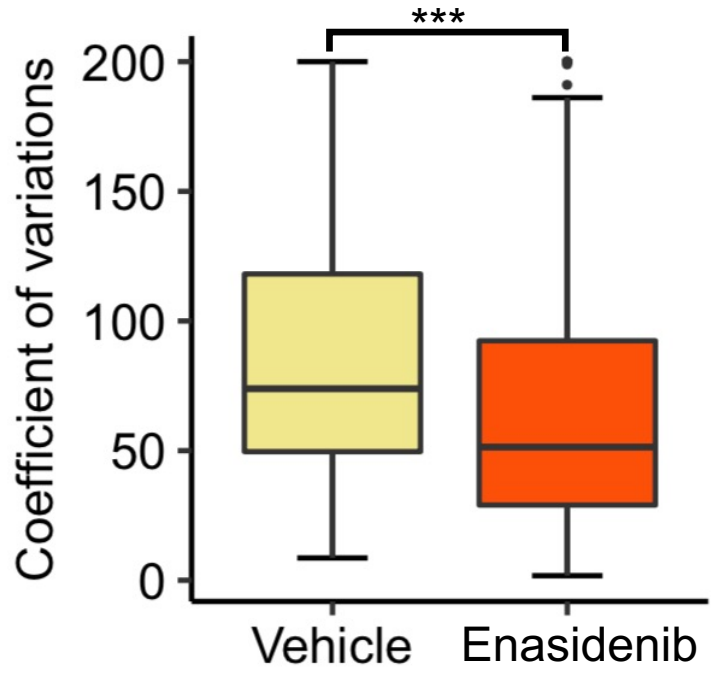
Mutant IDH2 inhibitor Enasidenib can suppress epigenetic and transcriptomic heterogeneity

***Idh2*^{R140Q};*Flt3*^{ITD} mouse model**

Epigenetic Heterogeneity

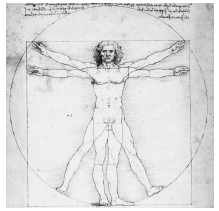


Transcriptomic Heterogeneity

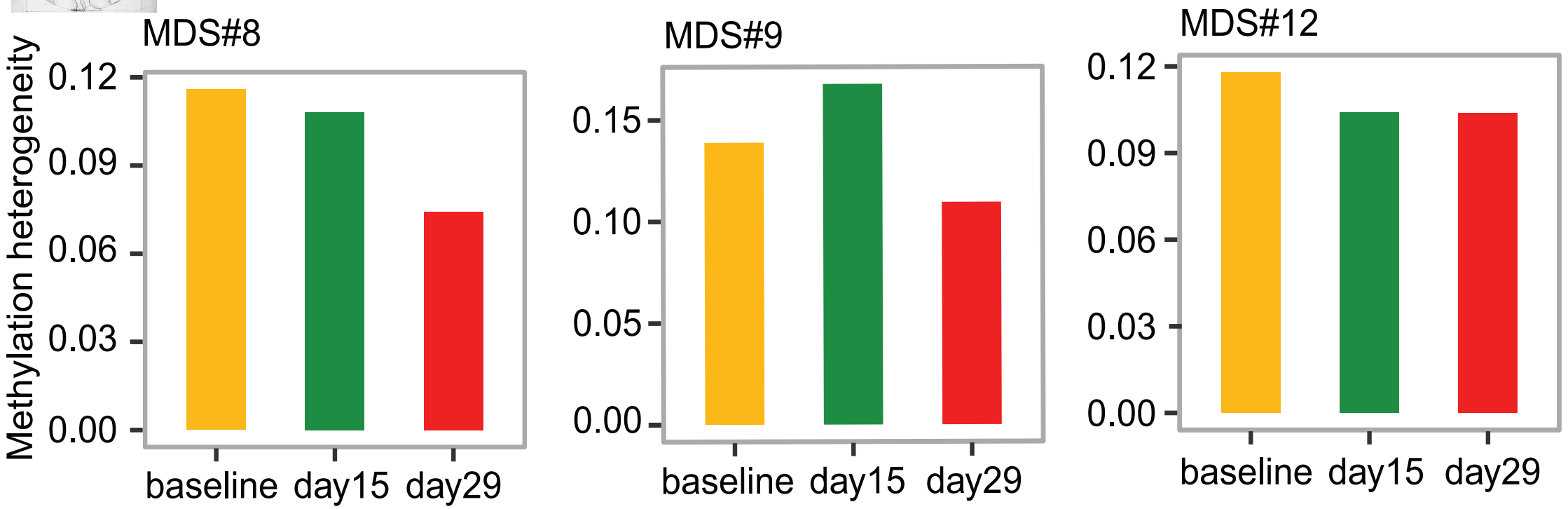


Li S*#, Chen X*, …, Melnick#, Cancer Discovery, 2020

Azacitidine can suppress epigenetic heterogeneity in MDS



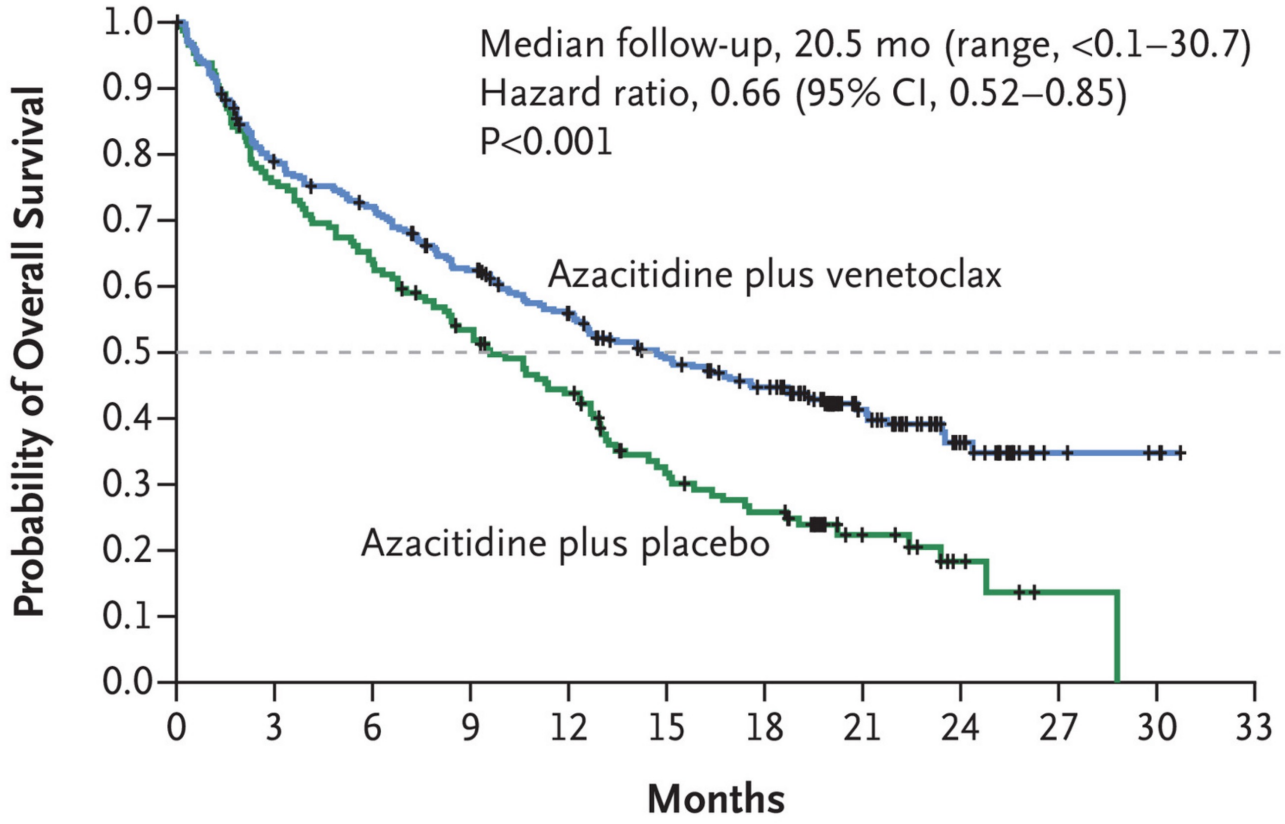
- AML can develop in patients with Myelodysplastic syndrome (MDS)



Li S*#, Chen X*, …, Melnick#, Cancer Discovery, 2020

Therapies that combine epigenetic drugs and standard therapy will become important clinical tools

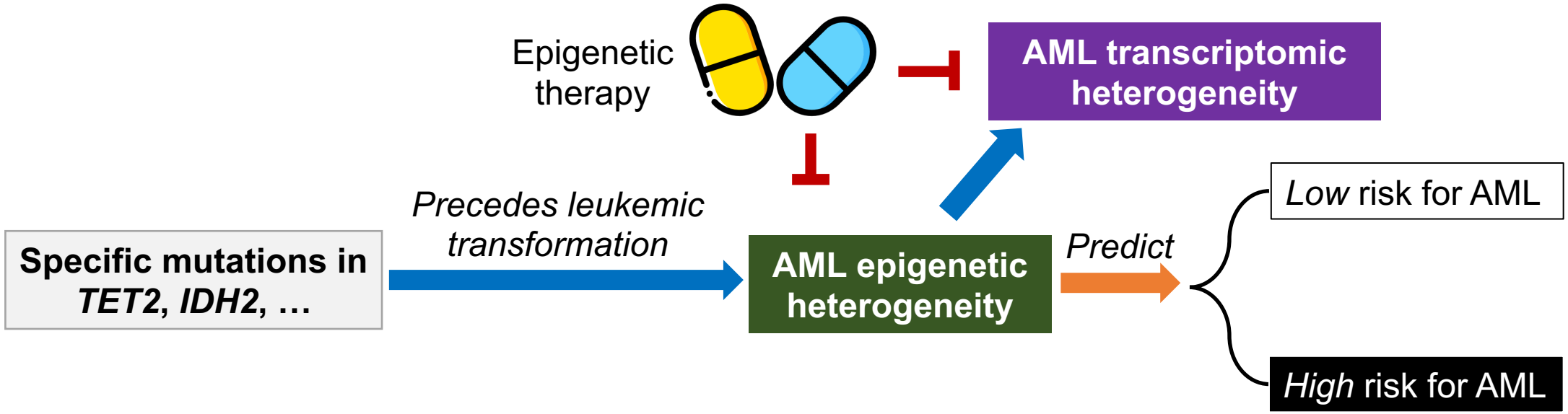
Azacitidine + venetoclax improves median overall survival by 5 months in elderly with AML



NiNardo, et al., NEJM. 2020

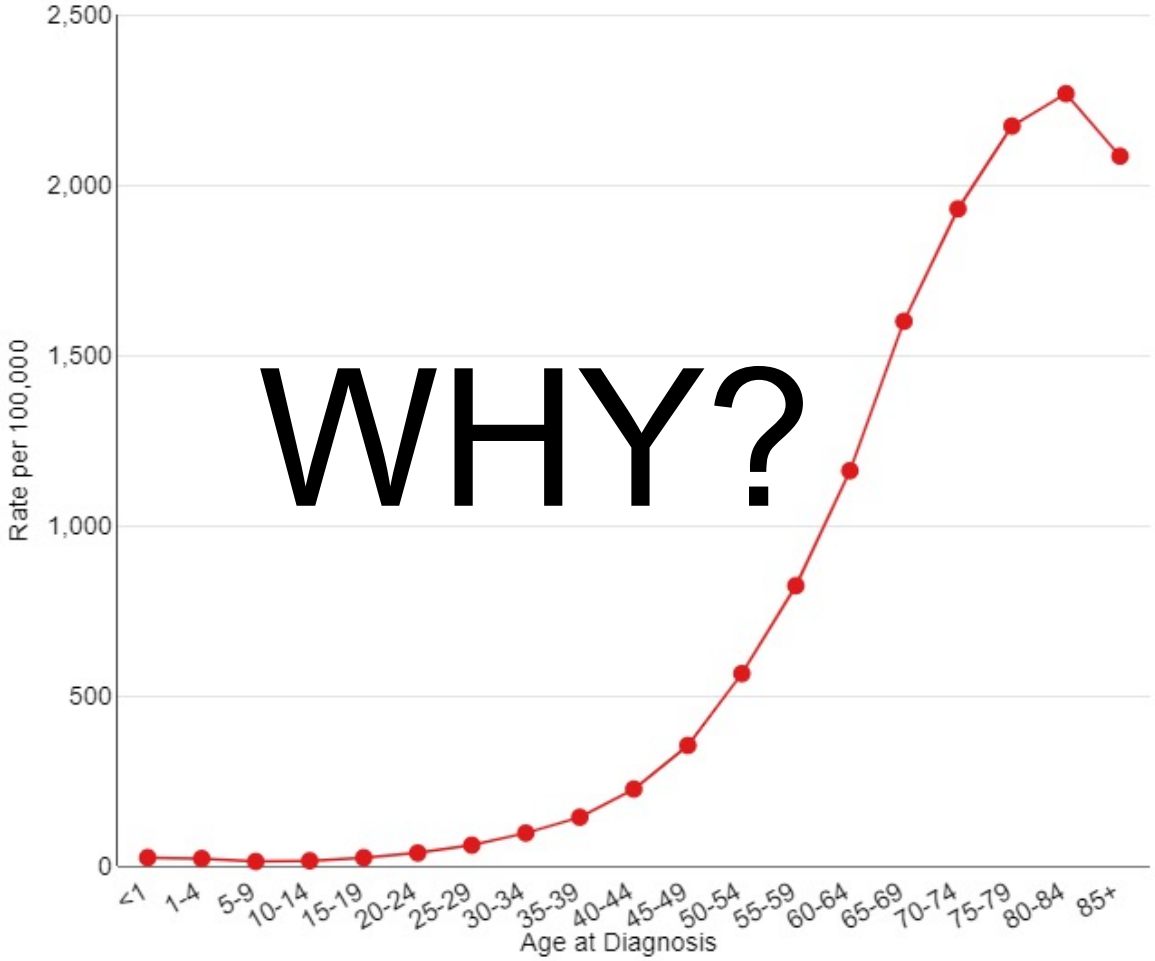
- Single-agent venetoclax: modest activity in AML
  - Souers, et al., Nat Med. 2013
  - Konopleva, et al., Cancer Discov. 2016

Somatic mutations drive specific, but reversible epigenetic heterogeneity states in acute myeloid leukemia (AML)



- Epigenetic evolution and heterogeneity can predict inferior clinical outcome
- Somatic mutations are the driving force of epigenetic heterogeneity
- Epigenetic and transcriptomic heterogeneity is reversible pharmaceutically
- Clonal reduction as a novel therapeutic target for AML

90% of cancers develop after the age of 50



Hematopoietic stem and progenitor cell (HSPC) functions

Immune system function

Inflammation

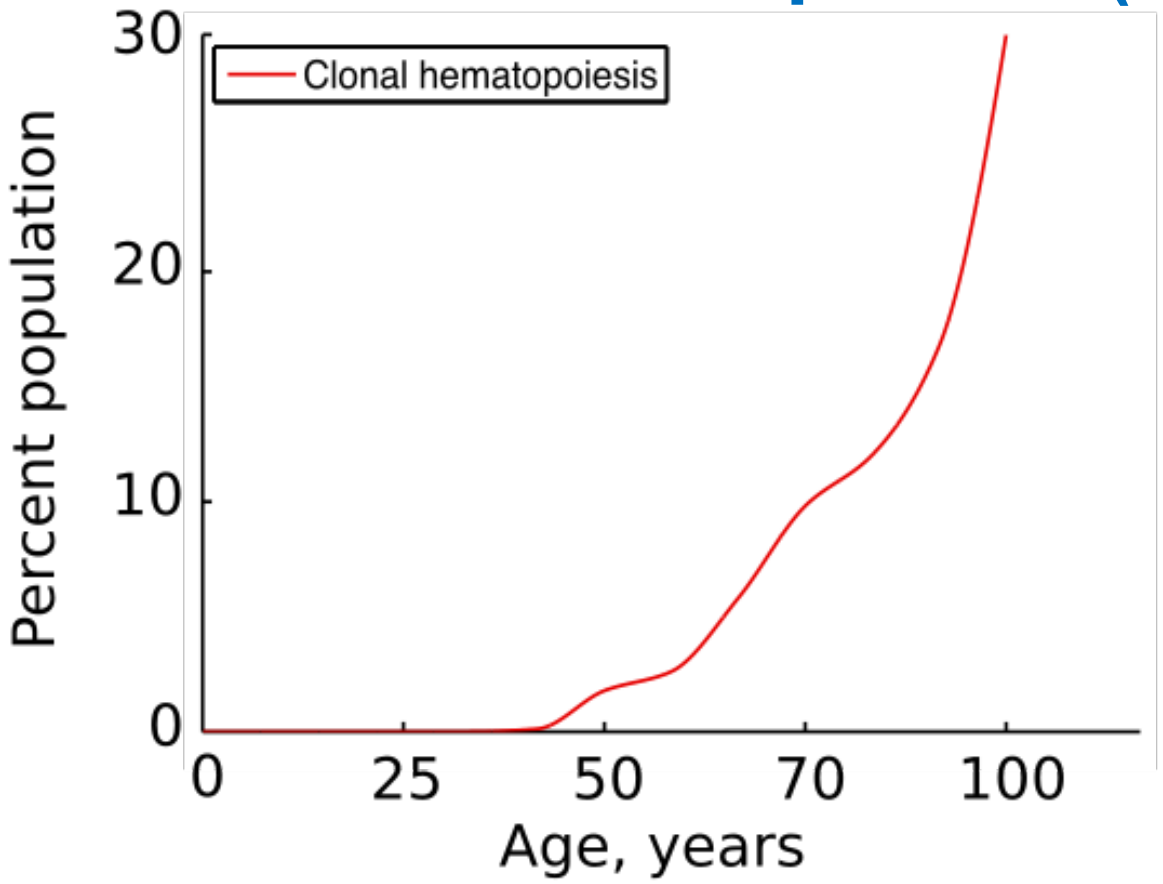
Epigenetic drift

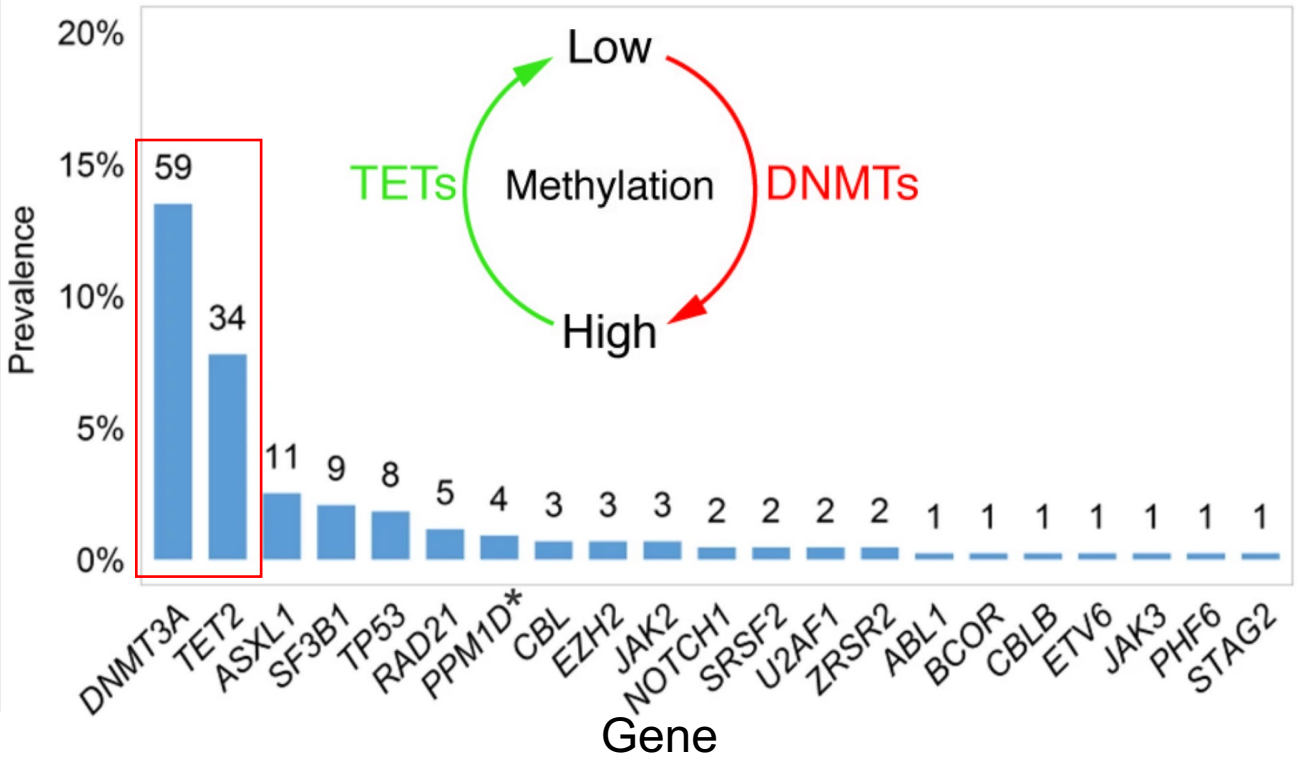
Mutation accumulation

Clonal hematopoiesis

NIH NATIONAL CANCER INSTITUTE
Surveillance, Epidemiology, and End Results Program

Clonal hematopoiesis (CH) is associated with aging



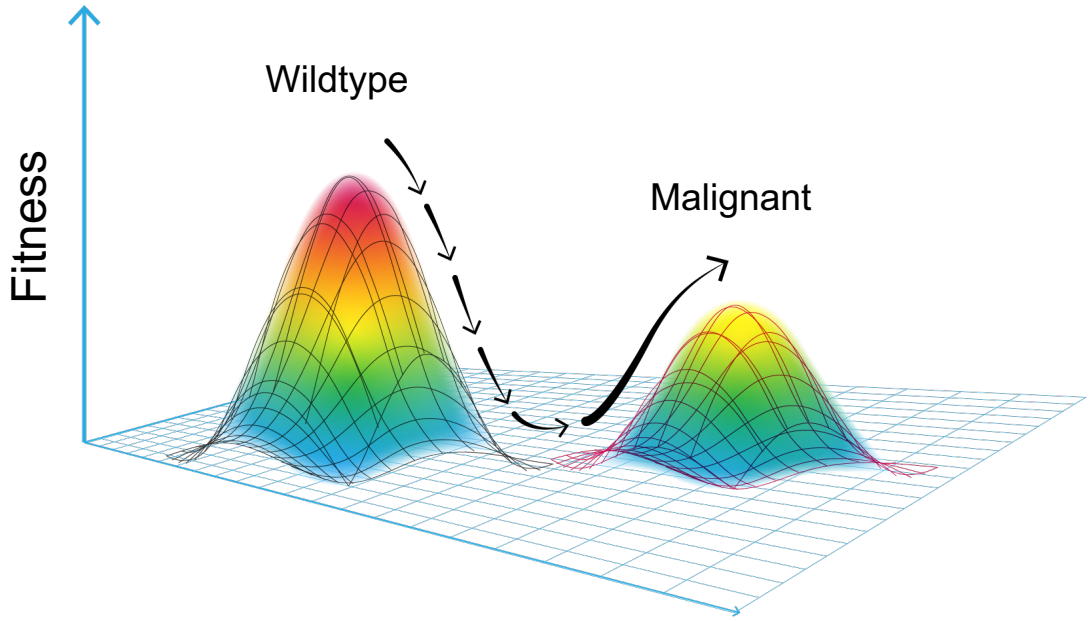


- Clonal expansion
- Mutated HSPC
- Epigenetic regulators
- Acute myeloid leukemia (AML)
- Cardiovascular disease and mortality

Issa, JCI, 2014
Arends, et al., Leukemia, 2018
Rozhok and DeGregori, eLife, 2019

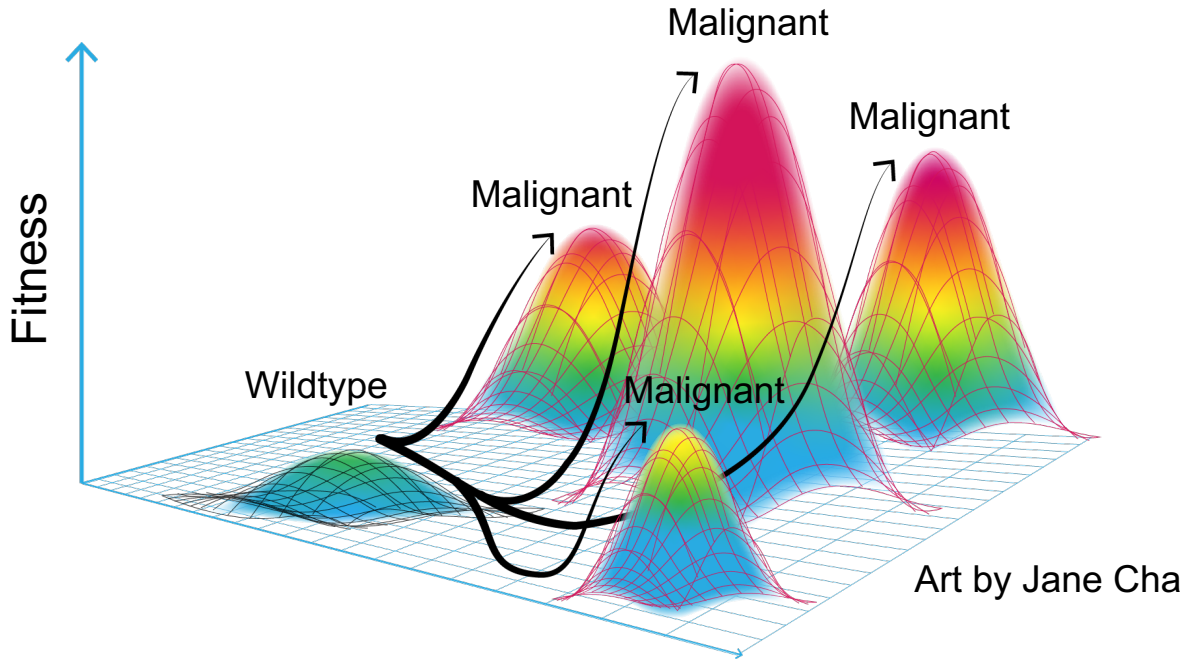
Evolution requires heritable phenotypic variation and directional selection, which is often driven by environmental change

- High stem cell fitness opposes somatic evolution, and thus promotes the status quo
- *Aging or damage alters the adaptive landscape*



Young, healthy stem cell pool and niche

Increased epigenetic heterogeneity

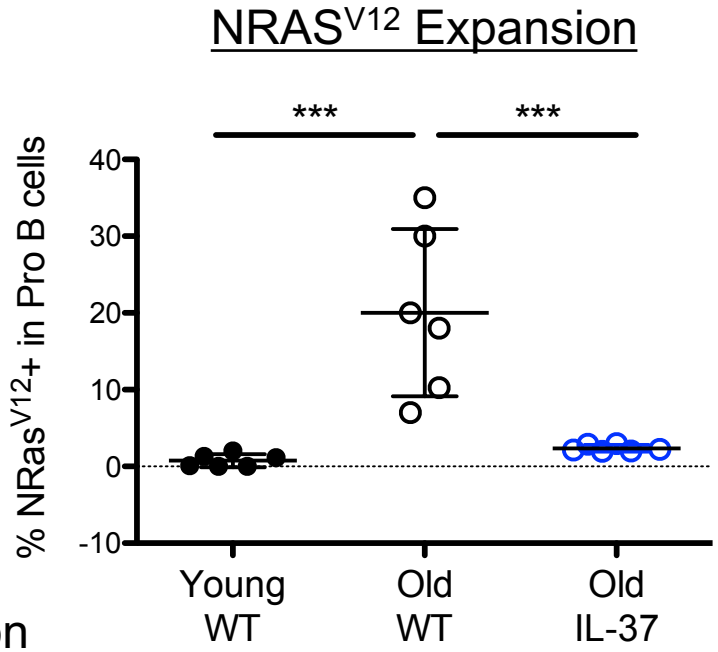


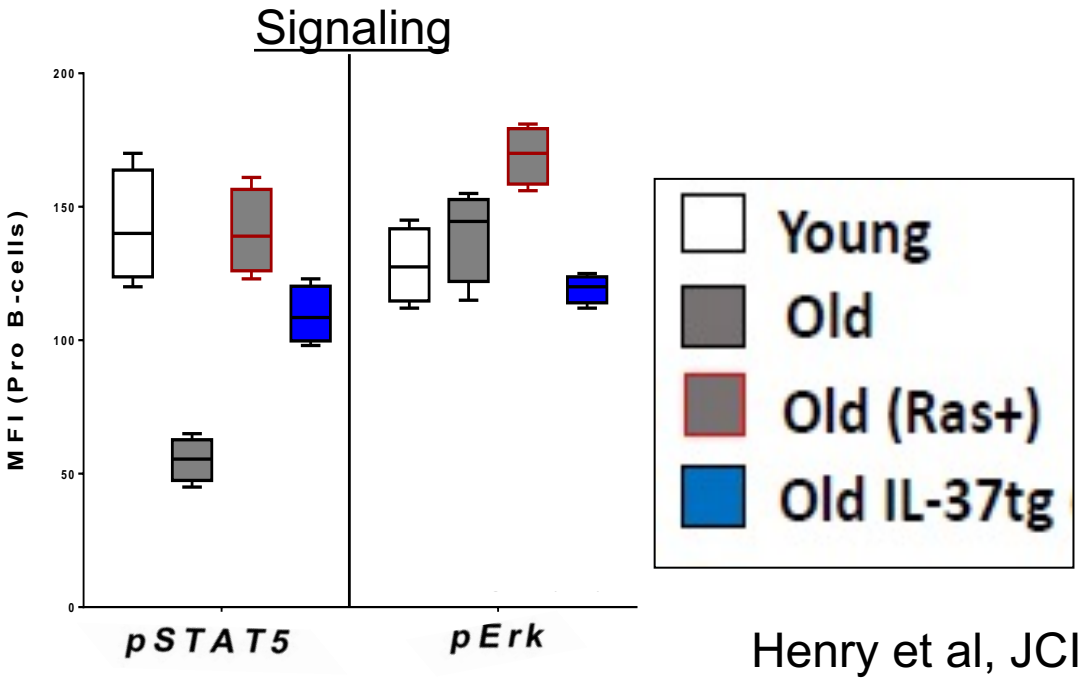
Old or damaged stem cell pool and niche

X-Y plane: potential phenotypes – dependent on genotypes and epigenotypes

- U01CA271830 (NCI/NIA OncoAging Consortium)
  - James DeGregori (MPI, U.Colorado)
  - Hideyuki Oguro (co-I, UCH)
  - Eric Pietras (co-I, U. Colorado)

Specific oncogenic events are selected for within *aged* hematopoietic contexts *dependent on inflammation*

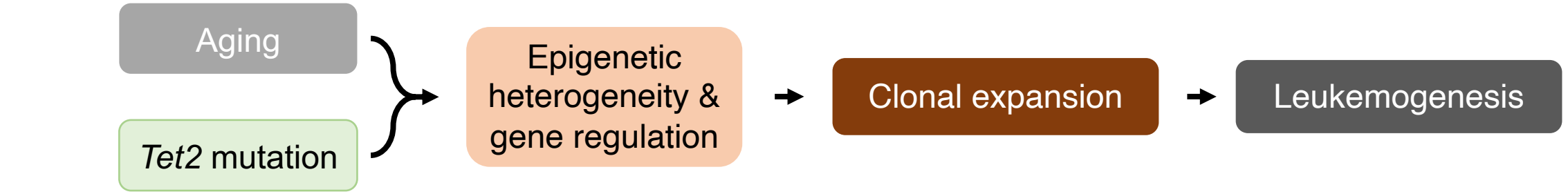


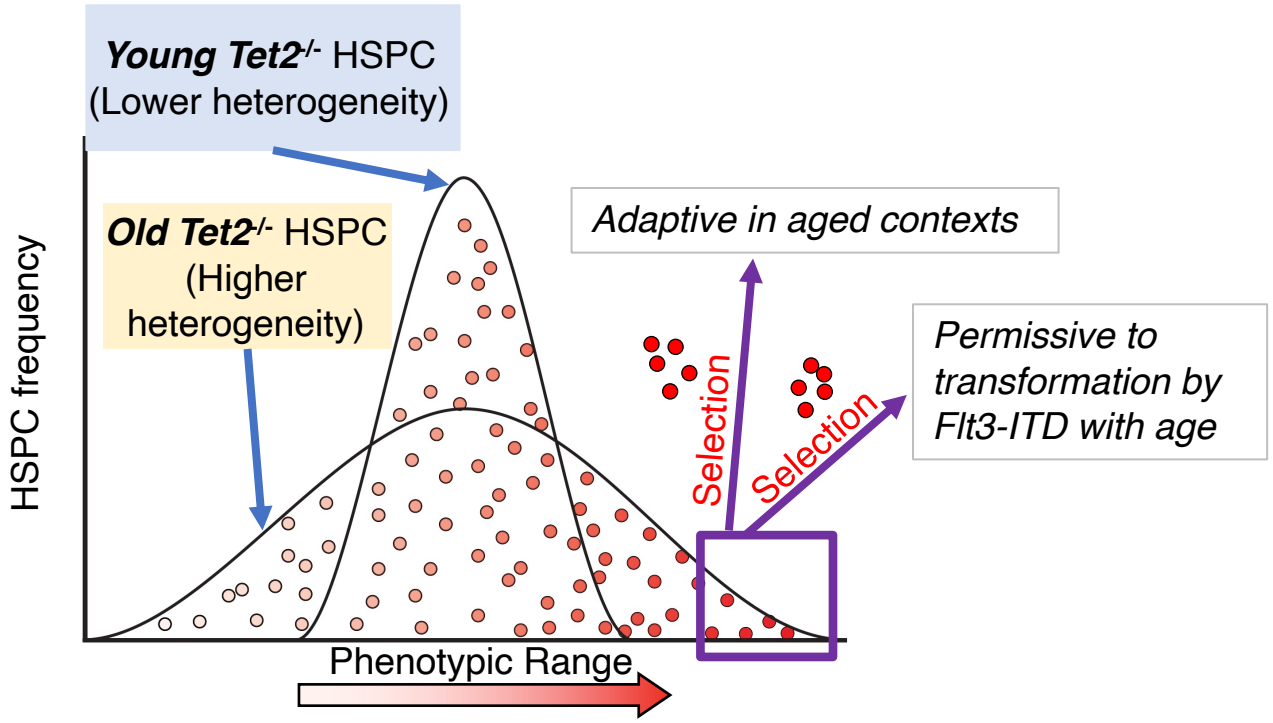


IL37 blocks inflammation

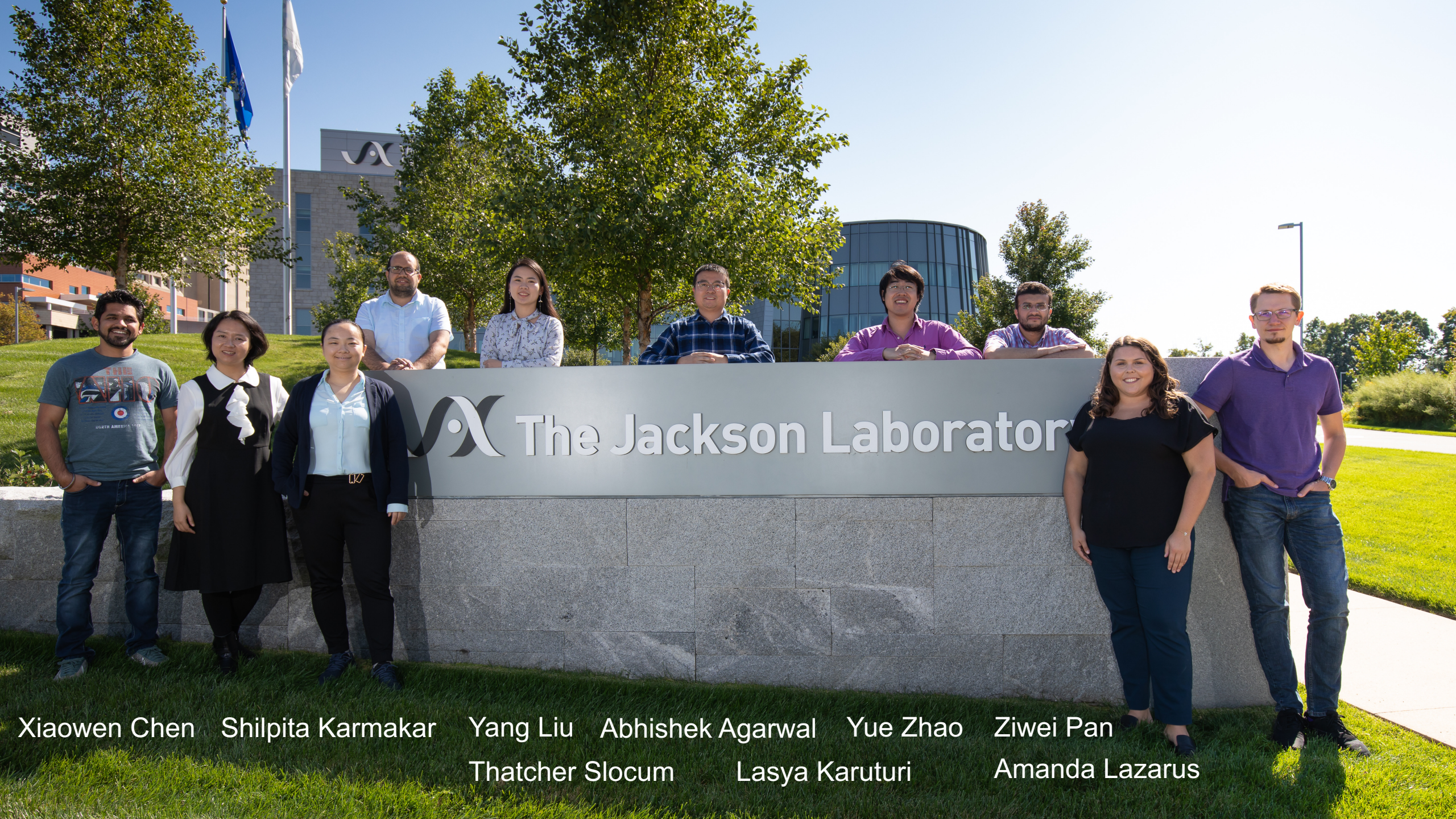
Henry et al, JCI 2015

How does age-related *Tet2*-mutant clonal hematopoiesis evolve to leukemia?





- Evolvability: the *propensity* of blood stem cells to adapt to altered environments
- Single-cell RNA-seq, single-nuclear ATAC-seq, and single-molecule bisulfite sequencing
- Leukemia onset
- Preventative interventions



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