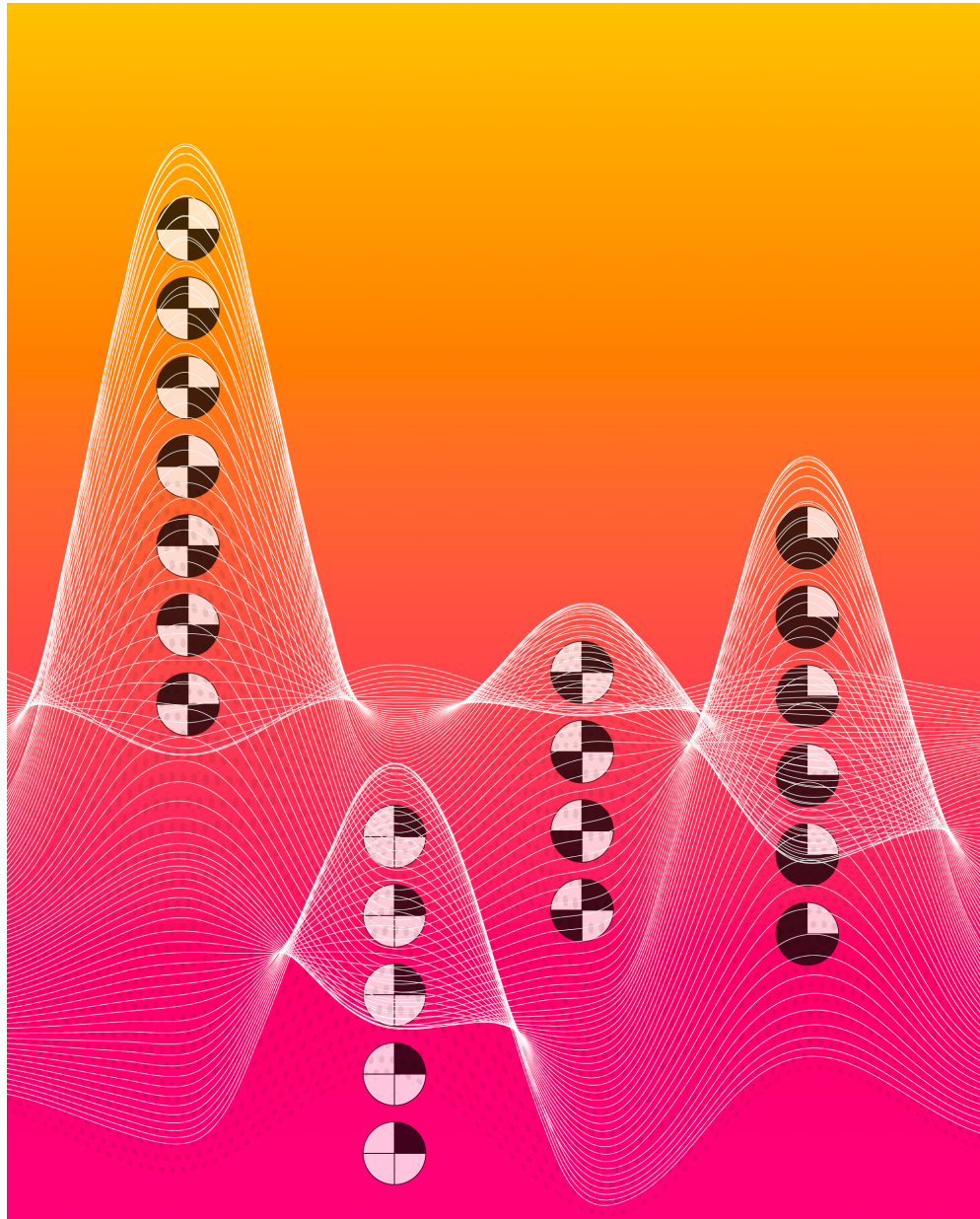
Genetic Fuel for Epigenetic Heterogeneity in Acute Myeloid Leukemia



March 23rd, 2021

Sheng Li

The Jackson Laboratory for Genomic Medicine

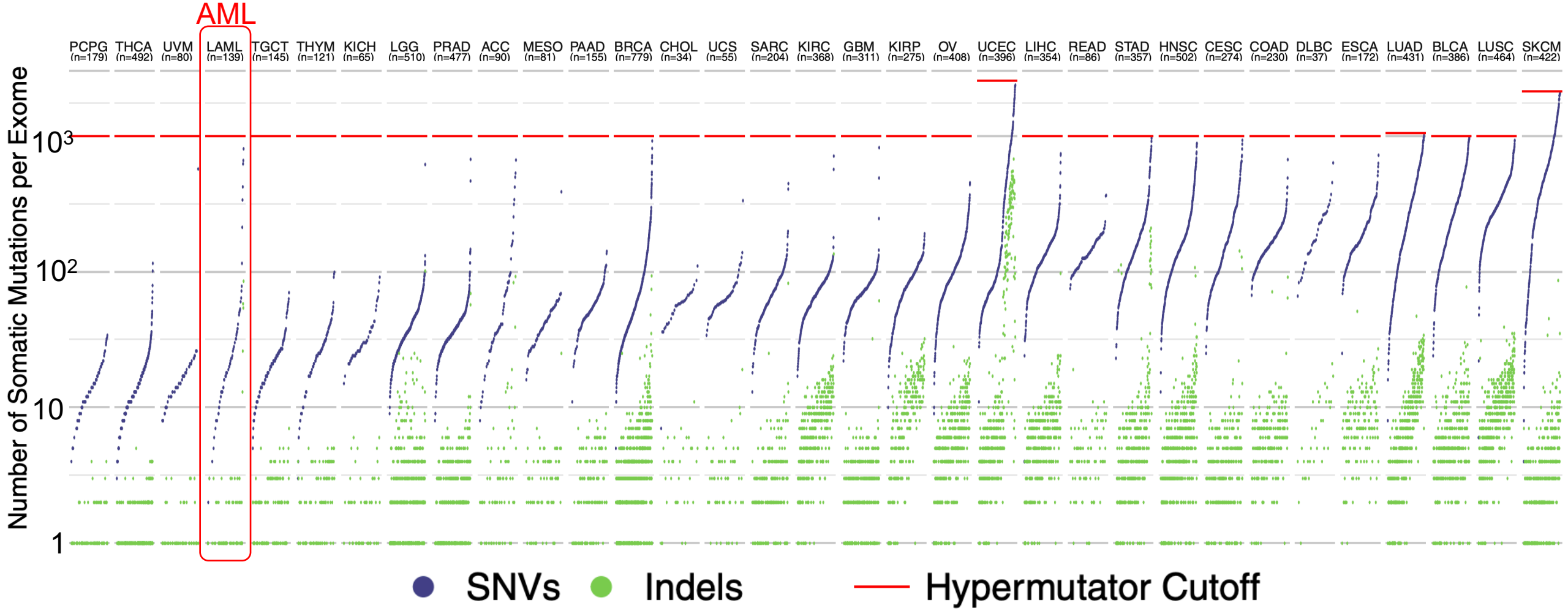
The Jackson Laboratory Cancer Center

Acute myeloid leukemia (AML) is heterogeneous disease

- An aggressive blood cancer starts in the bone marrow: morphologic, cytogenetic, and clinical heterogeneity
- In 2020, ~ 20,000 new cases, ~11,000 deaths from AML in US
- 5-year survival rate: ~ 26%; relapse rate: ~50%

American Cancer Society®

Low abundance of somatic mutation burden in AML



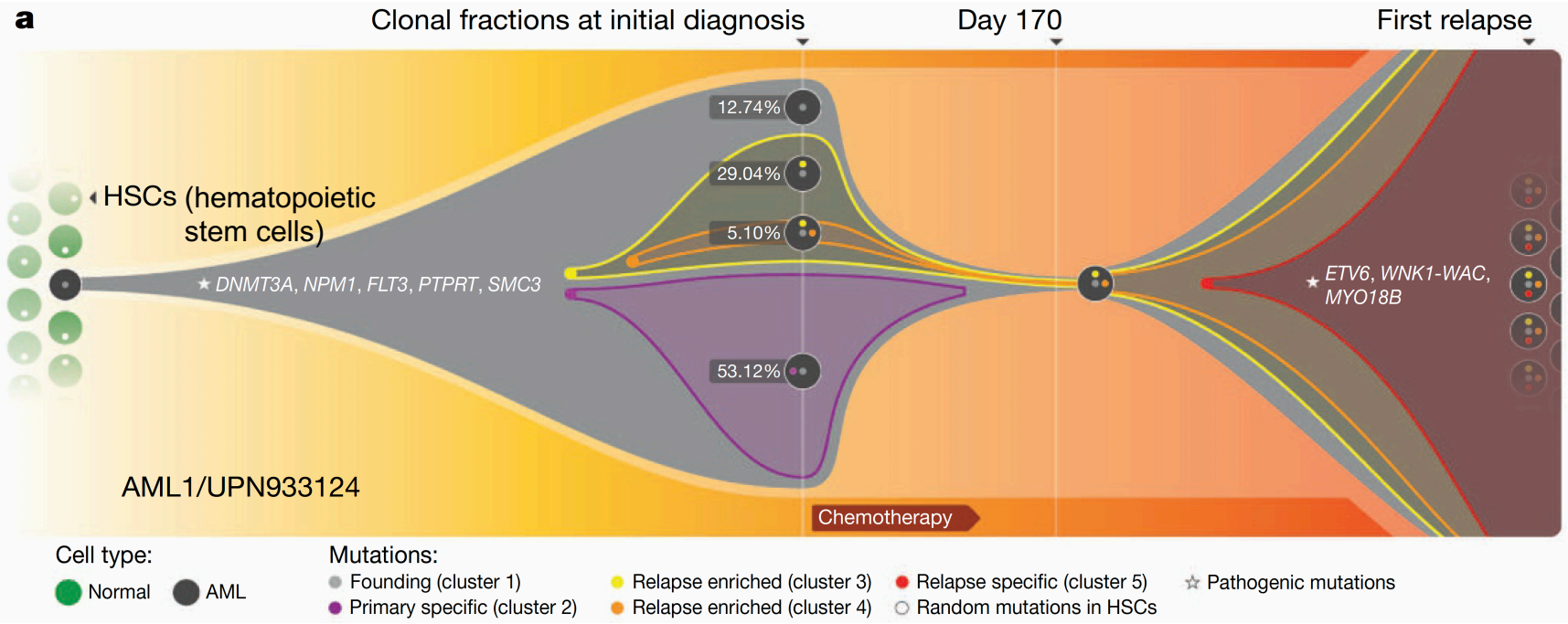
Bailey MH, et al., *Cell* 2018

Inter-patient genetic heterogeneity in AML and recurrent mutations in epigenetic modifiers

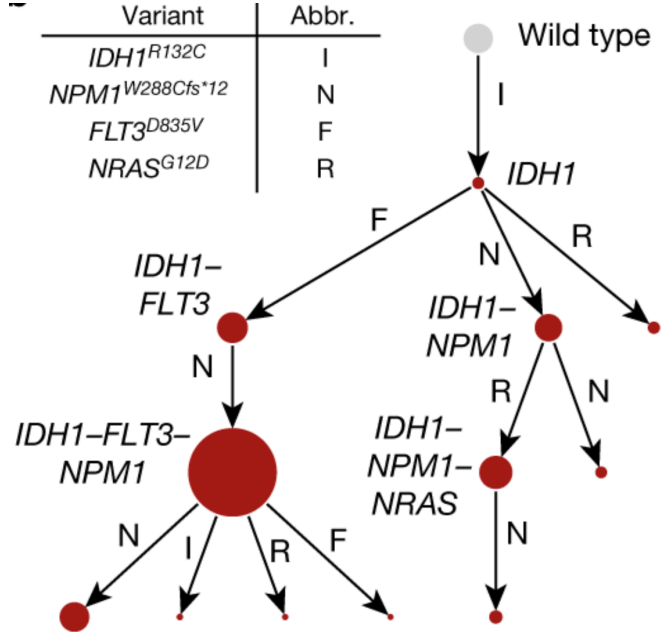
TCGA, *The New England Journal of Medicine* 2013

Leukemia evolution remains a key challenge for curative therapy

Genetic heterogeneity drives cancer evolution

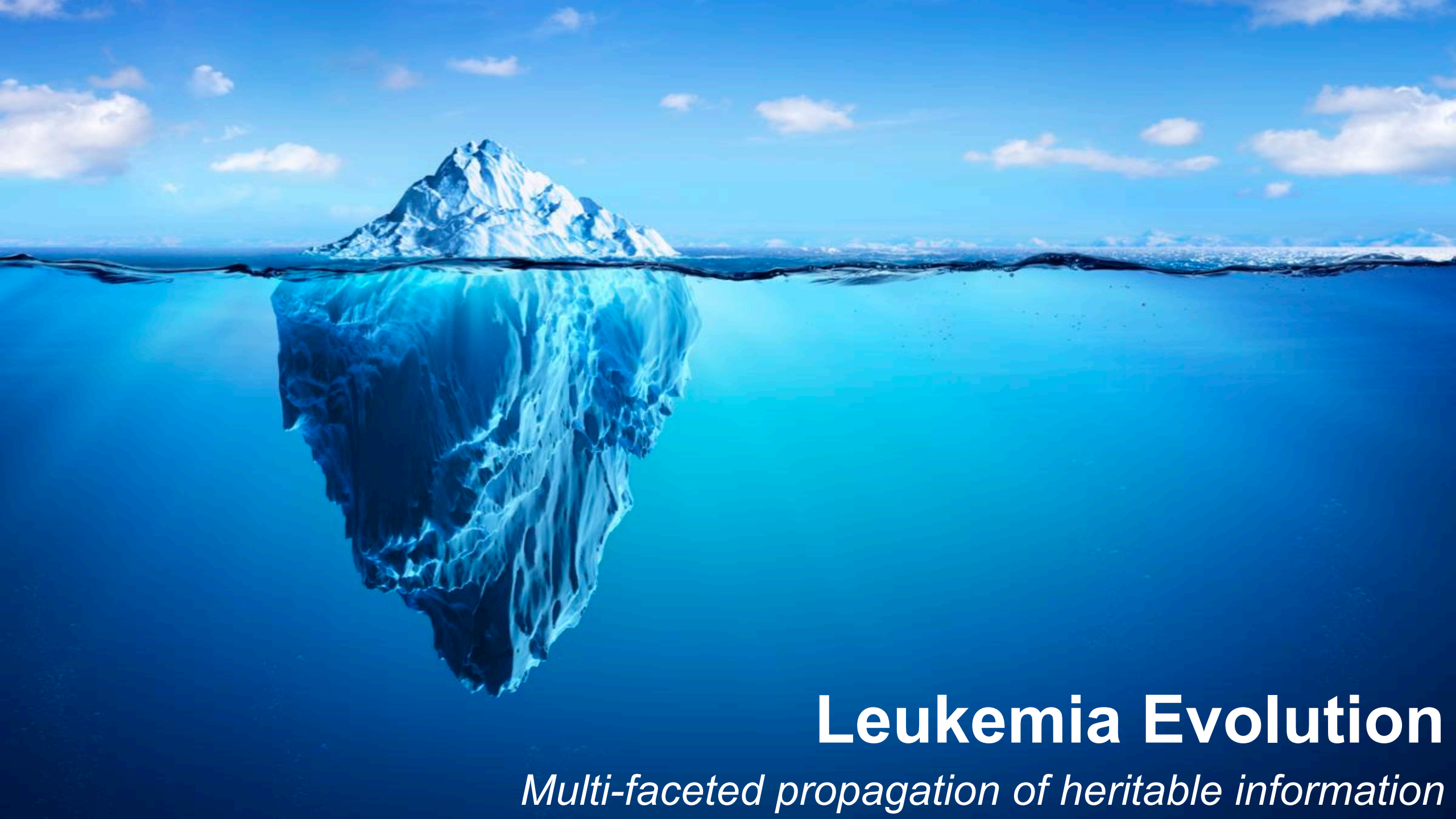


Bulk whole genome sequencing



Single cell DNA sequencing

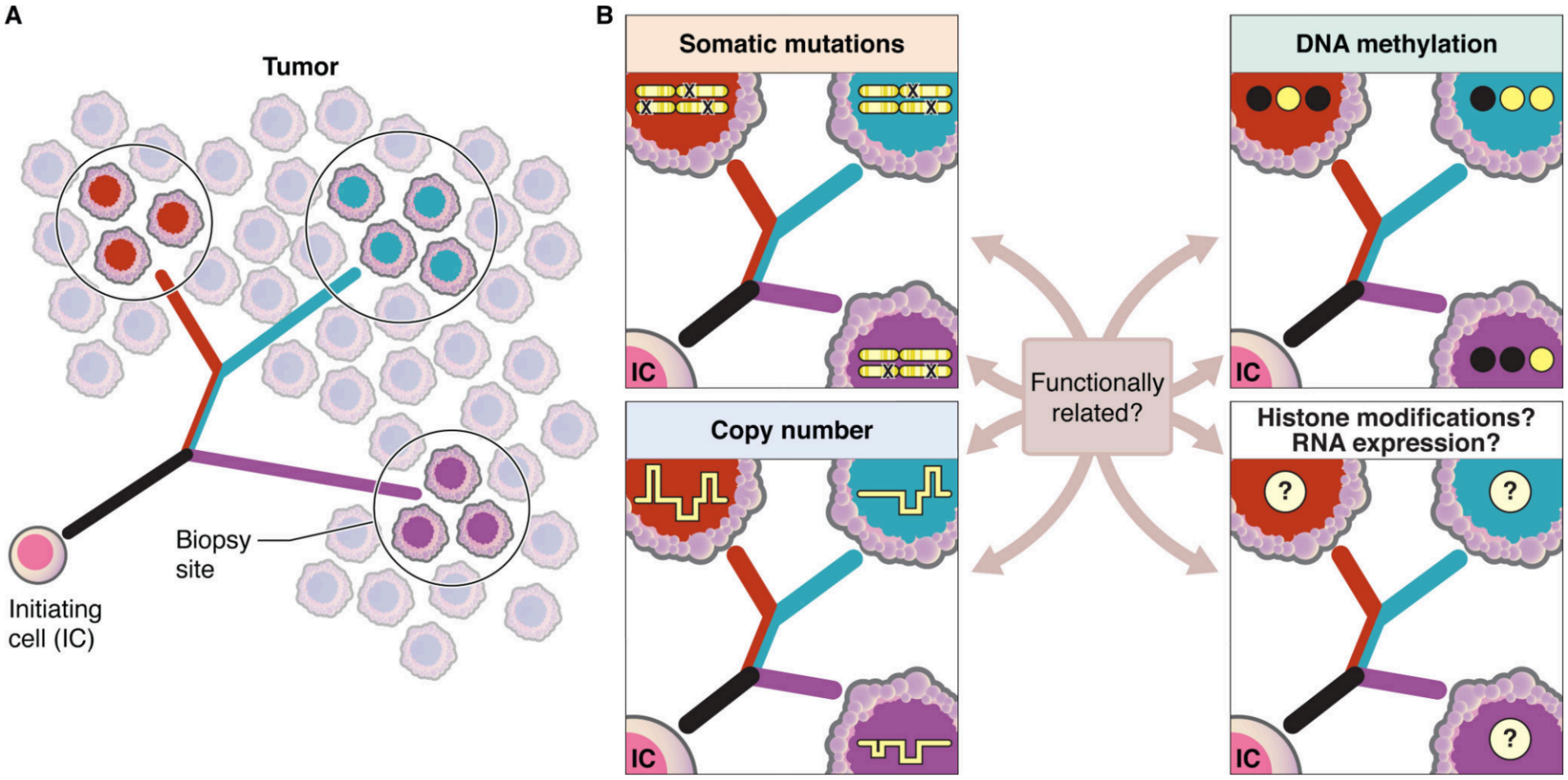
Ding L et al., *Nature* 2012
Miles LA et al., *Nature* 2020



Leukemia Evolution

Multi-faceted propagation of heritable information

Multi-omics heterogeneity drives cancer evolution



Mazor T, et al., *Cancer Cell*, 2016

Epigenomics:
instructions for activities of genes

Genomic information is constant

Genomics

Normal CGCA**G**TCTA

Change nucleic acid

Cancer CGCA**T**TCTA

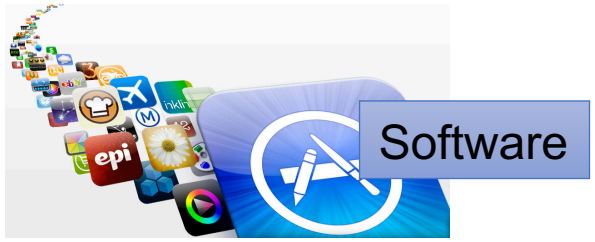
Epigenomics – DNA methylation

Normal CGCA**C**GCTA

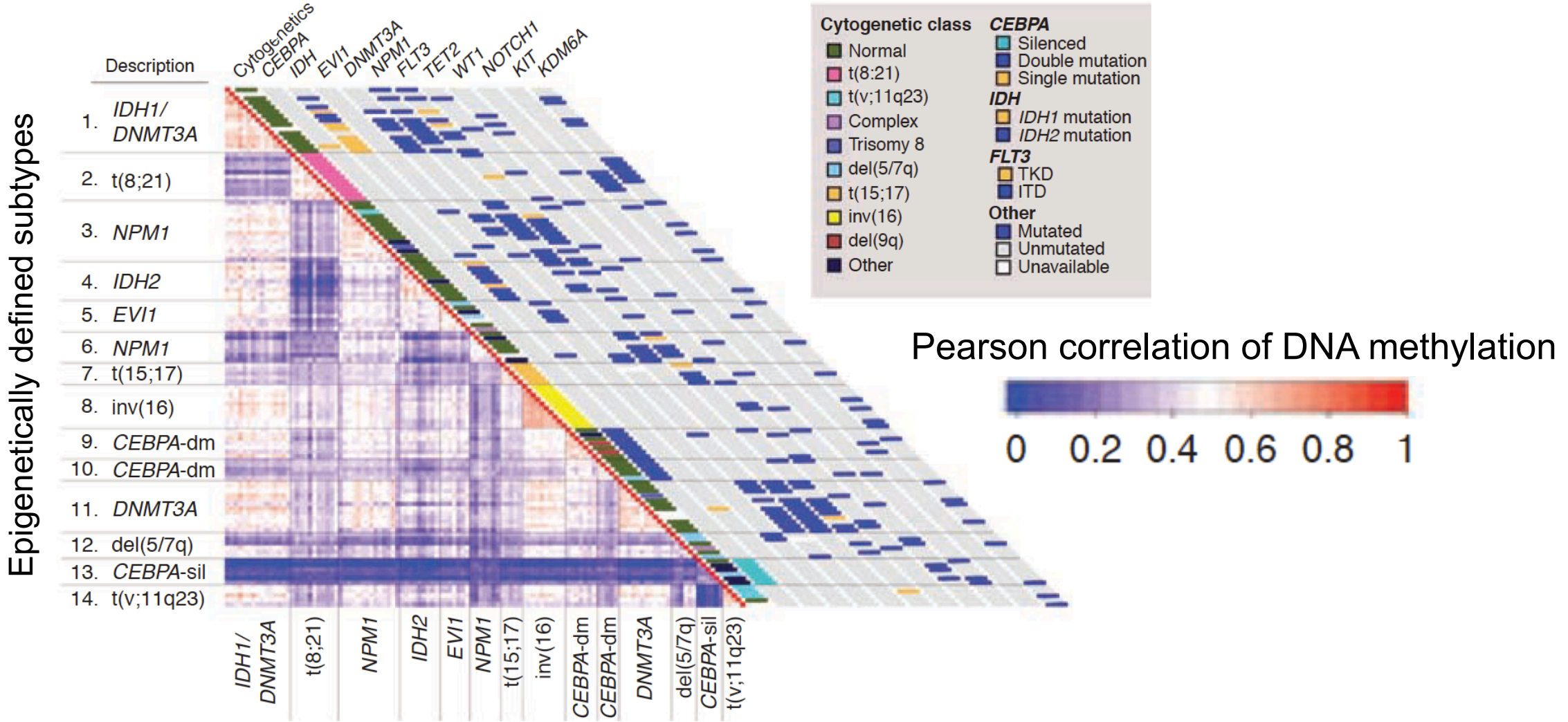
Adding Methyl group

Cancer CGCA**C**GCTA

Epigenomic information has plasticity



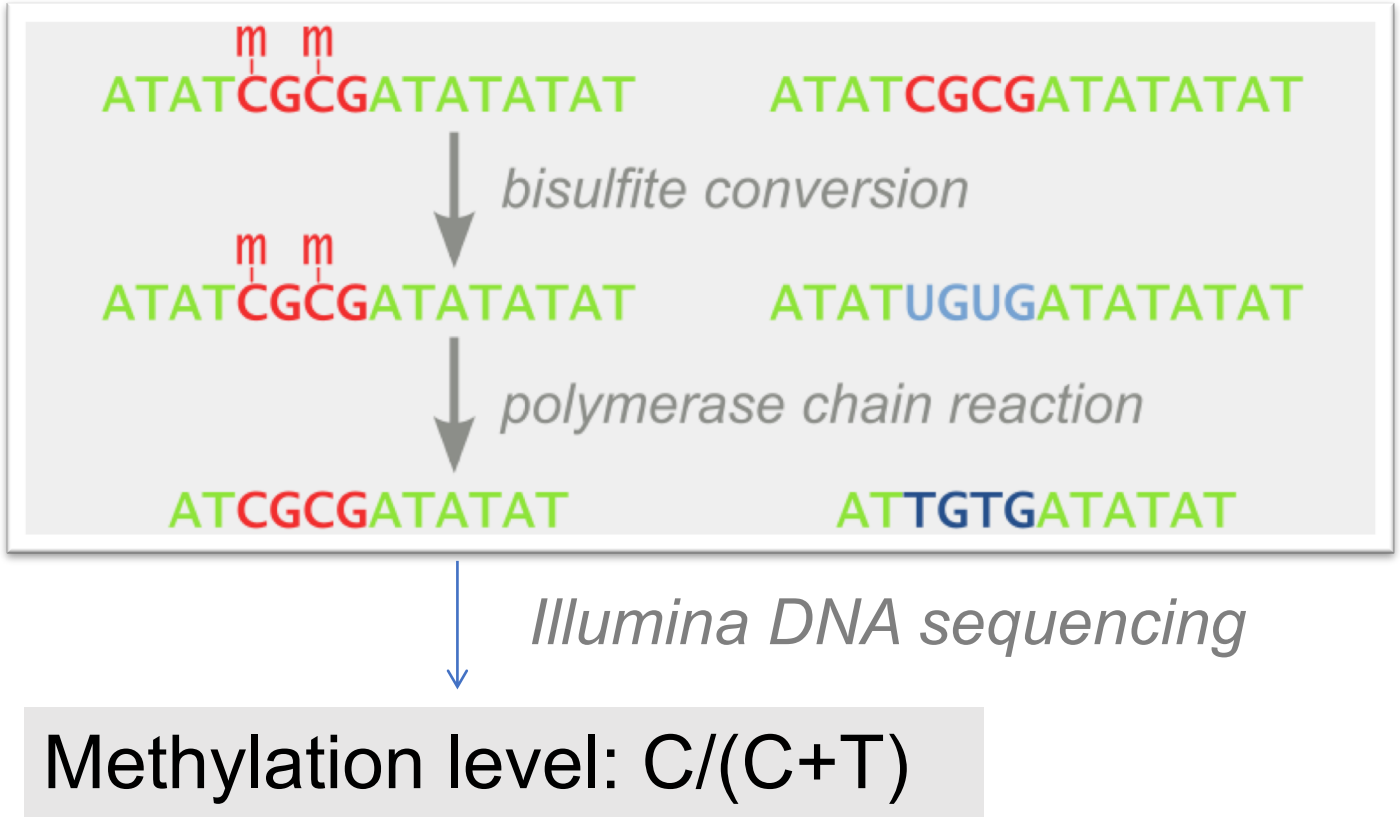
Aberrant epigenetic patterning is common and has emerged as a hallmark of AML



Wide-spread Abnormal DNA Methylation Landscape Across AML Patients

Glass J, et al., *Cancer Discovery*, 2017

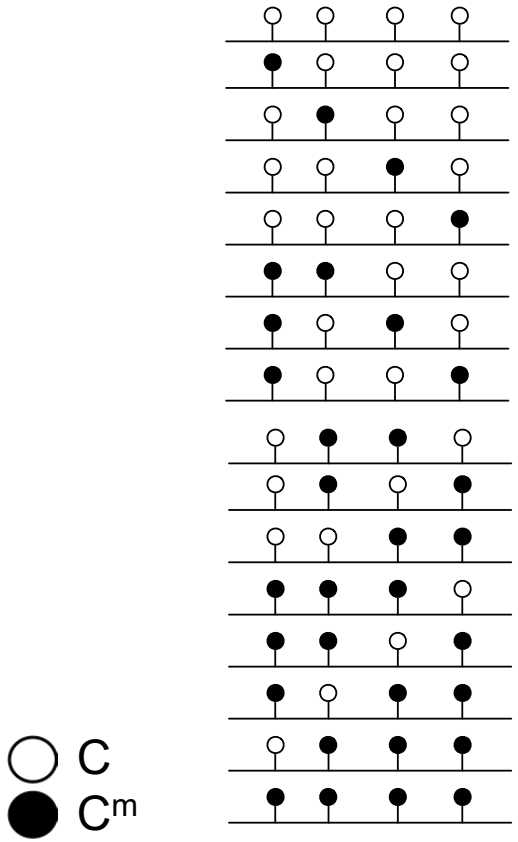
Bisulfite sequencing for single molecule DNA methylation profiling

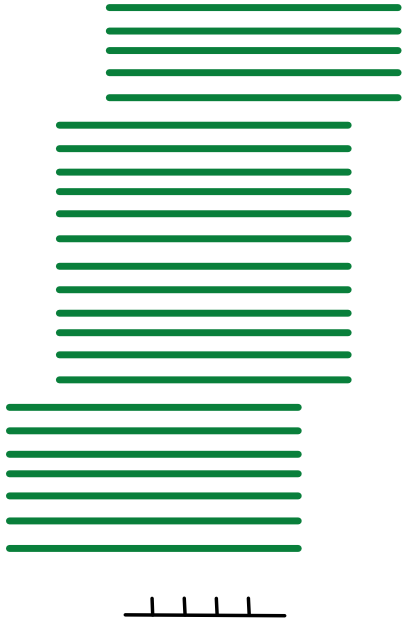


- Whole genome bisulfite sequencing (WGBS): 28 million CpGs
- Reduced Representation Bisulfite Sequencing (RRBS): 4-6 million CpGs

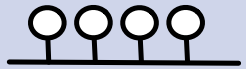
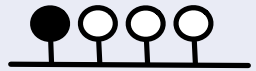
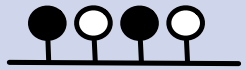
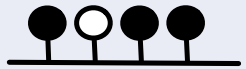
Epigenetic alleles (epiallele)

- The pattern of variation in methylation status among CpGs present in discrete sets
- Each epiallele can be tracked as a unit by virtue of the constituent CpGs being located adjacent to each other





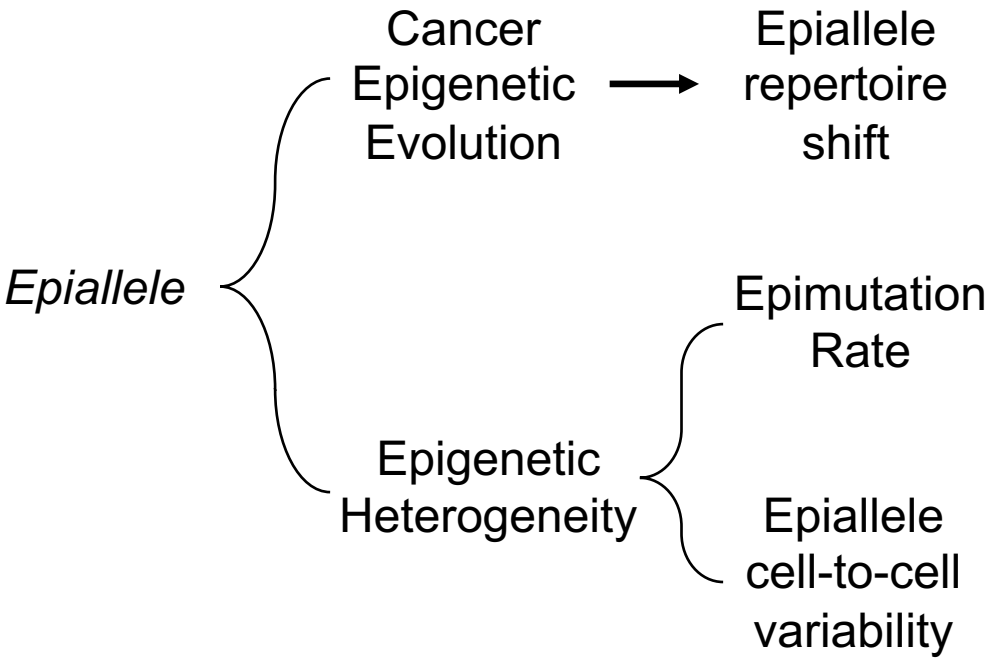
Genomic locus with four adjacent CpGs

Epiallele	Frequency/Read count
	60
	100
	20
	20

Epiallele

- Not identical in concept to genetic clones
- Measures population diversity among individual cancer cells
- Native barcode to trace cancer evolution

Utilize epiallele to trace cancer evolution and epigenetic heterogeneity



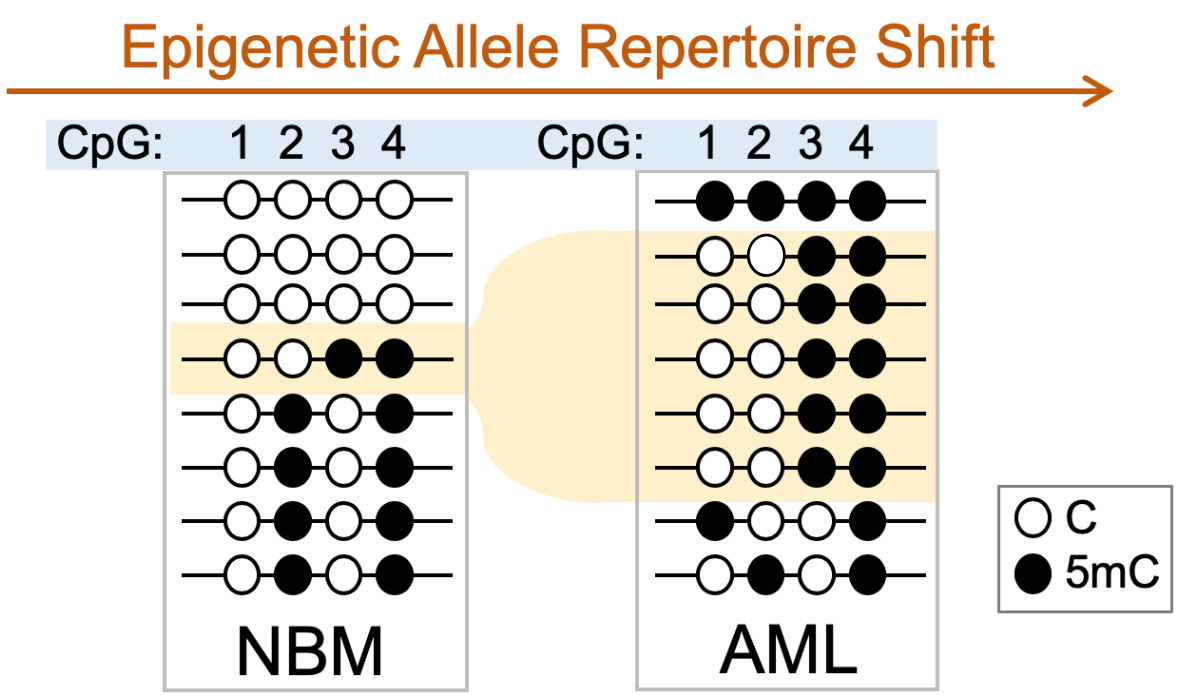
Landan G, et al., *Nature Genetics*, 2012
Li S, et al., *Genome Biology* 2014
Landau D, et al., *Cancer Cell*, 2014
Li S, et al., *Nature Medicine*, 2016
Gaiti F, et al., *Nature*, 2019
Li S[#], Chen X[#], et al, *Cancer Discovery*, 2020
Chen X, …, Li S, *Scientific Reports*, 2021

Utilize epiallele to trace cancer evolution and epigenetic heterogeneity

Epiallele

- **Cancer Epigenetic Evolution** → ***Epiallele repertoire shift***
- Epigenetic Heterogeneity
  - Epimutation Rate
  - Epiallele cell-to-cell variability

A molecular clock for cancer evolution



- *Methclone* measures the degree to which epiallele patterns shift at different time points.

Li S, et al., *Genome Biology* 2014
Li S, et al., *Nature Medicine*, 2016
Li S, et al, Cancer Discovery, 2020

Utilize epiallele to trace cancer evolution and epigenetic heterogeneity

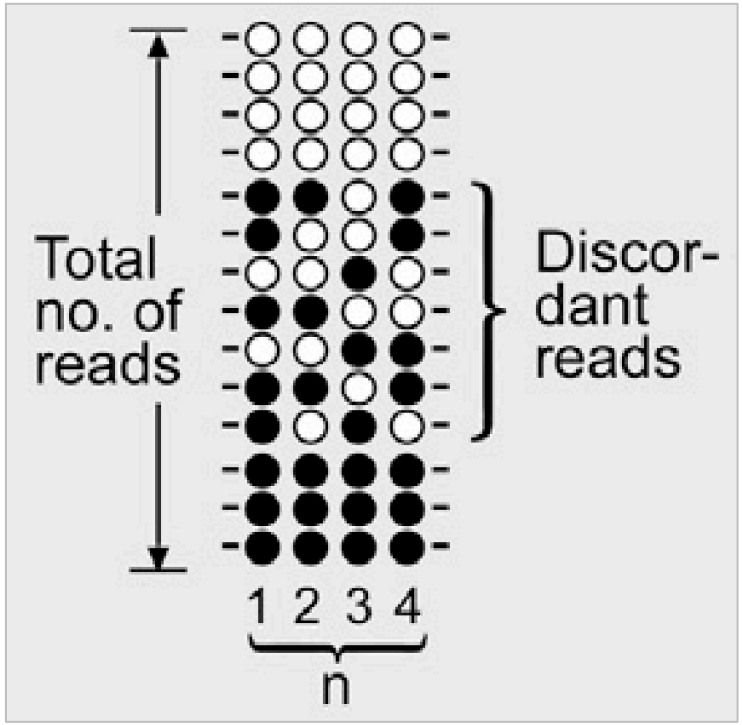
Epiallele

Cancer Epigenetic Evolution → Epiallele repertoire shift

Epimutation Rate

Epigenetic Heterogeneity

Epiallele cell-to-cell variability



$$\text{Epimutation rate} = \frac{Discordant\ read\ number}{Total\ number\ of\ reads}$$

Landau D, et al., *Cancer Cell*, 2014
Gaiti F, et al., *Nature*, 2019

Utilize epiallele to trace cancer evolution and epigenetic heterogeneity

Epiallele

Cancer Epigenetic Evolution → Epiallele repertoire shift

Epimutation Rate

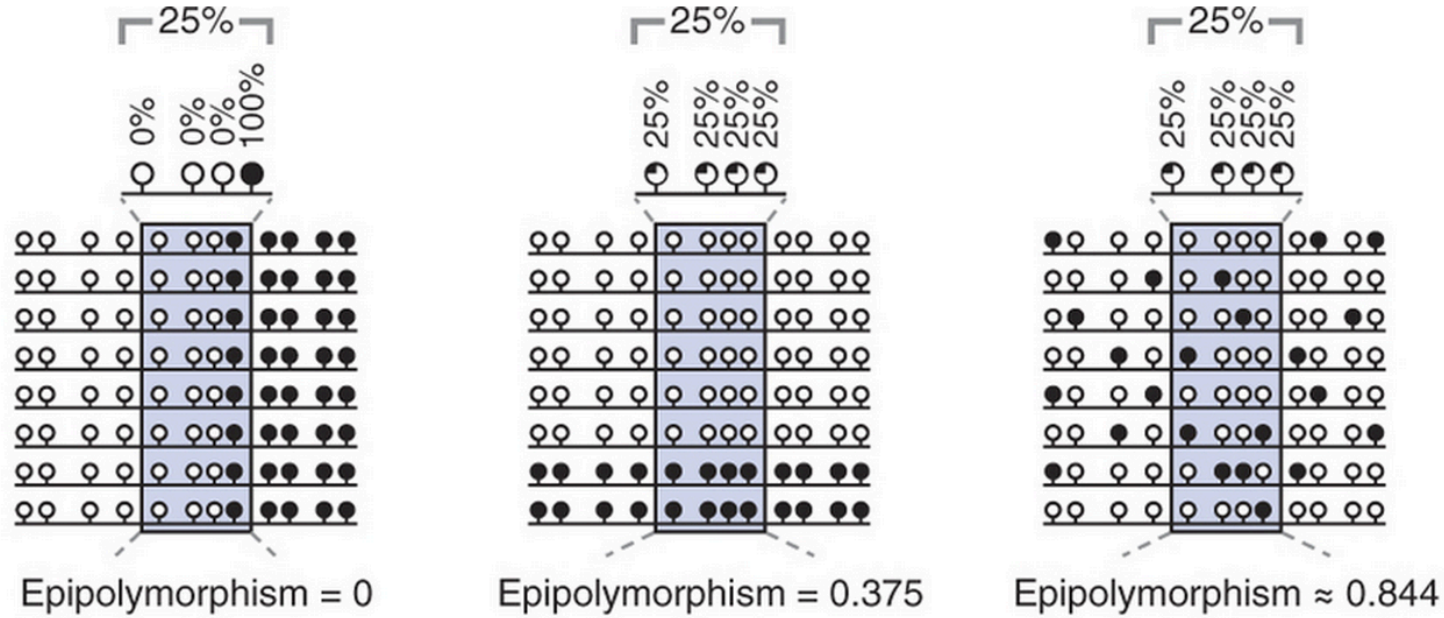
Epigenetic Heterogeneity

Epiallele cell-to-cell variability

Epipolymorphism

$p_{poly} = 1 - \Sigma(p_i^2)$

p_i: Epiallele percentage



Landan G, et al., *Nature Genetics*, 2012

Understanding evolution is essential for improving the efficacy of leukemia treatment and prevention

- Can epigenetic evolution and heterogeneity predict inferior clinical outcome?
- Is epiallele heterogeneity caused by the mutations or just by-product of leukemia transformation?
- Is epigenetic heterogeneity reversible?

Understanding evolution is essential for improving the efficacy of leukemia treatment and prevention

- **Can epigenetic evolution and heterogeneity predict inferior clinical outcome?**
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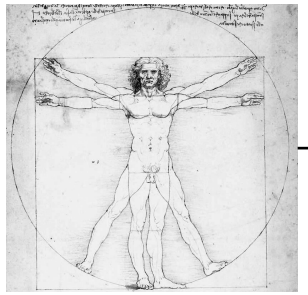
Assess the epiallele repertoire shift at diagnosis



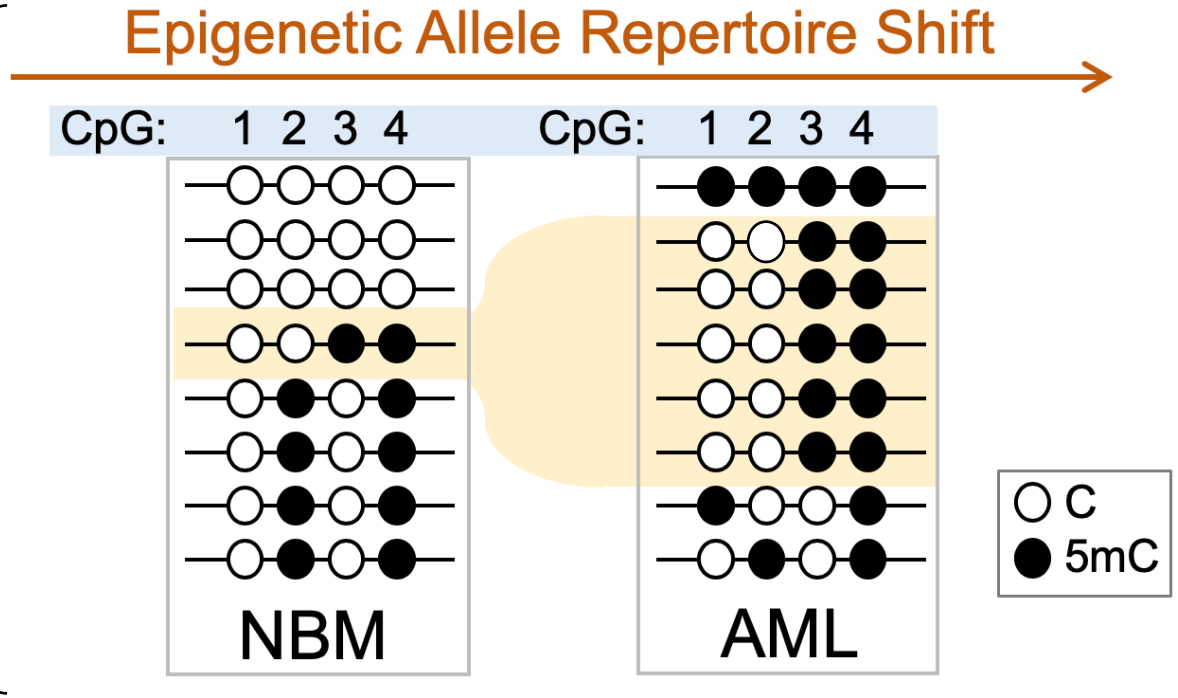
RRBS

Methclone

Loci with epigenetic allele repertoire shift

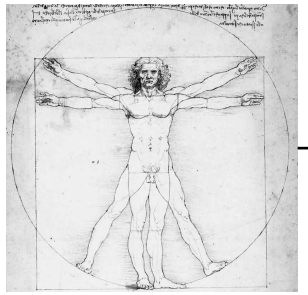
EPM

Cox proportional-hazards regression



Li S et al., *Genome Biology* 2014
Li S et al., *Nature Medicine* 2016

Assess the epiallele repertoire shift at diagnosis



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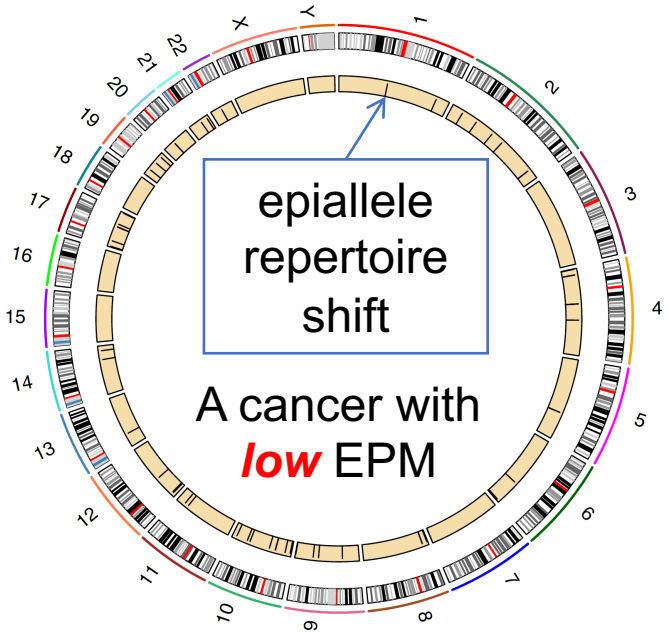
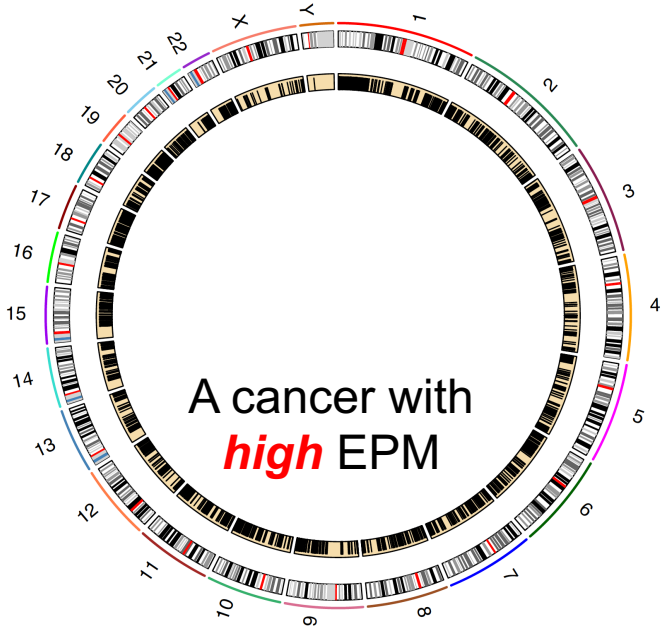
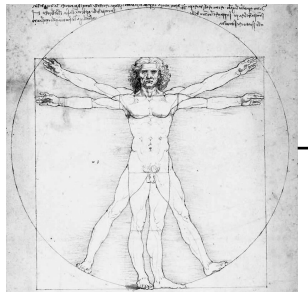




- EPM: number of loci with epiallele repertoire shift per million loci sequenced.

Li S et al., *Genome Biology* 2014
Li S et al., *Nature Medicine* 2016

The epiallele repertoire shift at diagnosis provides prognostic insight



RRBS

Methclone

Loci with epigenetic allele repertoire shift

EPM

Cox proportional-hazards regression

Table 1 Multivariate analysis of EPM association with time to relapse

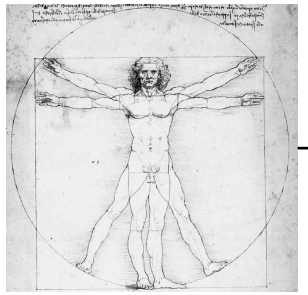
Variable	*P* value	Hazard ratio
EPM	0.024	1.559
Age	0.930	0.994
Sex	0.303	1.223
WBC count	0.339	0.999

Multivariate Cox proportional-hazards regression model using relapse time as response variable to test EPM and clinical parameters as variables in the entire cohort ($n = 127$).

- EPM: number of loci with epiallele repertoire shift per million loci sequenced.

Li S et al., *Genome Biology* 2014
Li S et al., *Nature Medicine* 2016

Epigenetic heterogeneity provides prognostic insight

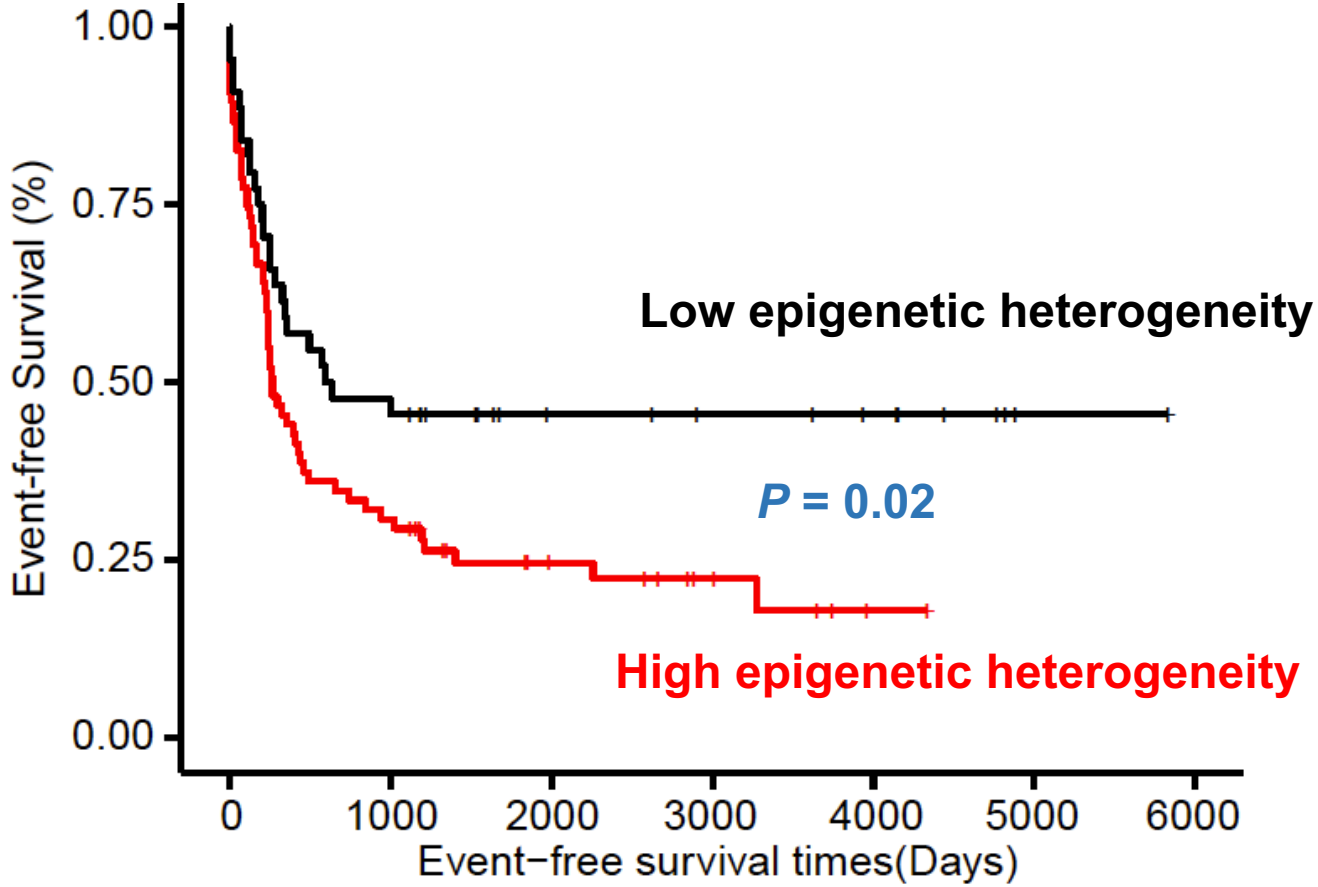


RRBS

Epihet

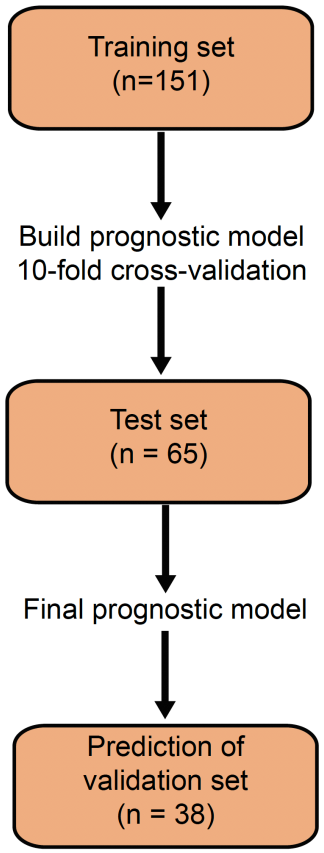
Epimutation Rate

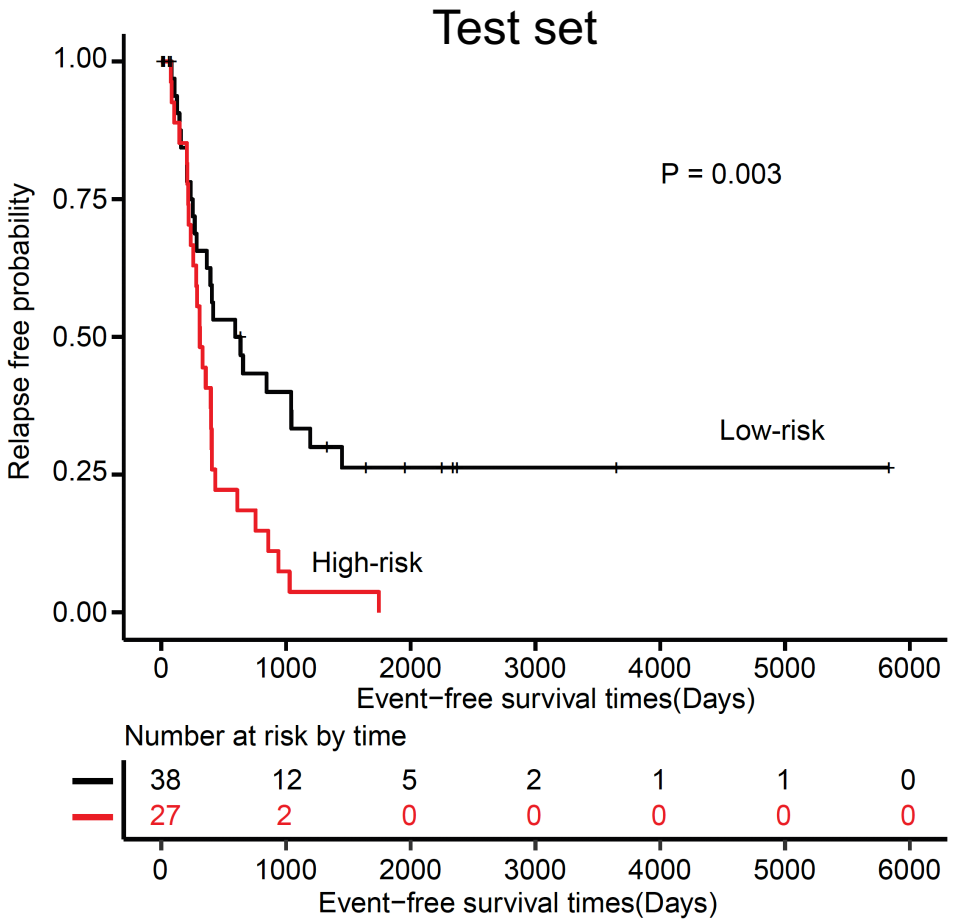
Log-rank test



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

An epiallele prognostic classifier predicts clinical outcome in AML





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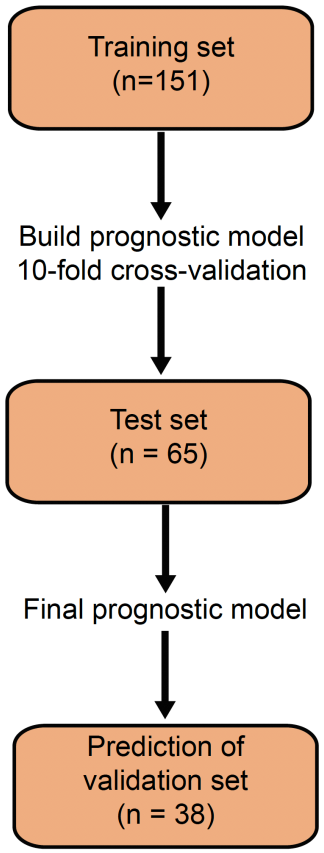
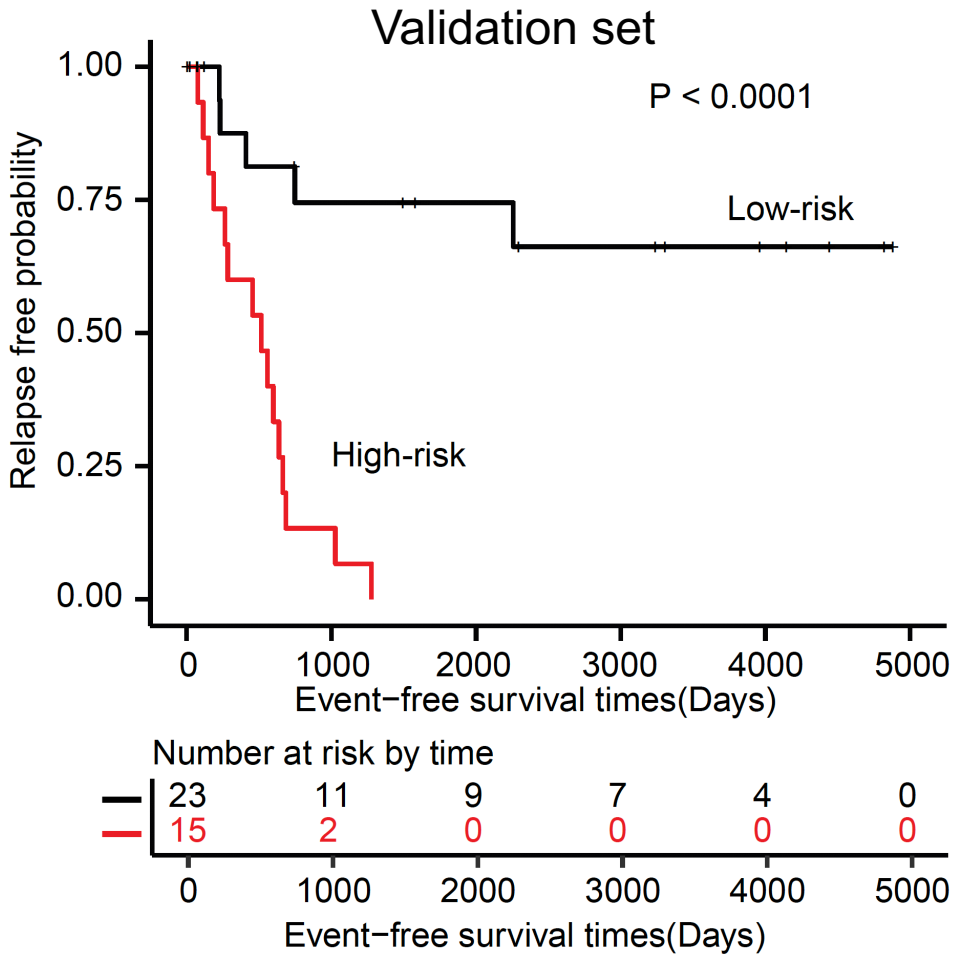
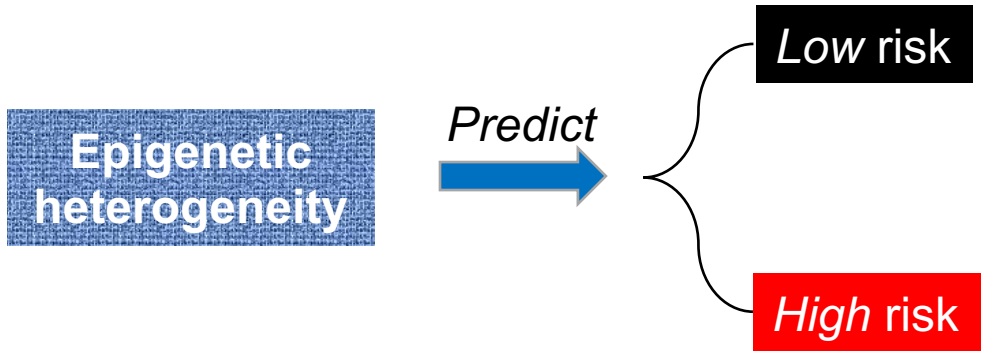




Li S#, Chen X#, …, *Cancer Discovery,* 2020

Summary I

- **Epigenetic evolution and heterogeneity can predict inferior clinical outcome**
  - The epiallele repertoire shift and epimutation rate at diagnosis provide prognostic insight.
  - The first epiallele prognostic classifier predicts clinical outcome in AML



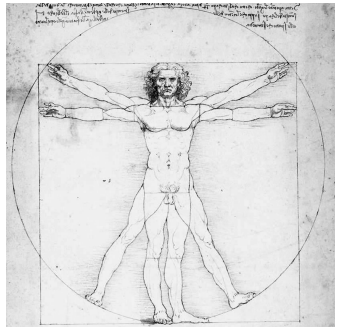
Understanding evolution is essential for improving the efficacy of leukemia treatment and prevention

- Can epigenetic evolution and heterogeneity predict inferior clinical outcome?
- **Is epiallele heterogeneity caused by the mutations or just by-product of leukemia transformation?**
- Is epigenetic heterogeneity reversible?

What are the driving forces of epigenetic heterogeneity in primary AML?

Hypothesis: particular somatic mutations, especially those affecting epigenetic modifiers induce epigenetic heterogeneity

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



67 Males; 52 Females;
Median Age: 44.

Blood or bone marrow

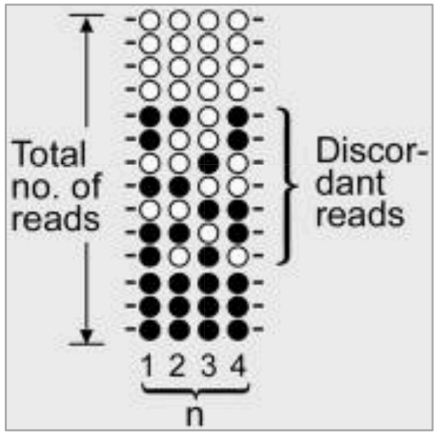
Lymphocytes depletion

Genomic Profiling

RRBS: single molecule DNA methylation profile

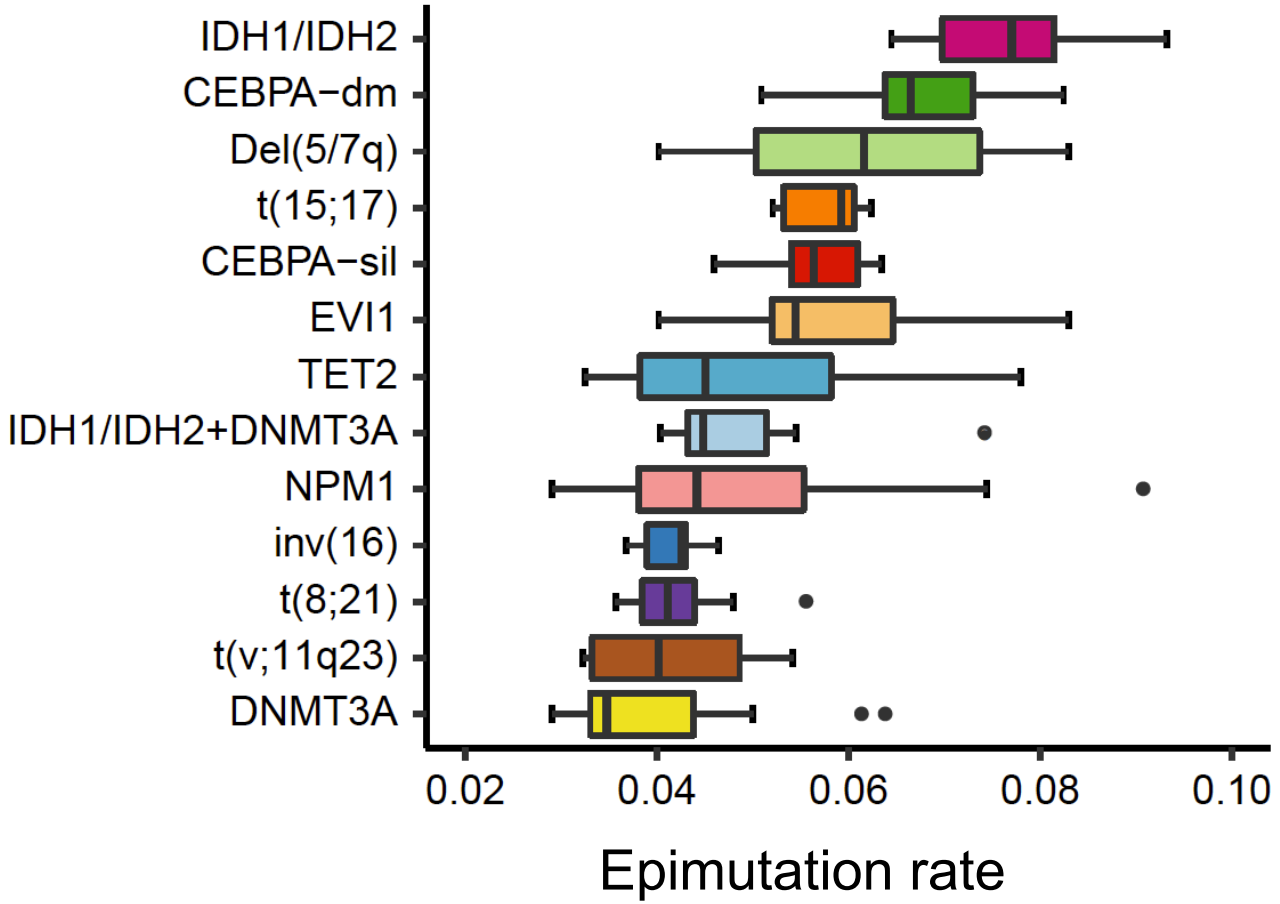
Targeted sequencing: genetic lesions profiles

Epigenetic Heterogeneity



Landau D, et al., *Cancer Cell*, 2014
Glass J, et al., *Cancer Discovery,* 2017
Li S#, Chen X#, …, *Cancer Discovery,* 2020

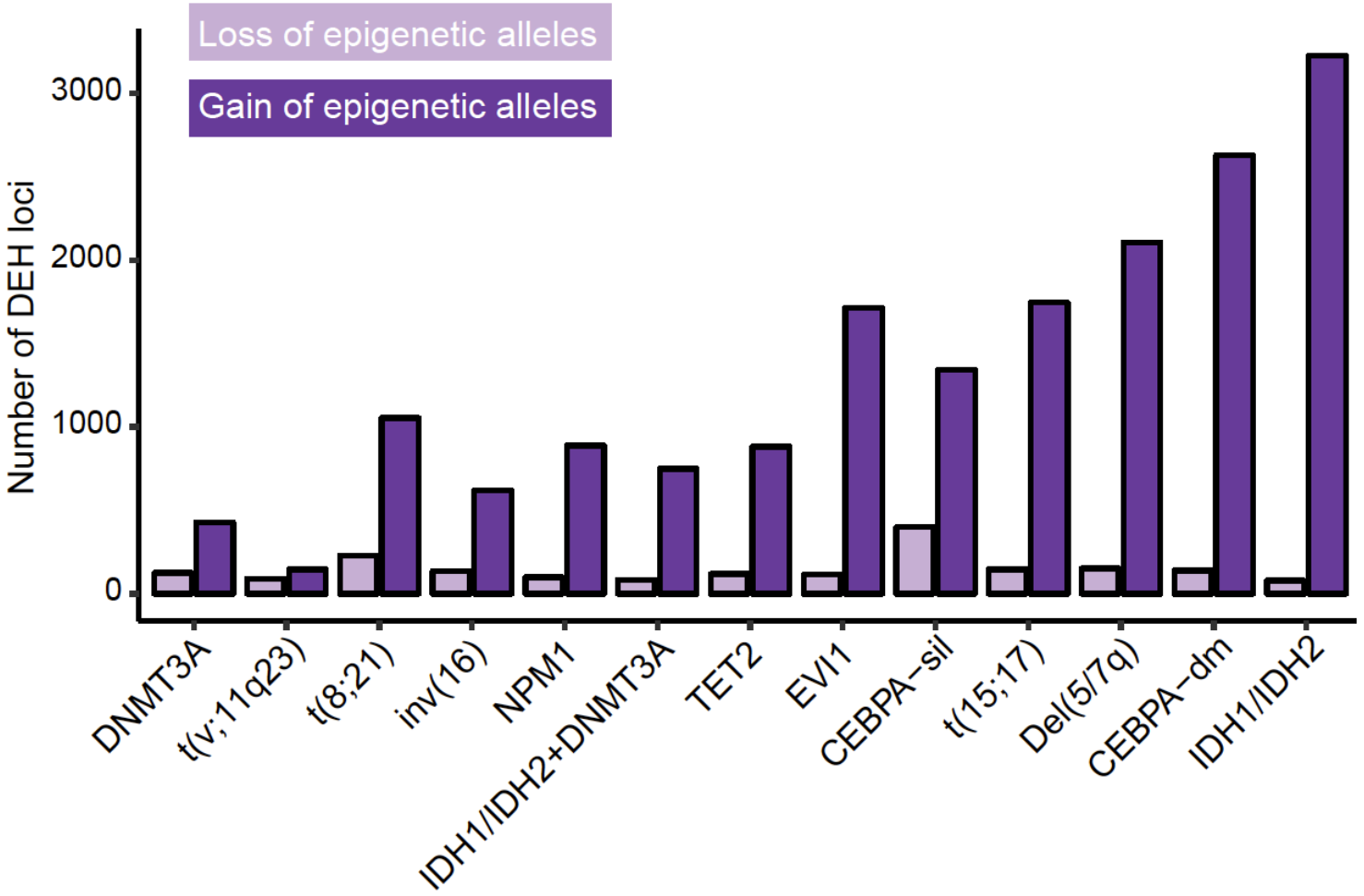
AML subtypes represent various levels of epigenetic heterogeneity



- Epigenetic heterogeneity is directly linked to specific mutations

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

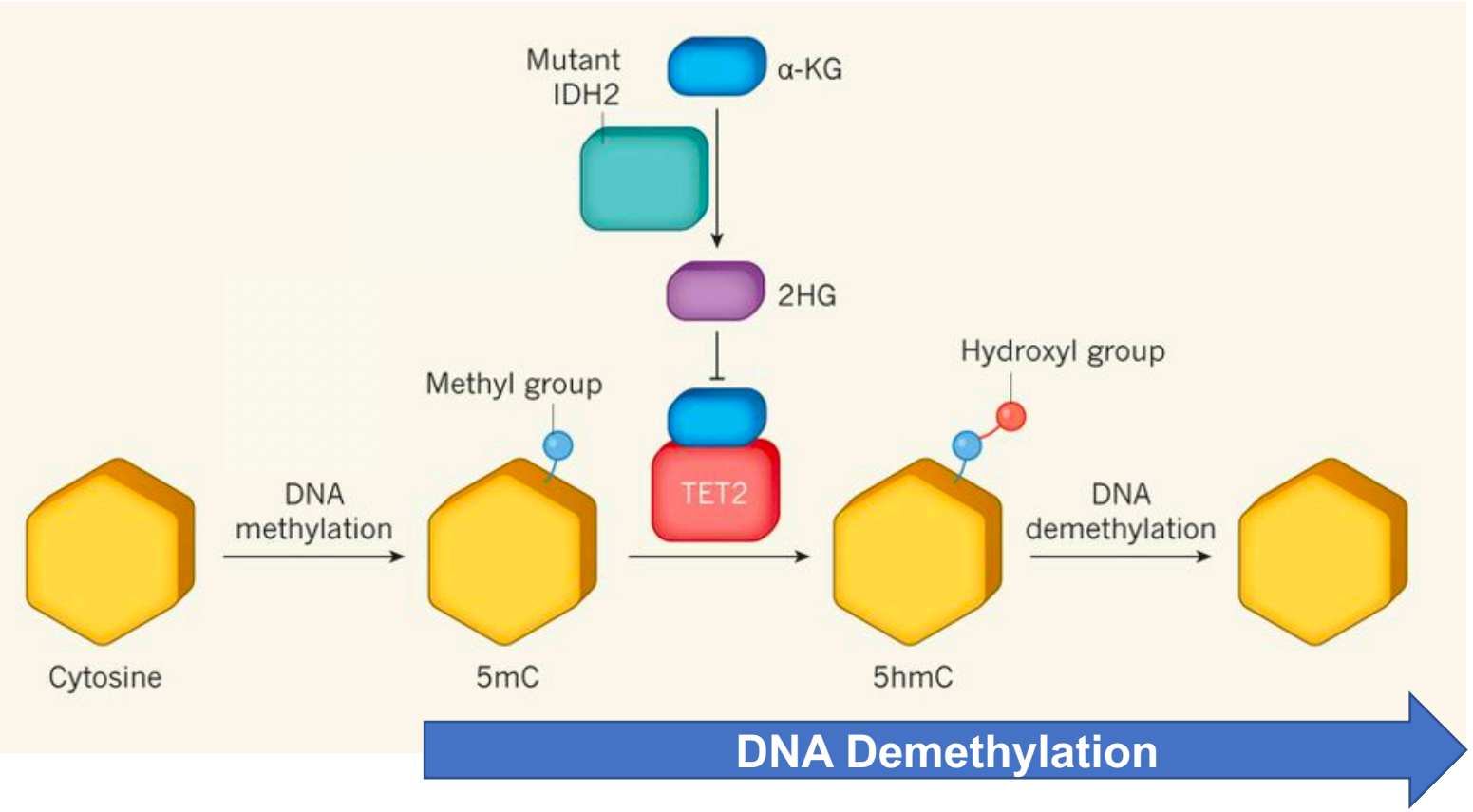
AML exhibits dominantly increased epigenetic heterogeneity loci than normal bone marrow

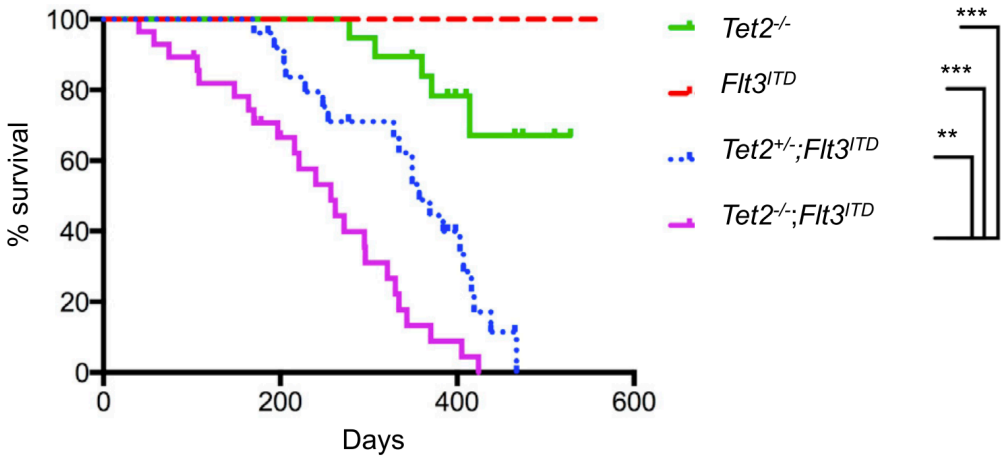


DEH Loci: Loci with Differential Epigenetic Heterogeneity

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

Is epiallele heterogeneity caused by the mutations or just by-product of leukemia transformation?



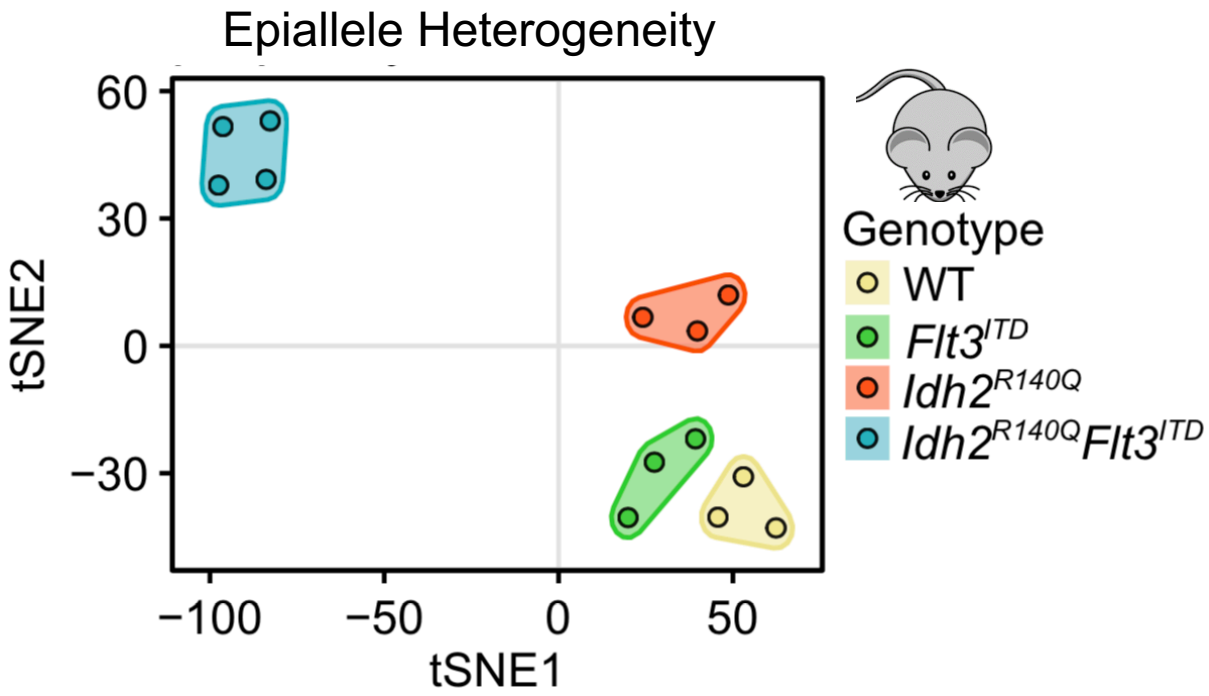


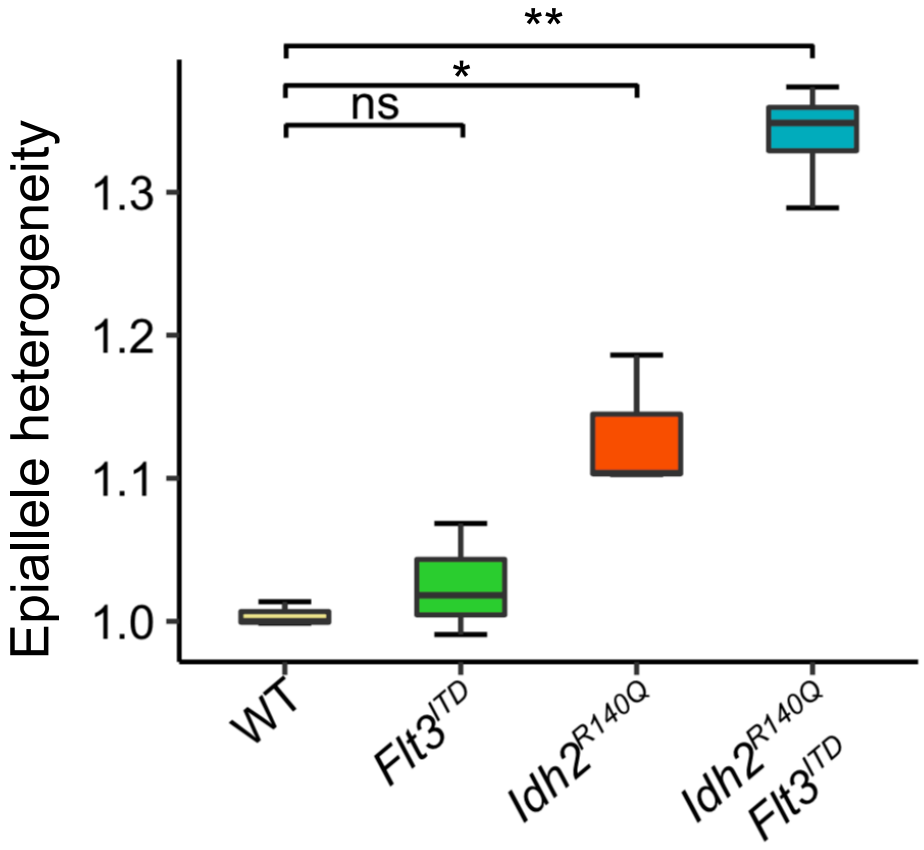
IDH2: Isocitrate dehydrogenase

TET2: Methylcytosine dioxygnase

Julie-Aurore L et al., *Nature,* 2017
Shih A et al., *Cancer Cell*, 2015 & *Cancer Discovery*, 2017

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

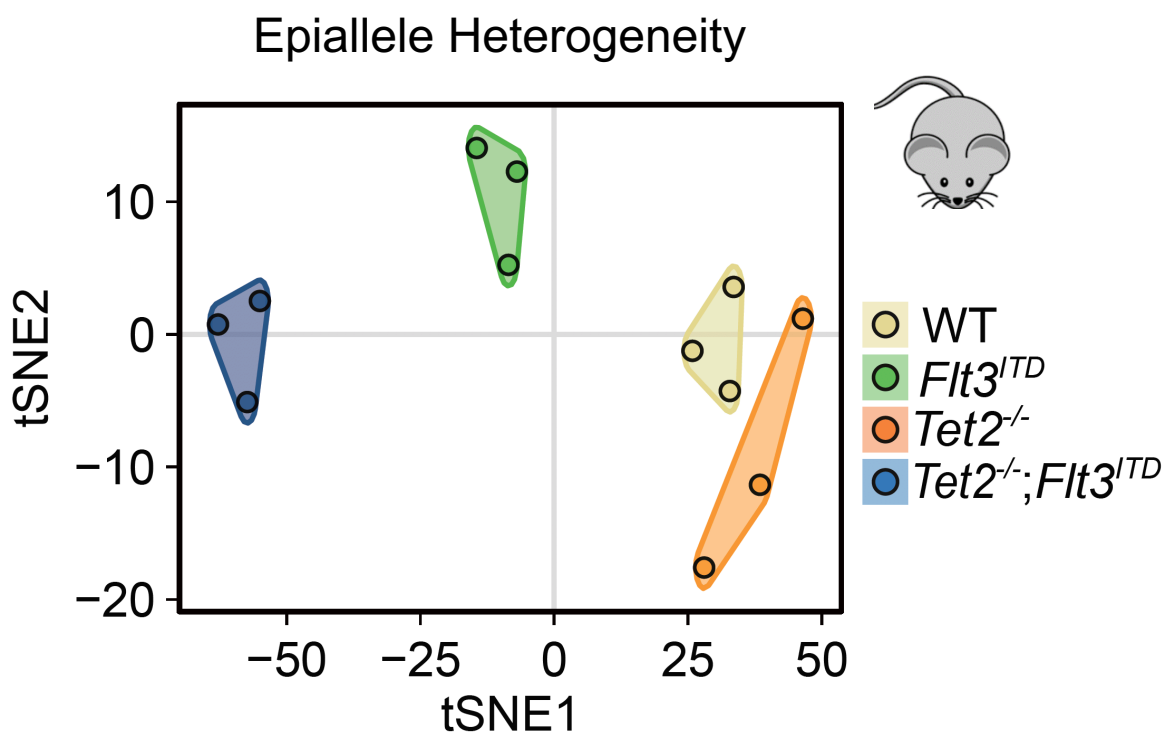


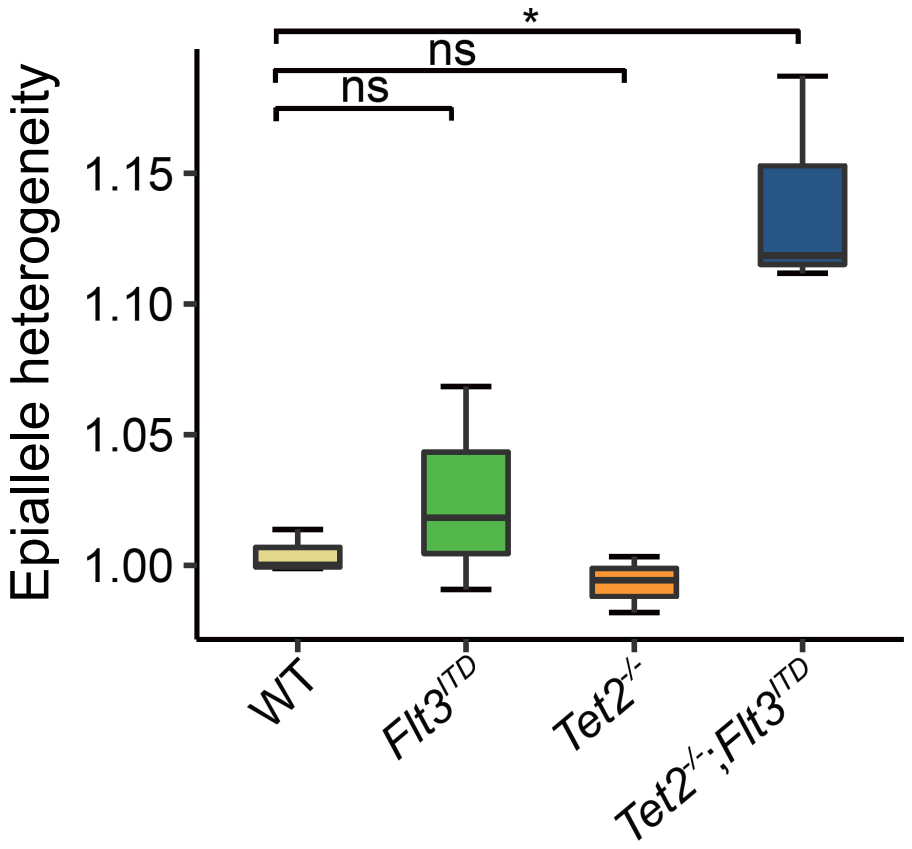


- Methylomes data: LSK (lin⁻Sca⁺cKit⁺) cells from ***healthy*** (non-leukemic) mice

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

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

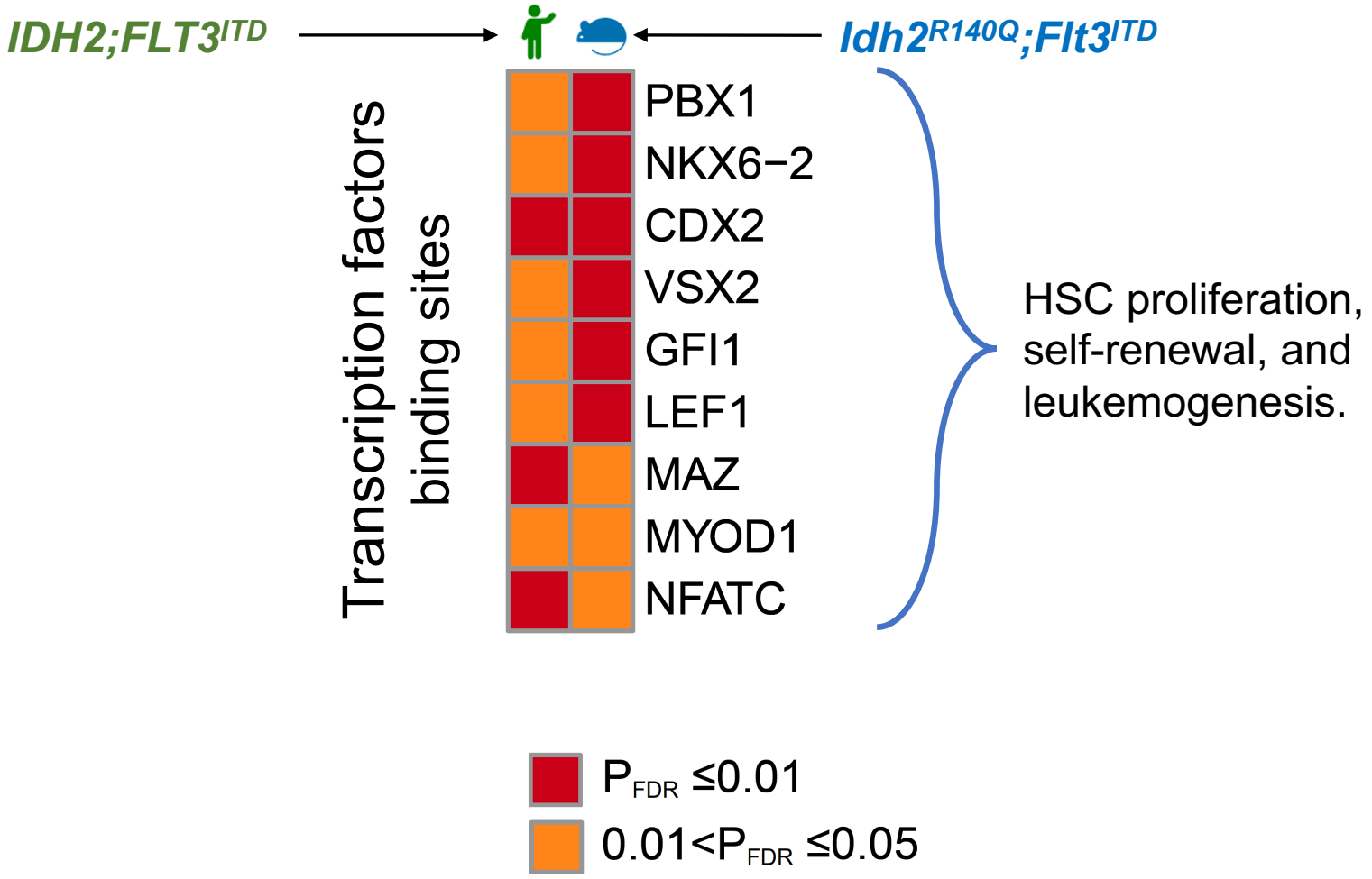




- Methylomes data: LSK (lin⁻Sca⁺cKit⁺) cells from ***healthy*** (non-leukemic) mice

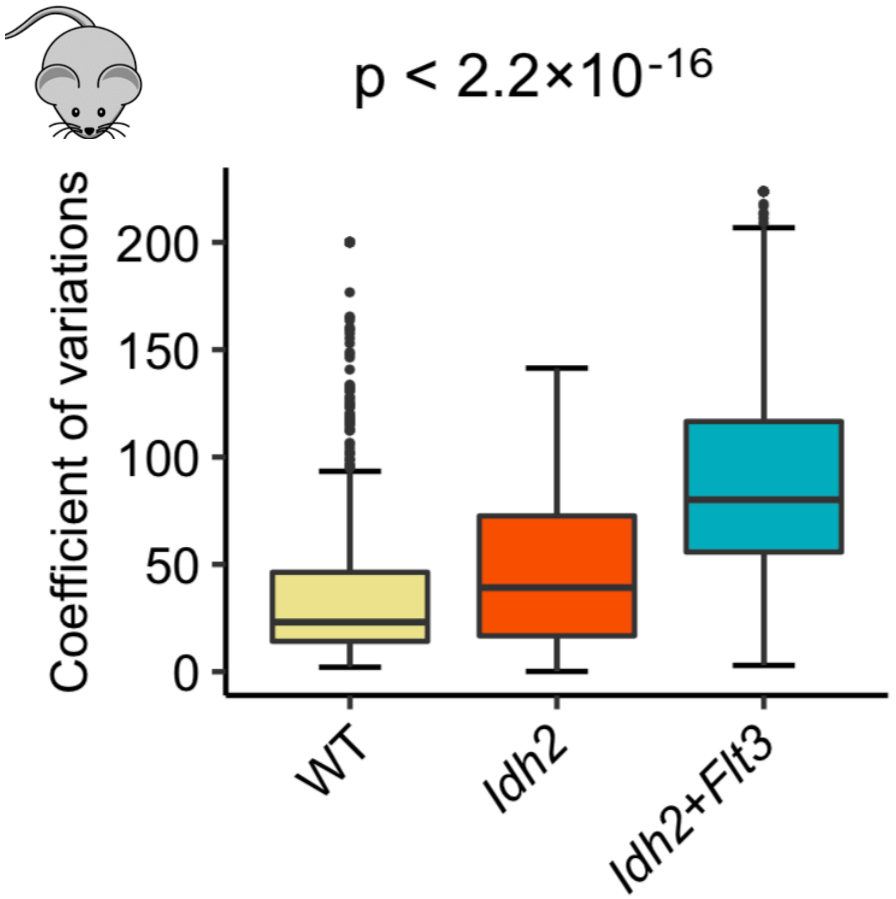
Li S#, Chen X#, …, *Cancer Discovery,* 2020

Epialleles arising in humans and mice may affect similar transcription factors with relevance to AML biology



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

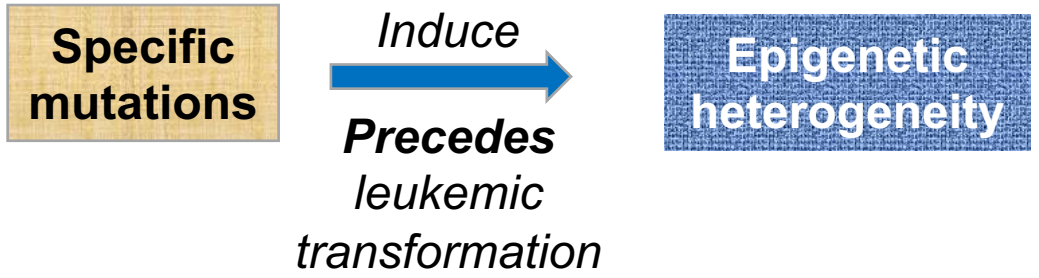
Transcriptional hyper-variability is linked to epigenetic allele diversity



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

Summary II

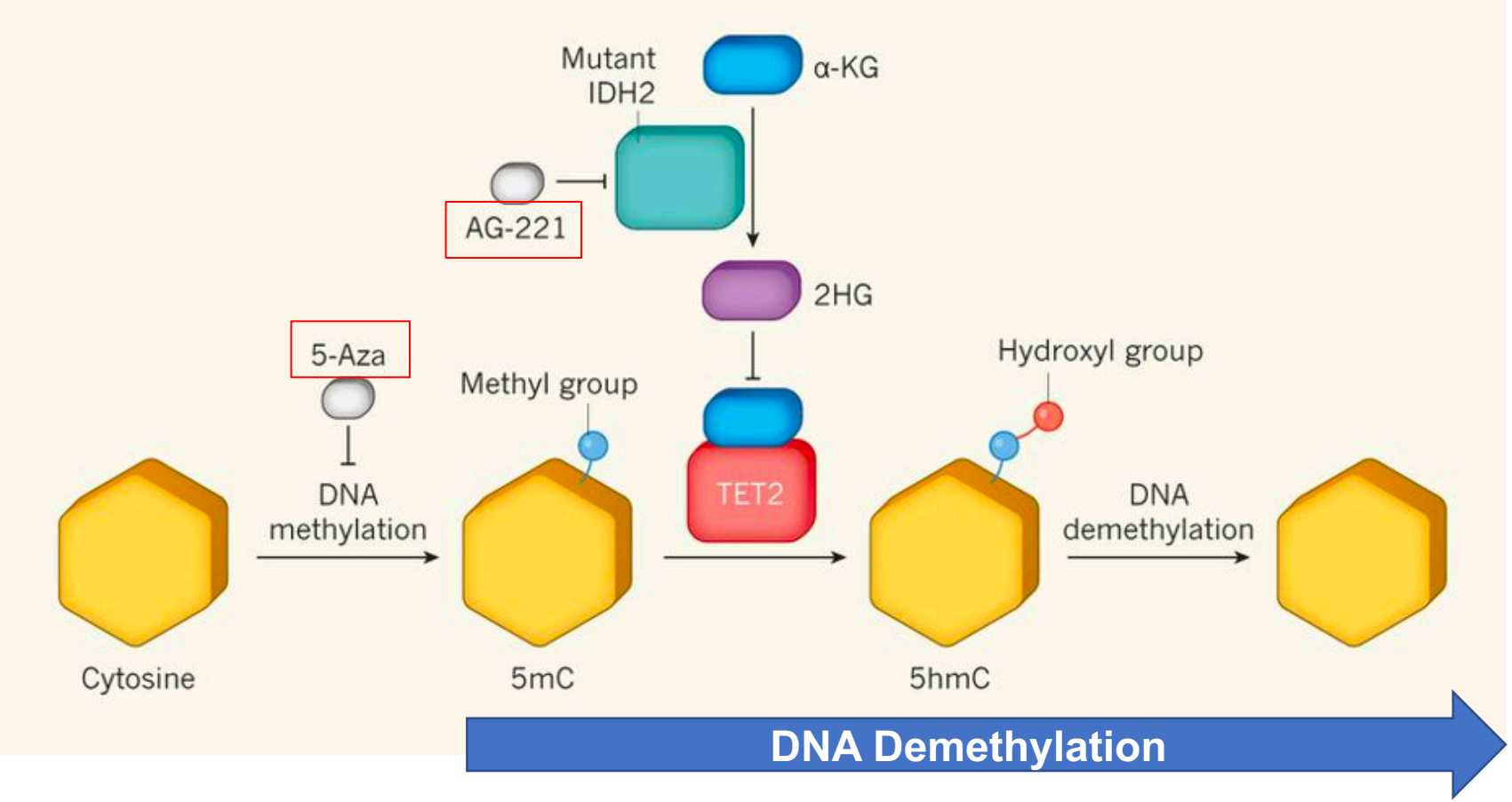
- **Somatic mutations are the driving force of epigenetic heterogeneity**
  - Genetically and epigenetically defined AML subtypes associate with specific levels of epigenetic heterogeneity
  - Somatic mutations cooperate to induce epigenetic heterogeneity before leukemic transformation. Thus, it is not a by-product of the transformation.
  - Transcriptional hyper-variability is linked to epigenetic heterogeneity



Understanding evolution is essential for improving the efficacy of leukemia treatment and prevention

- Can epigenetic evolution and heterogeneity predict inferior clinical outcome?
- Is epiallele heterogeneity caused by the mutations or just by-product of leukemia transformation?
- **Is epigenetic heterogeneity reversible?**

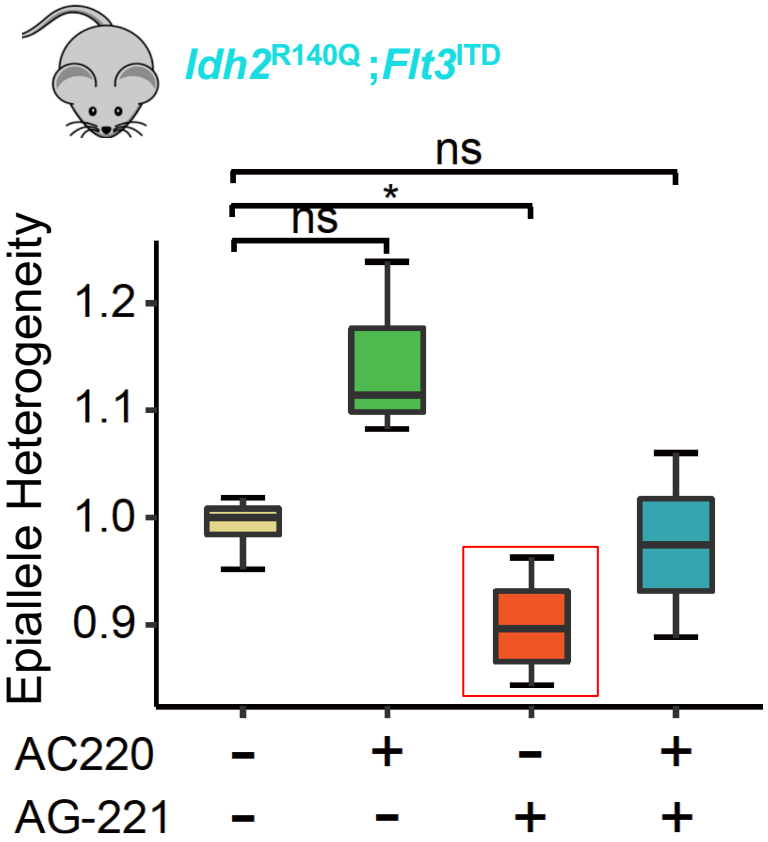
Can epigenetic therapy reverse intra-tumor epigenetic heterogeneity?



ac220: $FLT3^{ITD}$ inhibitor
ag221: mutant IDH2 inhibitor
5-Aza: hypomethylating agent

Julie-Aurore Losman, *Nature*, 2017; Shih et al., *Cancer Cell*, 2015

Can epigenetic therapy reverse intra-tumor epigenetic heterogeneity?



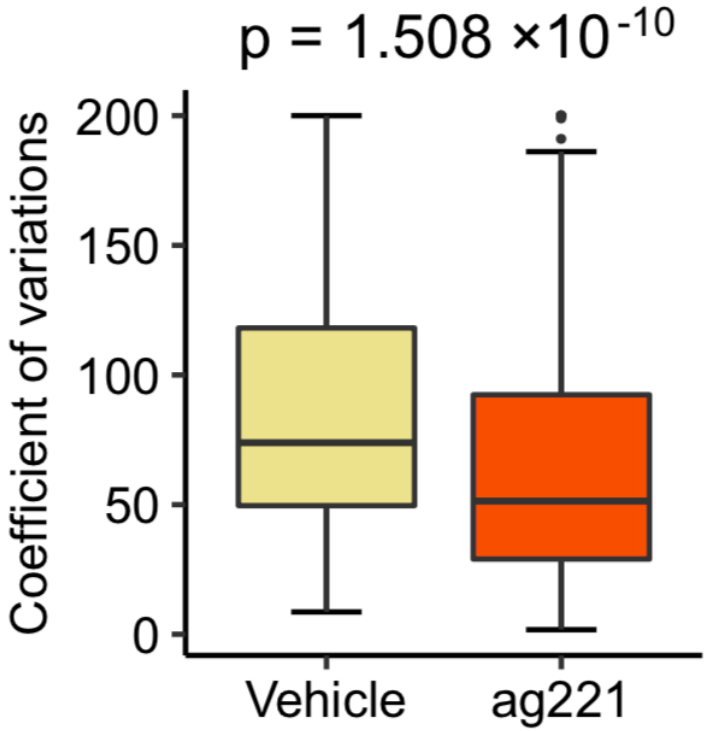
ac220: $FLT3^{ITD}$ inhibitor
ag221: mutant IDH2 inhibitor

- Mutant IDH2 inhibitor can suppress epiallele heterogeneity

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

Can epigenetic therapy reverse transcriptional hyper-variability?

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

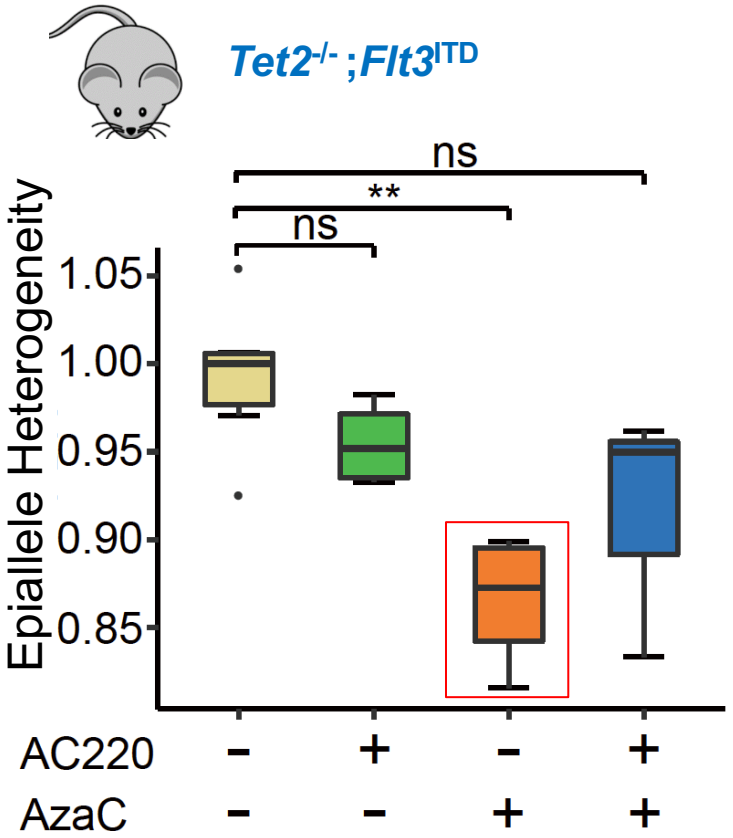


ag221: mutant IDH2 inhibitor

- Mutant IDH2 inhibitor can suppress transcriptome heterogeneity

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

Can epigenetic therapy reverse intra-tumor epigenetic heterogeneity?



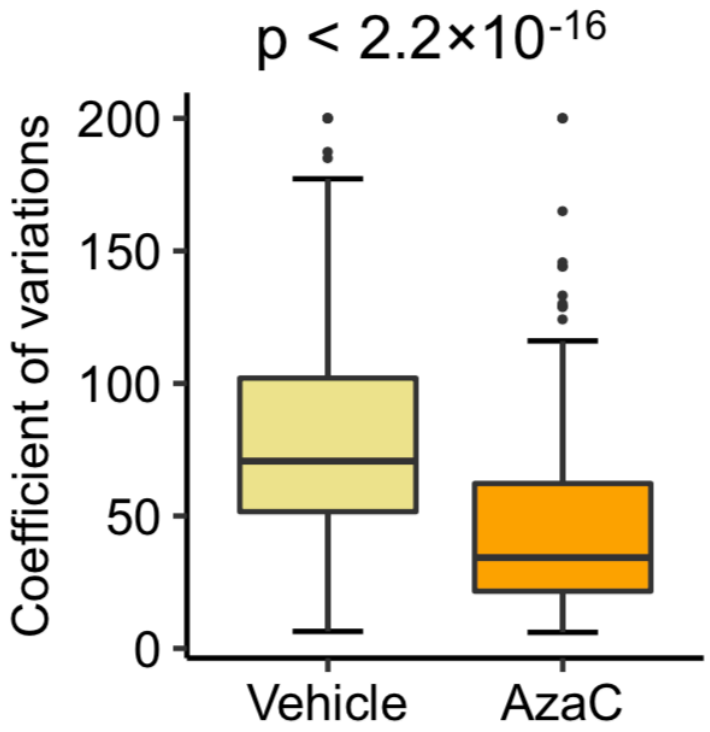
ac220: $FLT3^{ITD}$ inhibitor
5-Aza: hypomethylating agent

- DNA methyltransferase inhibitor can suppress epiallele heterogeneity

Li $S^{\#}$, Chen $X^{\#}$, …, *Cancer Discovery,* 2020

Can epigenetic therapy reverse transcriptional hyper-variability?

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

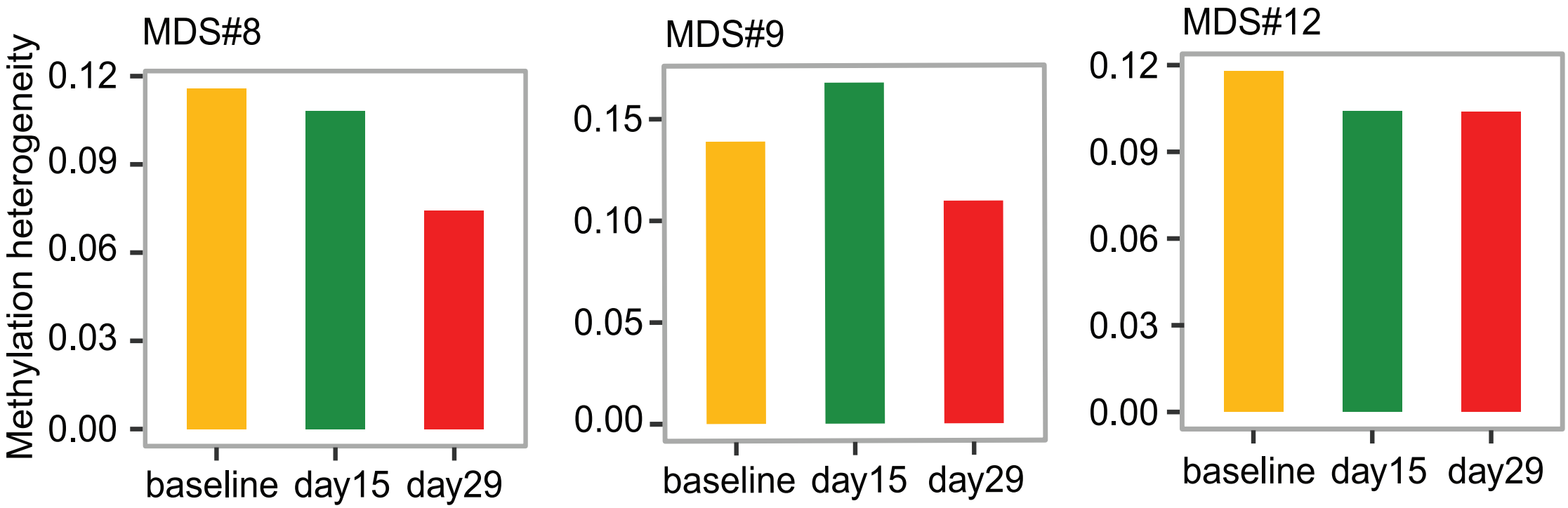


5-Aza: hypomethylating agent

- DNA methyltransferase inhibitor can suppress transcriptome heterogeneity

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

Can epigenetic therapy reverse intra-tumor epigenetic heterogeneity in human?

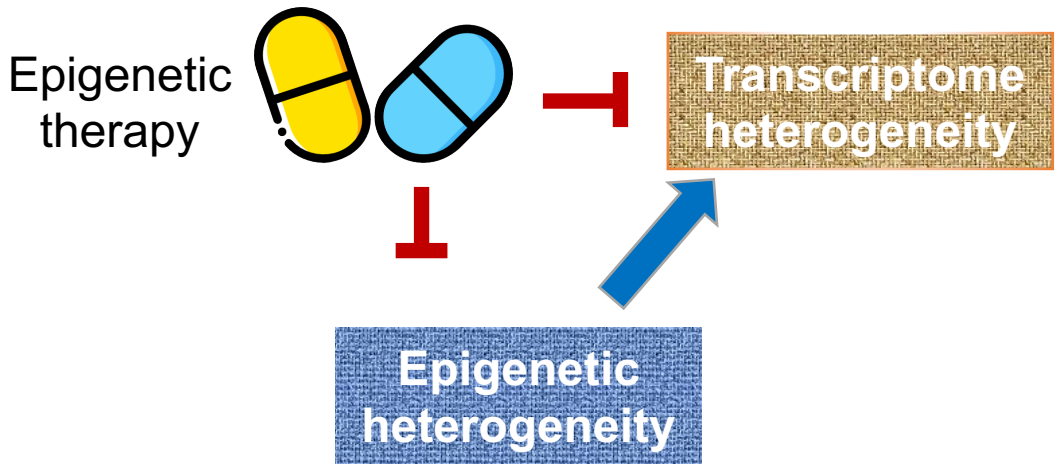


MDS: myelodisplastic syndrome
AML can develop in patients with MDS

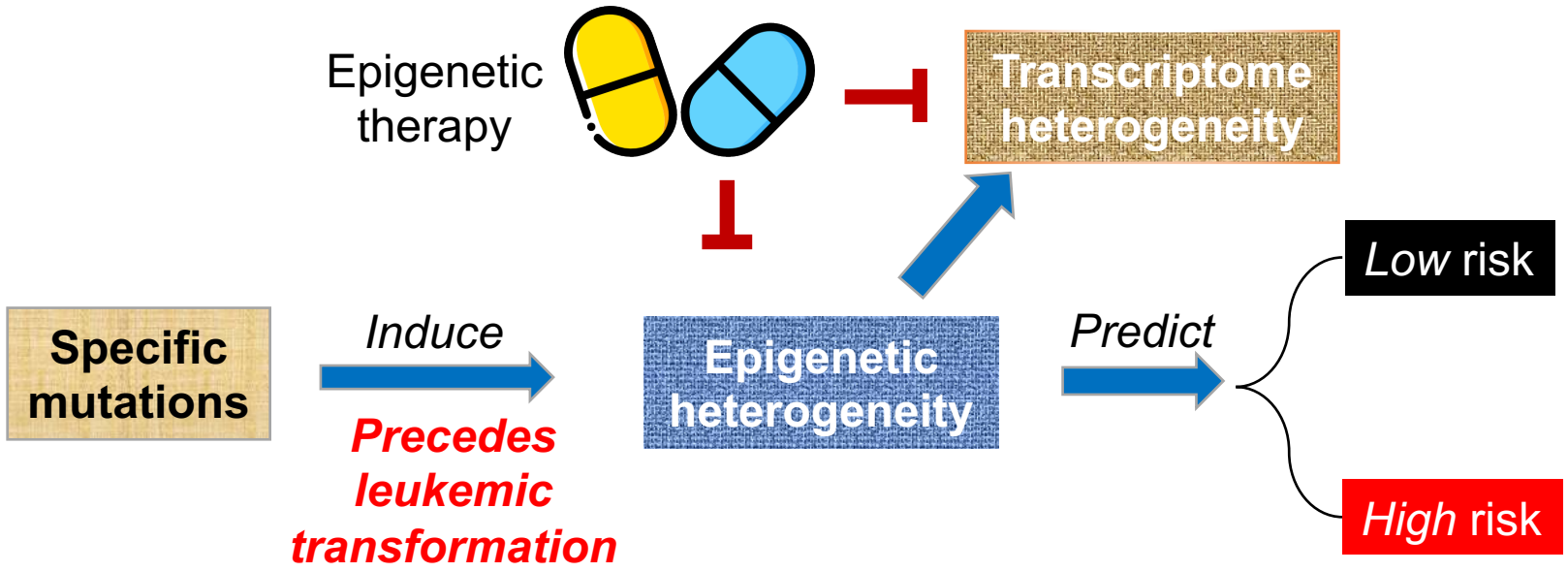
Li S#, Chen X#, …, *Cancer Discovery,* 2020

Summary III

- **Epigenetic heterogeneity is reversible pharmaceutically**
  - Epigenetic therapy can suppress epigenetic allele diversity.
  - Transcriptional heterogeneity associated with epialleles decreased significantly after epigenetic therapy.



Wrap-up



- Epigenetic evolution and heterogeneity can predict inferior clinical outcome
- AML recurrent somatic mutations are the driving force of epigenetic heterogeneity
- Epigenetic heterogeneity is reversible pharmaceutically

Next Steps

- Does epigenome evolution drive genome evolution? And if so, what are the key intrinsic factors mediating this process?
- Can we prevent cancer genome evolution by reverse epigenome evolution?
- Can epigenome heterogeneity be used as biomarker to monitor cancer initiation?

New Platforms to Study Intra-tumor Heterogeneity

- Single-cell multi-omics sequencing
  - Single-cell RNA-seq
  - Single-cell ATAC-seq
- Single-molecule long-read sequencing
  - Nanopore sequencing
  - Long-range epiallele detection
- 3D genome sequencing
  - Hi-C (Sub-Compartment Identifier)
  - ChIA-PET (ChIA-PIPE)

Ashoor H, … Li S, Nature Communications, 2020
Lee B, … Li S, Science Advances, 2020