COLUMBIA | COLUMBIA STEM CELL INITIATIVE

PATH 4500 CANCER BIOLOGY: HEMATOLOGICAL MALIGNANCIES

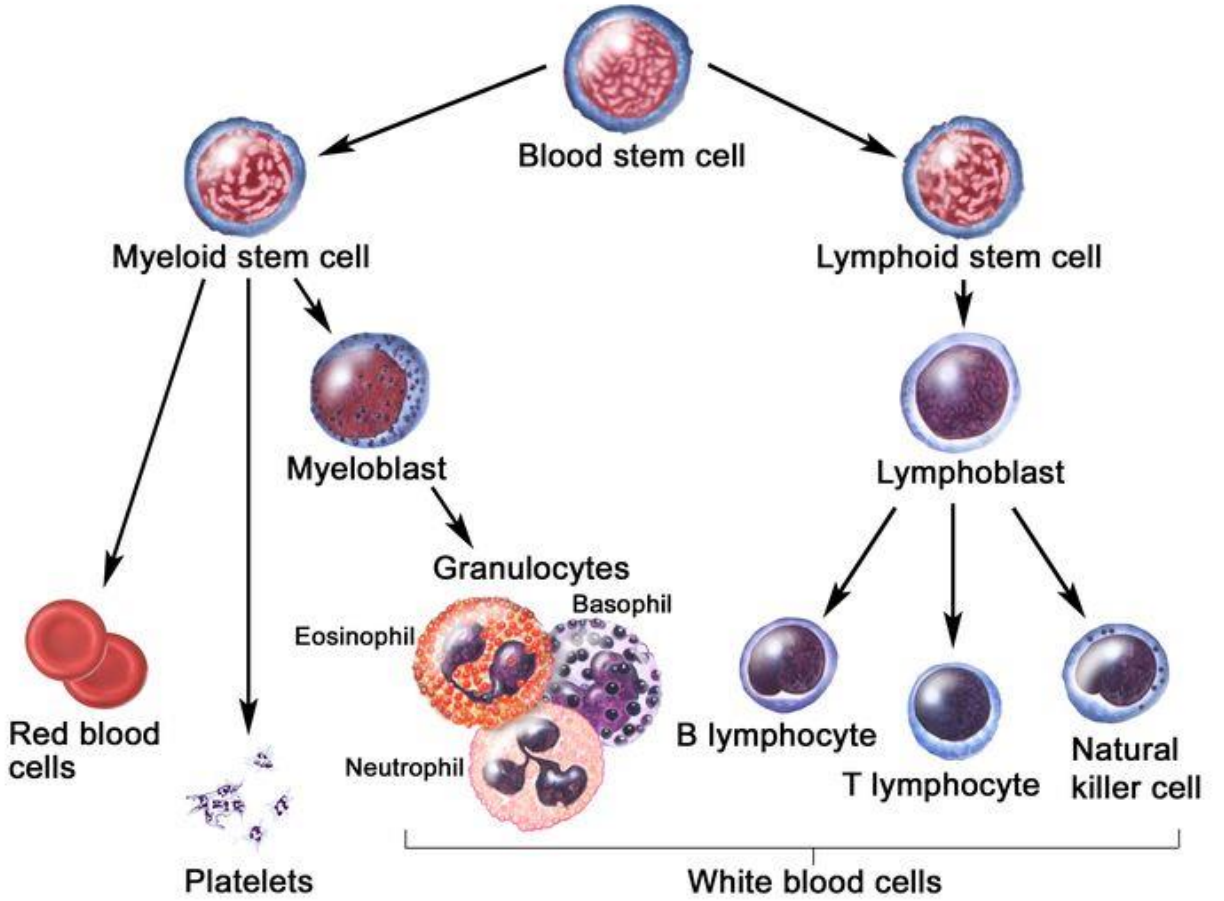
Aaron D. Viny MD MS
Columbia University Irving Medical Center
Division of Hematology & Oncology
Herbert Irving Comprehensive Cancer Center
Columbia Stem Cell Initiative

COLUMBIA | COLUMBIA UNIVERSITY IRVING MEDICAL CENTER

LEARNING OBJECTIVES

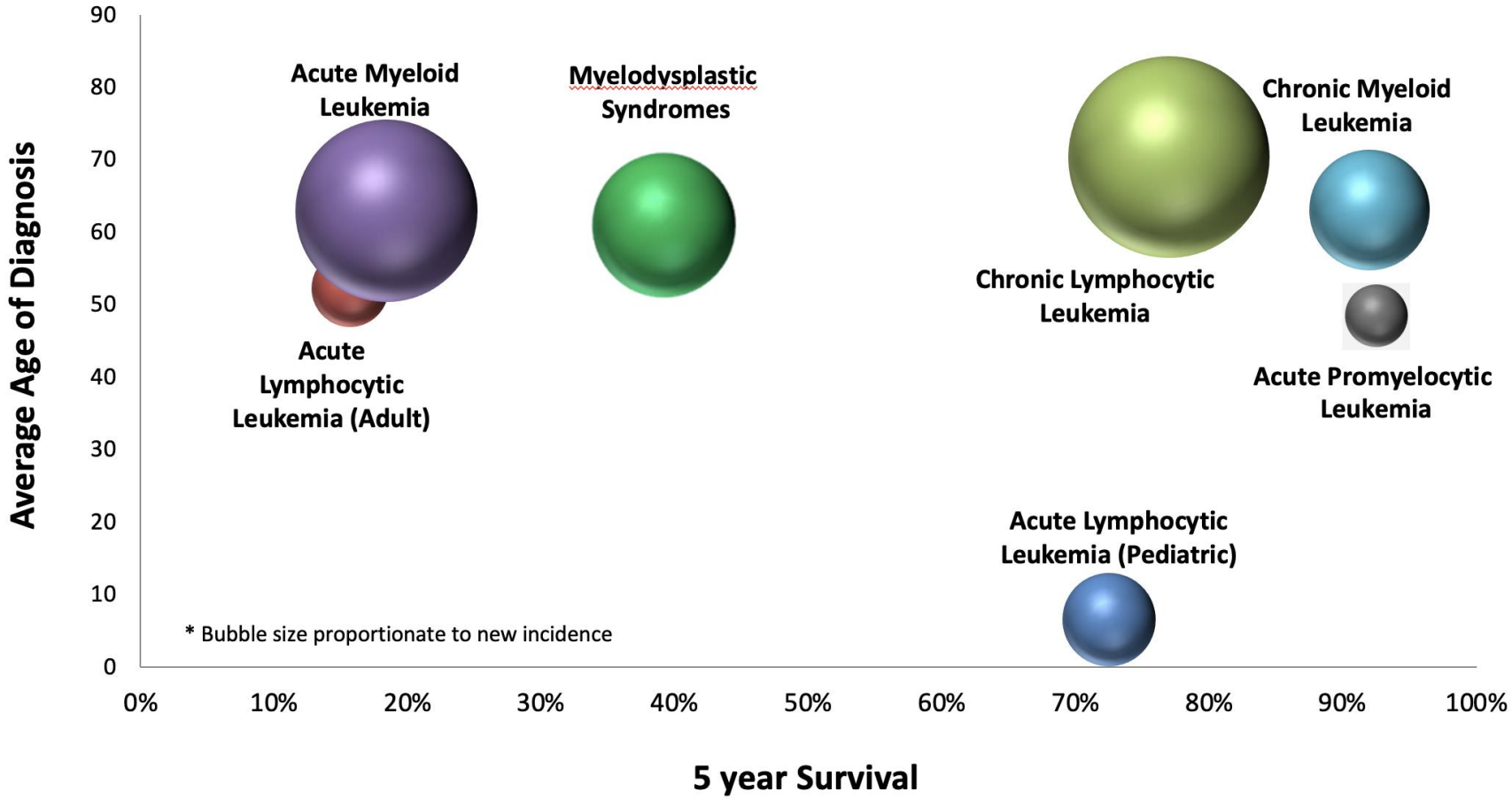
- Understand how the same molecular genetics can lead to distinct clinical phenotypes in clonal hematopoiesis,
- Contrast Stem Cell Fitness with Cancer
- Describe the concept of epigenetics and explain the role of epigenetic mechanisms in the regulation of gene expression and how it can influence disease. Recognize that some epigenetic modifications can change over time.
- Recognize the types of genetic and epigenetic changes that can result in gain-of-function of proto- oncogenes or loss-of-function of tumor suppressor genes (e.g., Knudson two-hit hypothesis) in hematological malignancies.
- Describe the application of current somatic/tumor and germline testing (cytogenetic, molecular, and epigenetic technologies) to clarify the mechanism of tumorigenesis, evolution, diagnosis, and prognosis of cancer.

The bone marrow is tasked with a lifetime of blood cell production!



https://www.cancer.gov/types/leukemia/

Cancer is really difficult to cure. Yet in blood cancers we have shown it is absolutely possible!



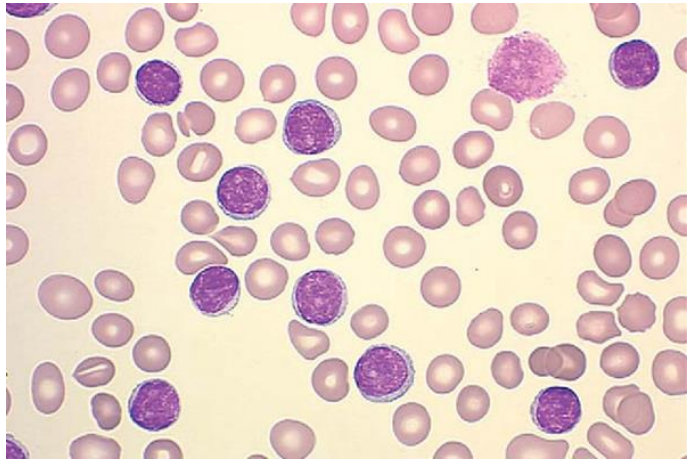
Risk Factors for hematological Malignancies

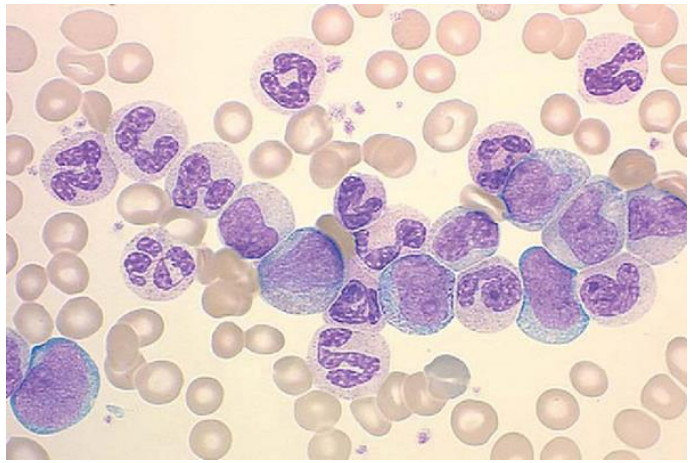
- Host factors
  - Chromosomal Instability (i.e. Li Fraumani, Fragile X, Fanconi's Anemia) or those with increased numbers of chromosomes (such as Down's syndrome)
  - Immunodeficiencies
  - Chronic marrow dysfunction such as those with myeloproliferative diseases, myelodysplastic syndromes, aplastic anemia, or paroxsymal nocturnal hemoglobinuria.
- Environmental factors:
  - Exposure to ionizing radiation
  - Exposure to mutagenic chemicals and drugs
  - Viral infections (HTLV, EBV)

Diagnosis

- CBC/Peripheral Smear
  - Failure of the bone marrow and normal hematopoiesis may result in pancytopenia
  - Finding of immature cells in the peripheral blood (including blasts, promyelocytes, promonocytes (>30% blasts is diagnostic)

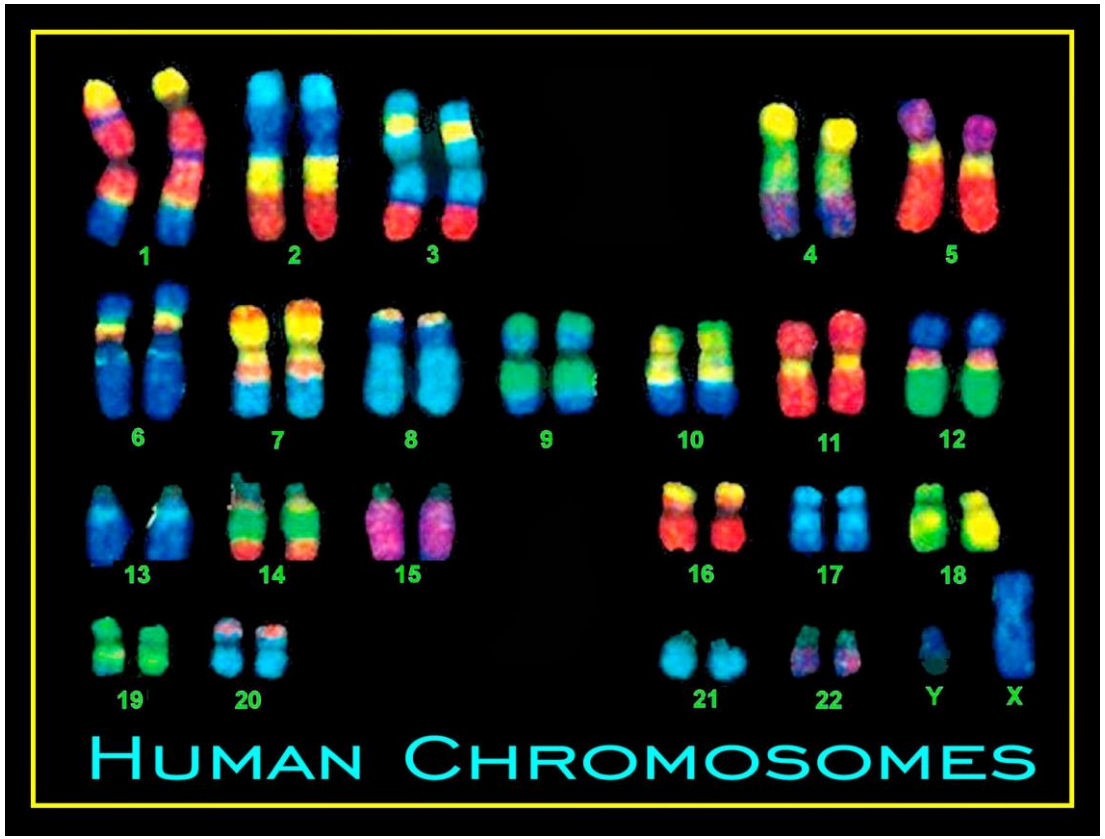
- Bone Marrow Examination
  - Morphologic dysplasia, abnormal increase in blasts
  - Incomplete maturation or maturation arrest
  - Hypercellularity (Normal is ~100 minus age)
  - Flow cytometry for abnormal cell population
  - Karyotype and Molecular Studies





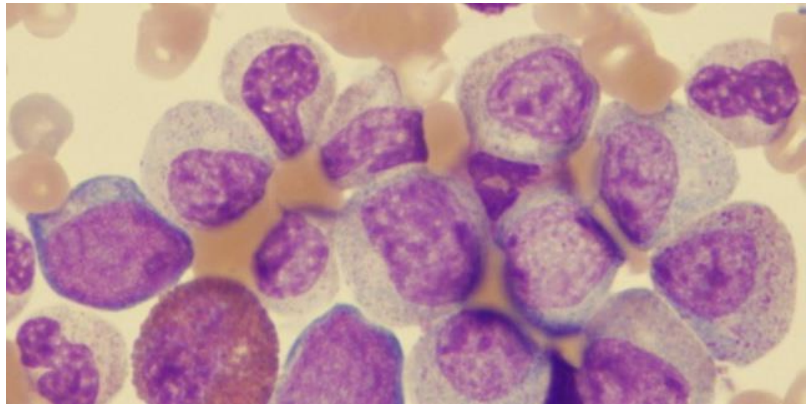
Assessing Chromosome Number: Metaphase Karyotyping

- What cell cycle stage do we need to be in to look at the chromosomes?
- What must these cells be able to “do” in order to run a karyotype?
- Common samples with dividing cells: blood, bone marrow, amniotic fluid, tumor
- What's the next step after metaphase?
- How do we keep them in metaphase?
- Standard metaphase karyotype testing counts 20 cells (count is in brackets)

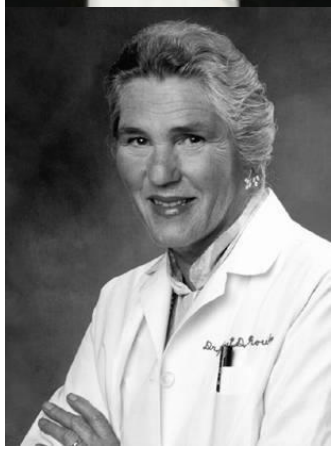


1960s, 1970s: Misplaced pieces of CHROMOSOMES as a marker for cancer

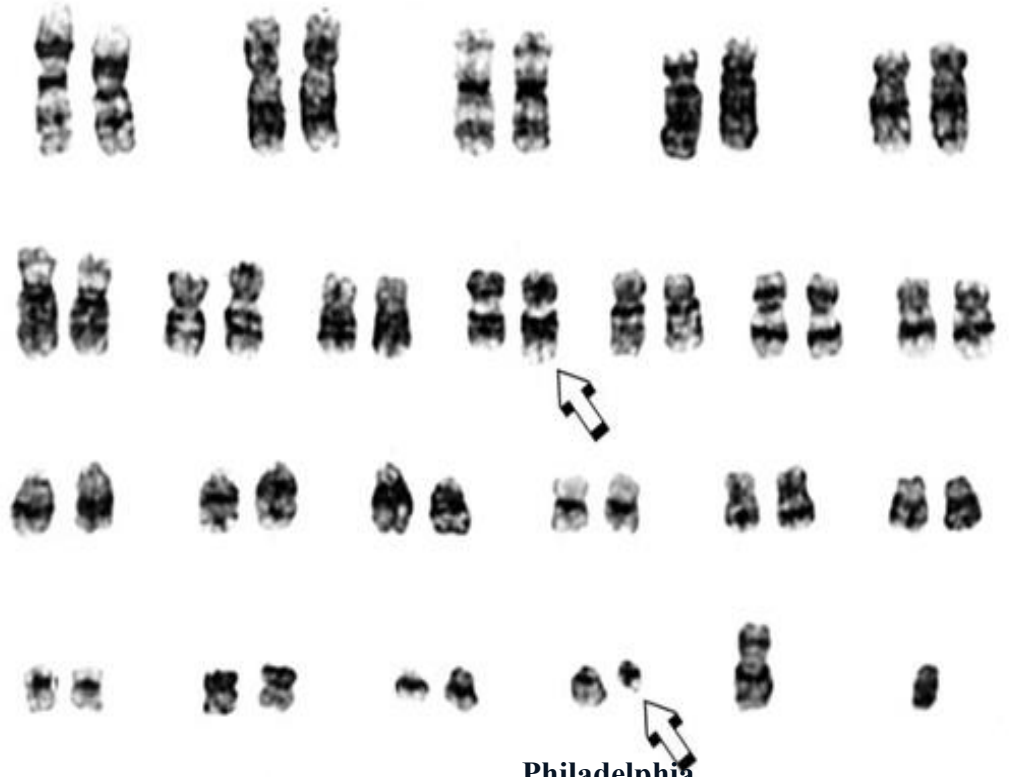
Chronic Myelogenous Leukemia



Peter Nowell, MD

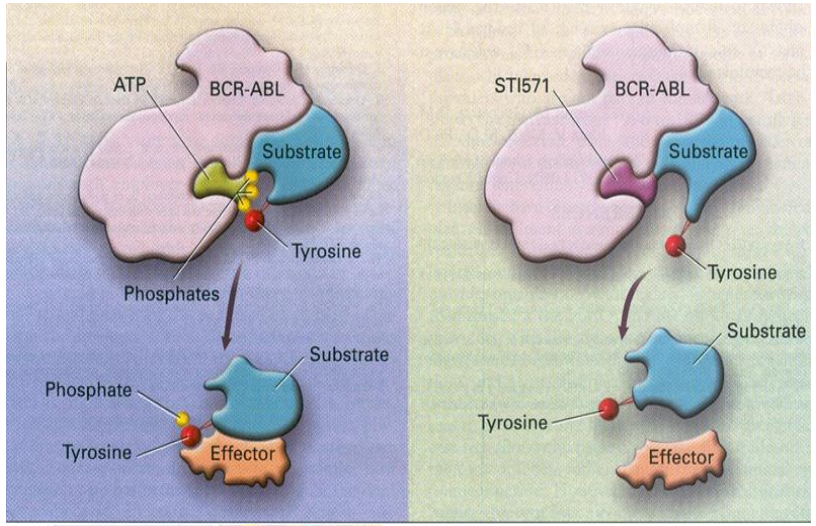


Janet Rowley, MD

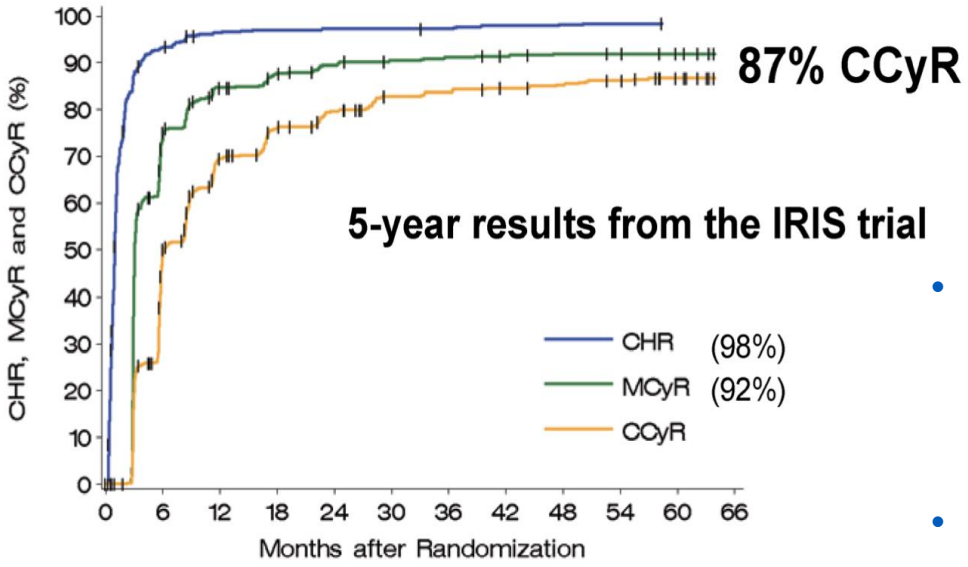


Philadelphia Chromosome

First targeted therapy of oncogene signaling: Imatinib in CML



Druker, Sawyers

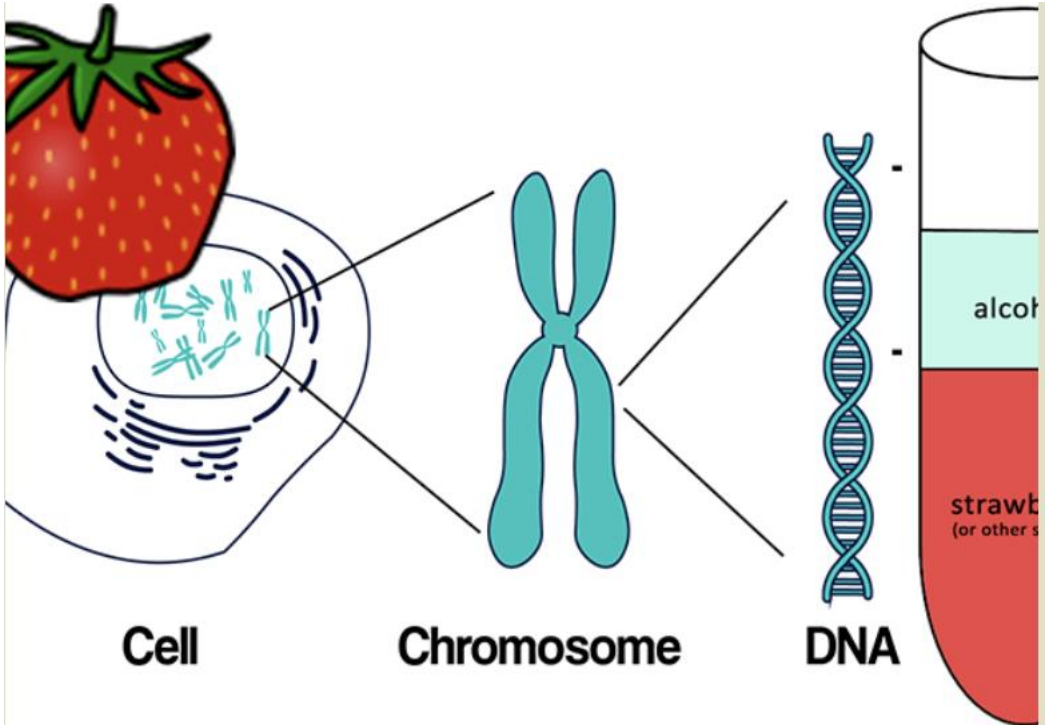


Goldman JM, Melo JV. *N Engl J Med.* 344:1084-1086.

- Precision cancer therapy, also known as precision medicine, is a treatment approach that uses a patient's tumor's genetic profile to tailor their cancer treatment. *CCyR = Complete Cytogenetic Remission
- BCR-ABL oncoprotein is phosphorylated on a tyrosine residue activating other downstream effector molecules.
- Imatinib occupies kinase pocket , the action of ATP is inhibited, and substrate cannot be phosphorylated, blocking leukemic signaling

Introduction to Genetics Testing (How big of a needle? How big of a haystack?)

- We need to “read” the genome looking for errors (ones we specifically suspect versus more of a fishing expedition)
- First things first: we need to isolate DNA!
- How are we going to get it? (Hint: did you ever isolate DNA from strawberries as a kid?)
- How do you break the cell and nuclear membranes?
- Is DNA polar or non-polar?



Molecular Methods and Technologies

Single Gene tests

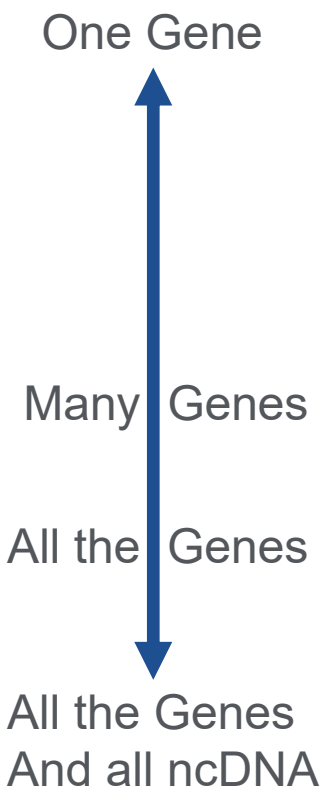
- Polymerase Chain Reaction (PCR)
- Trinucleotide Repeat Expansion by Capillary Electrophoresis
- Quantitative PCR (qPCR)
- Sanger Sequencing

Panel Testing

Exome Sequencing

Genome Sequencing

- Short reads
- Long reads



Sequencing Several Genes: Targeted Sequencing Panels

COLUMBIA | COLUMBIA UNIVERSITY DEPARTMENT OF PATHOLOGY AND CELL BIOLOGY

Genetic Testing

Genetic Testing is available in the Laboratory for Personalized Genomic Medicine links below for a description of tests offered. For more information, email pgminquiry@cumc.columbia.edu.

https://www.pathology.columbia.edu/diagnostic-specialties/personalized-genomic-medicine/genetic-testing

Next Generation Sequencing

- MOP-CWES - Cancer Whole Exome Sequencing (with Transcriptome)
- MOP-CSTP - Columbia Solid Tumor Panel (CSTP)
- MOP-CSTP - Columbia Solid Tumor Subpanels (CSTP)
- MOP-CCCP - Columbia Combined Cancer Panel (CCCP)
- MGP-CF Cystic Fibrosis Carrier Screening
- MGP-CONF Sanger Sequencing of Individual Variants
- MGP-FP Thrombophilia Risk Panel

Sequencing Several Genes: Targeted Sequencing Panels

Targeted Myeloid Panel (TMP)

Purpose

Myeloid neoplasms encompass a diverse array of blood cancers, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myeloproliferative neoplasm (MPN), amongst other less common entities. These cancers are characterized by a broad spectrum of clinical and pathologic abnormalities, including abnormal peripheral blood counts, morphologic dysplasia, and variable expansion of myeloblasts. Mutational characterization is used in the diagnostic and prognostic stratification of myeloid neoplasia, and may further inform personalized treatment strategies through the use of targeted and selective therapies. The Targeted Myeloid Panel includes 50 of the most commonly mutated genes in myeloid neoplasia, with an emphasis on genes with diagnostic, prognostic, and therapeutic significance.

https://www.pathology.columbia.edu/diagnostic-specialties/personalized-genomic-medicine/oncology-testing

Genes Tested (exons)

ABL1*(NM_005157 ex4-10)*, ***ANKRD26****(NM_014915 c.-113-c.-134)*, ***ASXL1****(NM_015338.5 ex1-13, NM_001164603.1 ex5)*, ***BCOR****(NM_017745 ex2-15, NM_001123385 ex8)*, ***BCORL1****(NM_021946 ex1-12)*, ***CALR****(NM_004343 ex8-9)*, ***CBL****(NM_005188 ex2-9,16)*, ***CBLB****(NM_170662 ex3,9,10)*, ***CBLC****(NM_012116 ex9-10)*, ***CEBPA****(NM_004364 ex1)*, ***CSF3R****(NM_156039 ex17, NM_172313 ex10,18, NM_000760 ex14-16)*, ***CUX1****(NM_001202543 ex15-24, NM_001913 ex1-23, NM_181552 ex1)*, ***DDX41****(NM_016222 ex1-17)*, ***DNMT3A****(NM_022552 ex2-3,5-23, NM_153759 ex1-2, NM_175630 ex4)*, ***ETNK1****(NM_018638 ex3)*, ***ETV6****(NM_001987 ex1-8)*, ***EZH2****(NM_004456, ex2-20)*, ***FLT3****(NM_004119 ex8-17,19-21)*, ***GATA2****(NM_032638 ex2-6)*, ***GNAS****(NM_000516 ex8-11)*, ***IDH1****(NM_005896 ex3-4)*, ***IDH2****(NM_002168 ex 4,6)*, ***JAK2****(NM_004972 ex12-16,19-25)*, ***KIT****(NM_000222 ex1-2,5,8-15,17-18)*, ***KMT2A****(NM_005933 ex1-13,15-36, NM_001197104 ex14)*, ***KRAS****(NM_004985 ex2-4)*, ***LUC7L2****(NM_016019 ex1-10, NM_001244585 ex2)*, ***MPL****(NM_005373, ex10,12)*, ***NF1****(NM_000267 ex1-14,16-57, NM_001128147 ex15, NM_001042492 ex31)*, ***NPM1****(NM_002520 ex12)*, ***NRAS****(NM_002524 ex2-5)*, ***PHF6****(NM_032335 ex2-8, NM_001015877 ex10)*, ***PIGA****(NM_002641 ex2-6, NM_020473 ex1)*, ***PPM1D****(NM_003620 ex6)*, ***PTPN11****(NM_002834 ex3-4,7-8,12-13, NM_080601 ex11)*, ***RAD21****(NM_006265 ex2-14)*, ***RUNX1****(NM_001754 ex2-3,5-9, NM_001122607 ex1,5)*, ***SETBP1****(NM_015559 ex4 p.799-p.950)*, ***SF3B1****(NM_012433 ex13-21)*, ***SH2B3****(NM_005475 ex2-8)*, ***SMC1A****(NM_006306 ex1-25, NM_001281463 ex2)*, ***SMC3****(NM_005445 ex10,13,19,23,25,28)*, ***SRSF2****(NM_003016 ex1-2)*, ***STAG2****(NM_006603 ex2-33, NM_001042749 ex32)*, ***TET2****(NM_001127208 ex4-11, NM_017628 ex3)*, ***TP53****(NM_000546 ex1-11, NM_001276696 ex10, NM_001276695 ex10)*, ***U2AF1****(NM_006758 ex2,6-7, NM_001025204 ex6)*, ***U2AF2****(NM_007279 ex1-12)*, ***WT1****(NM_000378 ex1-9, NM_001198552 ex8)*, ***ZRSR2****(NM_005089 ex1-11)*

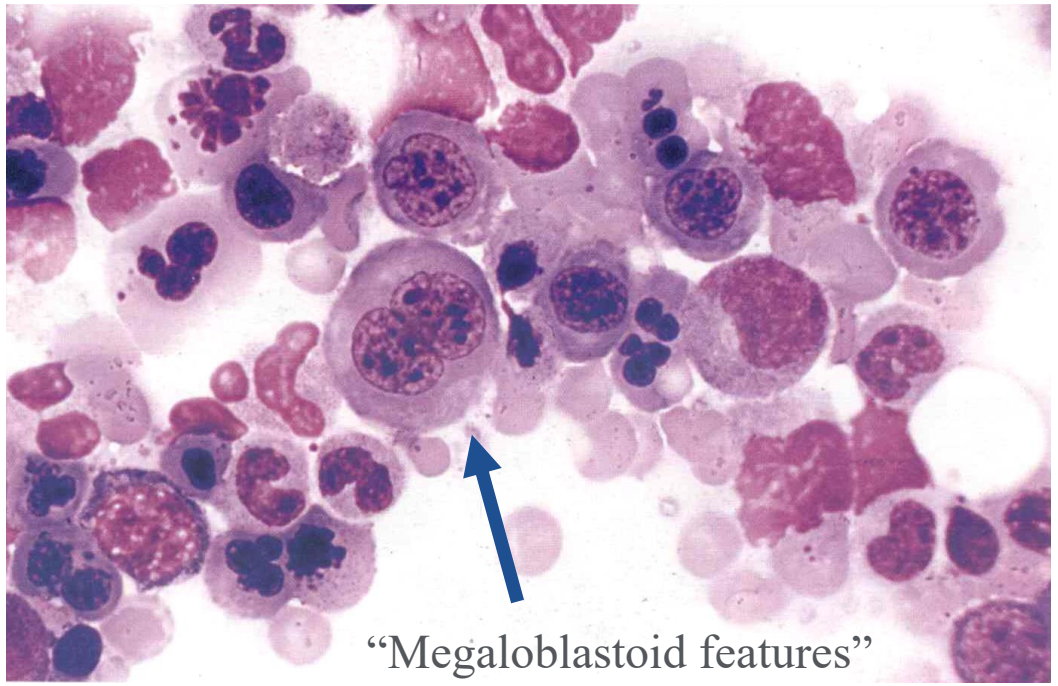
Myelodysplastic Syndrome and Acute Myeloid Leukemia: Two diseases within the same spectrum

Both conditions arise from somatic mutations in hematopoietic stem cell causing:

- Ineffective blood formation with accumulation of “blasts”
- Cytopenia(s) of all blood lineages (most often anemia)
- Qualitative disorders of blood cells and their precursors
- Stem/progenitor cell maturation is abnormal or blocked

Both conditions exhibit stem cells with a defective capacity for self-renewal and differentiation

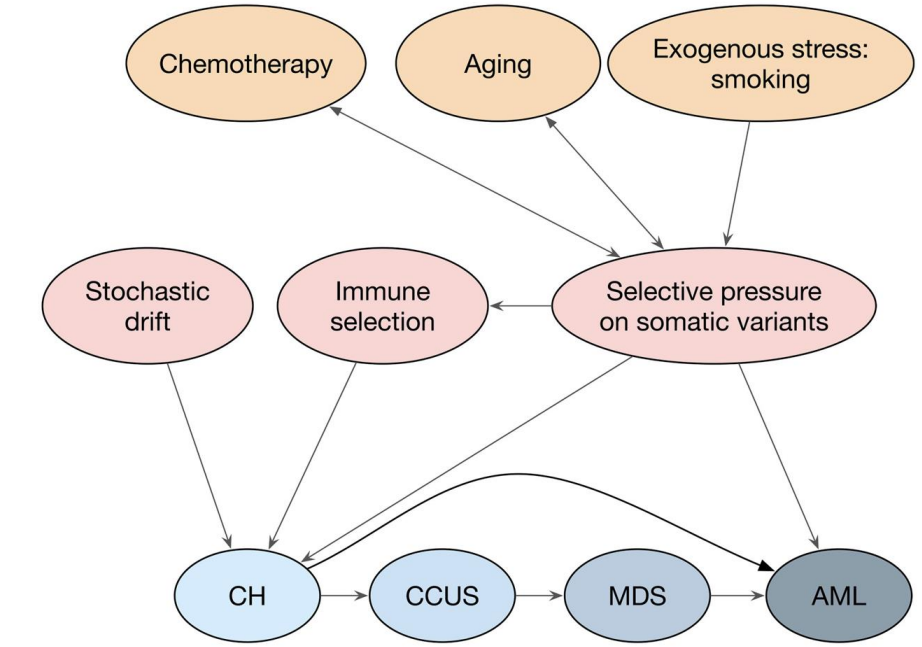
Both MDS and AML are hallmarked by recurrent mutations in genes that control epigenetic cellular processes (DNA methylation, chromatin structure, histone modification) and prevent the “right” genes from being turned on at the “right” time



“Megaloblastoid features”

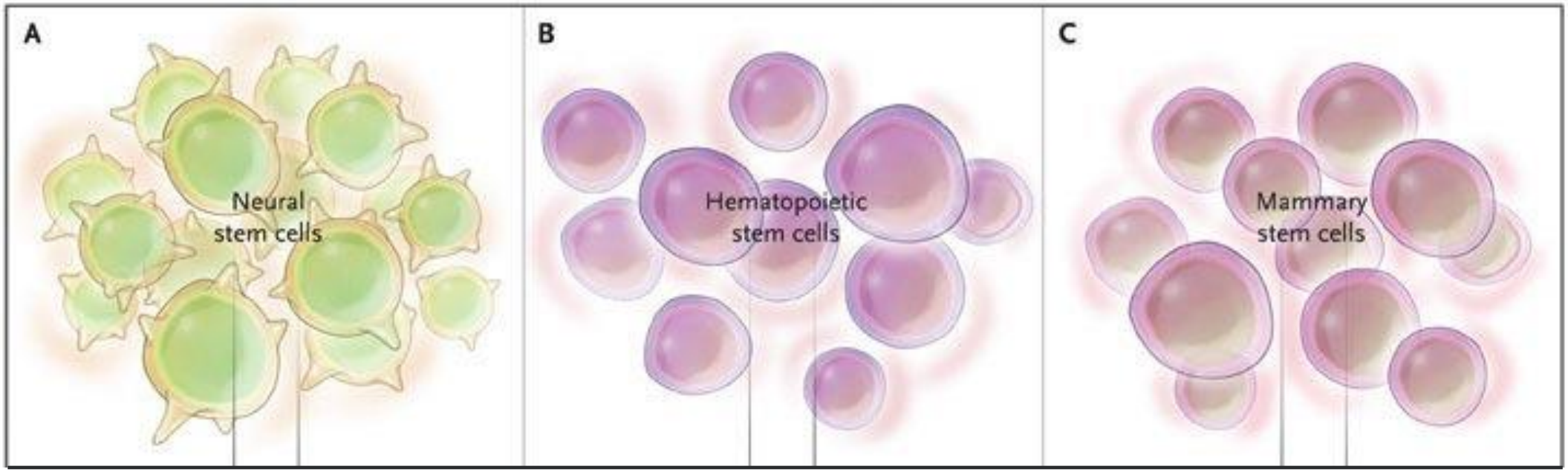
While MDS/AML affect blood formation, CH does not

- Clonal hematopoiesis is the development and persistence of an HSC clone that harbors a mutation that confers selective advantage within normal blood parameters
- Also known by other names: Clonal hematopoiesis of indeterminate potential (CHIP), age-related clonal hematopoiesis (ARCH)
- Almost exclusively has been studied in peripheral blood, thus potentially biasing towards alleles that can differentiate
- Vast majority of CH mutations are: DNMT3A, TET2, or ASXL1
- Only a 1-4% per year risk of progressing to MDS or AML



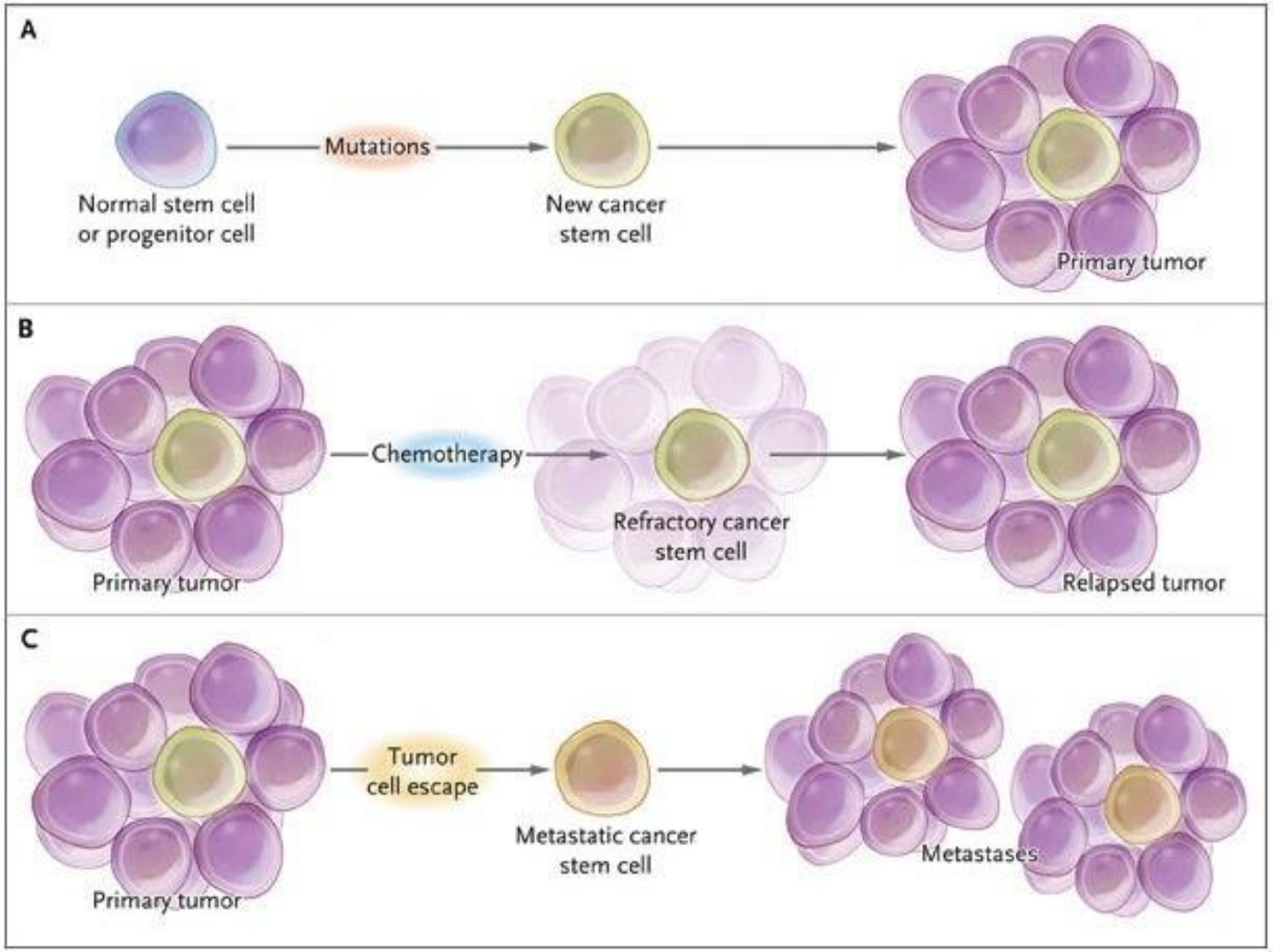
	CH	CCUS	MDS	AML
Somatic variation	+	+	+	+
Cytopenia	-	+	+	+
Dysplasia	-	-	+	+
Blast % in BM	<5%	<5%	<5-10%	>20%

What makes a stem cell a stem cell?



Pluripotency/Multipotency Quiescence Self-renewal Immortalization

Which of these features are applicable to cancer?



Jordan C et al, NEJM

Senescence and Immortalization—*in vivo*

Number of cell doublings of dermal fibroblasts declines with age

Fibroblasts from adults have lower replicative capacity than from newborns

Embryonic stem cells

- Have the ability to form all the cells of the body
- Have unlimited replicative potential under certain culture conditions

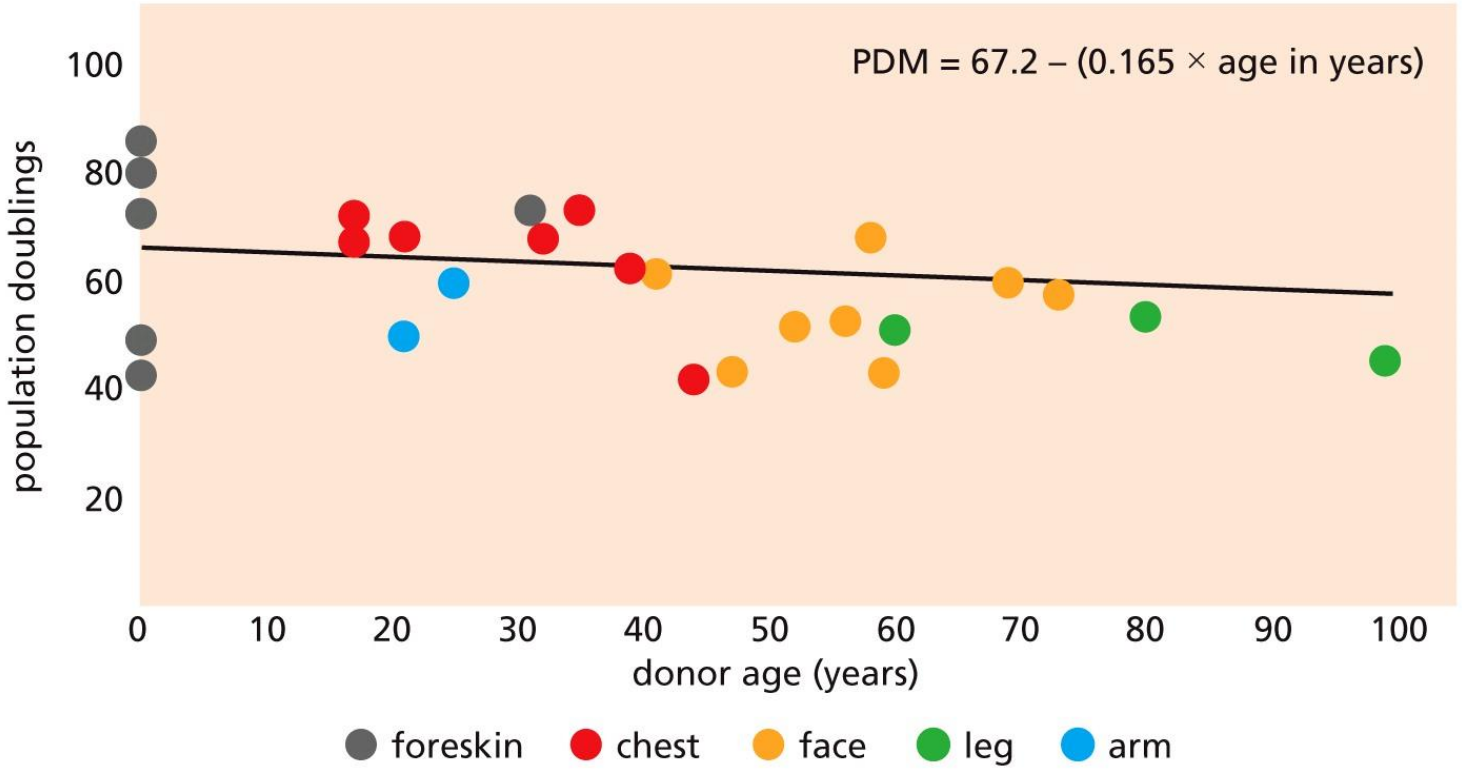
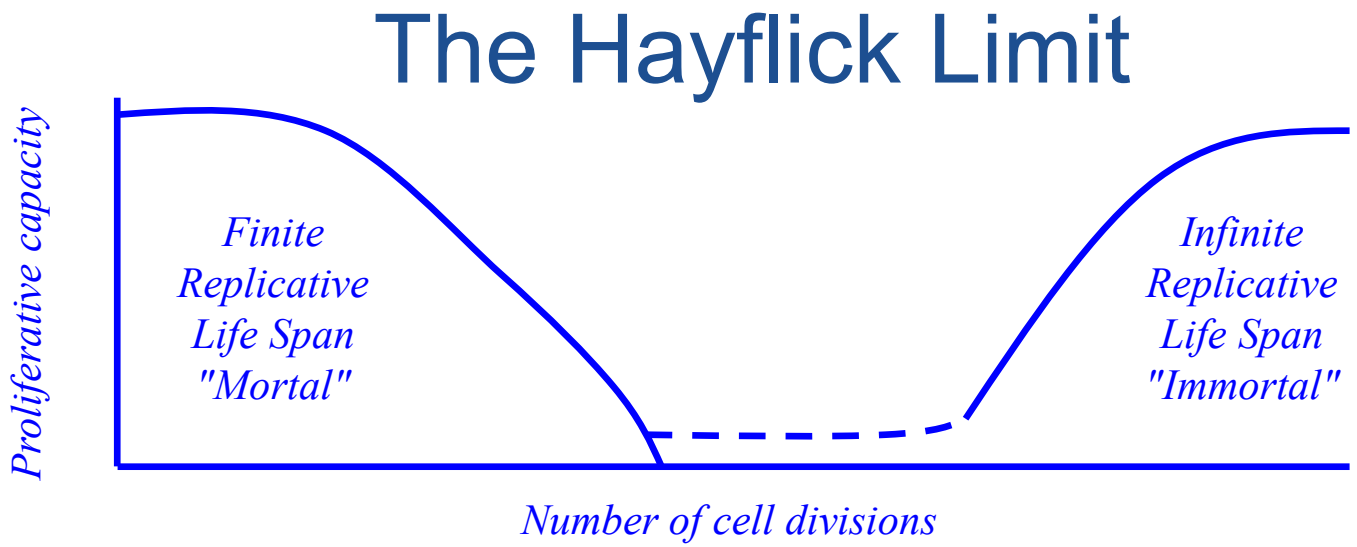


Figure 10.4a The Biology of Cancer (© Garland Science 2014)

What happens to cells when they reach their replicative limit?

- Irreversible arrest of cell proliferation (universal)
- Resistance to apoptosis (stem cells)
- Altered function (universal but cell type specific)



EXCEPTIONS

Germ line

Early embryonic cells (stem cells)

Many tumor cells

What happens when cells exhaust their replicative life span?

Cancer mutations overcome the Hayflick Limit

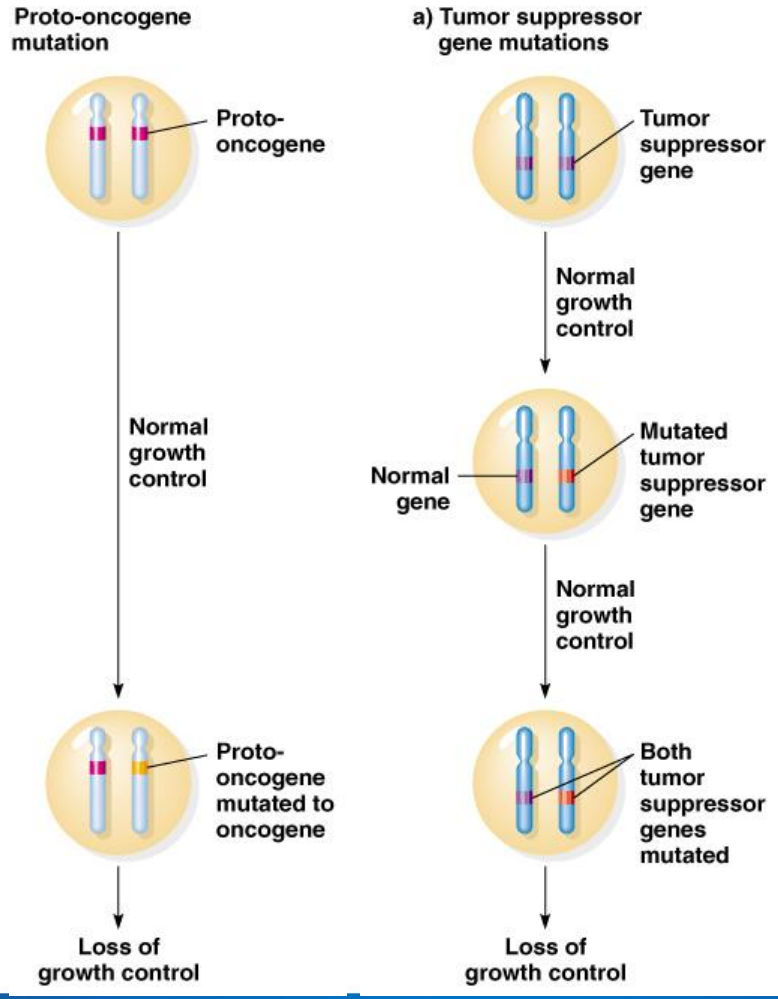
Oncogenes

All are involved in positive control of cell growth and division (think gas pedal is stuck)

- About 100 different oncogenes have been identified

Most commonly:

- Growth factors, regulatory genes involved in the control of cell multiplication.
- Protein kinases, add phosphate groups to target proteins, important in signal transduction pathways.



Tumor Suppressor Genes

All are involved in negative of cell growth and division (think loss of brake pedal)

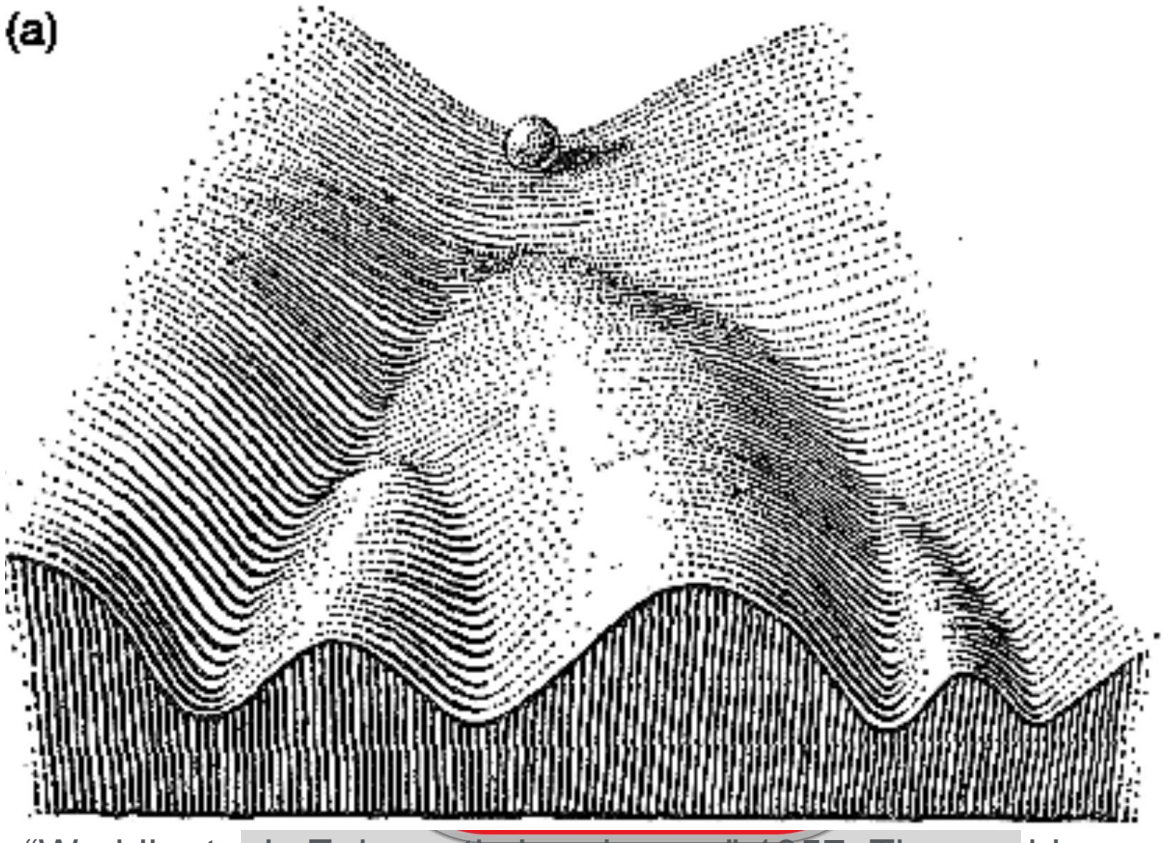
Most commonly affect:

- Cell cycle progression checkpoint, regulatory genes involved in the control of cell cycle.
- Apoptosis, genes that sense and facilitate programmed cell death.

Mutations are commonly loss of function, but typically both alleles must be lost

What is epigenetics

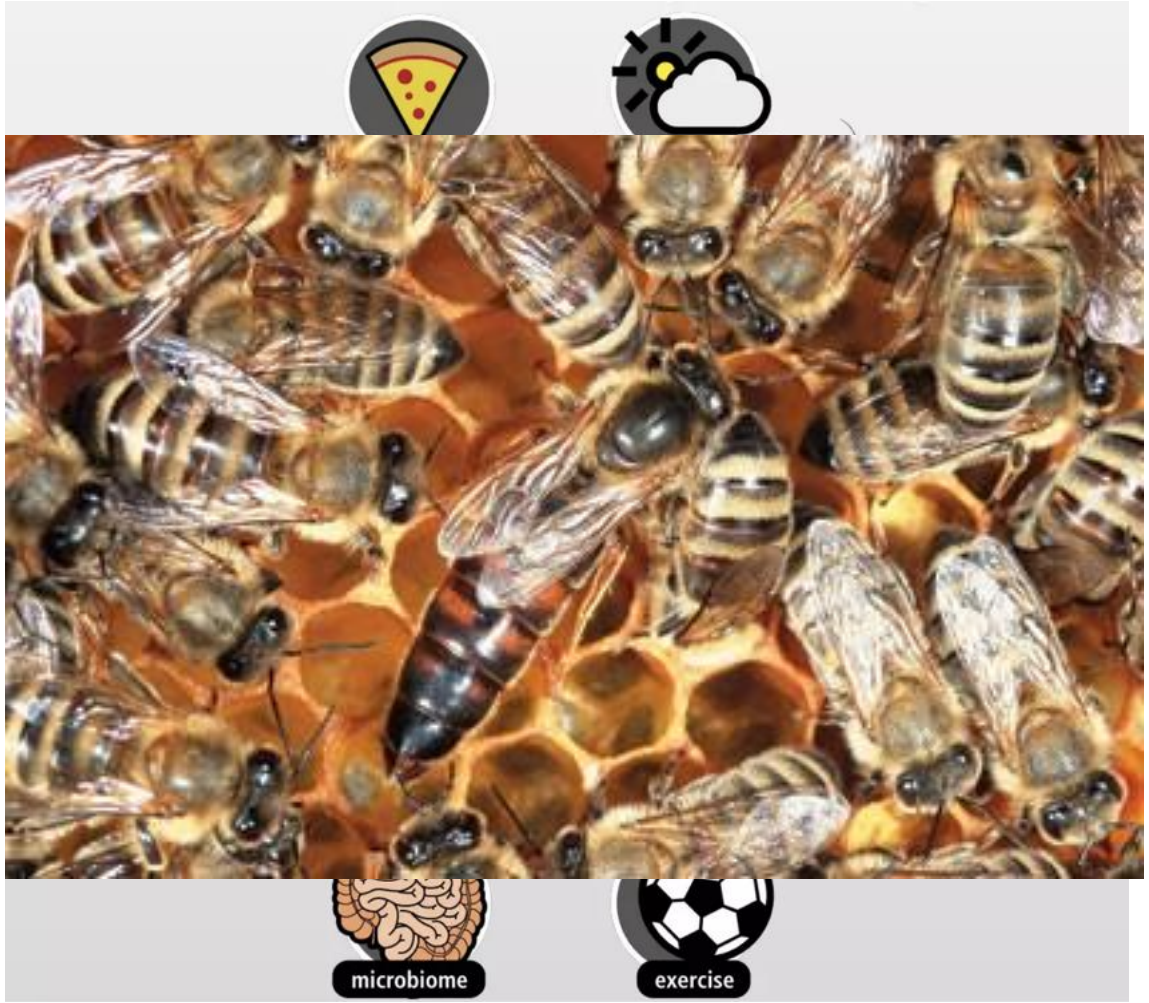
- Epigenetics is the study of heritable changes in gene expression that do not involve alterations to the underlying DNA sequence
- Heritable here can mean inherited by offspring or inheritance by daughter cells
- These changes are often caused by chemical modifications to DNA or associated proteins, which can turn genes on or off
- The term epigenetics was coined by Conrad Waddington and Ernst Hadorn in 1942, originating from the latin word *epigenesis:* influence of genetic processes on development



"Waddington's Epigenetic Landscape" 1957. The marble represents a biological system (e.g. a cell) at the verge of taking a developmental path toward one of a set of alterative more differentiated states represented by the three ending depressions at the base of the slope

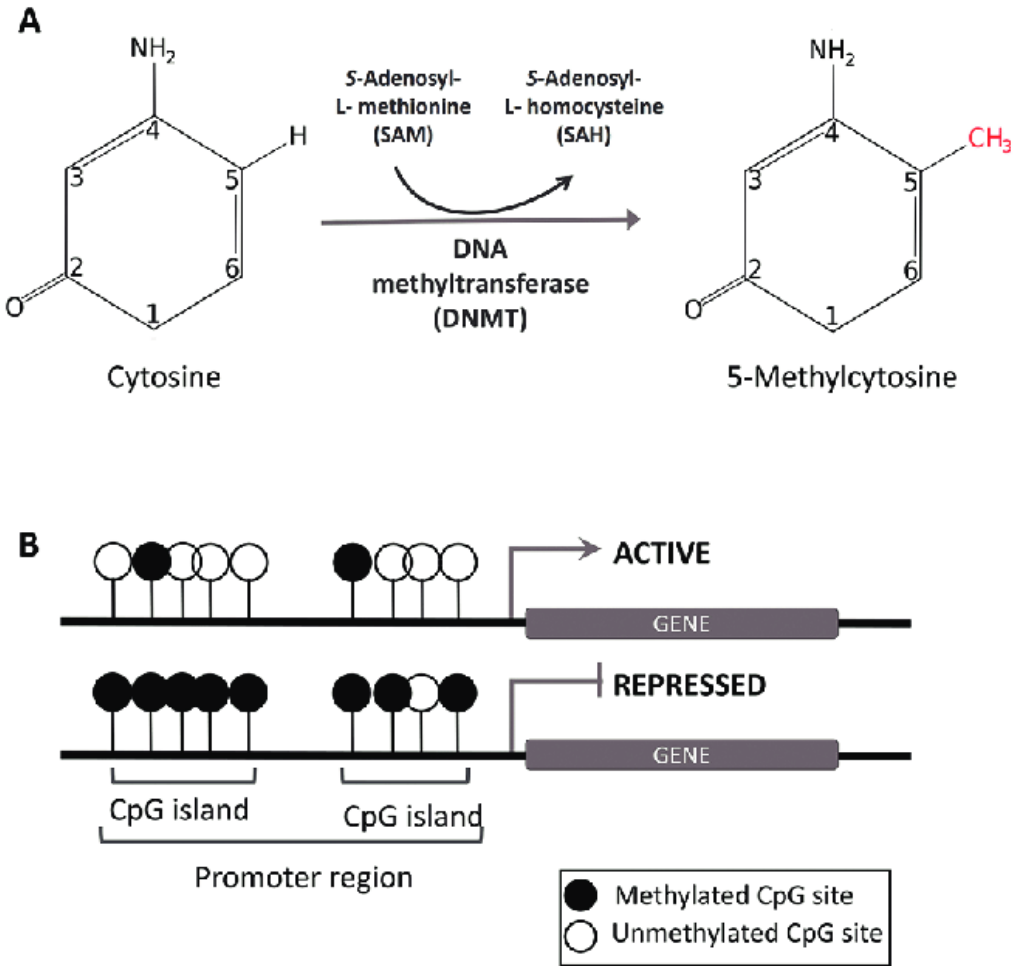
Why epigenetics?

- Changing DNA just takes too long! (generations to millions of years!)
- DNA is the same in every cell, but each cell type requires cell type-specific gene expression patterns
- At different times over a lifespan or in response to environmental signals and other stimuli epigenetic controls can modulate gene expression
- These epigenetic modifications to chromatin are copied to the daughter cells
- Bees. Go to the Gilder Center at the Natural History Museum.



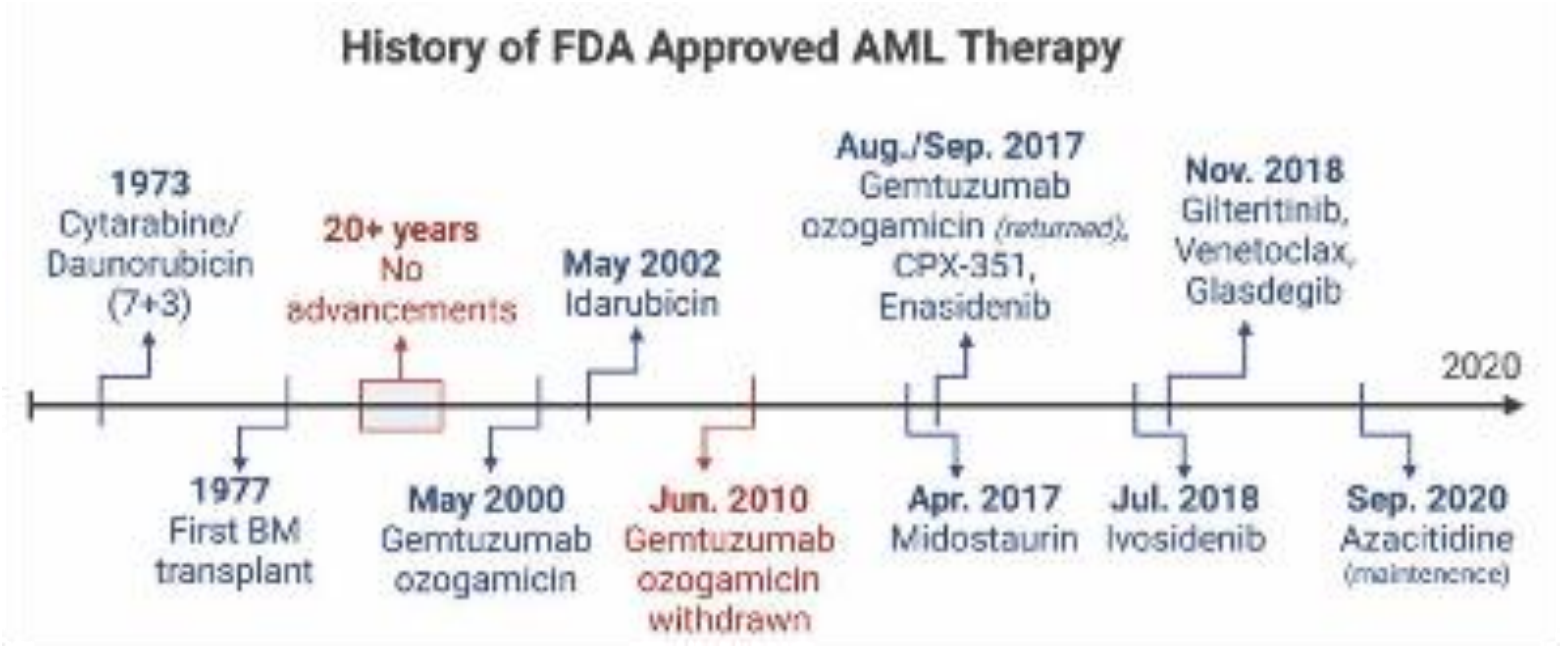
Why is DNA methylated

- THE ANSWER IN 2025: Methylation of cytosines (particularly C-G near promoters) likely silences gene expression through inhibiting TF binding
- BUT: Only ~60% of promoters even have C-G repeats, which means only ~13,000 genes COULD be methylated
- That represents only 0.4% of the methylome yet all 5mC is copied during cell division
- The other “stuff” likely matters as this is a HUGE energy expenditure
- *Royal Jelly is a potent DNMT inhibitor



Alarcon and Figuroa, 2017

New therapies emerging, but “7+3” remains the standard 40 years later…



Carter et. al 2020

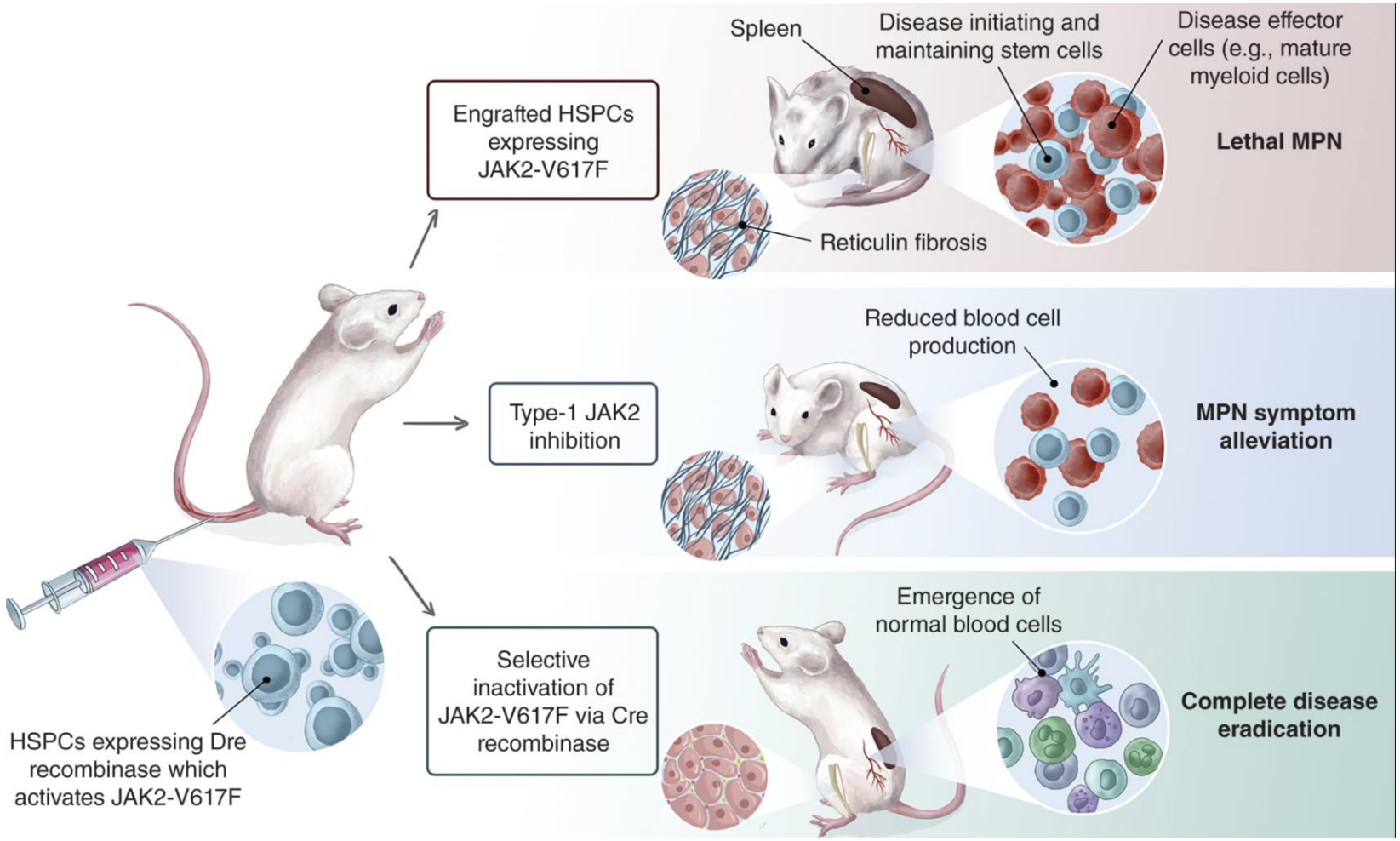
“Oh honey, there ain’t nothing complete about a complete remission”

Professor Judy Karp

Head of Leukemia Program, JHMI
(Source: lecture from Chris Hourigan ~2019 stating this was heard on ward-rounds ~2008)

Challenges in targeting oncogenes in cancer

- > 88 specific targeted inhibitors have been FDA approved for use in cancer – the ABL1 kinase inhibitor imatinib for CML is the rare example of curative therapy (iSTOP trial)
- Yet, Small molecule inhibitors are largely non-curative therapy, with low response rates even in biomarker positive patients
- Limitations in the promise of small molecule inhibitors face two major challenges: resistance mutagenesis (on and off target) and target “quality”
- Often toxicity limiting dose escalation precludes answering a fundamental question: Bad Drug or Bad Target?



Chromosomal Status is most important prognostic feature in acute myeloid leukemia

National Comprehensive Cancer Network®

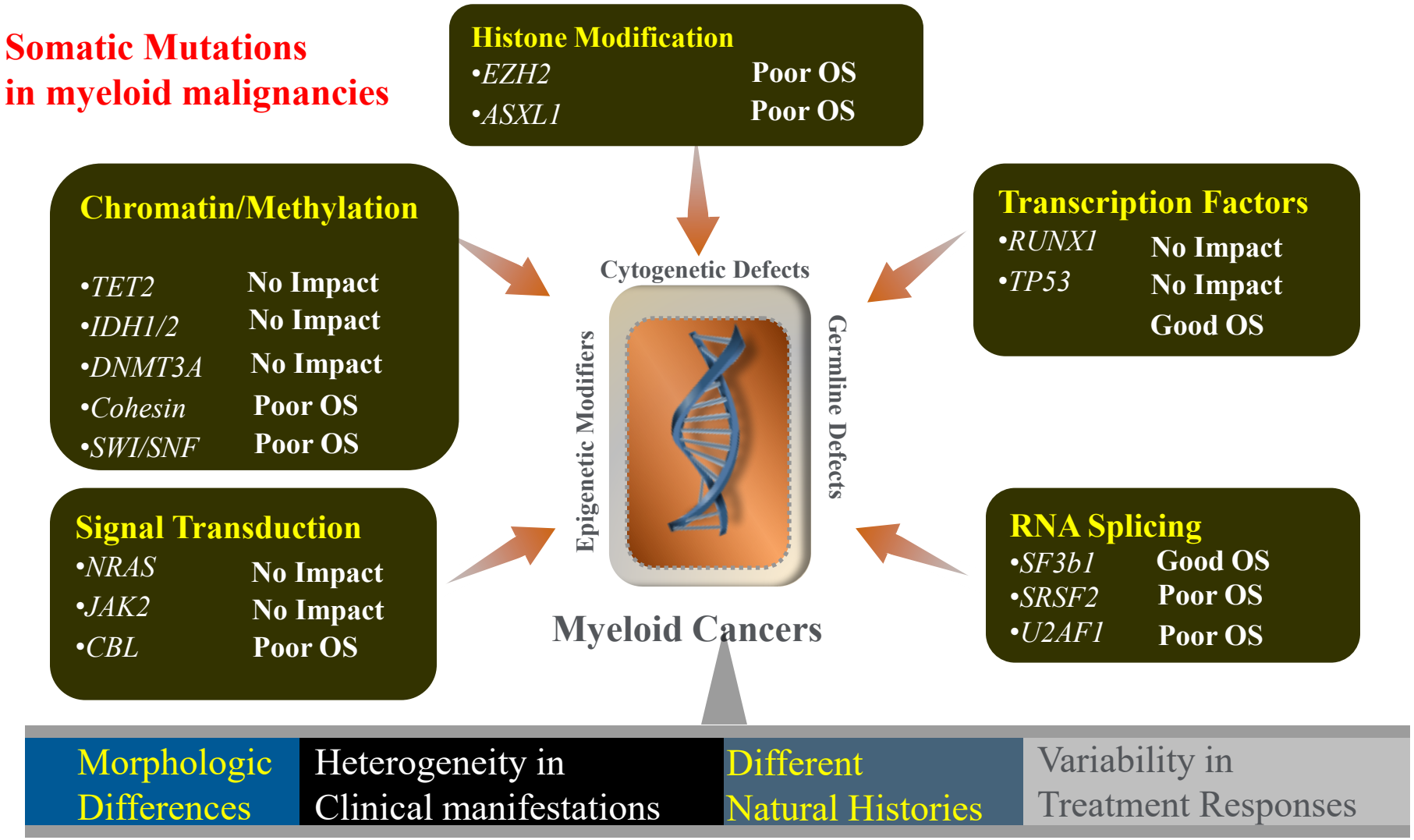
NCCN Guidelines Version 2.2013
Acute Myeloid Leukemia

NCCN Guidelines
AML Table of Co
Disc

RISK STATUS BASED ON VALIDATED CYTOGENETICS AND MOLECULAR ABNORMALITIES[1]

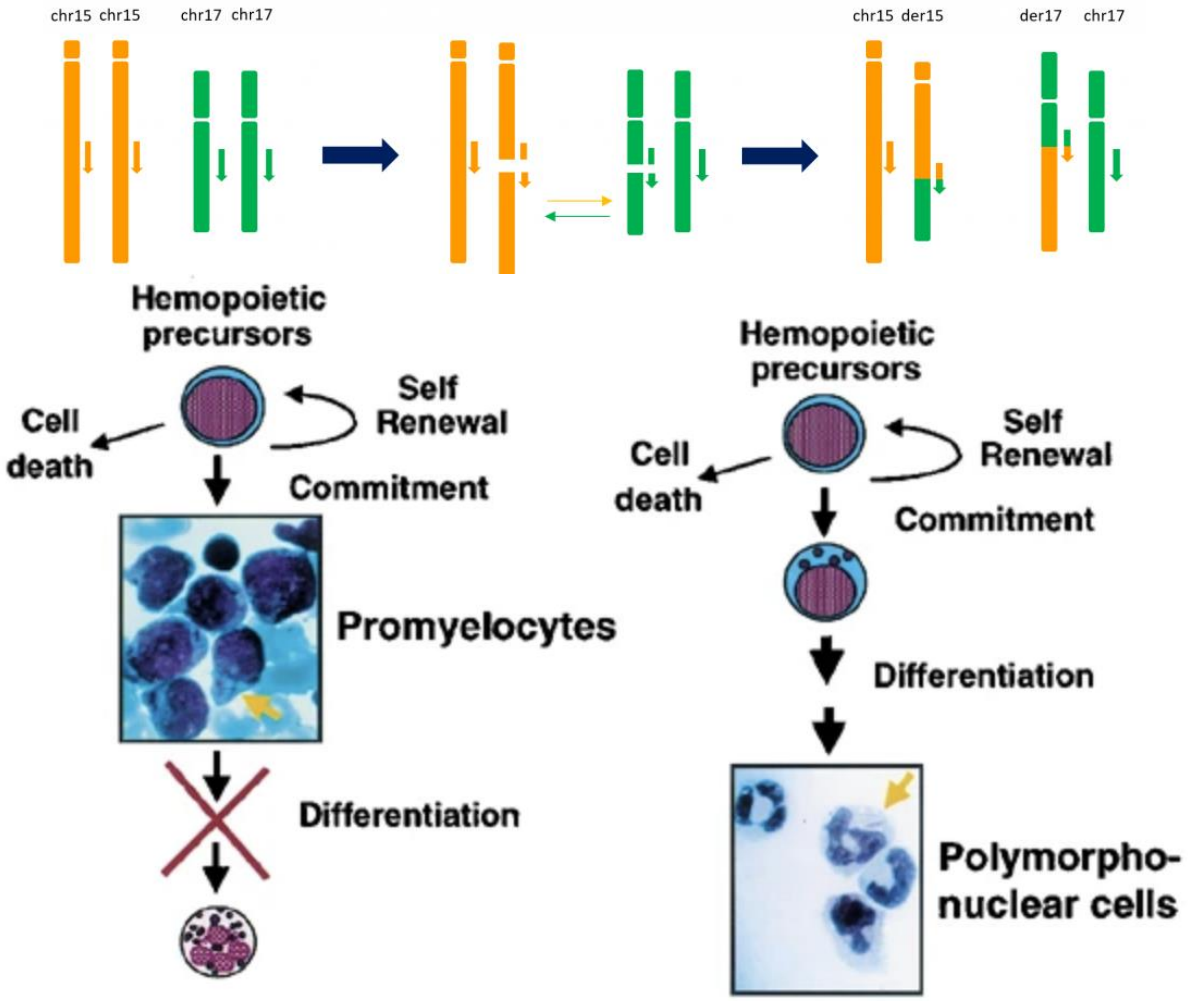
RISK STATUS	CYTOGENETICS	MOLECULAR ABNORMALITIES
Better-risk	inv(16)[2,3] or t(16;16)[2] t(8;21)[2] t(15;17)	Normal cytogenetics: NPM1 mutation in the absence of FLT3-ITD or isolated biallelic CEBPA mutation
Intermediate-risk	Normal cytogenetics +8 alone t(9;11) Other non-defined	t(8;21), inv(16), t(16;16): with c-KIT[5] mutation
Poor-risk	Complex (≥3 clonal chromosomal abnormalities) Monosomal karyotype -5, 5q-, -7, 7q- 11q23 - non t(9;11) inv(3), t(3;3) t(6;9) t(9;22)[4]	Normal cytogenetics: with FLT3-ITD mutation[6]

Genotypic Diversity in Myeloid Malignancies



Reprogramming Leukemia Cells as Therapy

- In acute promyelocytic leukemia there is a DNA fusion that prevents a key gene (PML) from activating its target genes and accumulating in specific aggregates in the nucleus
- Using high dose vitamin A (retinoic acid, or ATRA) the abnormally fused gene is released, and the leukemia cells are now able to differentiate!
- New therapies that inhibit IDH2 or IDH1 have a similar effect in AML patients with mutations in these genes



He, Merghoub, and Pandolfi 2019

Just have the “right” transcription factor, its so easy

Transcription factors bind to specific DNA sequences to “read” the DNA and turn on specific sets of genes

BUT

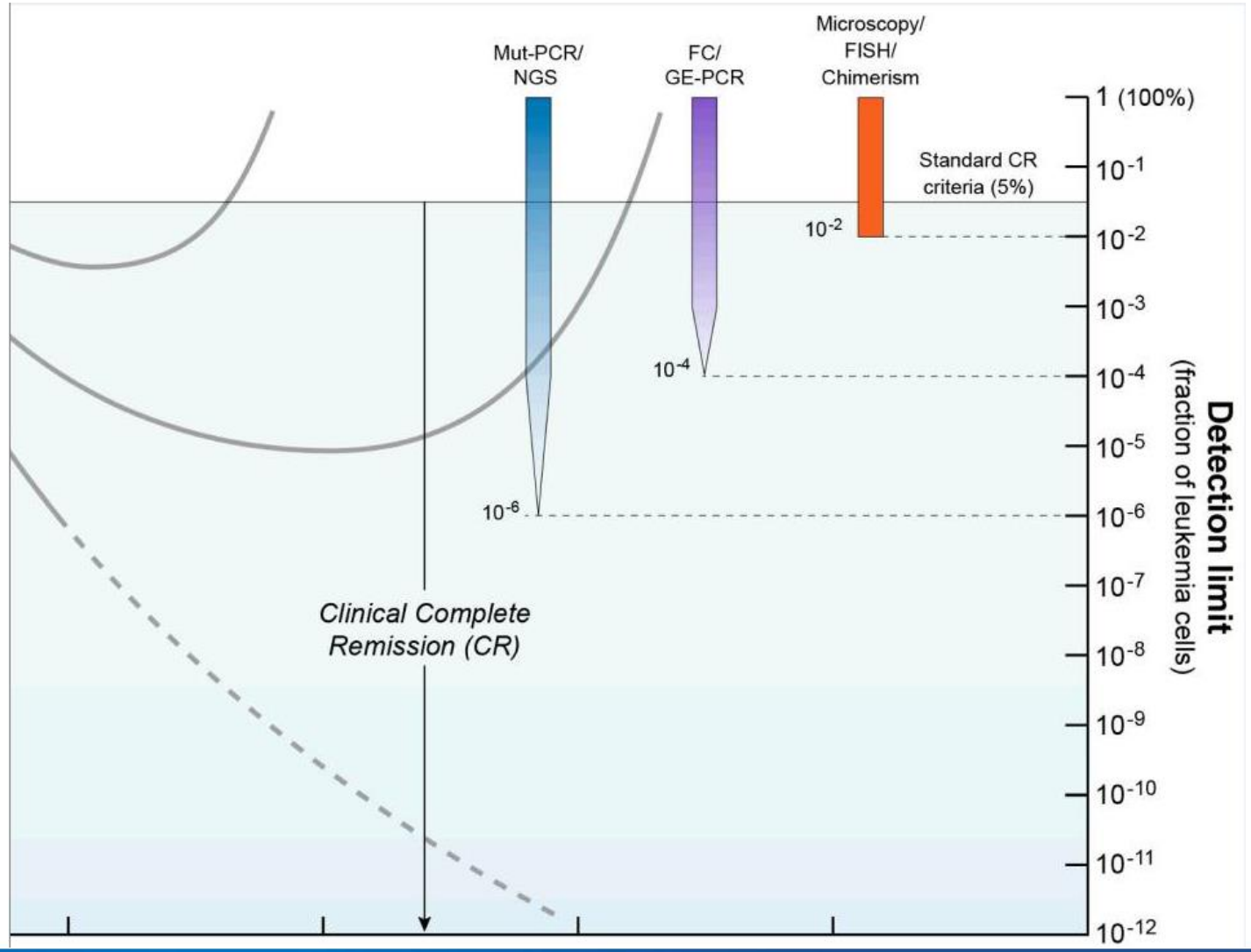
- The same factors have different target genes in different cells
- This does not account for controlling gene “volume”
- Regulating DNA “accessibility” through its 3D structure adds new layers of complexity in our understanding of blood formation

“It's like dandelions in the back yard: You can cut the leaves off all you want, but unless you kill the root, it will keep growing back.”

John Dick, inventor of the term “cancer stem cell”

MRD: Not minimal, but MEASURABLE residual disease

- Can detect a depth of 1:1M for gene fusions and RT-PCR amenable markers, but this only represents a small portion of AML patients
- Clinical NGS sequencing typically can resolve mutations to 1:10K depth
- MRD simply isn't a binary variable



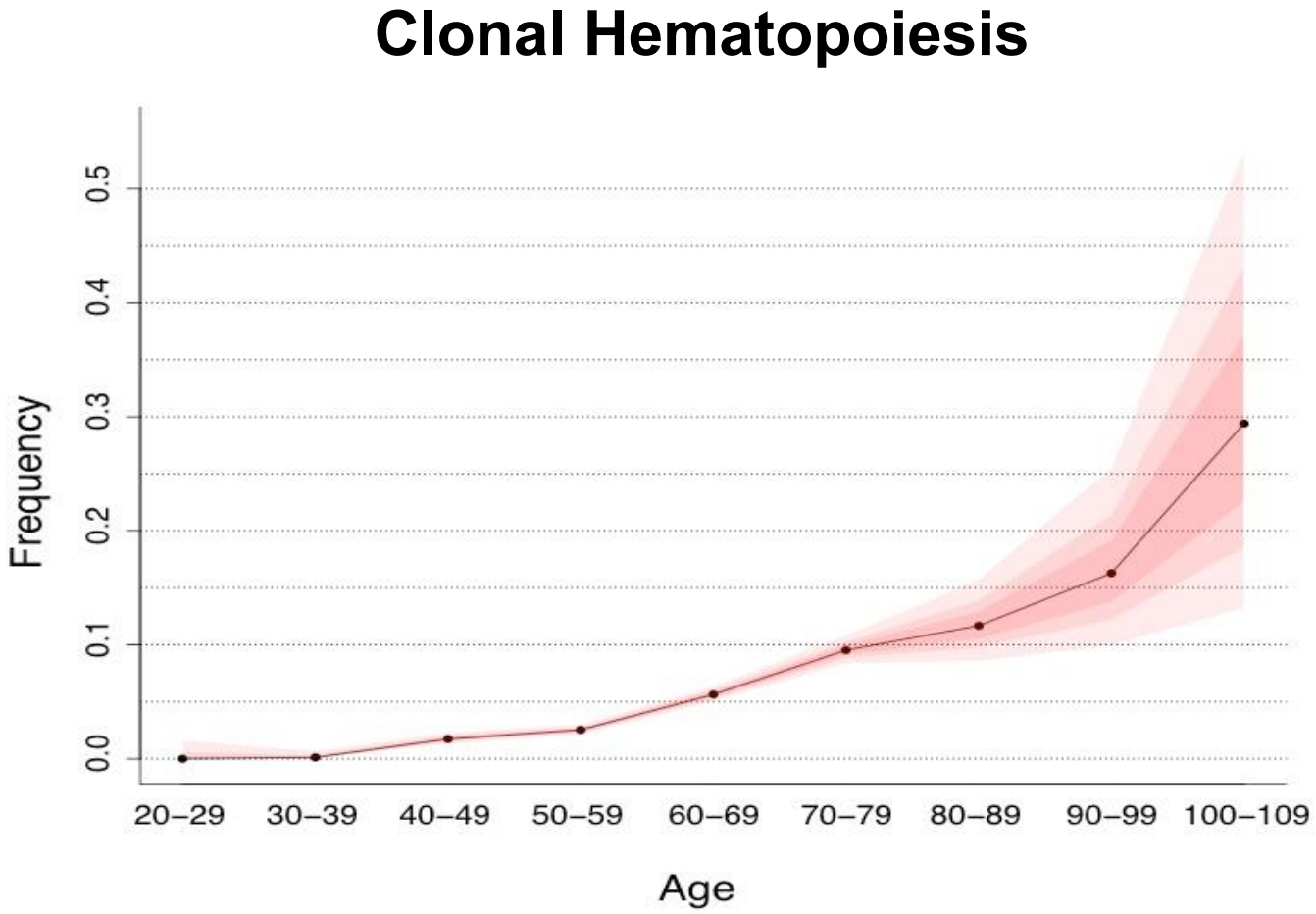
Hourigan and Karp, Nature Reviews Clinical Oncology, 2013

Do all mutations matter? Somatic mutations accumulate with age

Most common mutations:
DNMT3A, TET2, ASXL1, and JAK2

Increase in risk:
Hematologic cancer (HR 11.1)
All-cause mortality (HR 1.4)
Transition to acute leukemia 0.5-1%/year

*THE VAST MAJORITY OF PATIENTS DO NOT GET CANCER

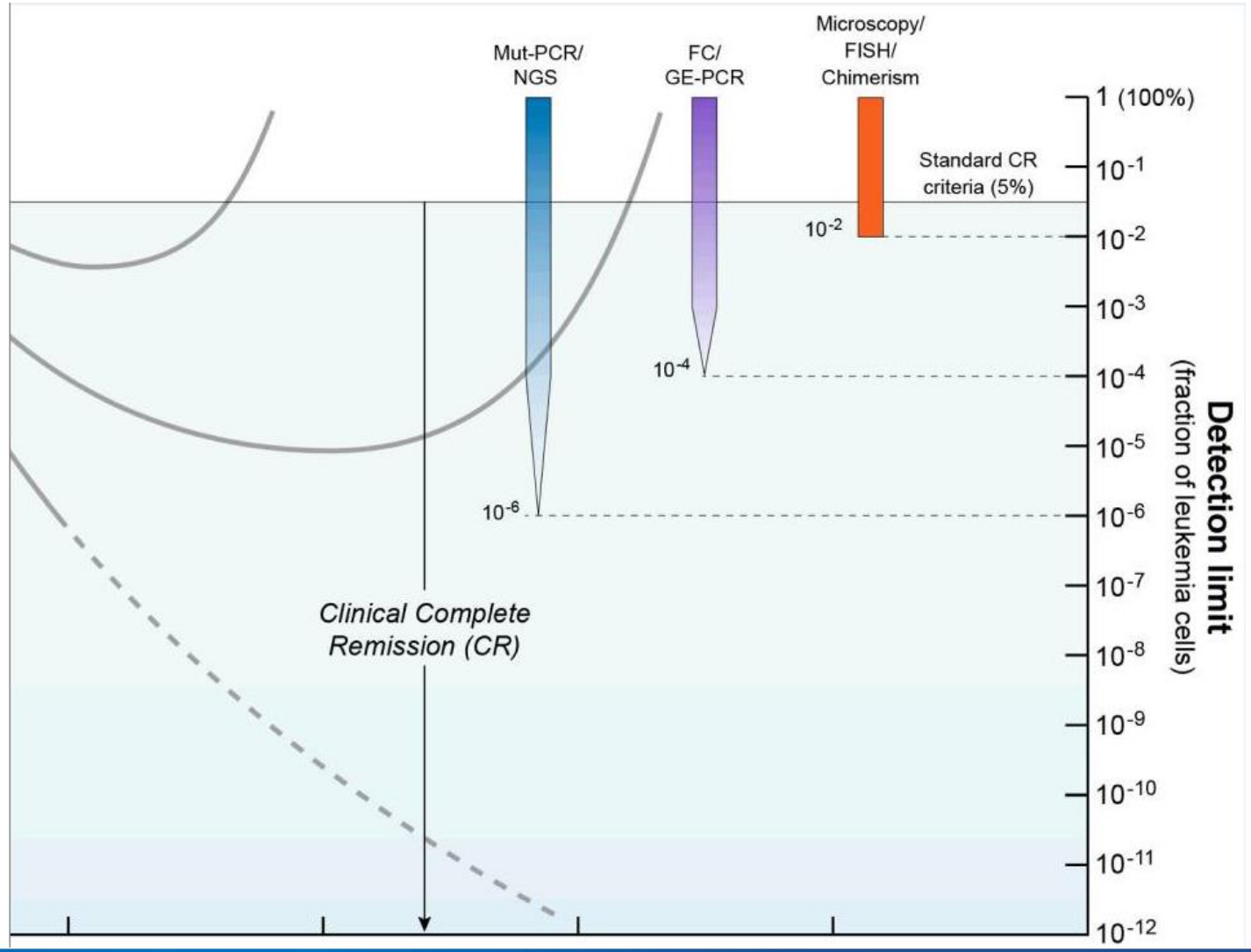


HR=Hazard ratio

Jaiswal et al NEJM 2014

MRD: Not minimal, but MEASURABLE residual disease

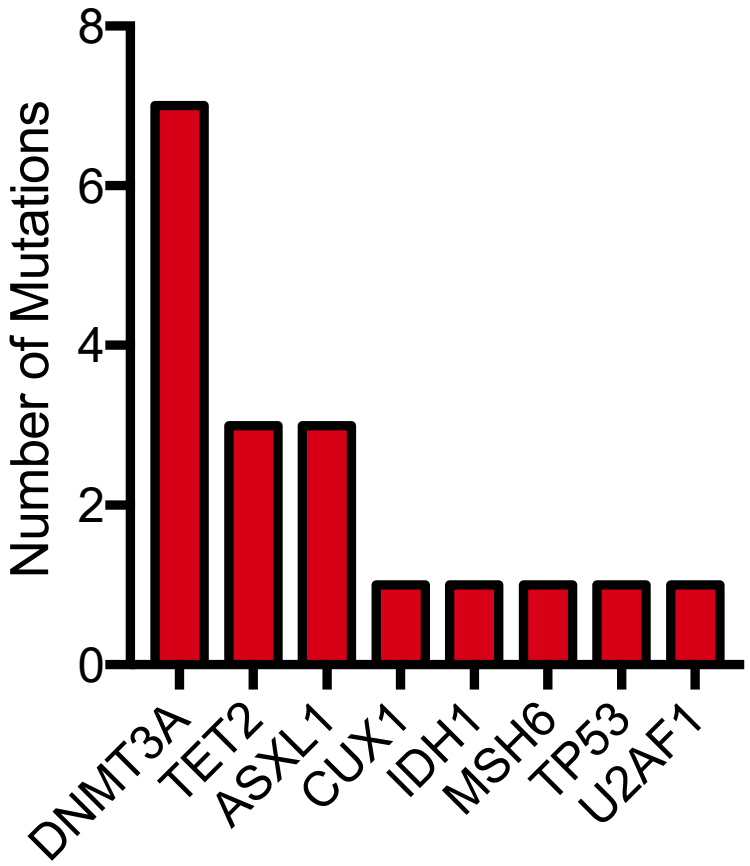
- Can detect a depth of 1:1M for gene fusions and RT-PCR amenable markers, but this only represents a small portion of AML patients
- Clinical NGS sequencing typically can resolve mutations to 1:10K depth
- MRD simply isn't a binary variable



Hourigan and Karp, Nature Reviews Clinical Oncology, 2013

CUIMC Biobank– Normal Bone Marrow

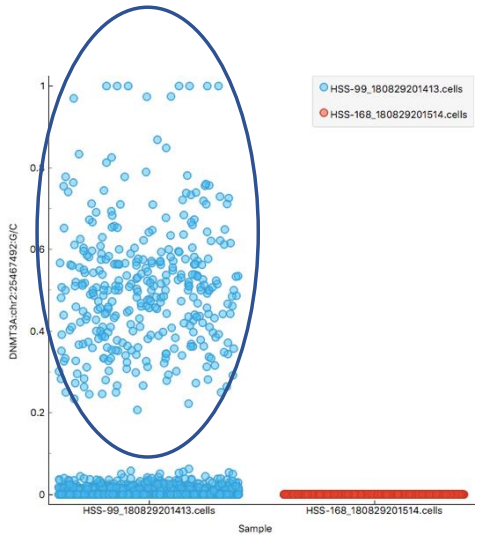
- Normal bone marrow from individuals undergoing total hip arthroplasty
- Exclusion Criteria: HIV, Active or history of malignancy (except cutaneous BCC/SCC), History of radiation or chemotherapy exposure
- Viable cells banked in significant numbers
- Since 2021: 267 Patients, 69 Sequenced by PGM
- Non-CH samples have been used for a “normal” reference genome
- CH mutations identified in 34 patients (22%)

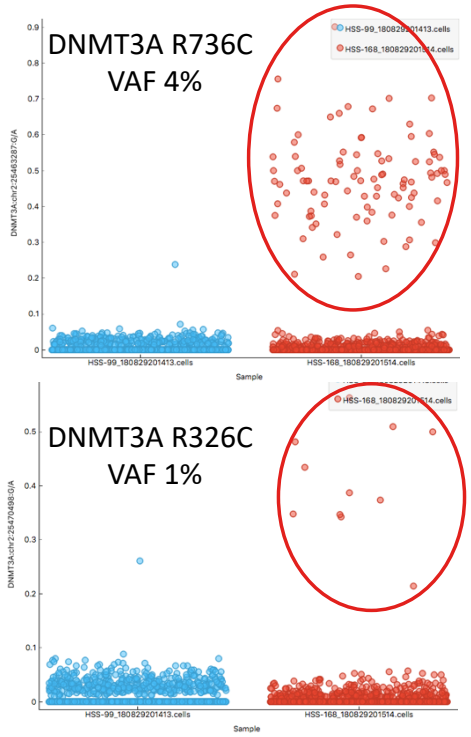


Mission Bio Tapestri-Based DNA-sequencing to delineate Mutations in Single Cells

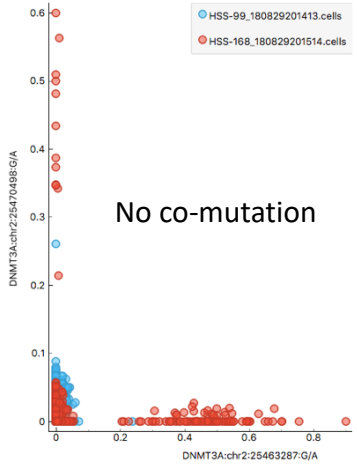
HSS-99

DNMT3A Y528*
VAF 9%





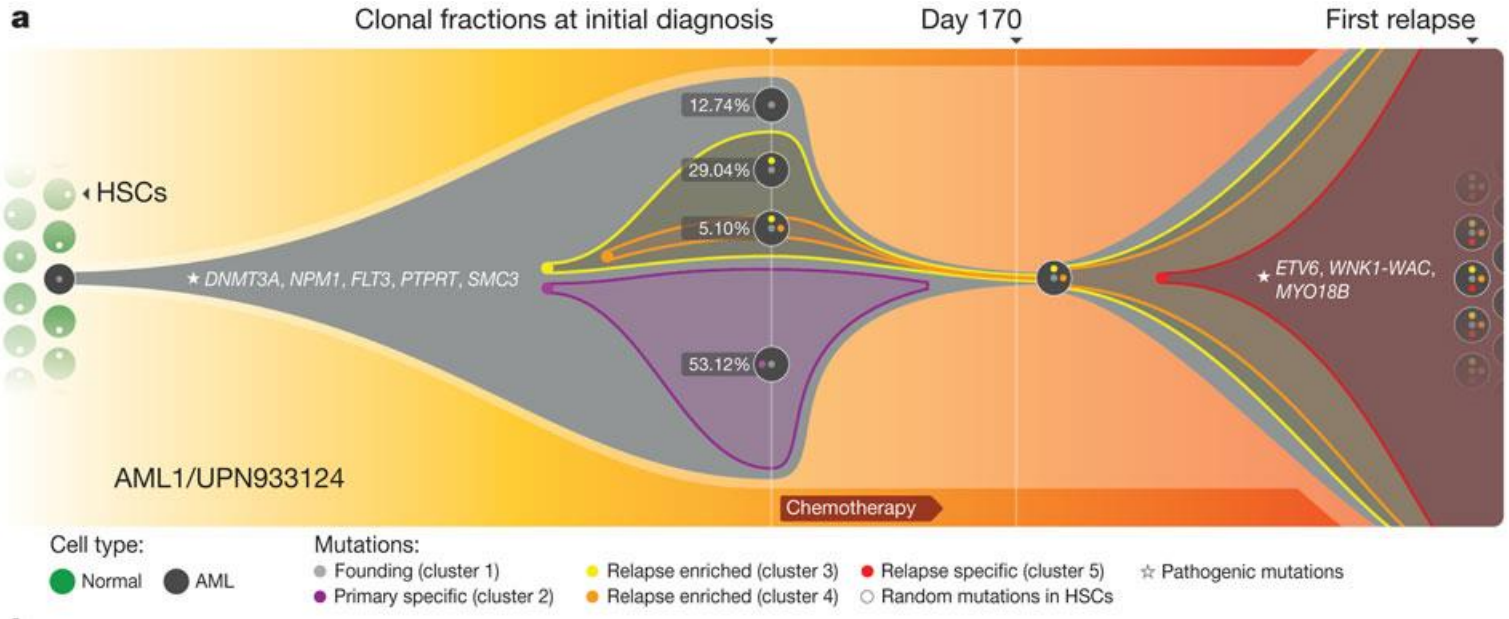
HSS-168



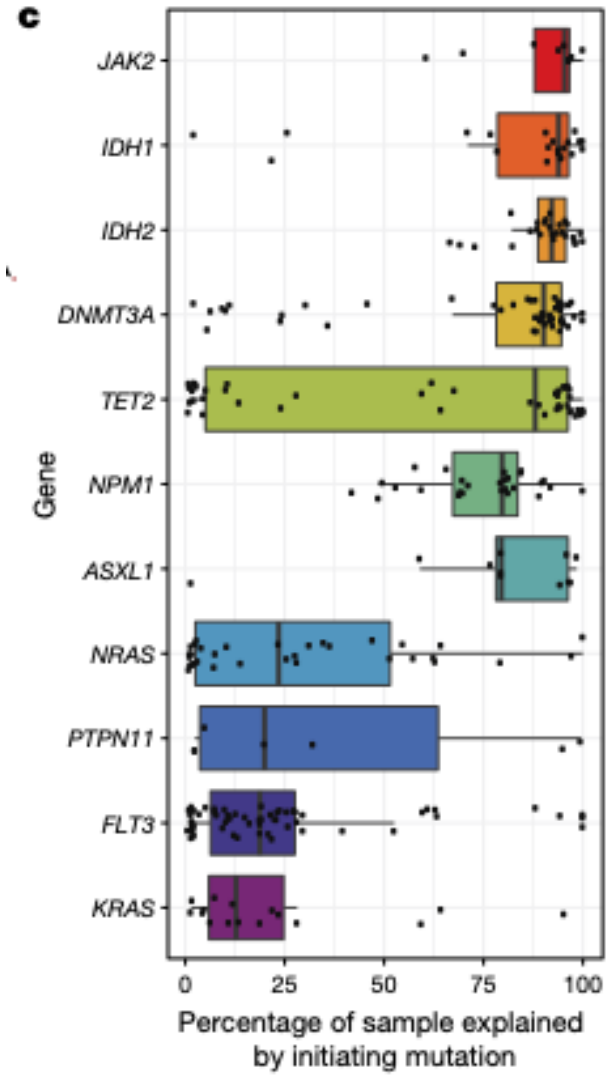
- Barcode based sequencing of single cells
- Designed a custom panel of 200 amplicons for top CH/myeloid genes
- Not inexpensive (700-800/sample + sequencing) but may be ways to multiplex
- Can be used for informative samples to provide definitive evidence of clonal architecture and mutant order

Clonal Heterogeneity/Complexity in AML

The clinical course of myeloid malignancies changes with the clonal evolution and serial acquisition of mutations



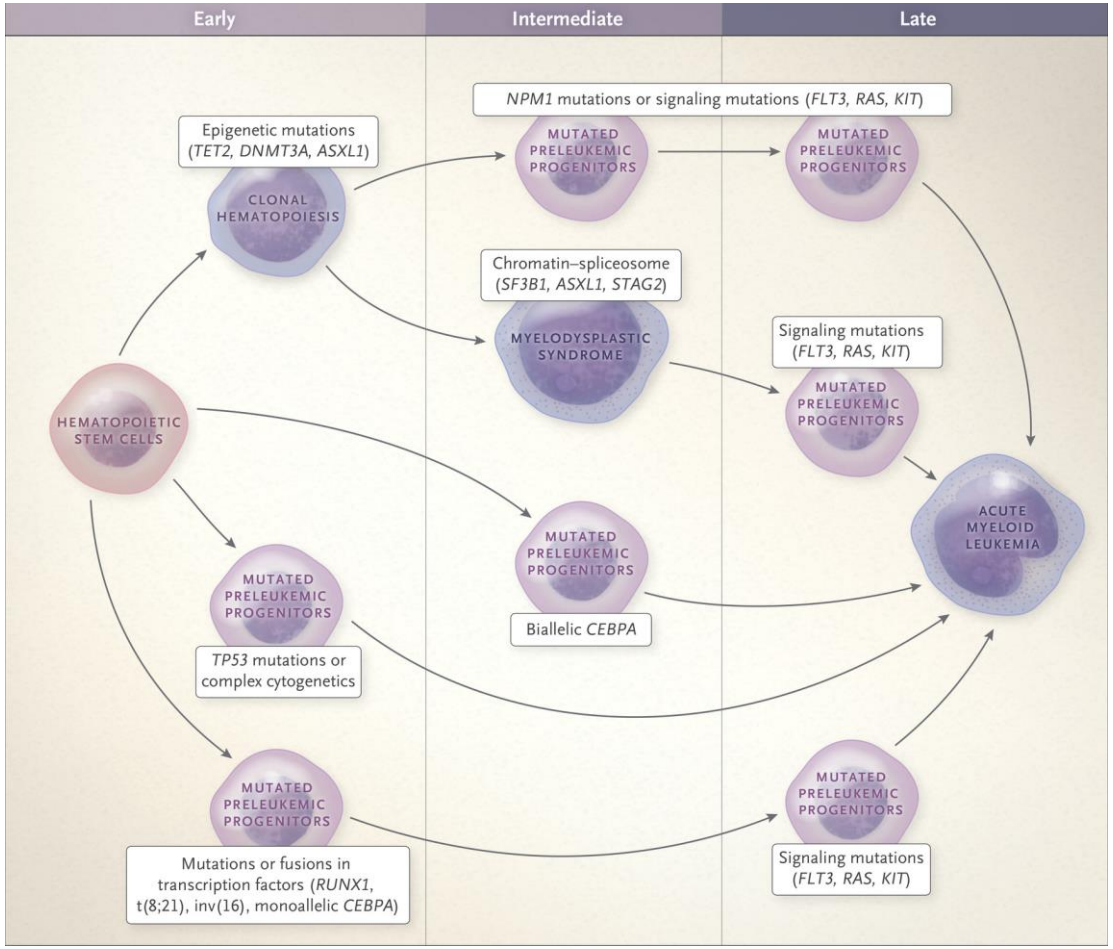
Combinatorial genomic events affect proliferation, quiescence, and epigenomic factors that influence chemotherapeutic sensitivity



L Ding *et al*. *Nature* **2012**

Miles and Bowman *et al*. *Nature* **2020**

“Roads” leading to leukemia: Clonal Hematopoiesis vs Leukemia, is this the original cancer stem cell?



- Just because we can detect a “cancer mutation” does NOT make it cancer
- What hallmarks of cancer are necessary and sufficient?
- What is your “functional” definition of a cancer stem cell
- How would you prove it?

Viny and Levine, *NEJM* 2016

Overcome Hayflick through Oncogenes and TSGs. Simple!

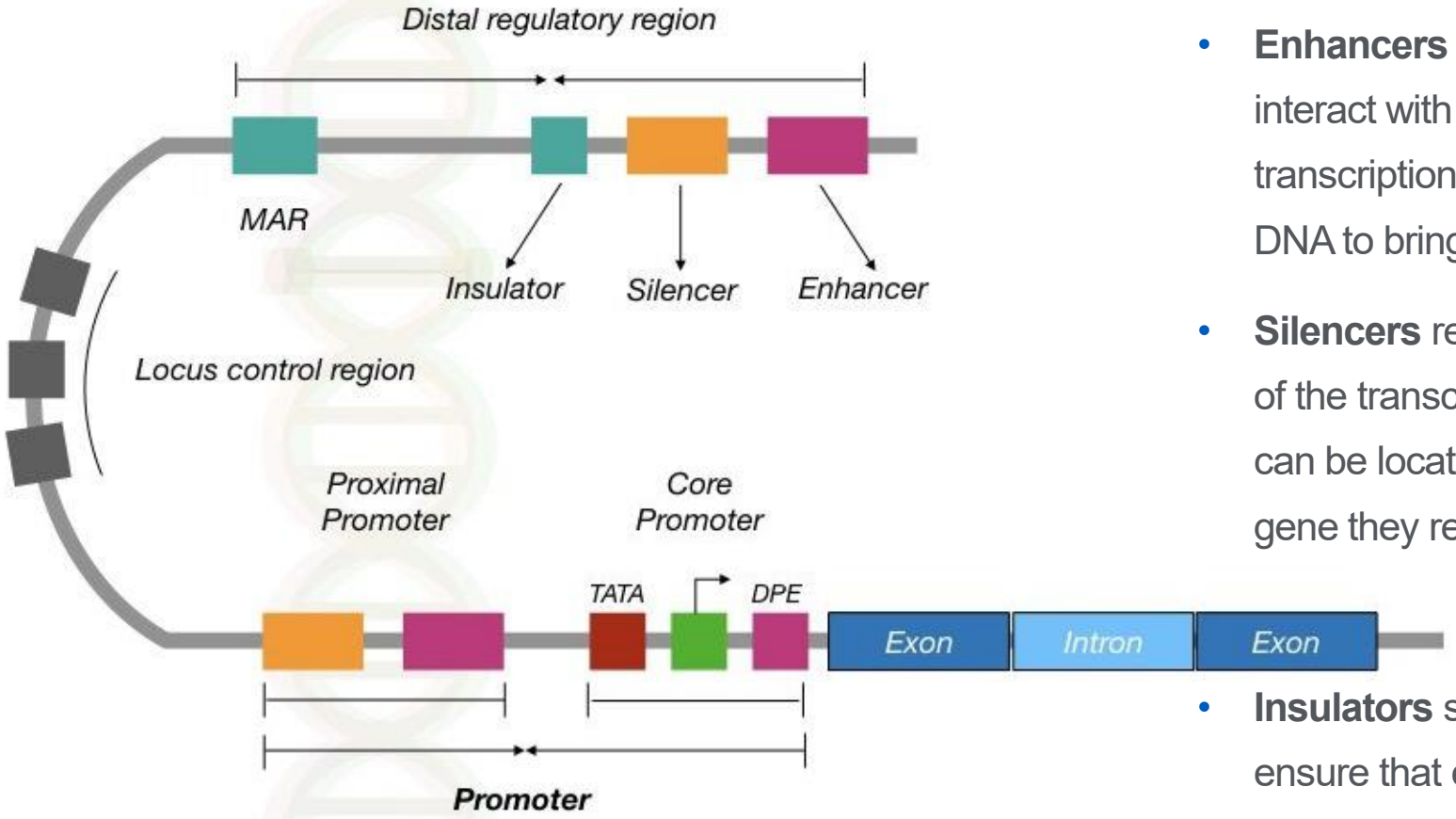
Oncogenes: “gas pedal is stuck to the floor”

TSGs: ”brake pedal is broken”

BUT: Which “driver’s seat” changes the mechanisms of acceleration and braking!

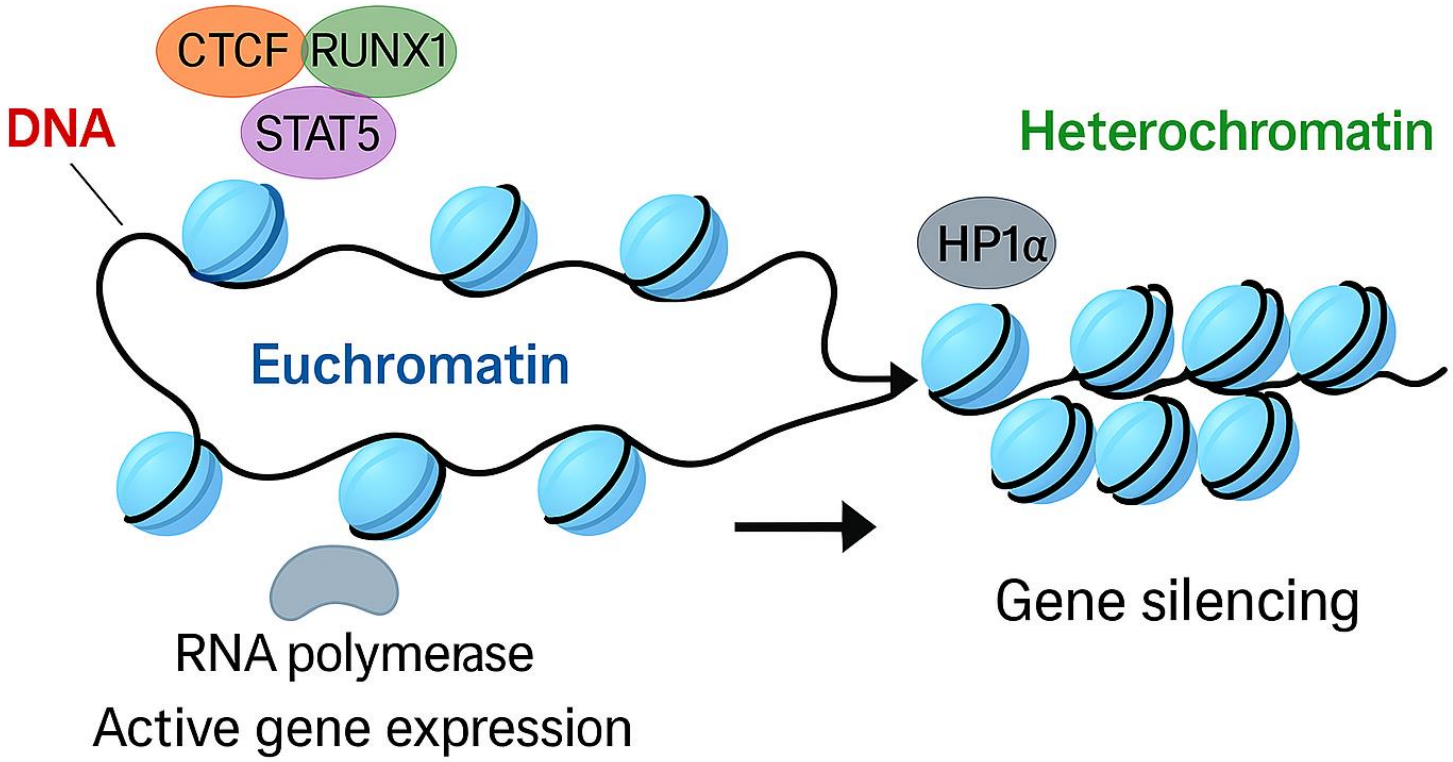
Different cell types express different genes (*pleiotropy*) so how does it turn on only the “right” ones?

Anatomy of a Gene (Redux)



- **Enhancers** work by binding coactivation factors, which then interact with the promoter region of the gene to enhance its transcription. This interaction often involves looping of the DNA to bring the enhancer in close proximity to the promoter
- **Silencers** recruit repressor proteins and inhibit the assembly of the transcriptional machinery at the promoter. Silencers can be located upstream, downstream, or even within the gene they regulate
- **Insulators** separate genomic neighborhoods. Insulators ensure that enhancers activate only their target genes and not neighboring genes

Chromatin Accessibility

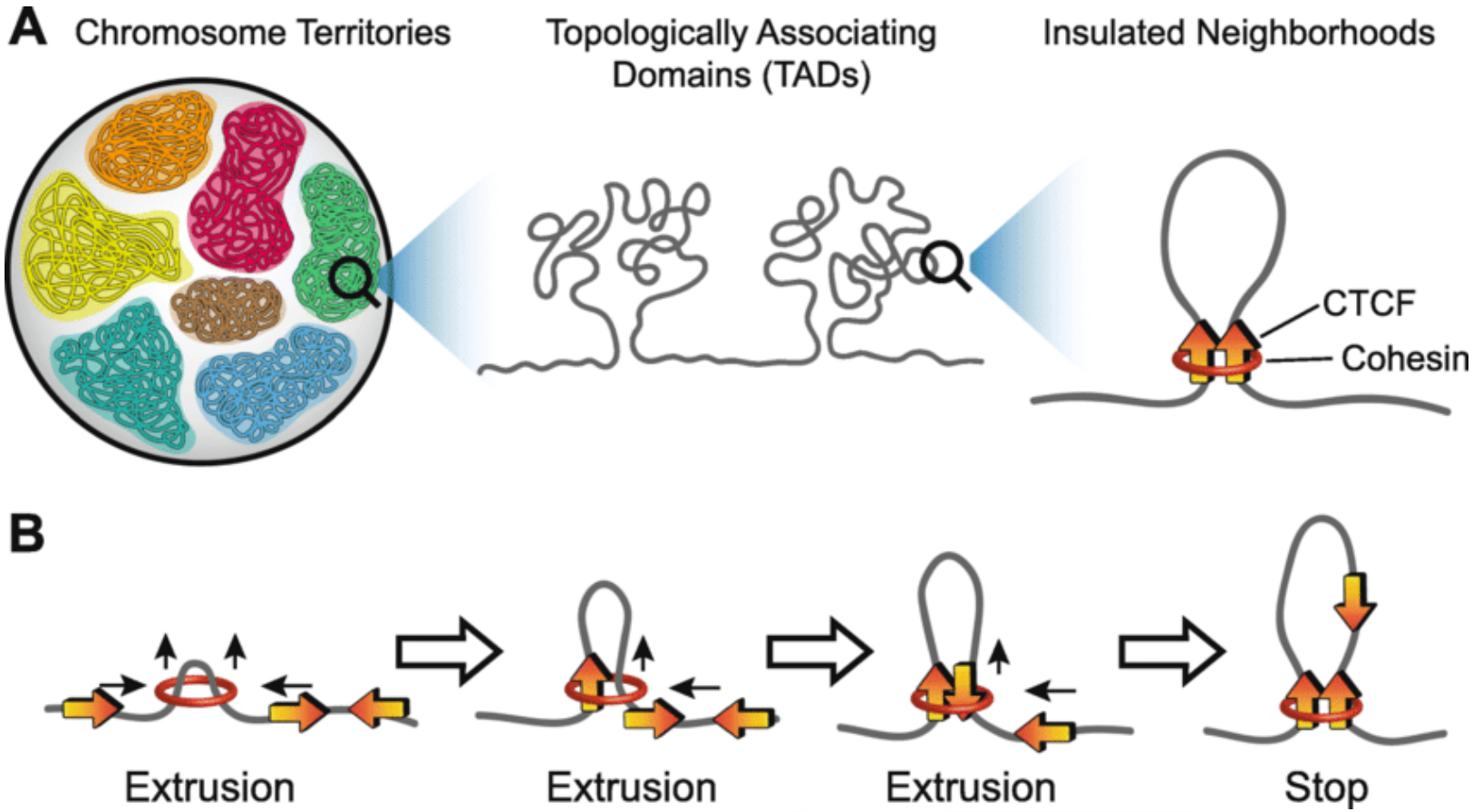


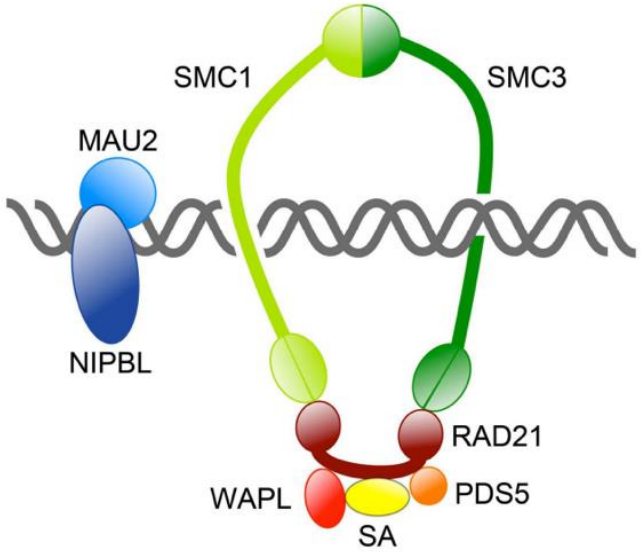
Chromatin accessibility refers to how open or closed regions of chromatin are, which affects the ability of transcription factors and other proteins to bind DNA.

Open chromatin regions are typically associated with active gene expression, while closed regions are often silenced.

ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) is a powerful technique used to identify accessible regions of the genome.

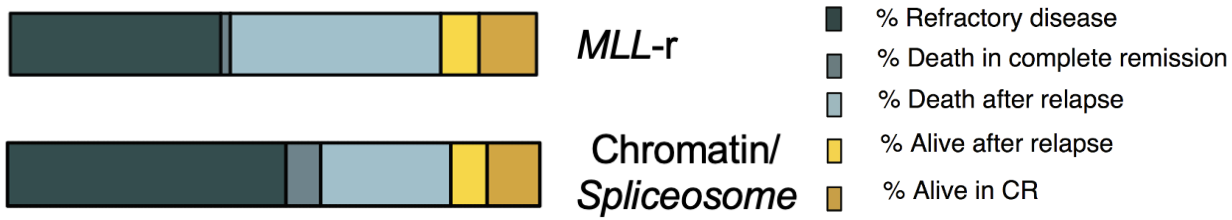
Overview of the interphase 3D nuclear architecture





Fu et. al 2018

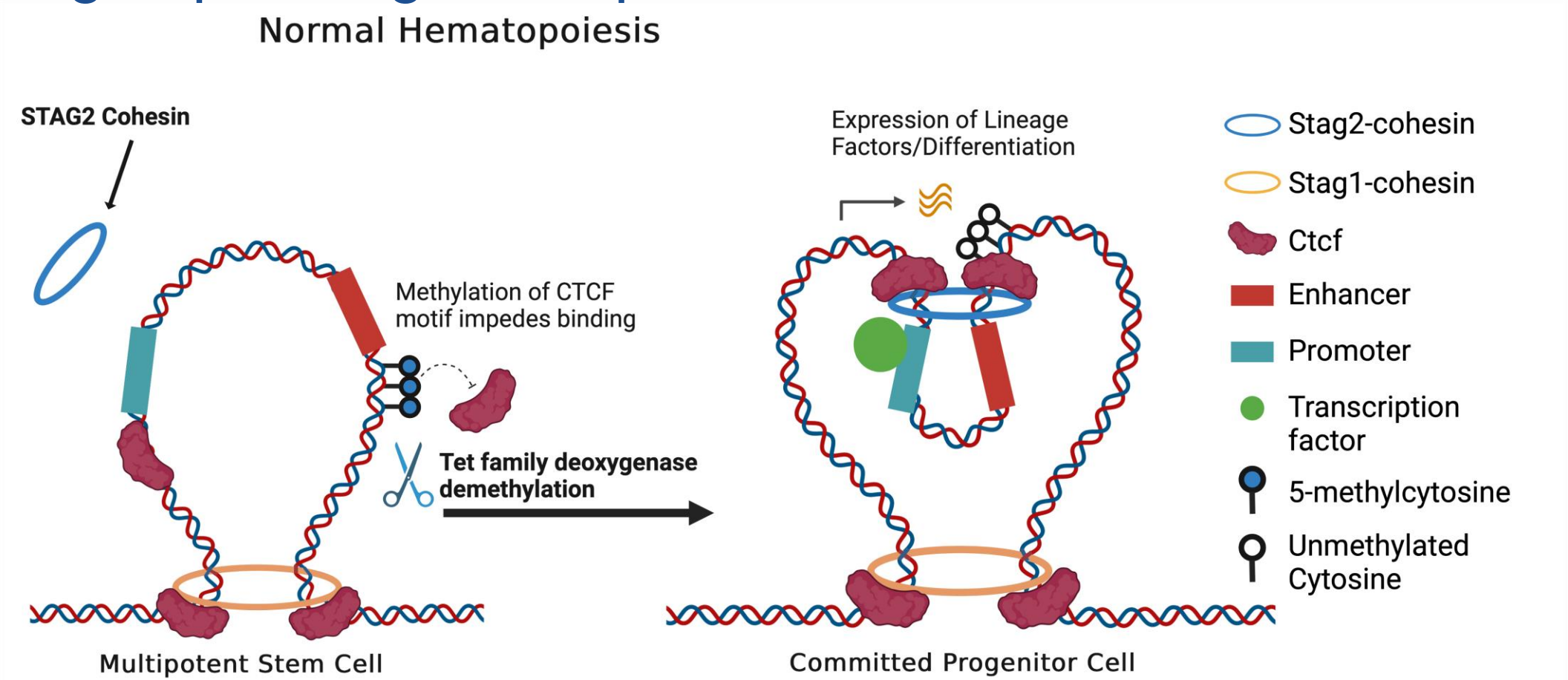
Beyond Oncogenes: Chromatin regulators are frequently altered in MDS/AML



Driver Mutations	Pathway/Functions	Approximate Frequency (%)		
		de novo AML	sAML	MDS
STAG2	Cohesin	2–7	10–14 *	3–8 †
RUNX1	Transcription factor	5–20	20–31 *	6–14
EZH2	Chromatin modification	2–4	5–9 *	4–15 †
BCOR	Chromatin modification	2–3	7–8 *	2–6 †
ASXL1	Chromatin modification	5–15	19–32 *	10–23 †
MLL-PTD	Chromatin modification	5–8	14	4–5

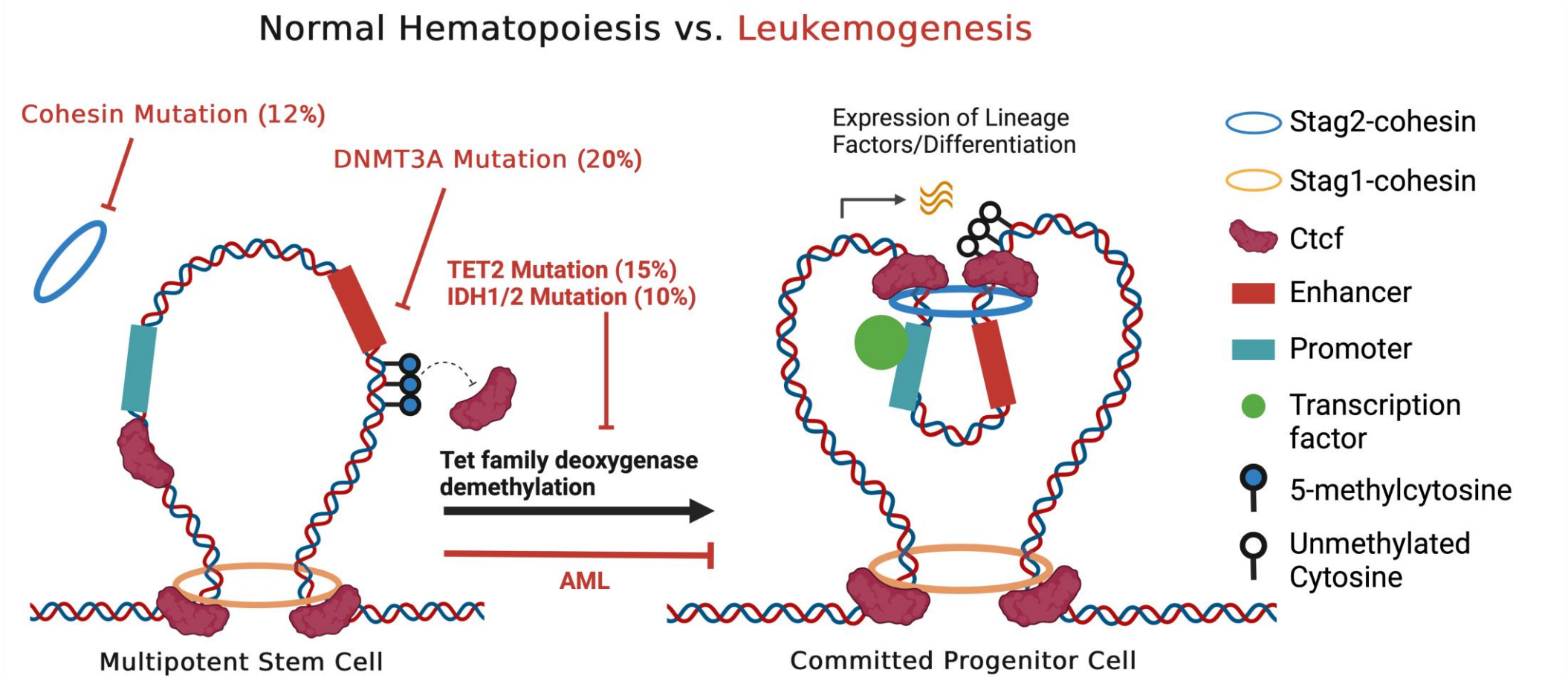
- Chromatin regulators, including transcription factors are recurrently mutated
- High rates of primary induction failure and relapse
- Mutations are more frequent in MDS† and sAML* (except MLL^{PTD})
- Aberrant chromatin structure influences cell state/identity and can result in disordered transcriptional expression and leukemogenesis

Cis-chromatin interactions facilitate key identity and lineage-specific gene expression



created with biorender.com

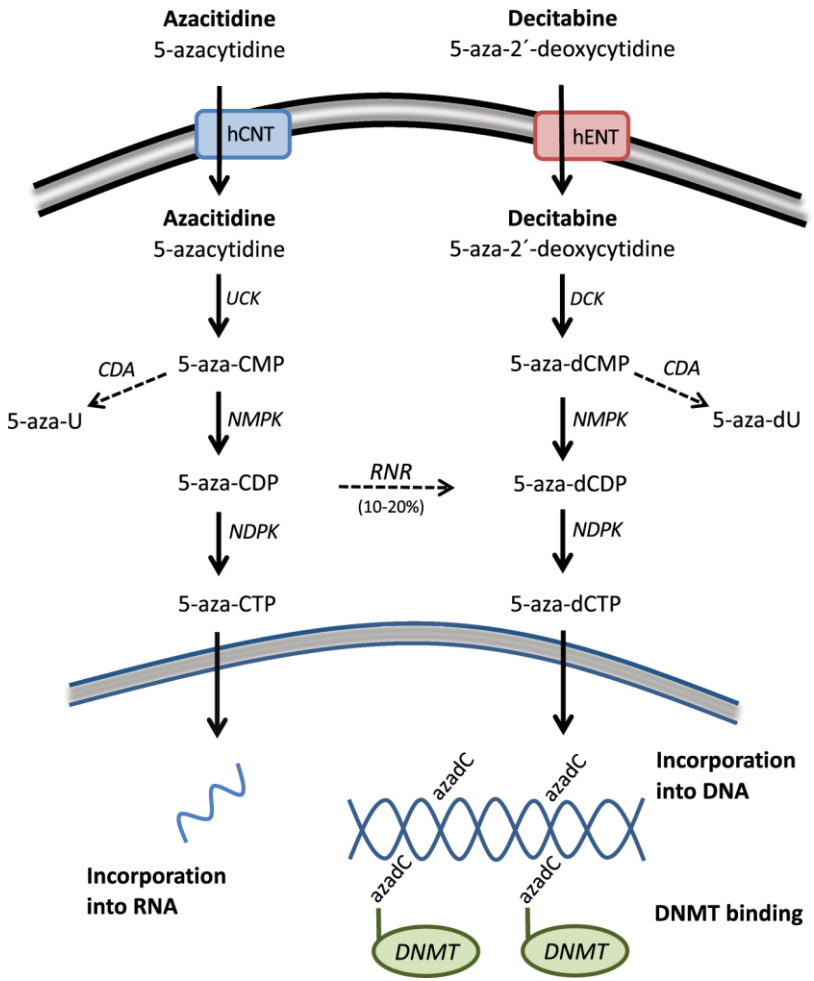
Possible convergent pathophysiology of chromatin aberrations in leukemogenesis



created with biorender.com

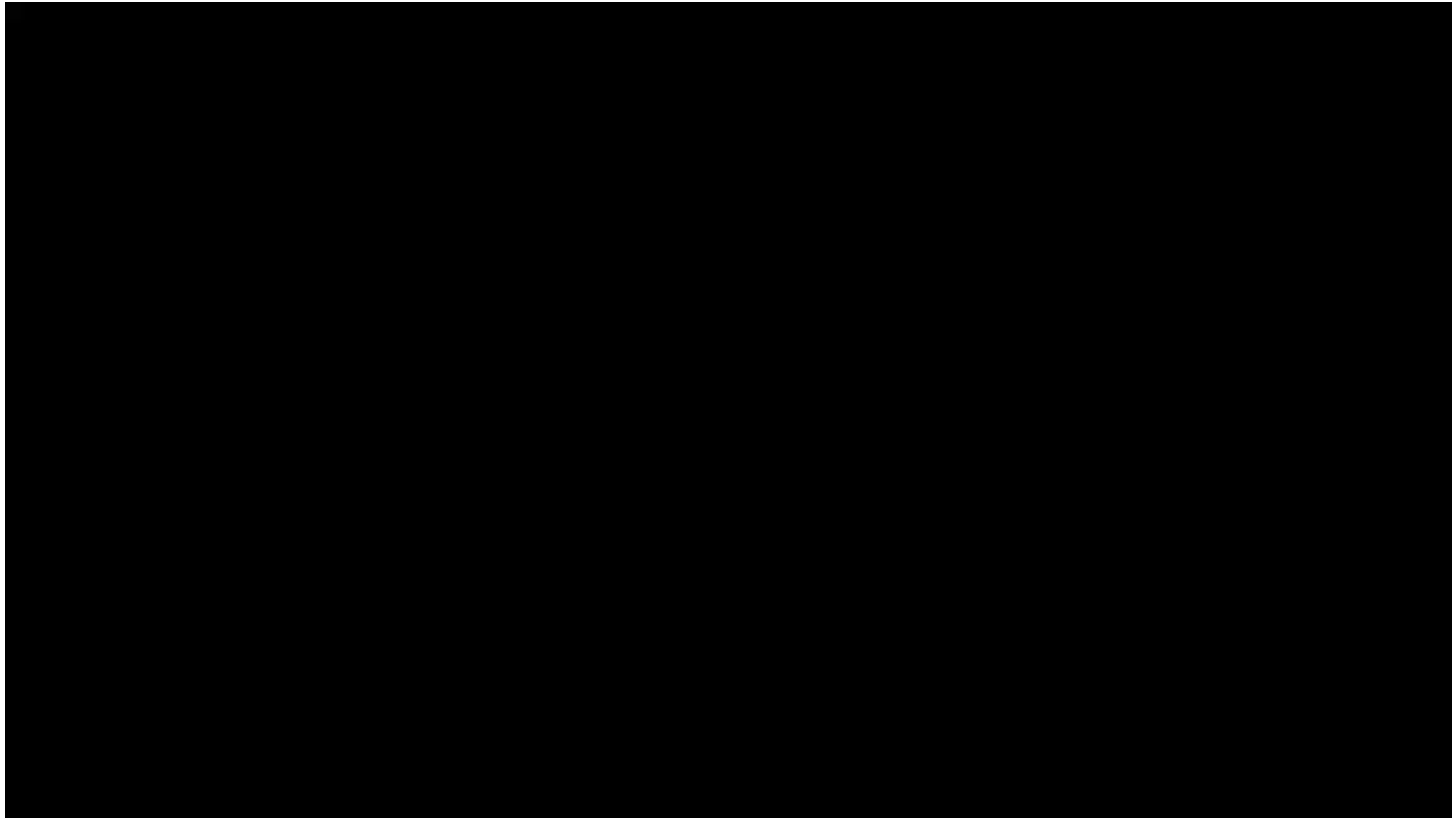
Hypomethylating Agents as (maybe) a chromatin directed therapy

- At high concentrations HMAs cause DNA damage, apoptosis
- DNMT1 depletion at lower levels, lending credence to the hypothesis that global hypomethylation may activate TSGs
- No genetic predictors of response, which is perplexing as the biology might suggest TET2 patients would be favorable
- Activation of endogenous retroviruses, IFN-responsiveness leading to differentiation are also proposed mechanisms
- Single agent HMAs have ~20% CR rate in MDS/AML (increases to ~37% with Venetoclax)
- Typically requires at least 4-6 cycles to gauge response

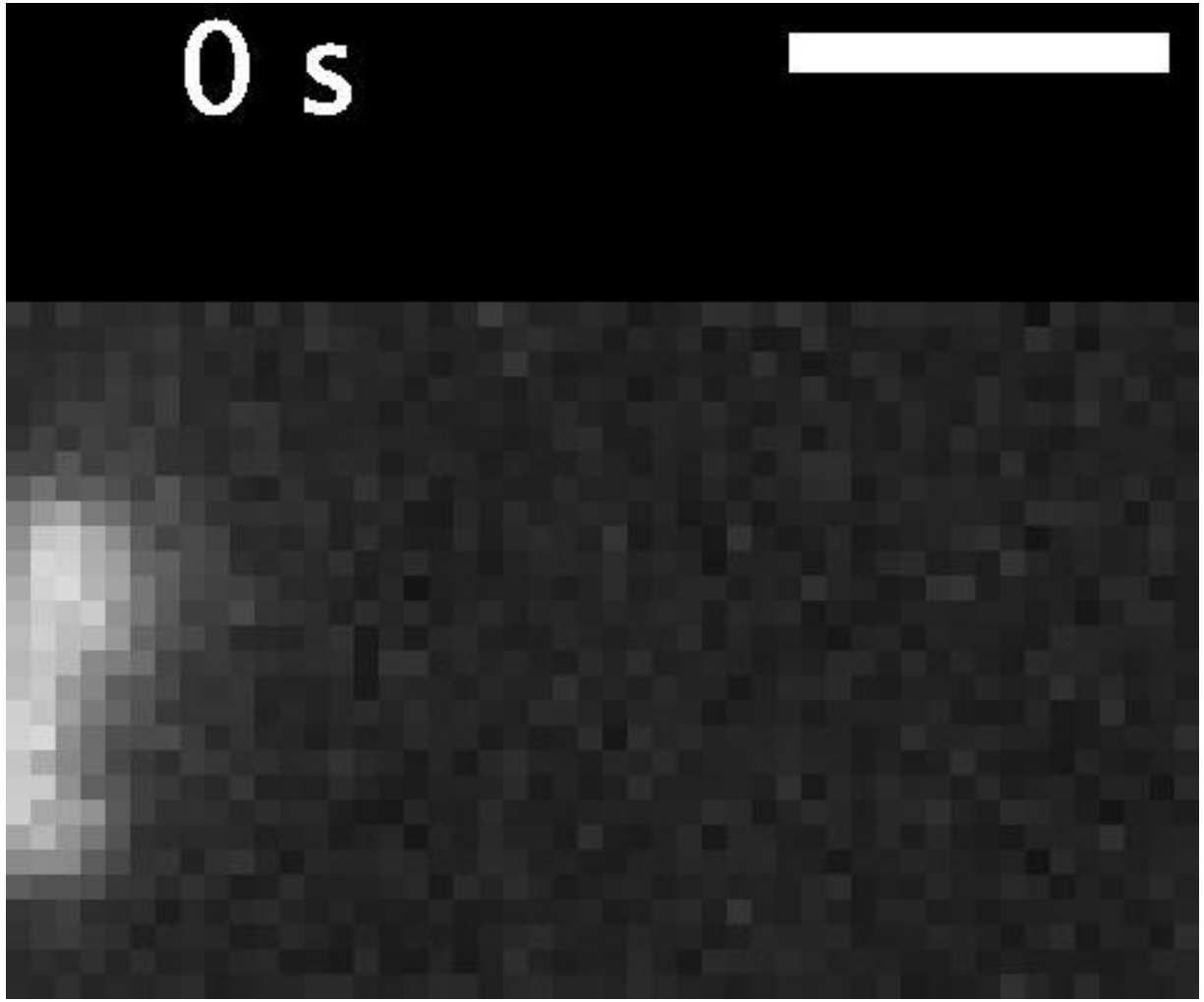


Stomper et al Leukemia 2021

Cohesin tethered CTCF organizes the 3D genome

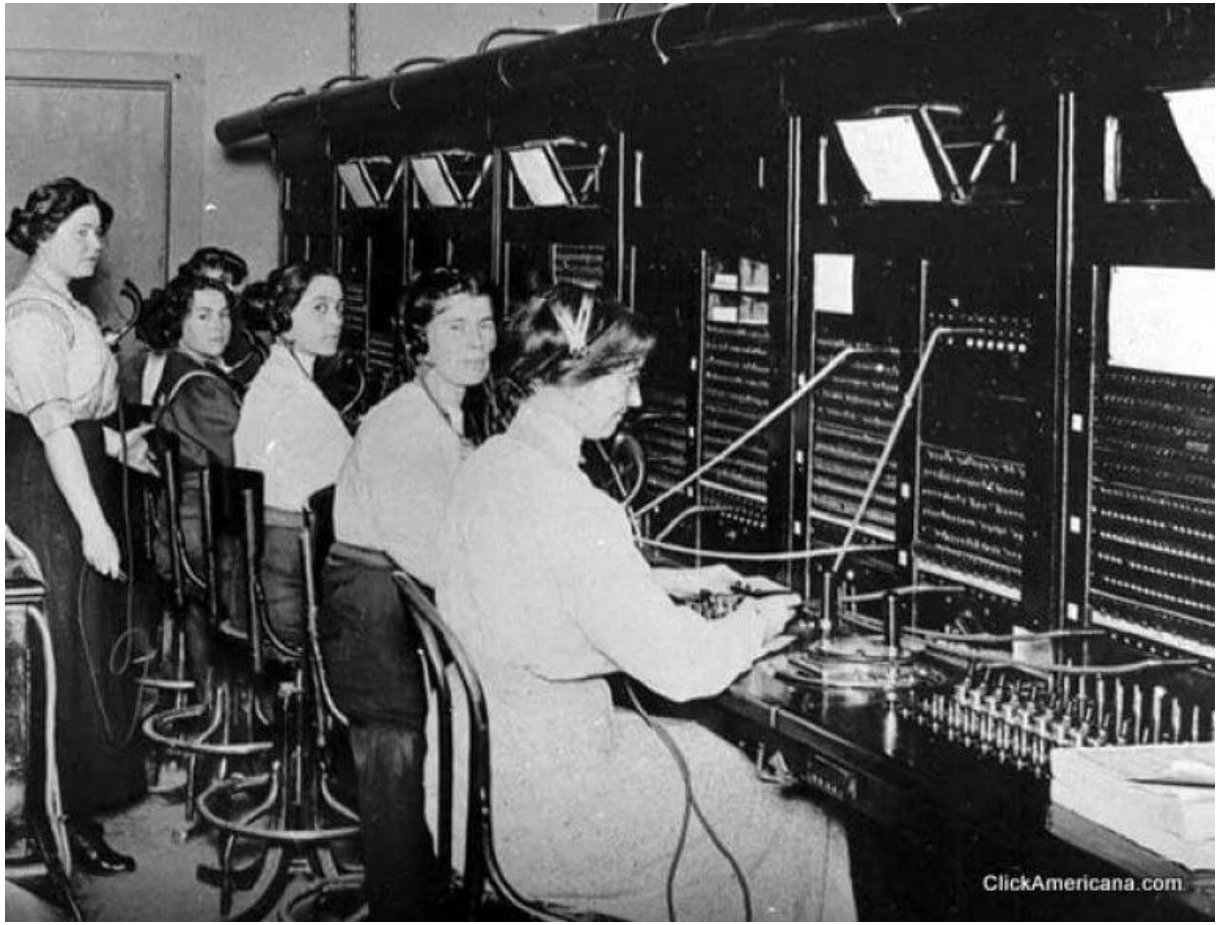


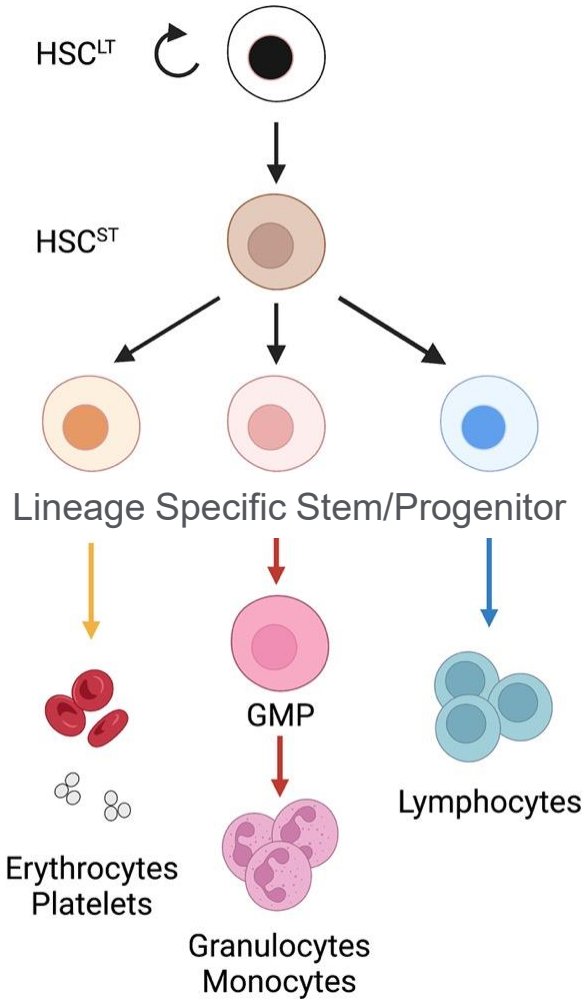
Cohesin tethered CTCF organizes the 3D genome



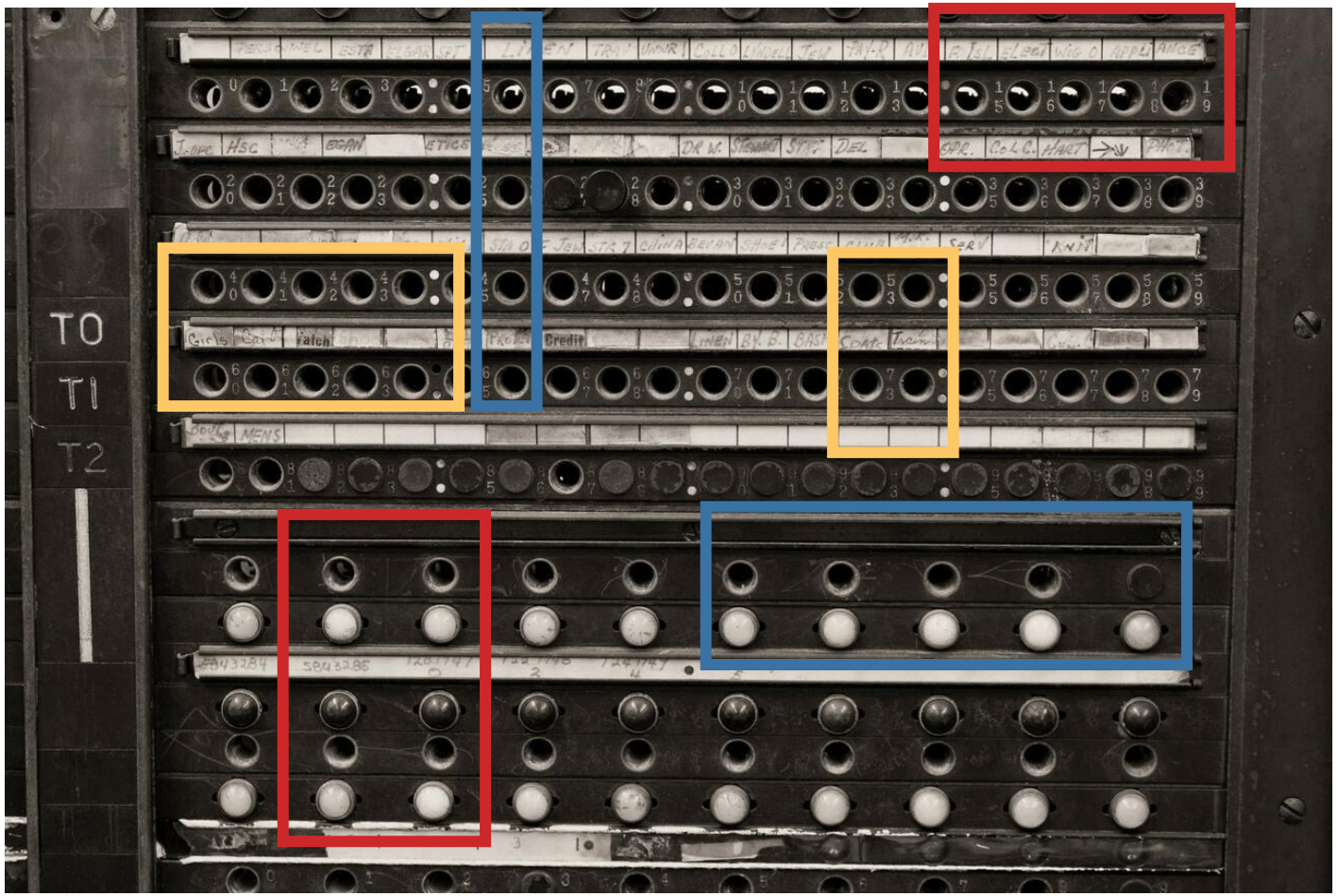
Mirny Lab YouTube Channel, Davidson I. et al. *Science* 2019

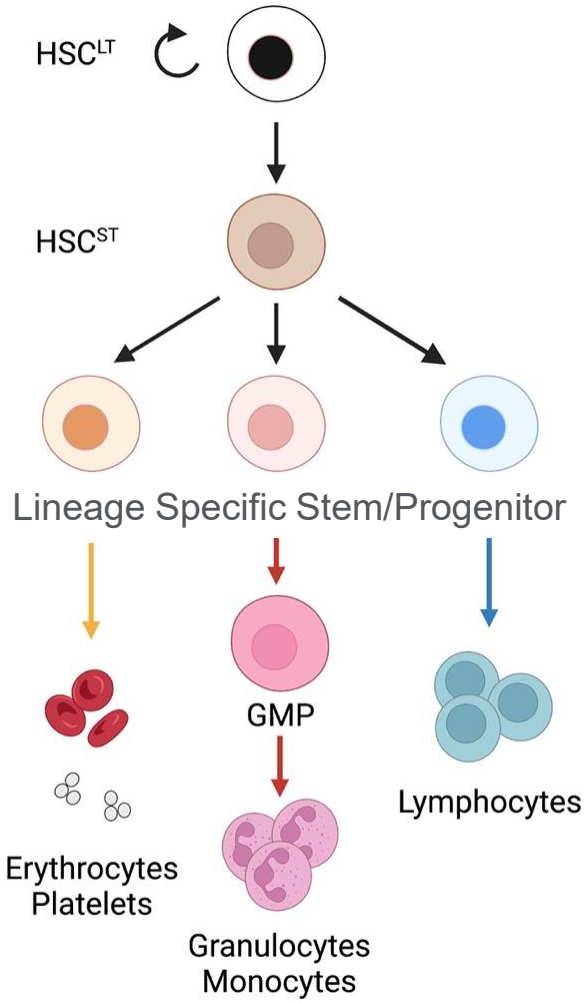
An “operator” switchboard control model of TF binding



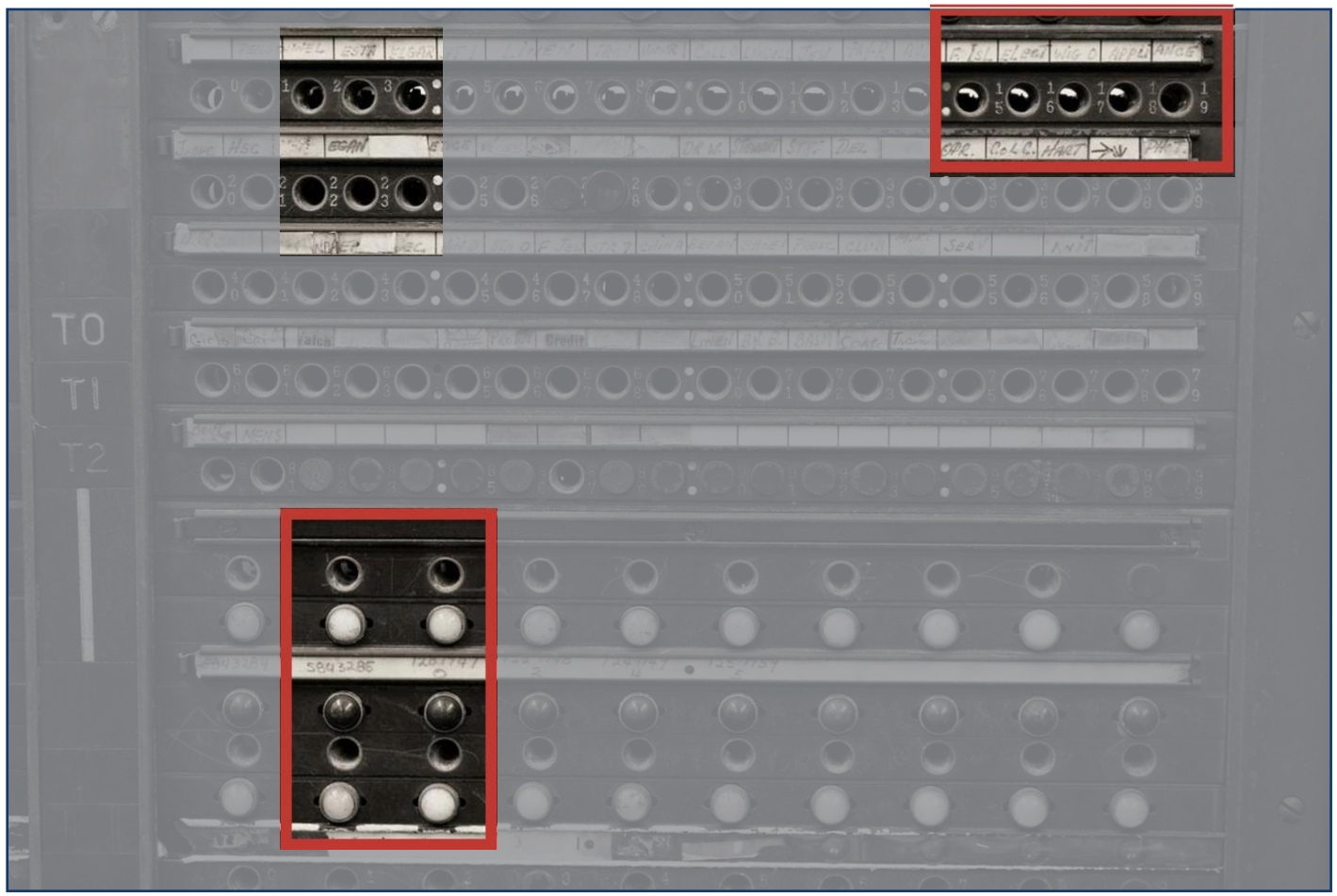


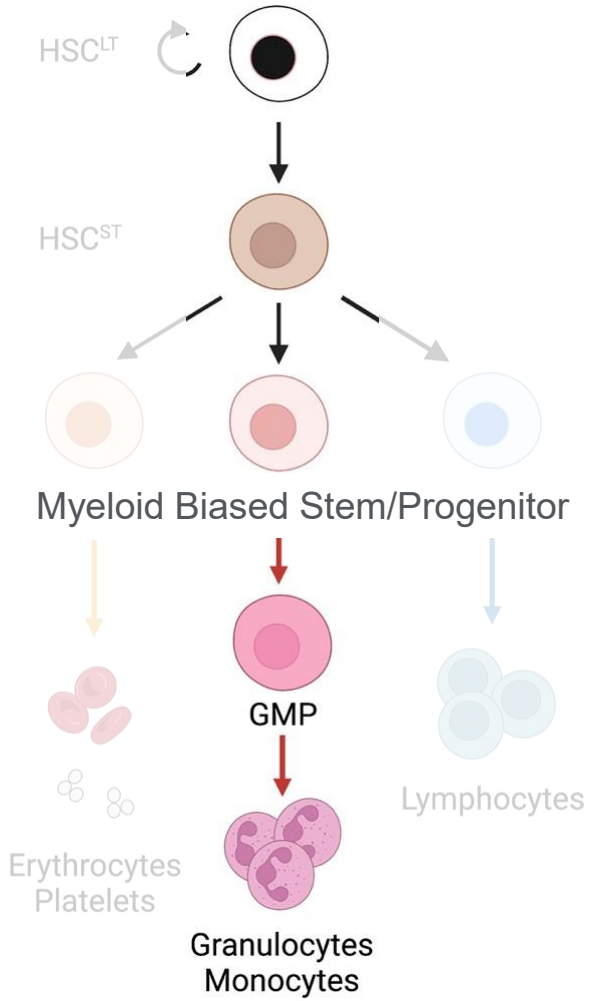
There are over 22,000 genes in the genome to choose



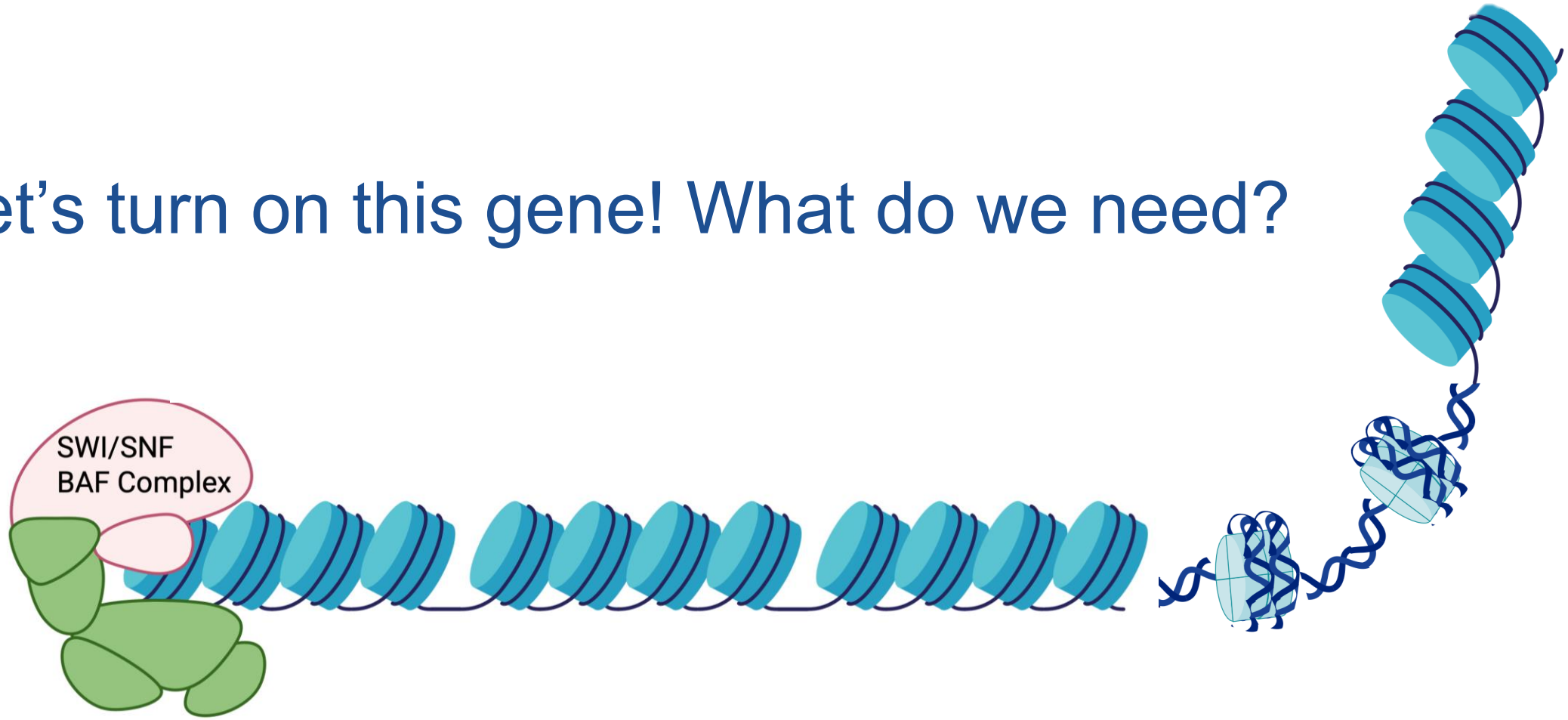


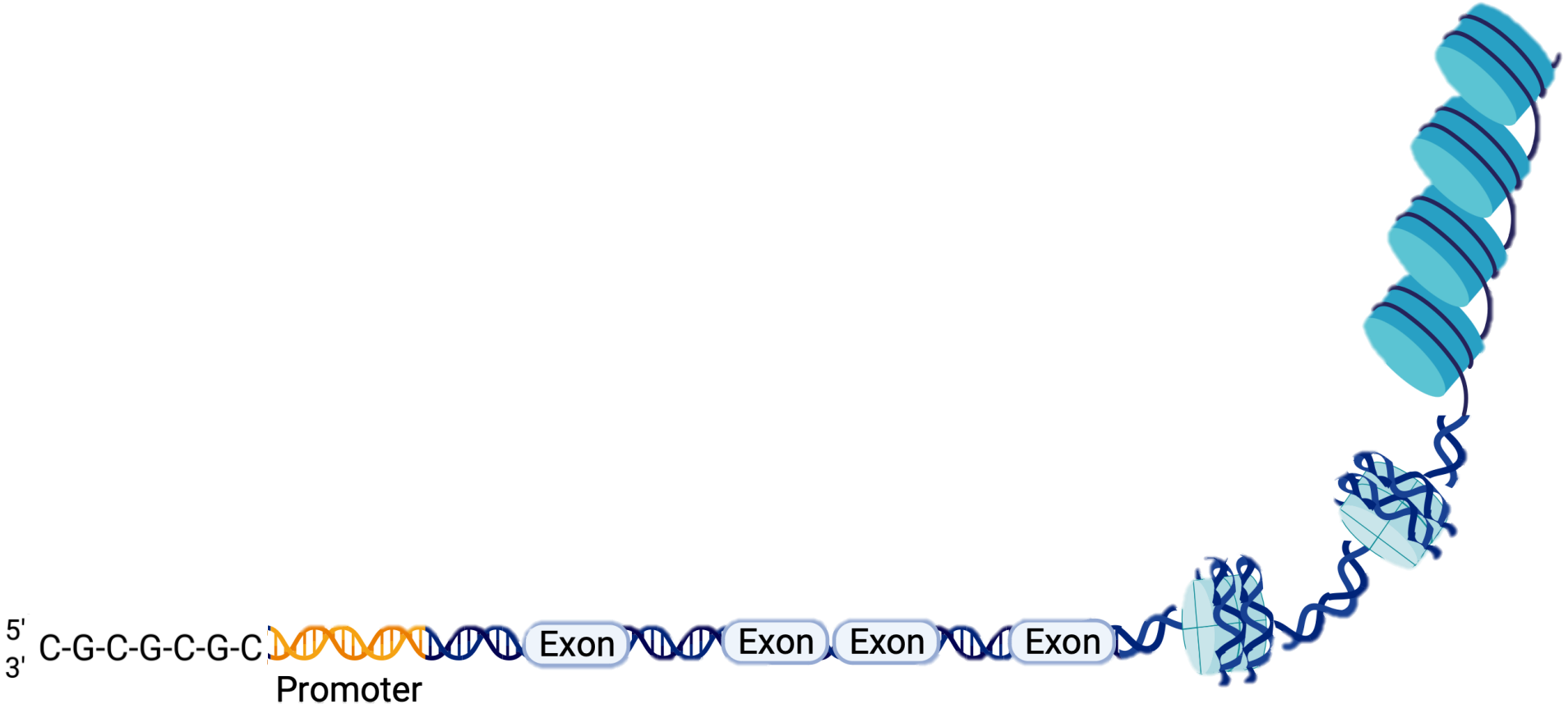
Chromatin accessibility and methylation restrict the options

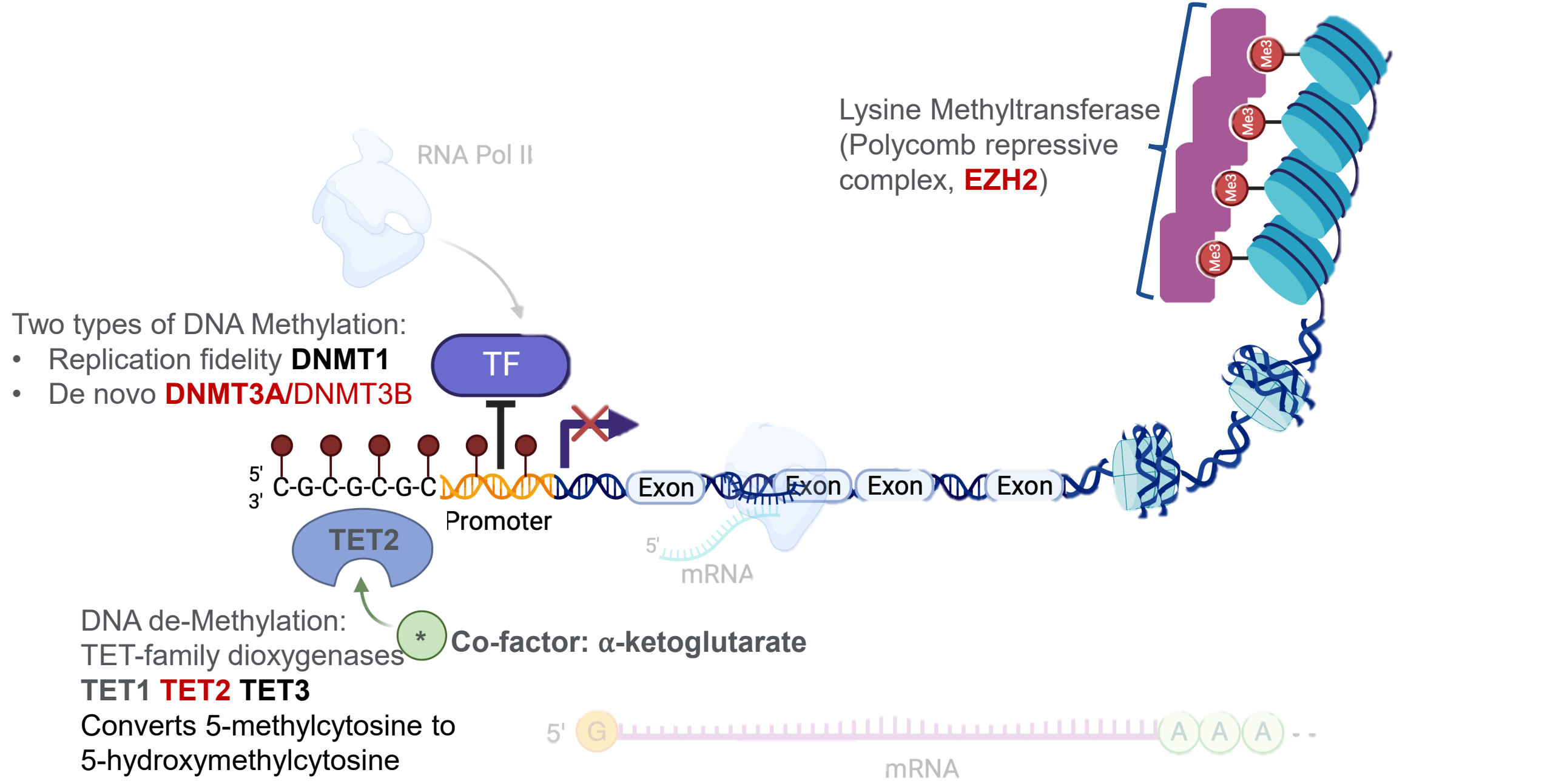




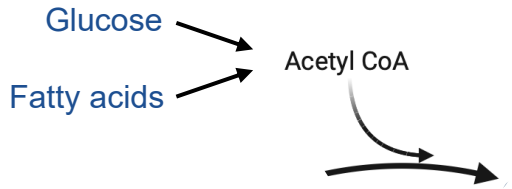
Let’s turn on this gene! What do we need?



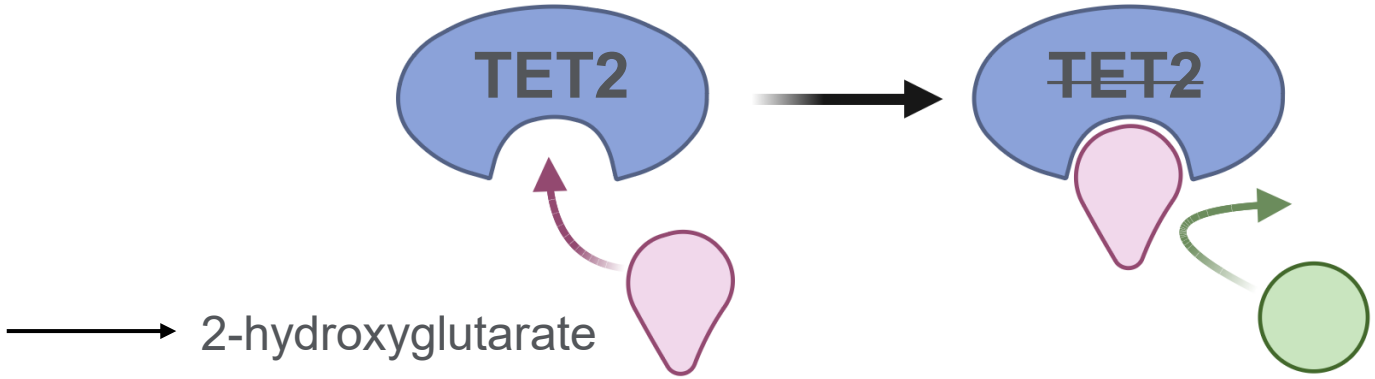

5'
3'
C-G-C-G-C-G-C
Promoter
Exon
Exon
Exon
Exon


Lysine Methyltransferase
(Polycomb repressive
complex, EZH2)
Me3
Me3
Me3
Me3
RNA Pol II
Two types of DNA Methylation:
• Replication fidelity DNMT1
• De novo DNMT3A/DNMT3B
TF
5'
3'
C-G-C-G-C-G-C
Promoter
Exon
Exon
Exon
Exon
5'
mRNA
TET2
DNA de-Methylation:
TET-family dioxygenases
TET1 TET2 TET3
Converts 5-methylcytosine to
5-hydroxymethylcytosine
*
Co-factor: α-ketoglutarate
5'
G
A A A
mRNA

Loss of epigenetic control in cancer – Krebs strikes back!

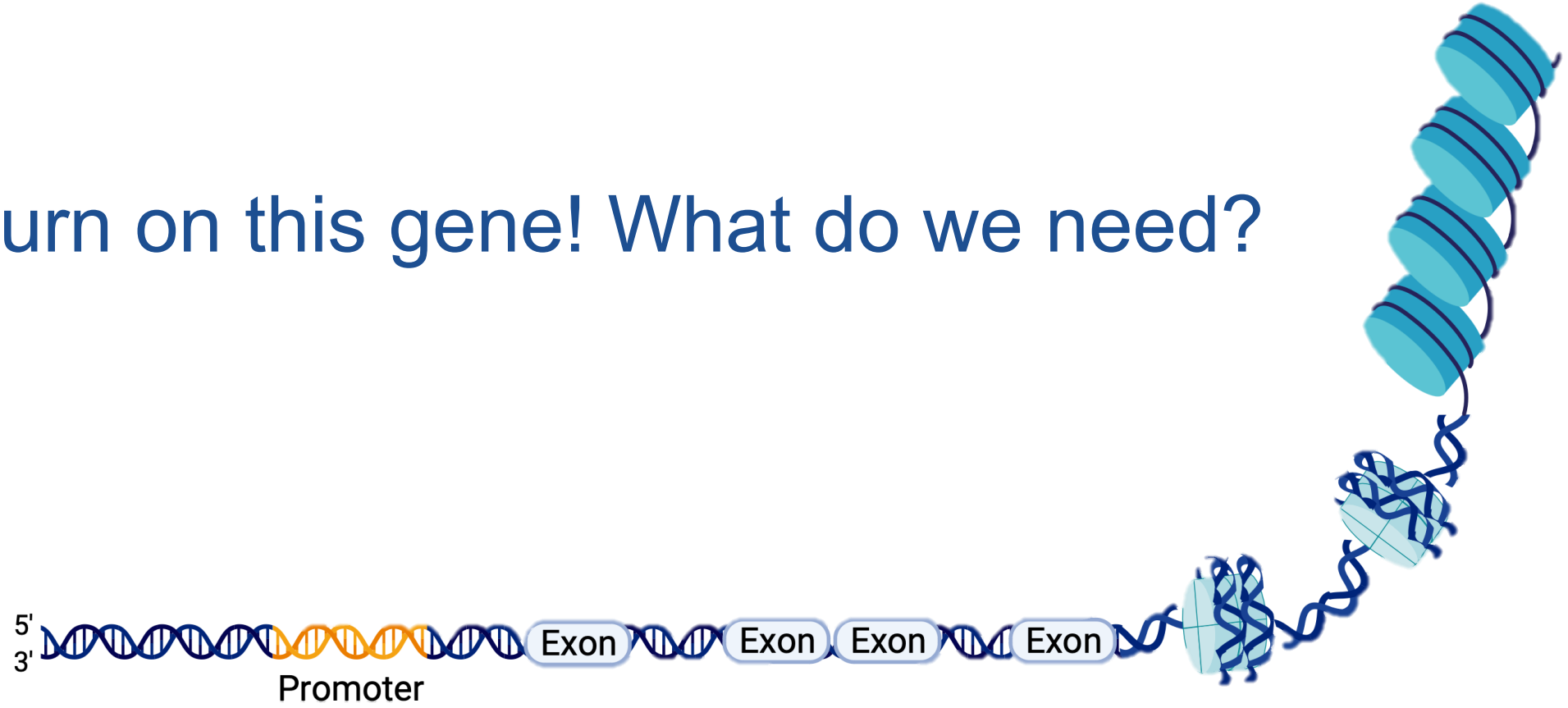


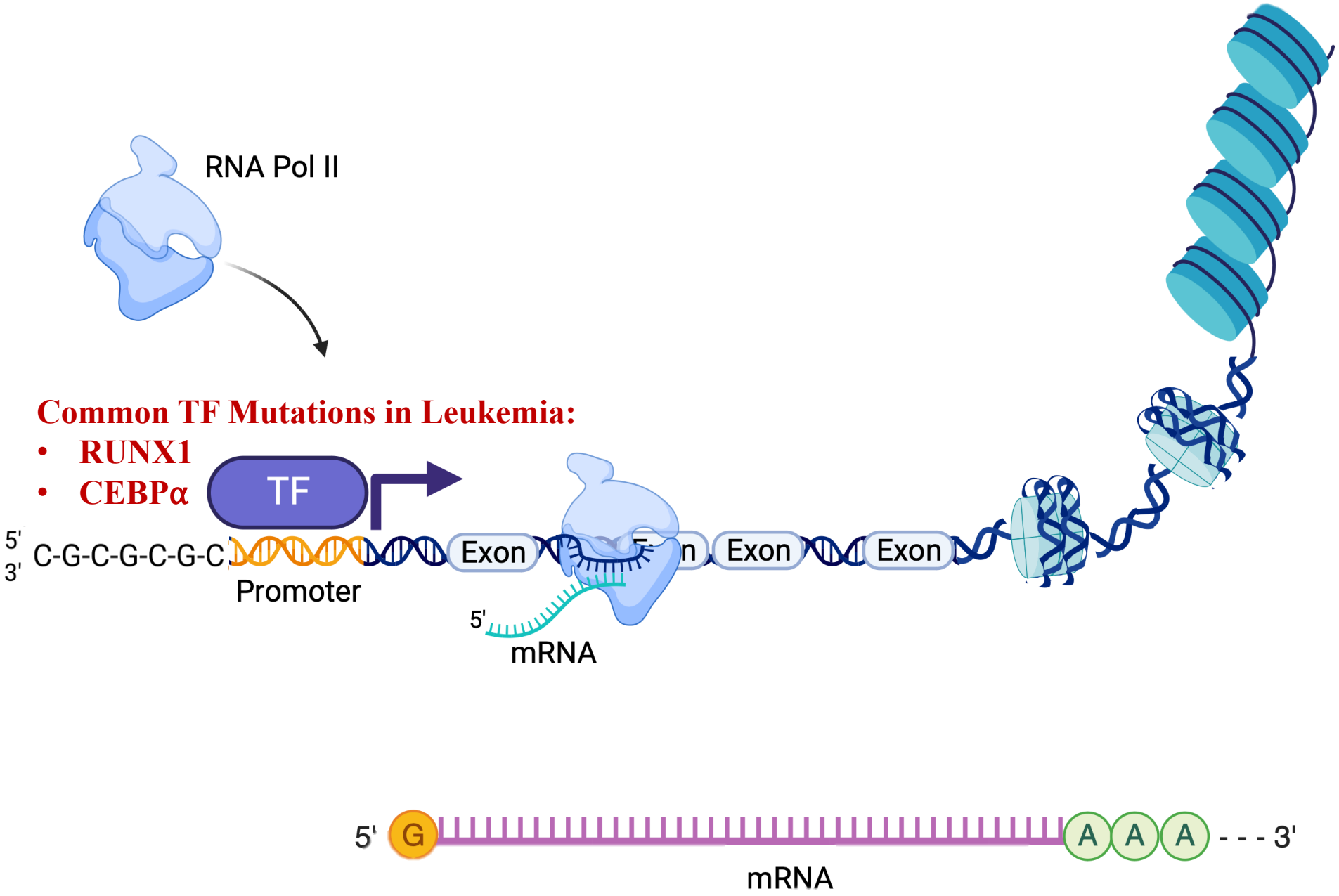
Krebs Cycle

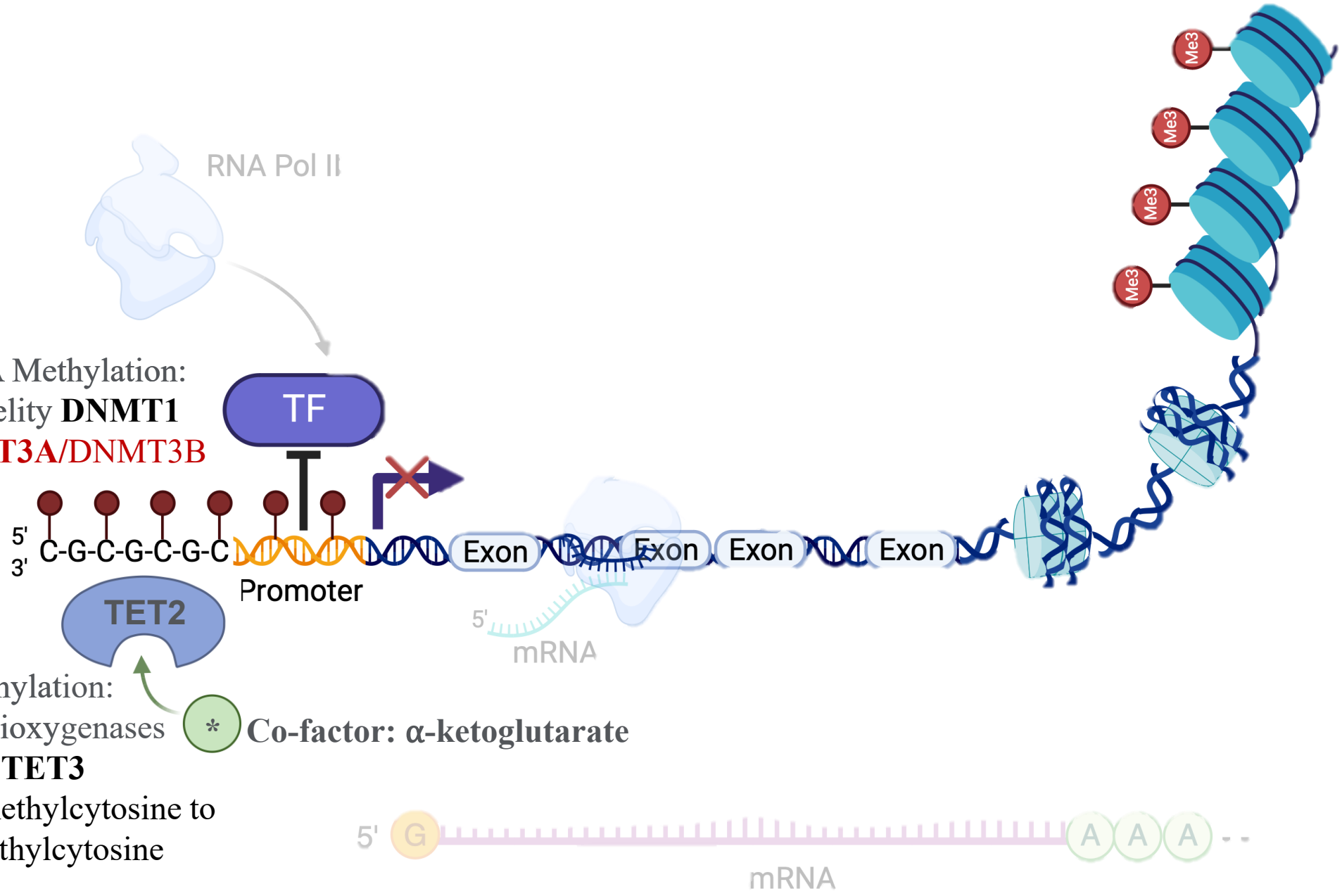


Isocitrate Dehydrogenase

Let’s turn on this gene! What do we need?




RNA Pol II
Common TF Mutations in Leukemia:
• RUNX1
• CEBPα
TF
5'
3'
C-G-C-G-C-G-C
Promoter
Exon
Exon
Exon
5'
mRNA
5'
G
A A A
- - - 3'
mRNA

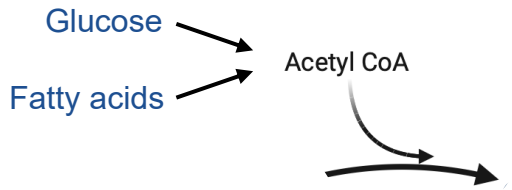


Two types of DNA Methylation:

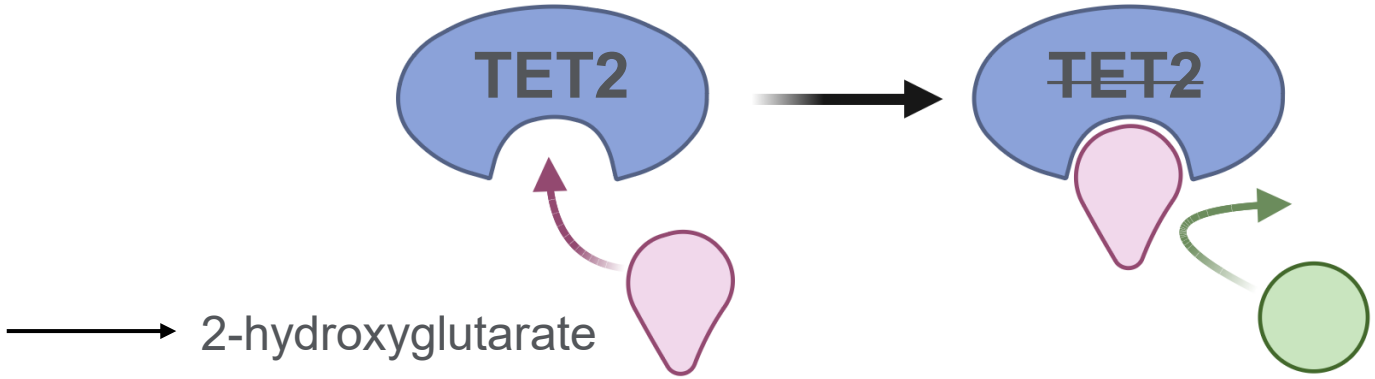
- Replication fidelity **DNMT1**
- De novo **DNMT3A**/DNMT3B

DNA de-Methylation:
TET-family dioxygenases **Co-factor: α-ketoglutarate**
TET1 TET2 TET3
Converts 5-methylcytosine to
5-hydroxymethylcytosine

Loss of epigenetic control in cancer – Krebs strikes back!

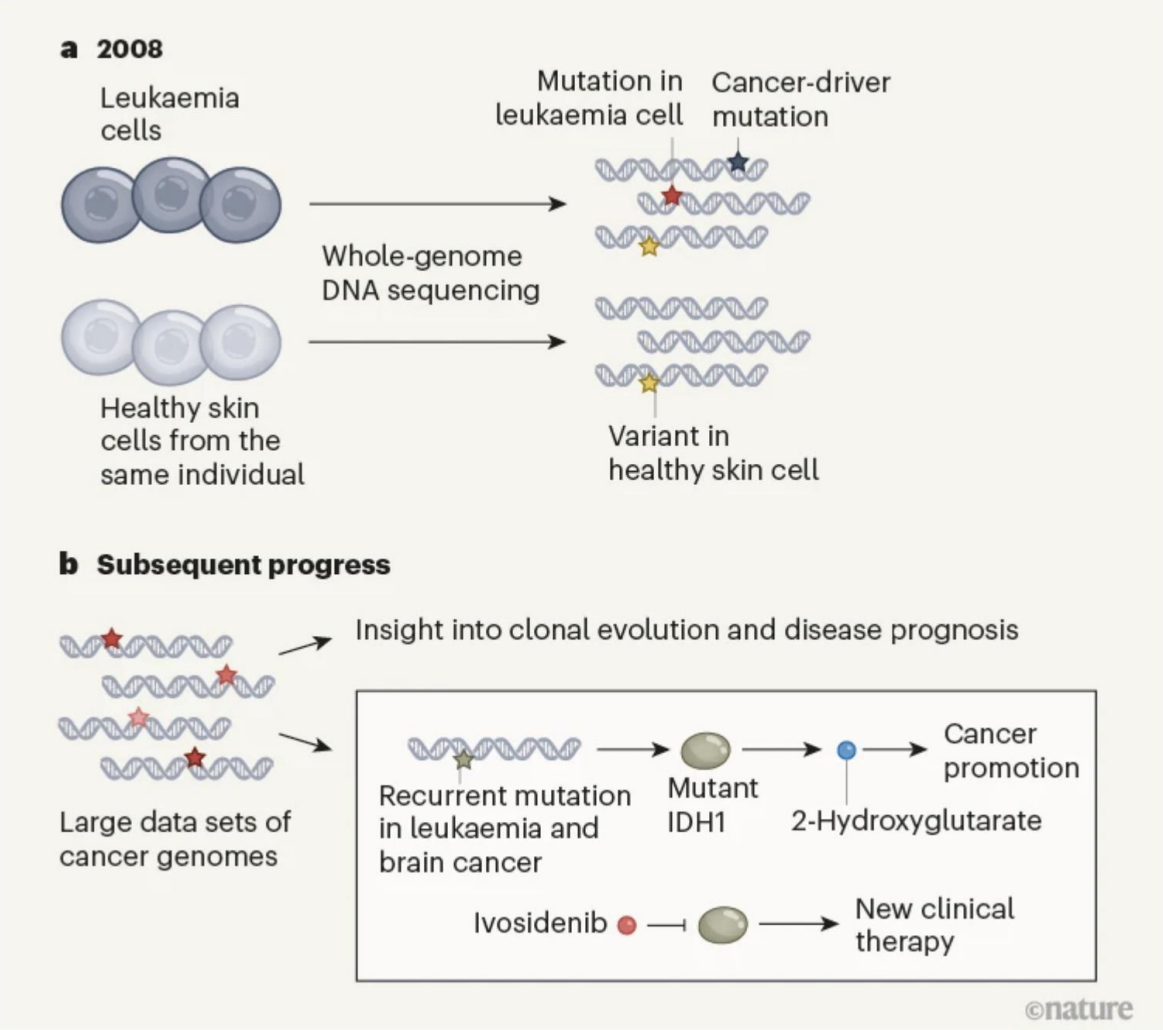


Krebs Cycle



Isocitrate Dehydrogenase

This year is the 15th anniversary of sequencing the first cancer genome!



Cai and Levine, *Nature*, December 2023

National Marrow Donor Program

- Please consider becoming a bone marrow donor
- Swab your cheek, save a life, and if you'd like you can do it right now! Scan the QR code and you'll be mailed a swab