MDS: What Do My Mutations Mean?

Casey O’Connell, MD

Objectives

- Refresh your basic understanding of blood, bone marrow, stem cells and “Mutations”
- Review the MDS diagnosis, distinguish it from CCUS and AML
- Discuss how or why MDS develops
- Compare and contrast germline and somatic mutations
- Consider how genetic mutations may lead to cancer
- Outline the treatment approach to MDS
- Identify “targetable” mutations in MDS

How Does a Factory Making 310,000,000,000 cells Daily Function for 80+ Years?????

1. **<u>Needs Ingredients</u>**: proteins, iron, oxygen, B12, Folate, copper, etc.

2. **<u>Needs Stimulus</u>**:

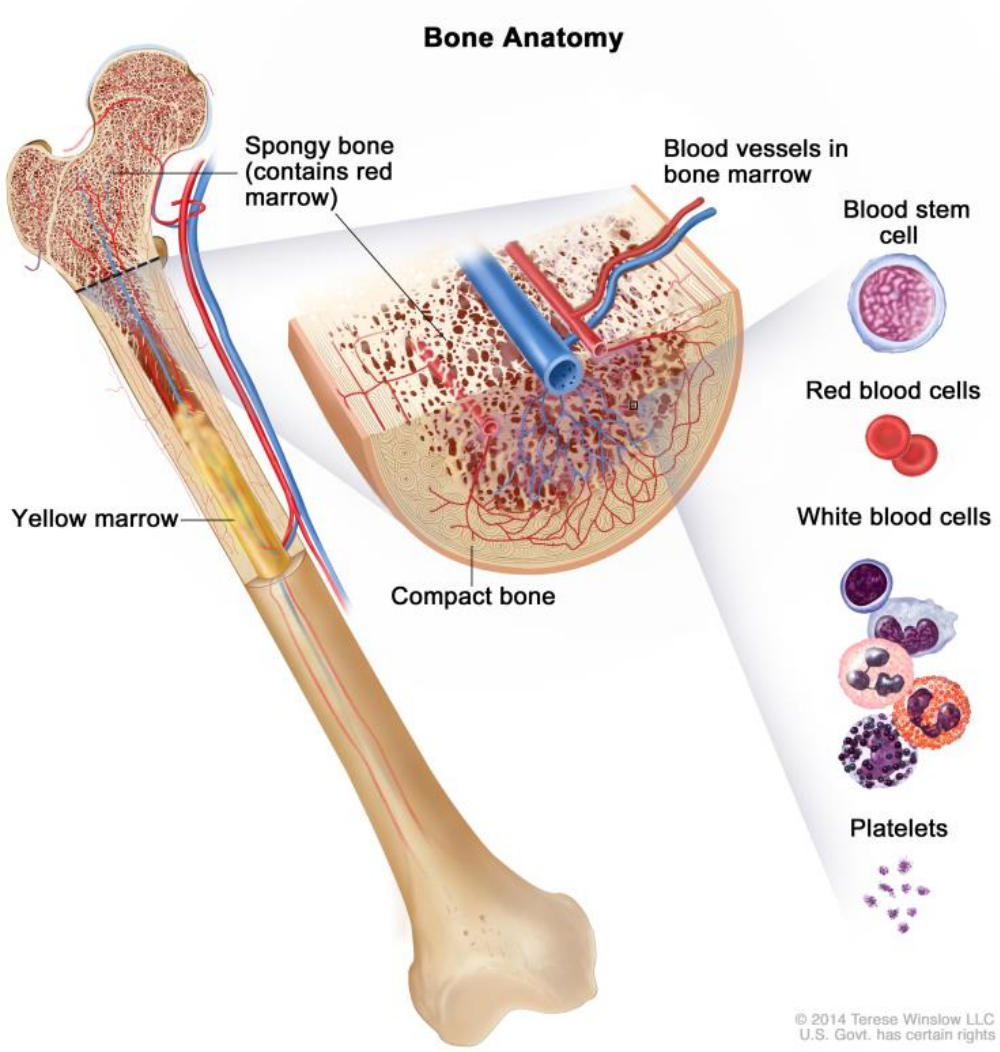
   Erythropoietin – from the kidneys stimulates Red Cell Production

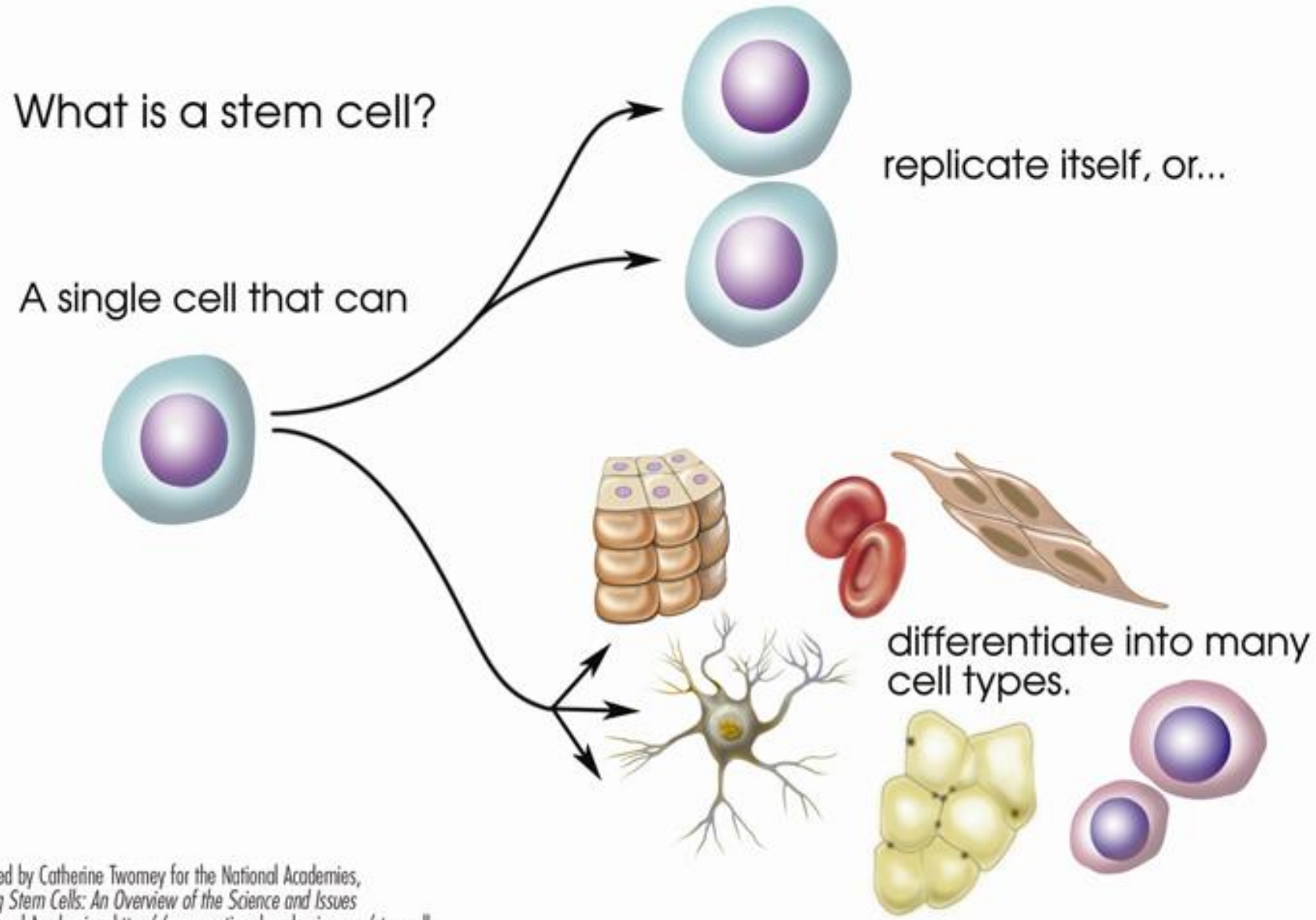
   GCSF – from blood vessel lining, immune cells stimulates WBC Production

   Thrombopoietin – from the liver stimulates Platelet Production

3. **<u>Needs Source</u>**:

   STEM CELLS

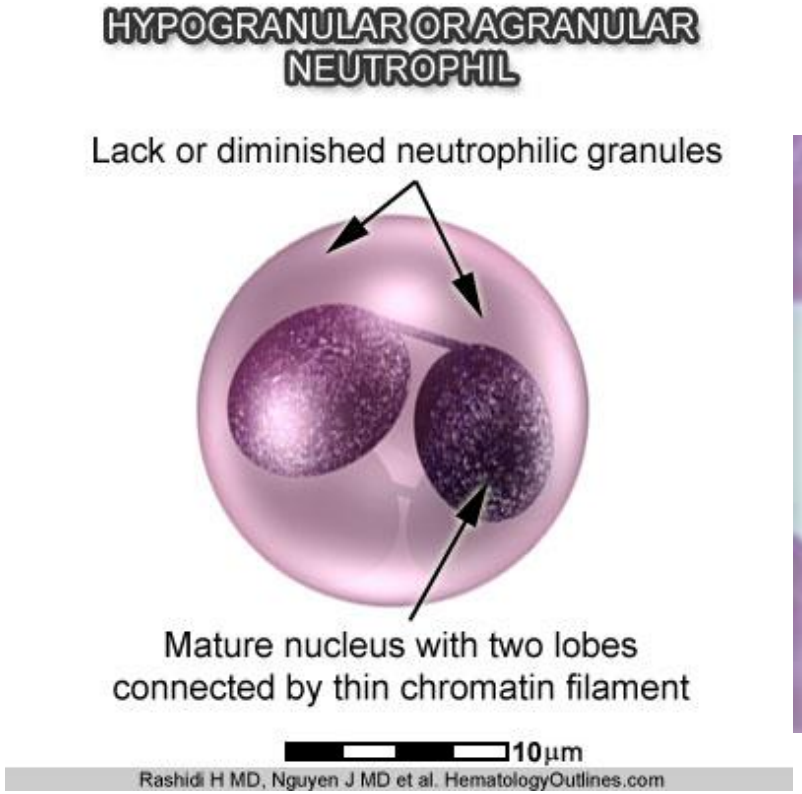


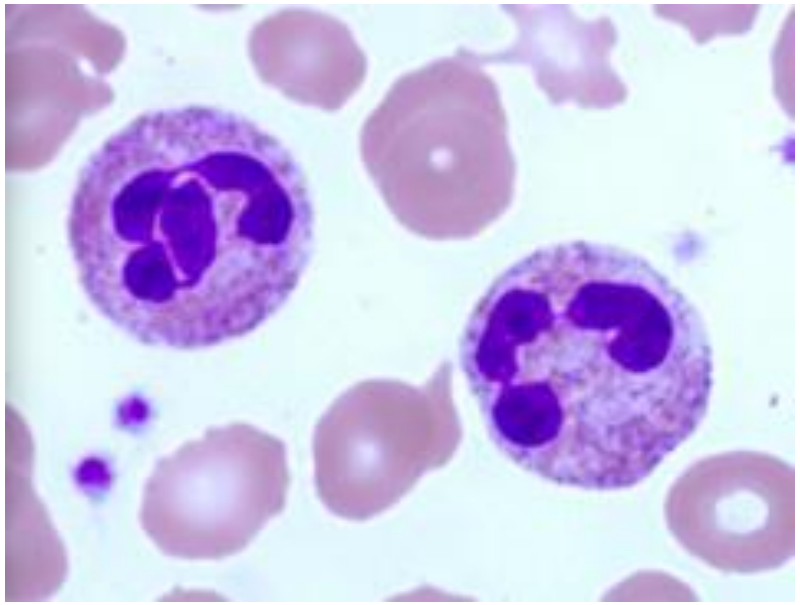


What Is Myelodysplastic Syndrome (MDS)?

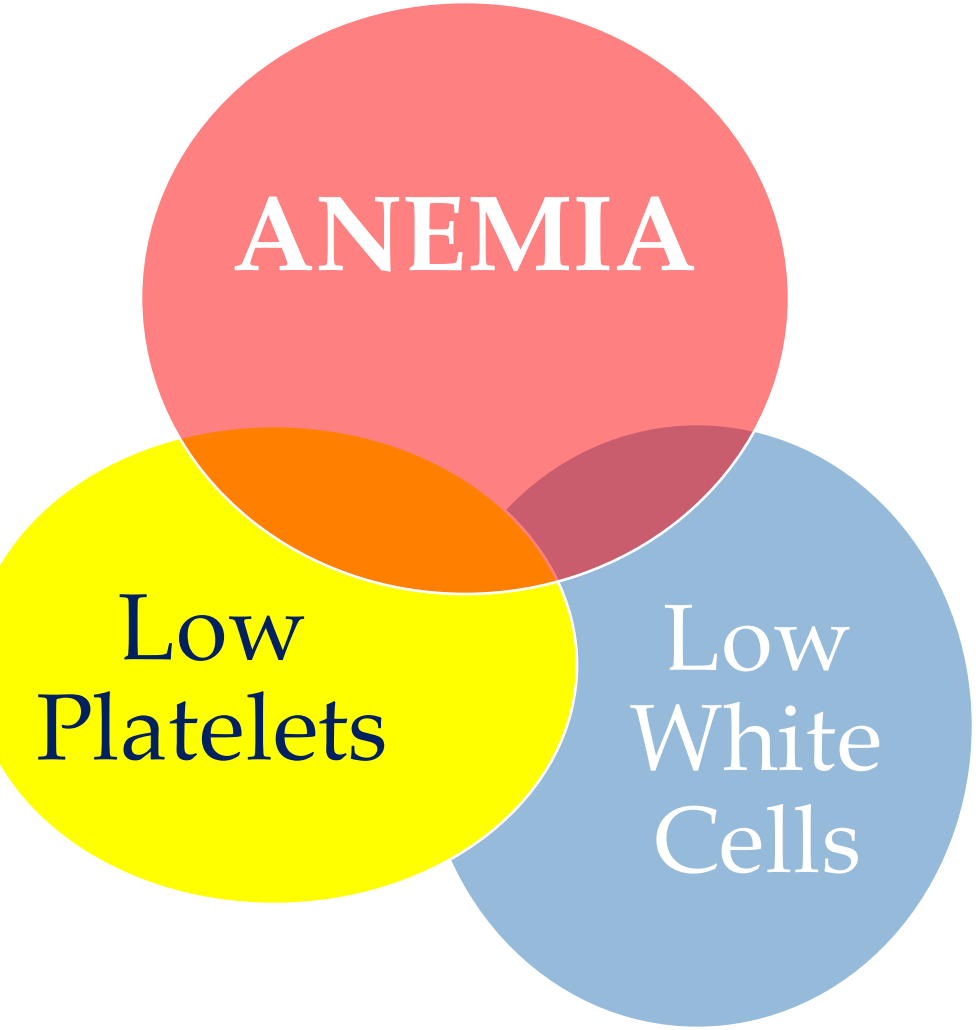
- MYELO = Greek myelos = marrow

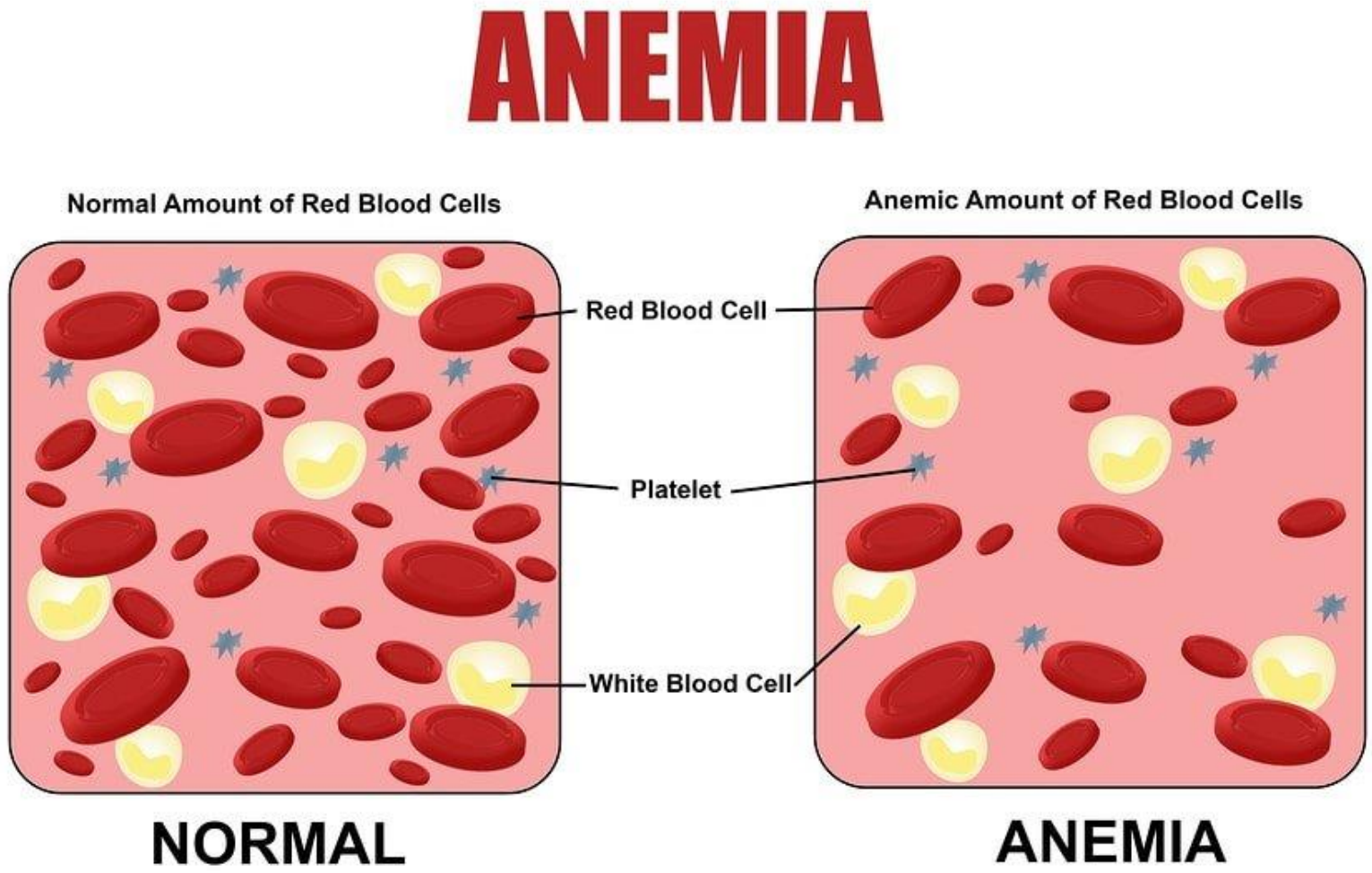
- DYSPLASIA= dys (ABNORMAL) + plasia (GROWTH or DEVELOPMENT)





MDS Leads to Abnormal Blood Cell Production





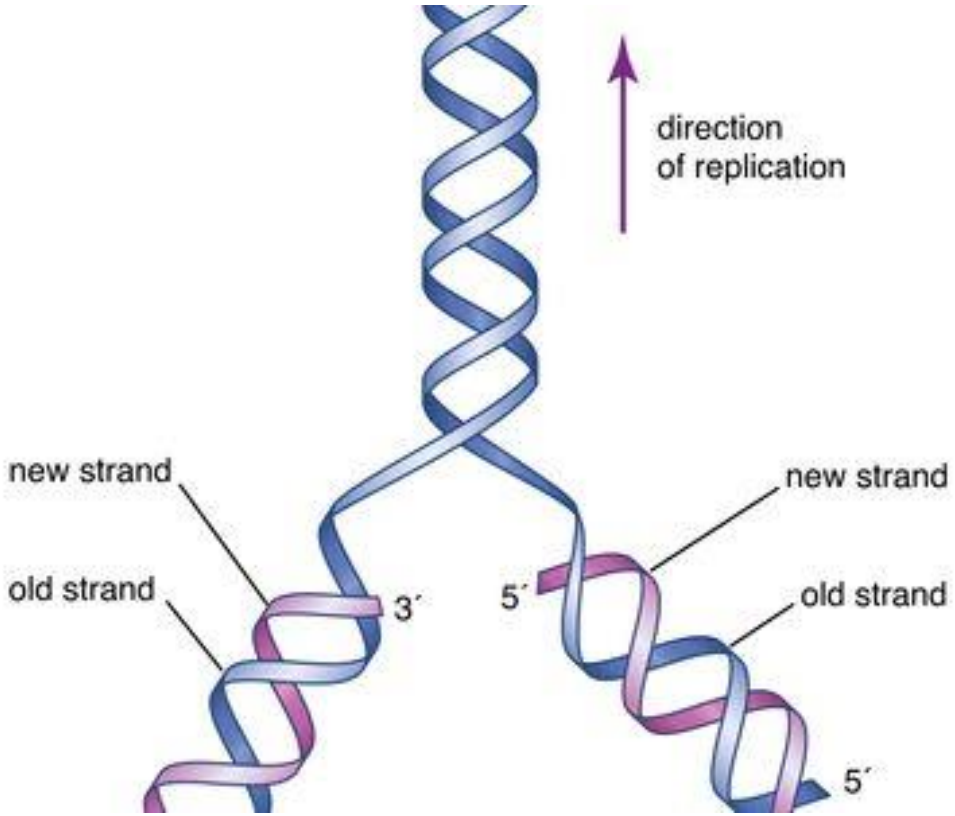
DNA/Gene Mutations in Blood-Producing Precursors Cause MDS

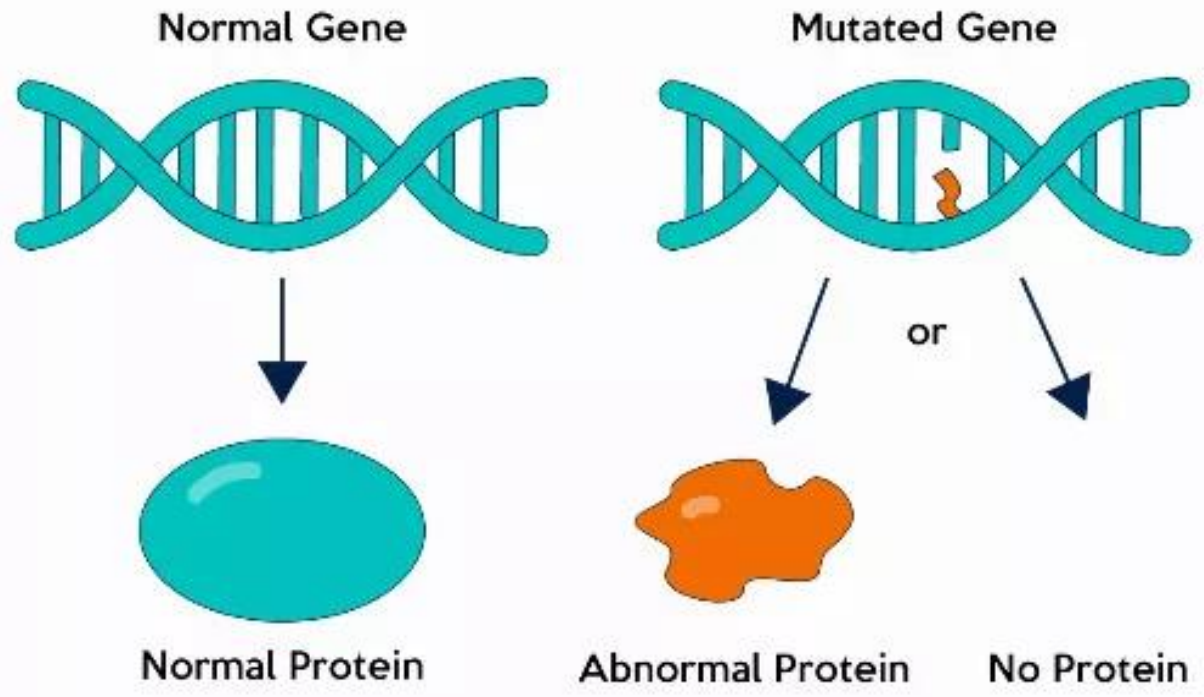
WHY?????

- Exposure to gene-damaging agents
  - Chemotherapy
  - Chemicals
  - Radiation
- Spontaneous Mutations in DNA
- Inherited Predisposition Genes or Mutations
- Environmental exposure Causing New Mutations in DNA

REROUTING.... What Are Mutations???

Mutations = DNA Changes That Affect the Protein Genes Produce





Mutations Can Be Congenital or Acquired During Life

CONGENITAL (Germline) – present at birth, inherited or acquired during development, *mutation is in every cell*

Examples:

Sickle Cell Anemia

Hemophilia

Down's Syndrome

Familial Cancer Syndromes (BRCA, Polyposis, etc)

ACQUIRED (Somatic) - happen after birth, spontaneous or due to genotoxic exposure or immunodeficiency, *mutation is only in certain cell types*

Examples:

MDS & AML

Most Solid Tumors

AIDS-related Cancers

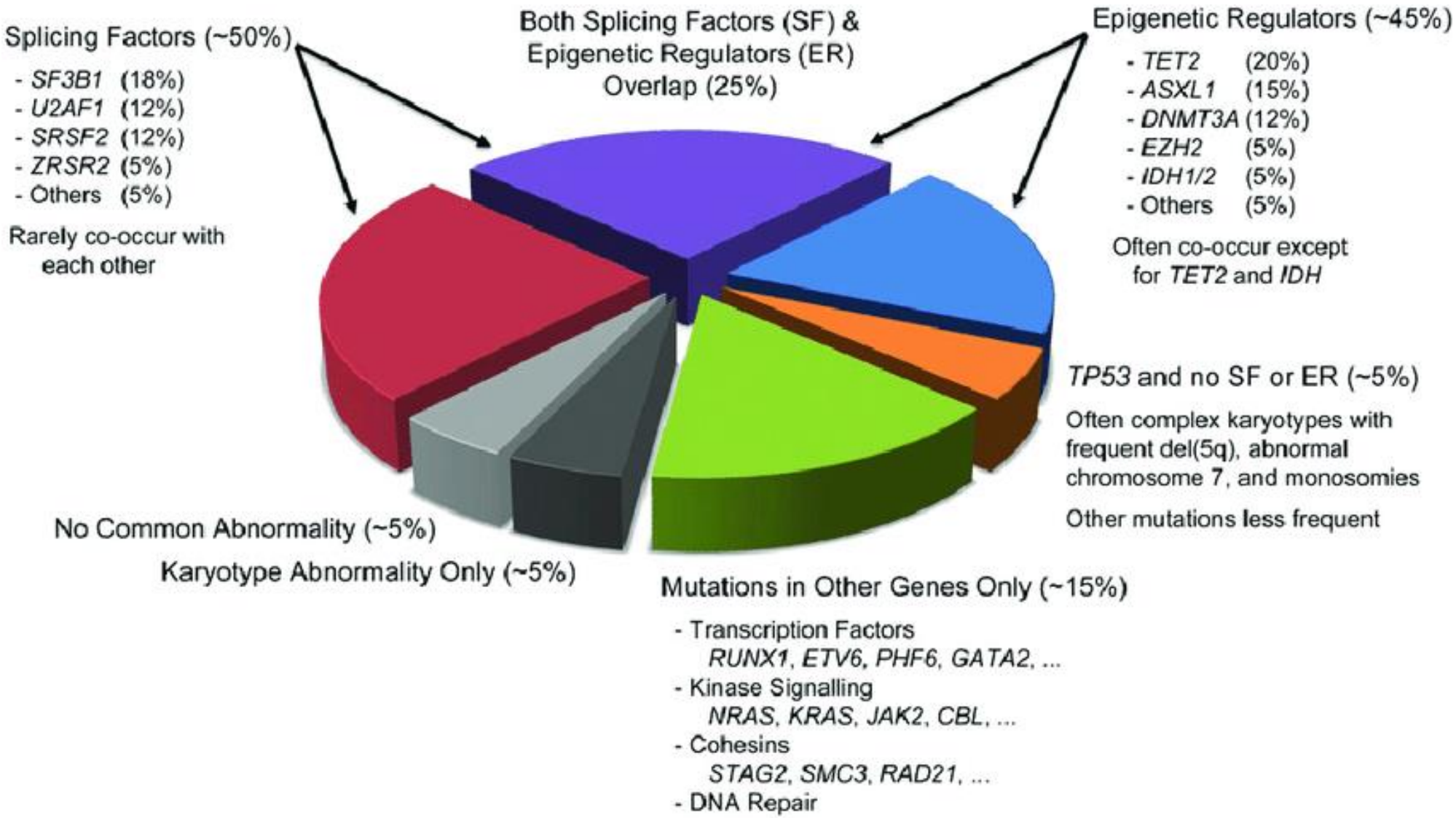
Post-transplant lymphoproliferative disorders

Testing For Mutations

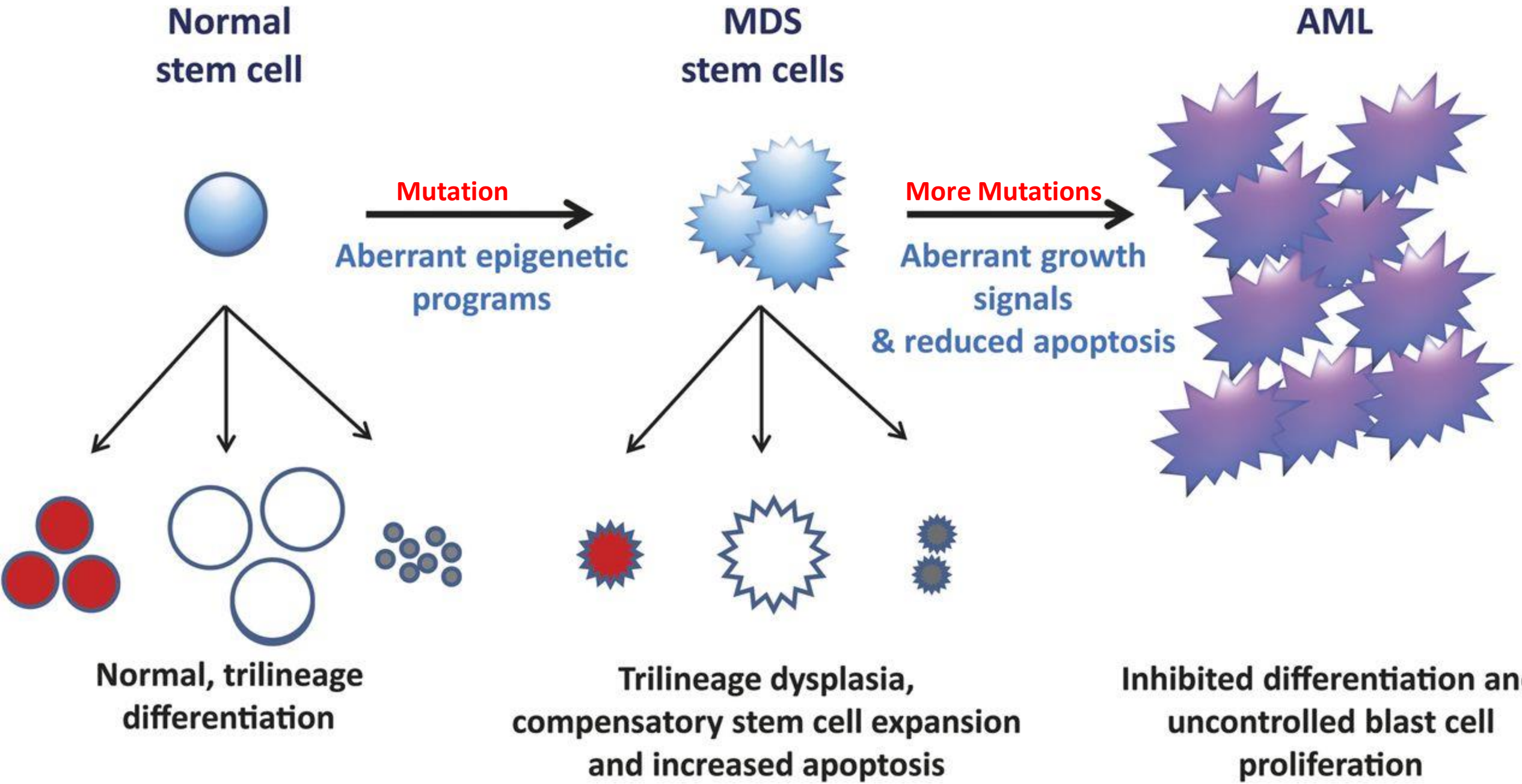
KARYTOPE	FISH	PCR ("NGS")
Normal = 46 XX, XY Picks up 1/20 Cells with a structural mutation <u>Examples:</u> 5q- Monosomy 7 Deletion 20 Translocations	Can Detect 1/200 Cells Carrying a Specific Mutation <u>Examples:</u> 5q- Monosomy 7 Deletion 20 Translocations	Can Detect 1/10,000+ Cells Carrying a Small, Specific DNA Change <u>Examples:</u> See next slide

Back to Mutations and MDS

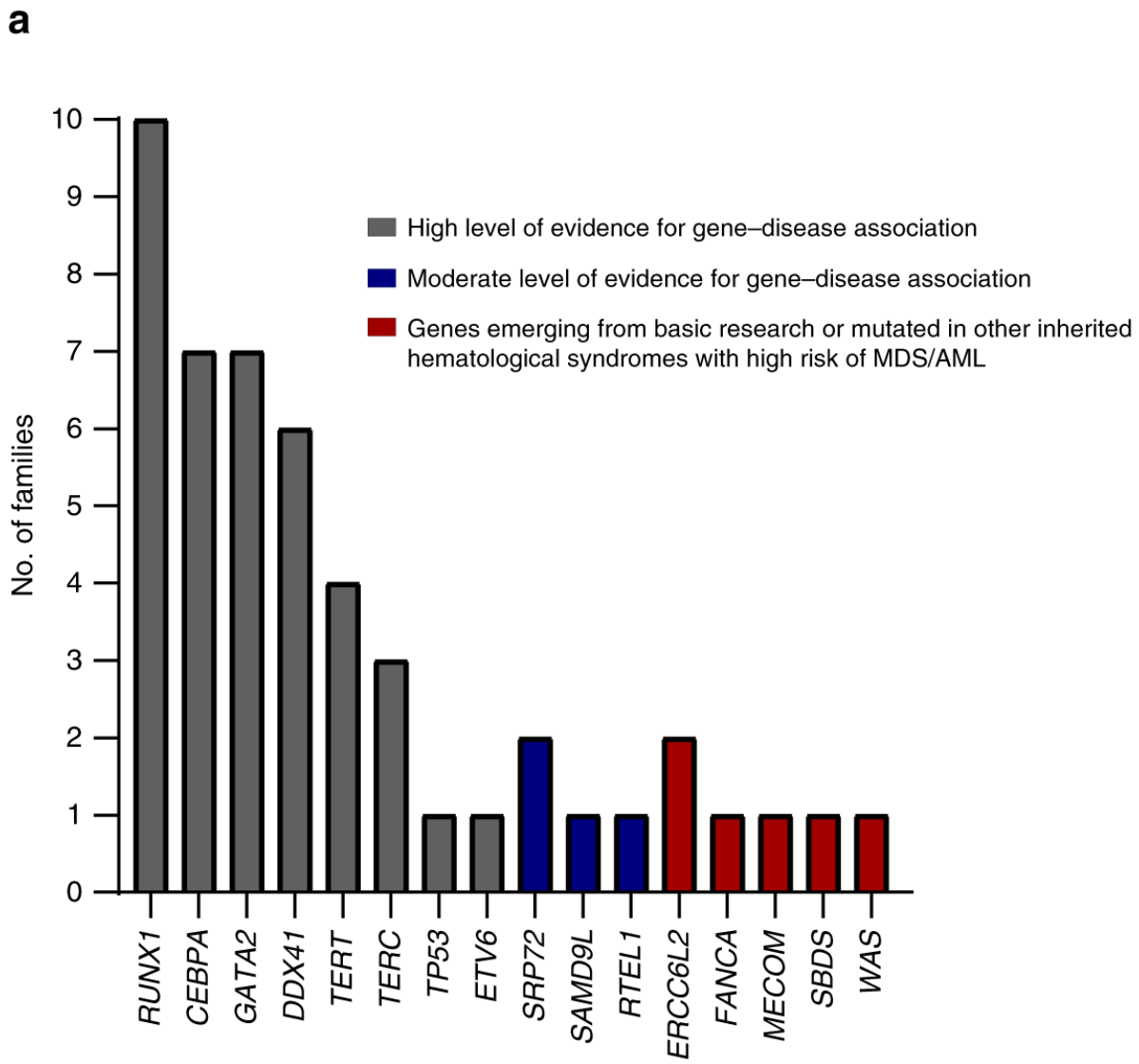
MDS Molecular Mutations Categorized

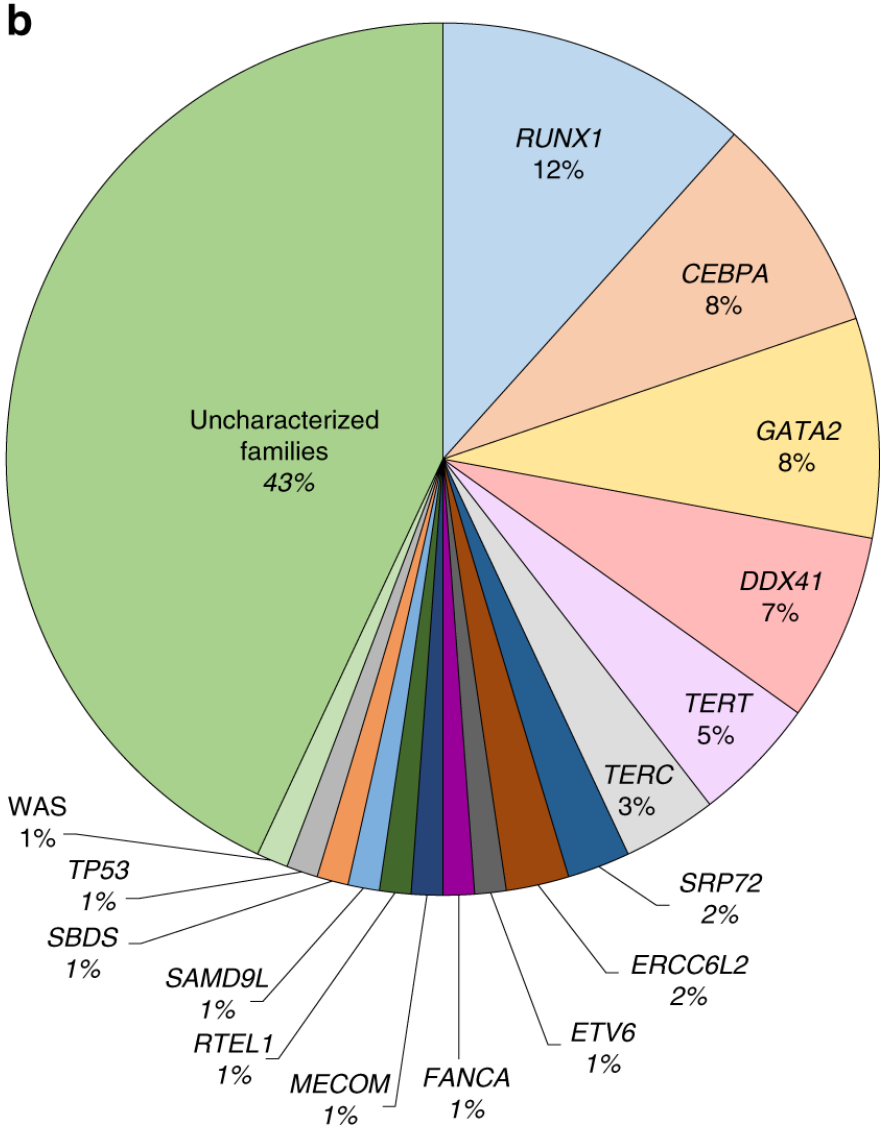


Epigenetic Genes Influence Other Genes: Turn on/off, Increase/Decrease Protein Production

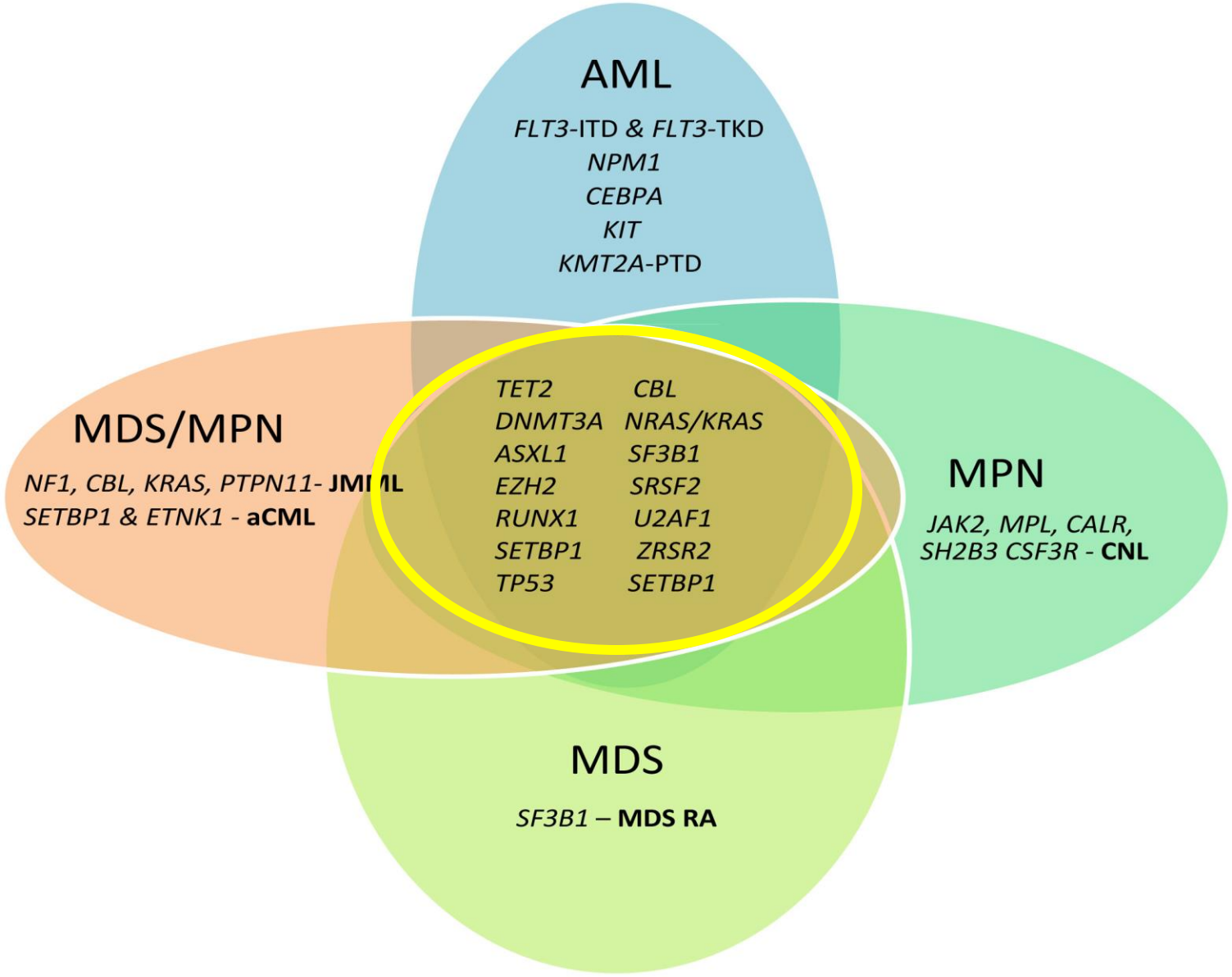

Normal stem cell
MDS stem cells
AML
Mutation
Aberrant epigenetic programs
More Mutations
Aberrant growth signals & reduced apoptosis
Normal, trilineage differentiation
Trilineage dysplasia, compensatory stem cell expansion and increased apoptosis
Inhibited differentiation and uncontrolled blast cell proliferation

RUNX1, DDX41, GATA2, TERT, TP53 Mutations Can be Acquired or Inherited in Families with MDS





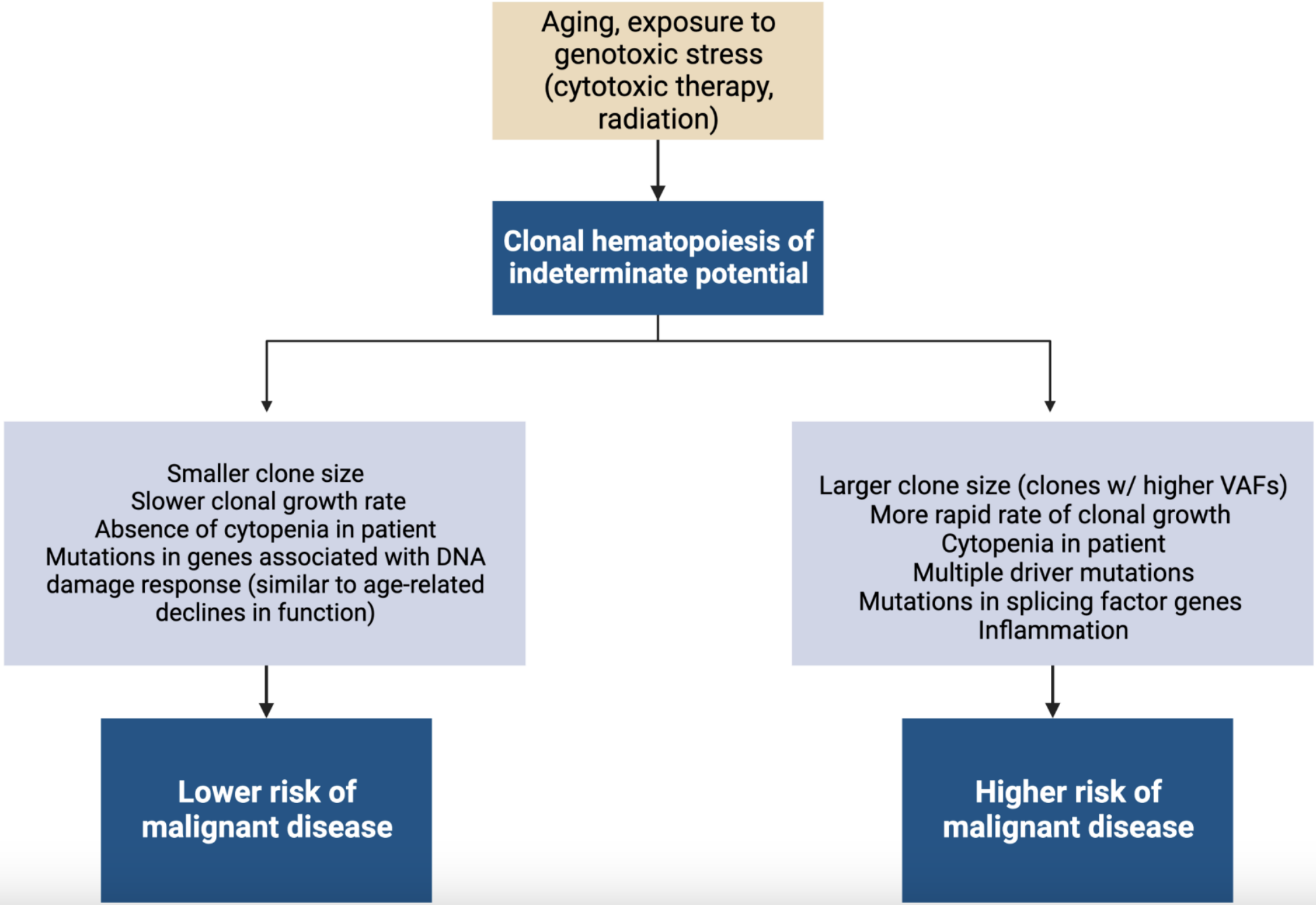
MDS Mutations Can Co-Occur in the Same Person AND They Are Found in Other Blood Cancers…



Mutations Precede MDS

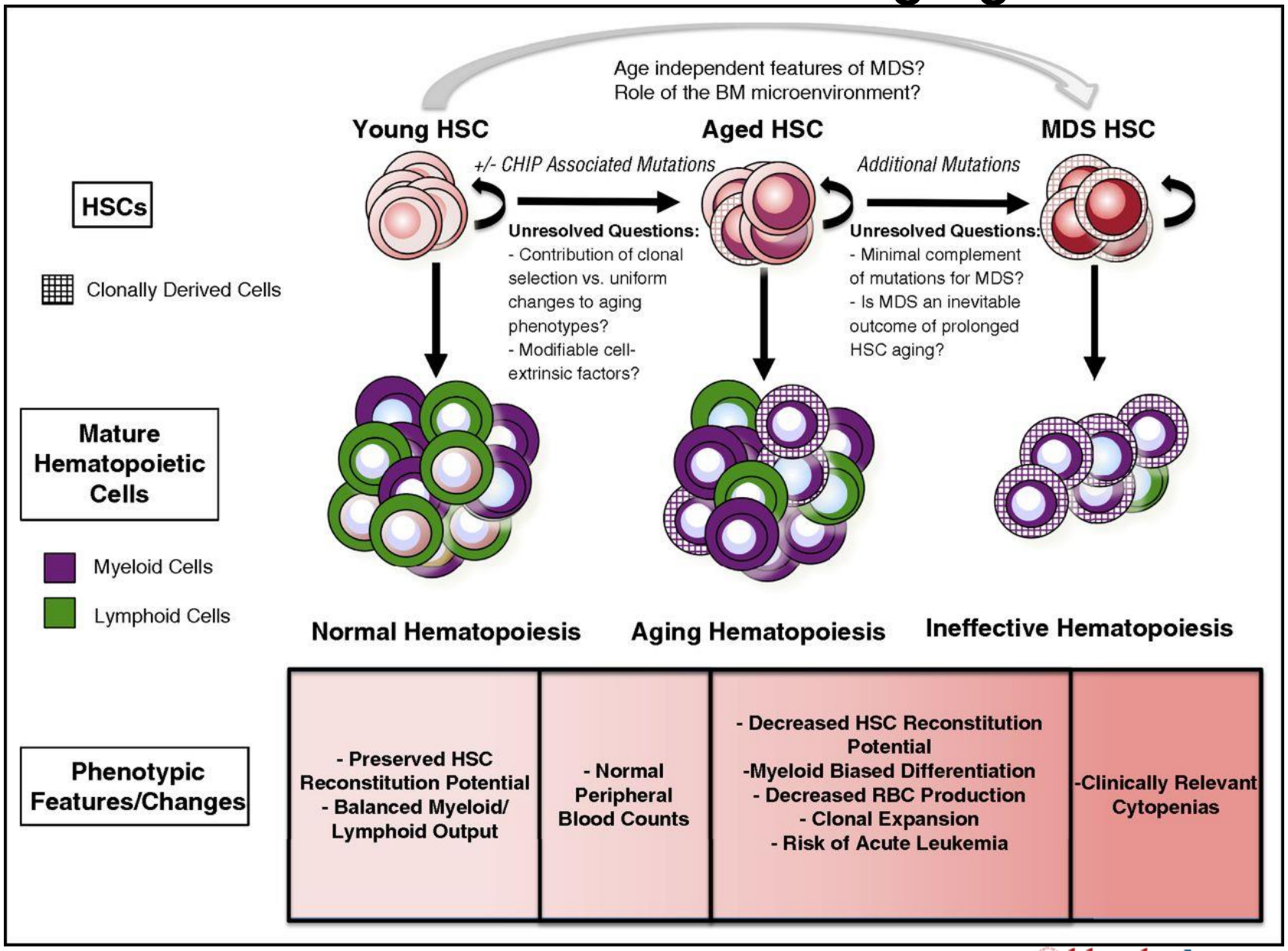
Abbreviation	Full-form	Operational definition
CH ARCH	Clonal hematopoiesis Age-related clonal hematopoiesis	This is an all-encompassing term to describe detection of somatic pathogenic variants identified in the hematopoietic compartment (originating in the hematopoietic stem and progenitor cells), typically present at higher frequencies in aging individuals.
CHIP	Clonal hematopoiesis of indeterminate potential	Somatic pathogenic variants detected at a variant allele fraction (VAF) of ≥2%, and in the absence of cytopenias, cytosis, or bone marrow dysplasia. The 2% threshold was chosen as it is the lower limit of detection for most sequencing assays.
CCUS	Clonal cytopenia of undetermined significance	Somatic pathogenic variants in the presence of cytopenias,[a] typically at higher VAF (≥10%–20%) in the absence of MDS diagnostic criteria (≤10% dysplasia cells from any hematopoietic cell lineage on bone marrow evaluation, absence of excess blasts, and absence of MDS-defining cytogenetic abnormalities)
MDS	Myelodysplastic syndromes	Persistent cytopenia(s) in one or more peripheral blood lineages, and morphologic dysplasia (≥10% dysplastic cells) in one or more bone marrow blood cell lineages OR one of MDS-defining cytogenetic abnormalities.
CMUS	Clonal monocytosis of undetermined significance	CH plus monocytosis defined as absolute monocyte count >0.5 × 109/L and > 10% of white blood cells (WBCs) in the absence of bone marrow (BM) findings suggestive of CMML.
CCMUS	Clonal cytopenia and monocytosis of undetermined significance	CH plus cytopenia plus monocytosis (defined as above).
CMML	Chronic myelomonocytic leukemia	Myeloid neoplasms with absolute (≥0.5 × 10^9/L) and relative (≥10%) peripheral blood monocytosis with blasts <20% of cells in peripheral blood and bone marrow, and with characteristic bone marrow findings.
CCsUS	Clonal cytosis of undetermined significance	Elevated peripheral cell counts (other than monocytosis) in the absence of bone marrow morphological features diagnostic of a myeloid neoplasm.
Mosaic chromosomal abnormality	-	Clonal, structural (deletions, duplications or copy number neutral loss of heterozygosity), somatic alterations identified in hematopoietic stem and progenitor cells

Magnaokar & Patnaik. AJH 2023.



Goldman et al. Cold Spr Harbor Mol Case Stud 2023.

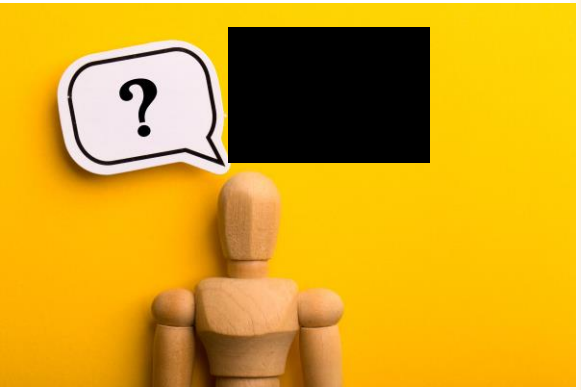
The features of HSCs in the context of aging and MDS are shown.

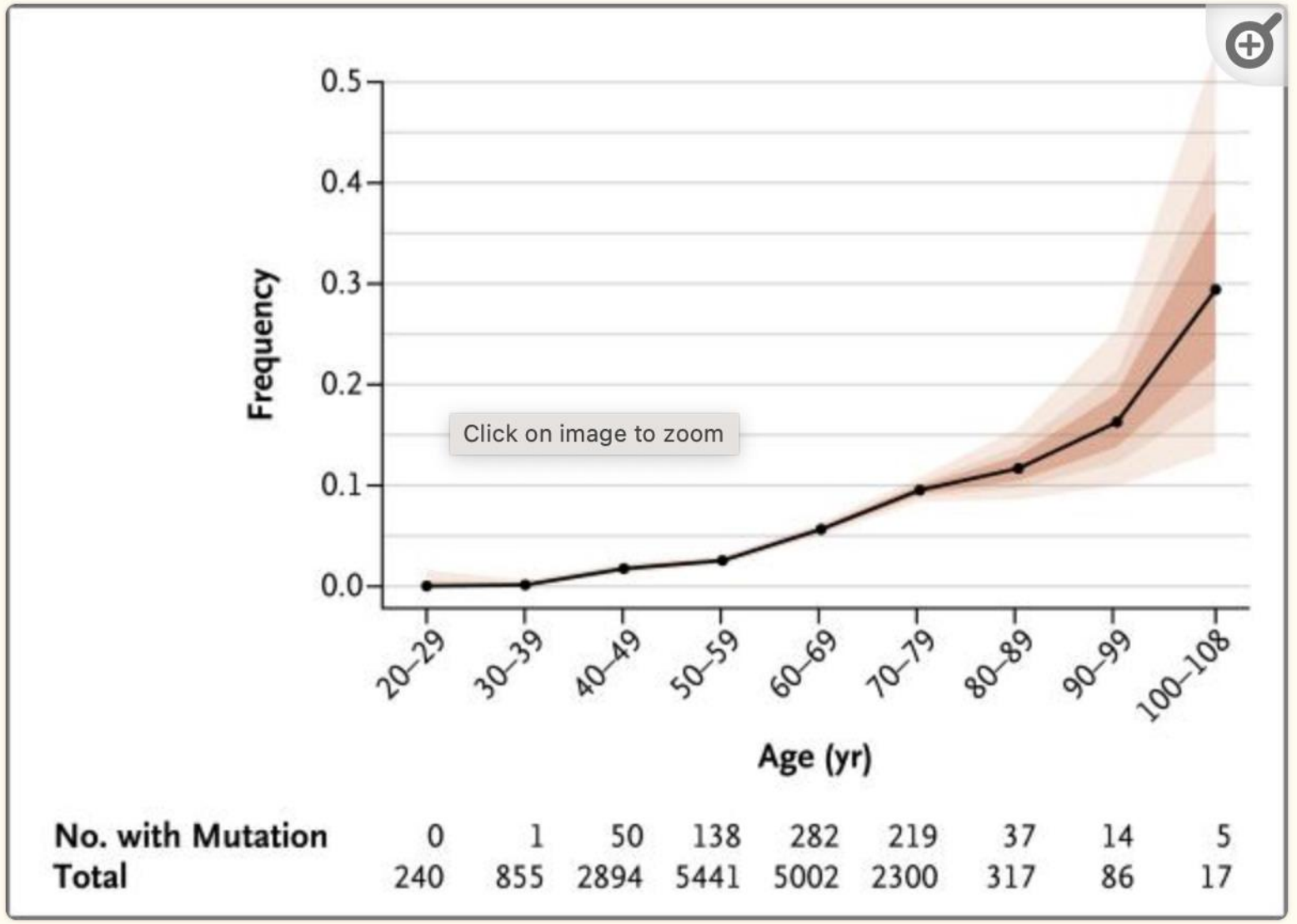


Stephen S. Chung, and Christopher Y. Park Blood Adv 2017;1:2572-2578

blood advances

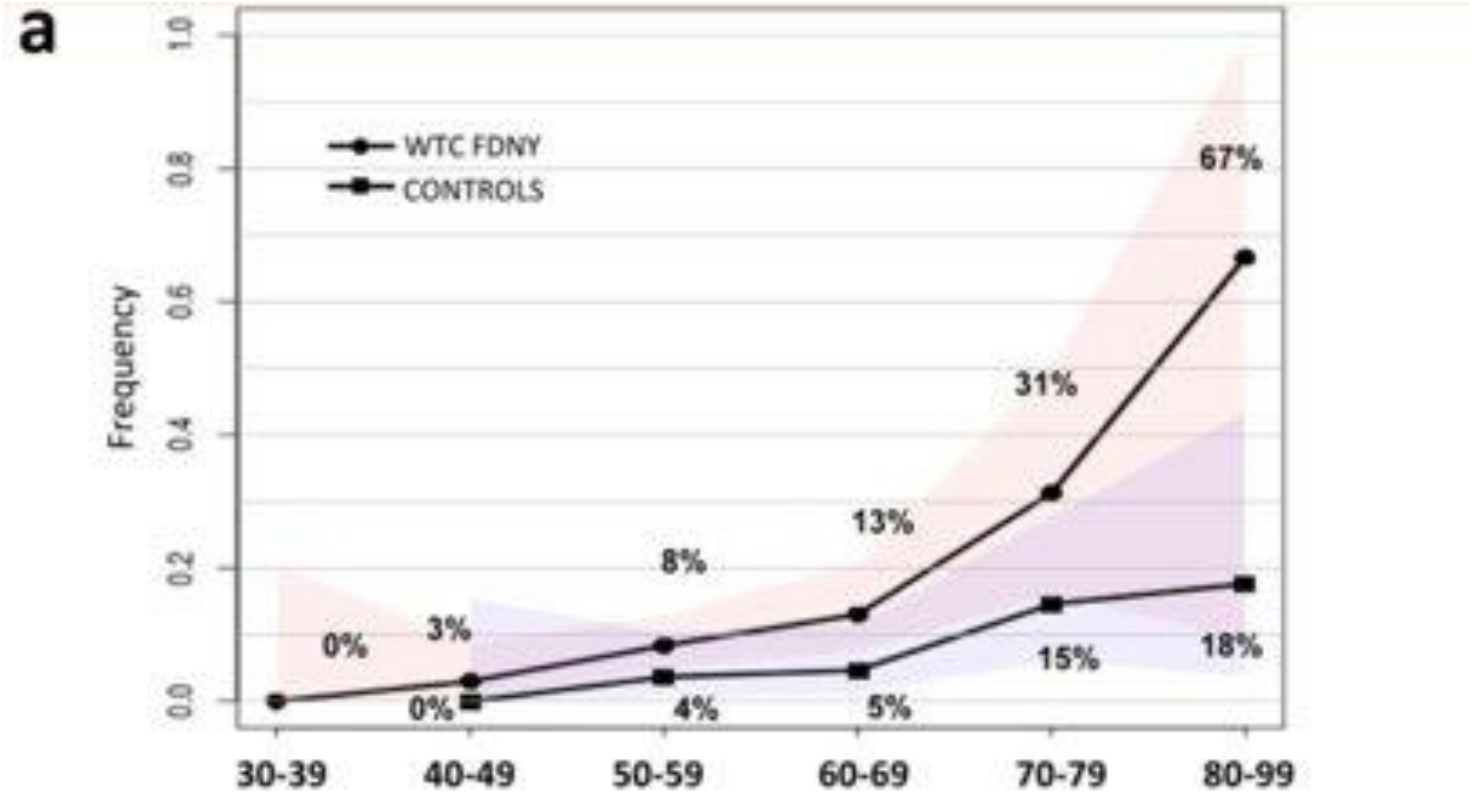
Prevalence of Mutations Increases With Age





Jaiswal et al. NEJM 2014.

WTC First Responders Developed CHIP



		30-39 yrs	40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs	80-99 yrs	Total
WTC FDNY	*CH*	0	3	18	15	10	2	48
	TOTAL	16	100	215	115	32	3	481
CONTROLS	*CH*	0	0	3	4	7	3	17
	TOTAL	0	22	82	86	48	17	255

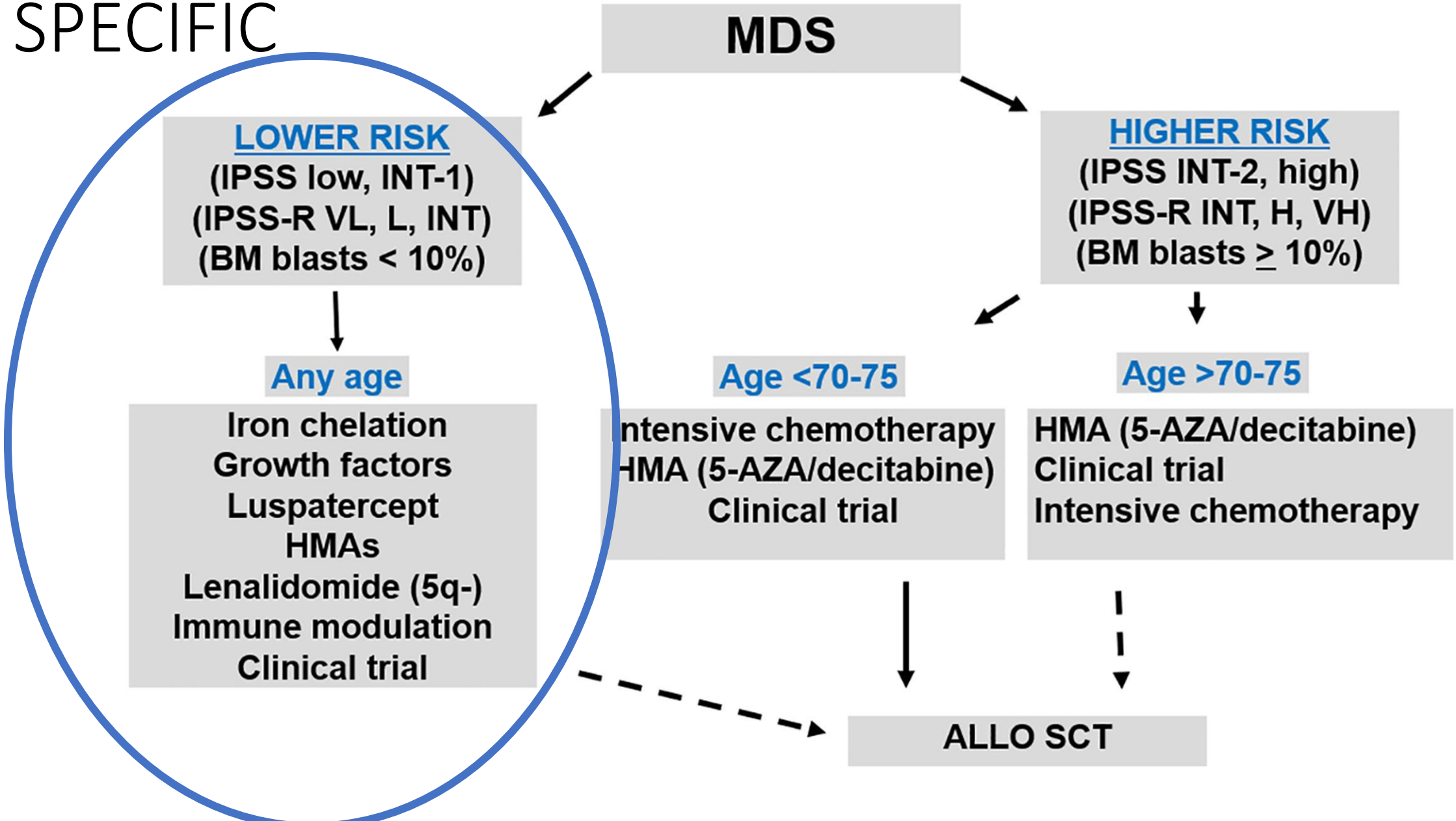
Are There Treatments For CHIP?

- Clinical Trials Exploring:
  - Anti-inflammatories
  - Vitamin C
  - Curcumin
  - Low-risk MDS: oral azacitidine causes more harm than benefit

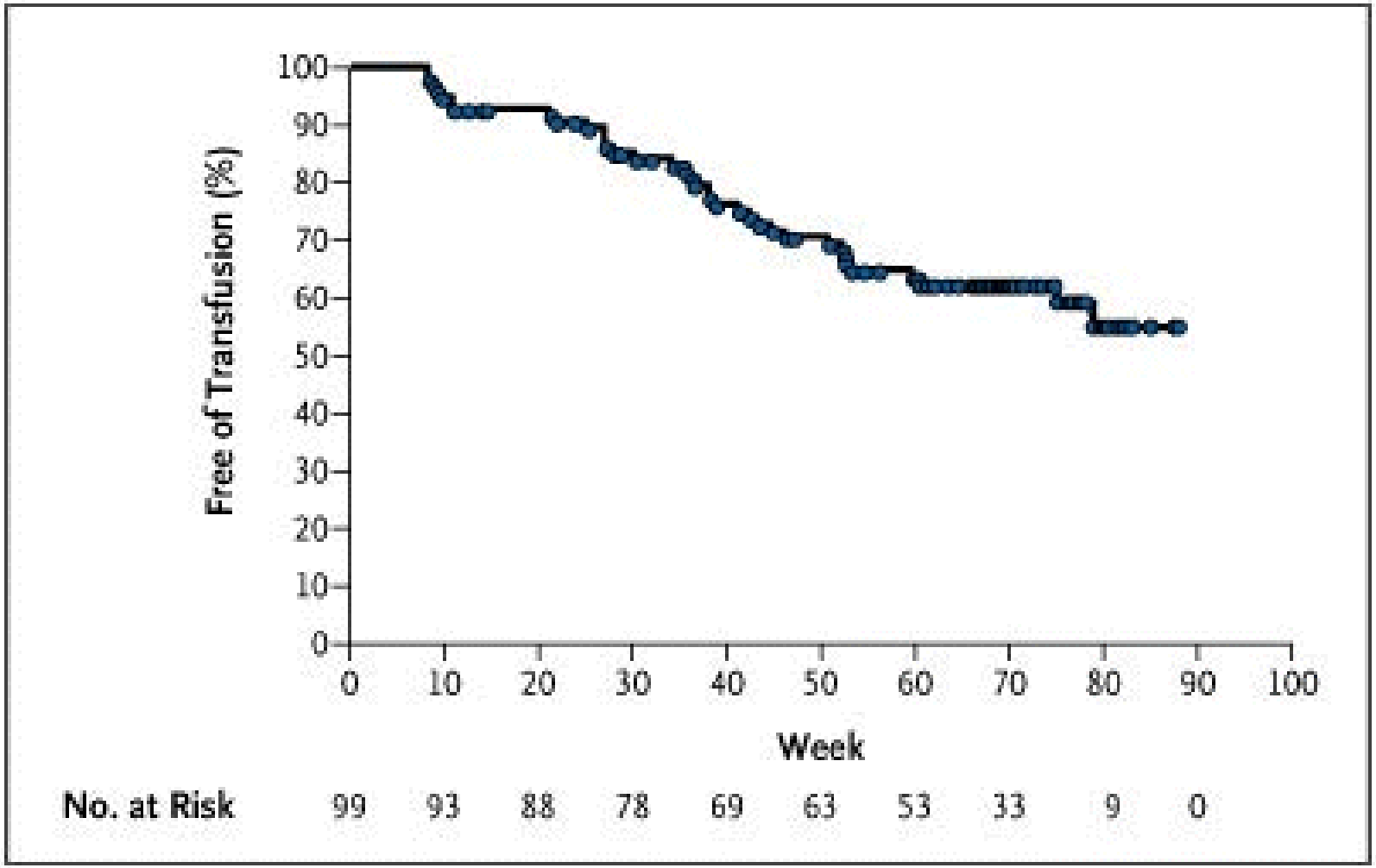
Can We Target the Mutations To Treat MDS?

DRIVER MUTATIONS = CONTINUE CAUSING DISEASE

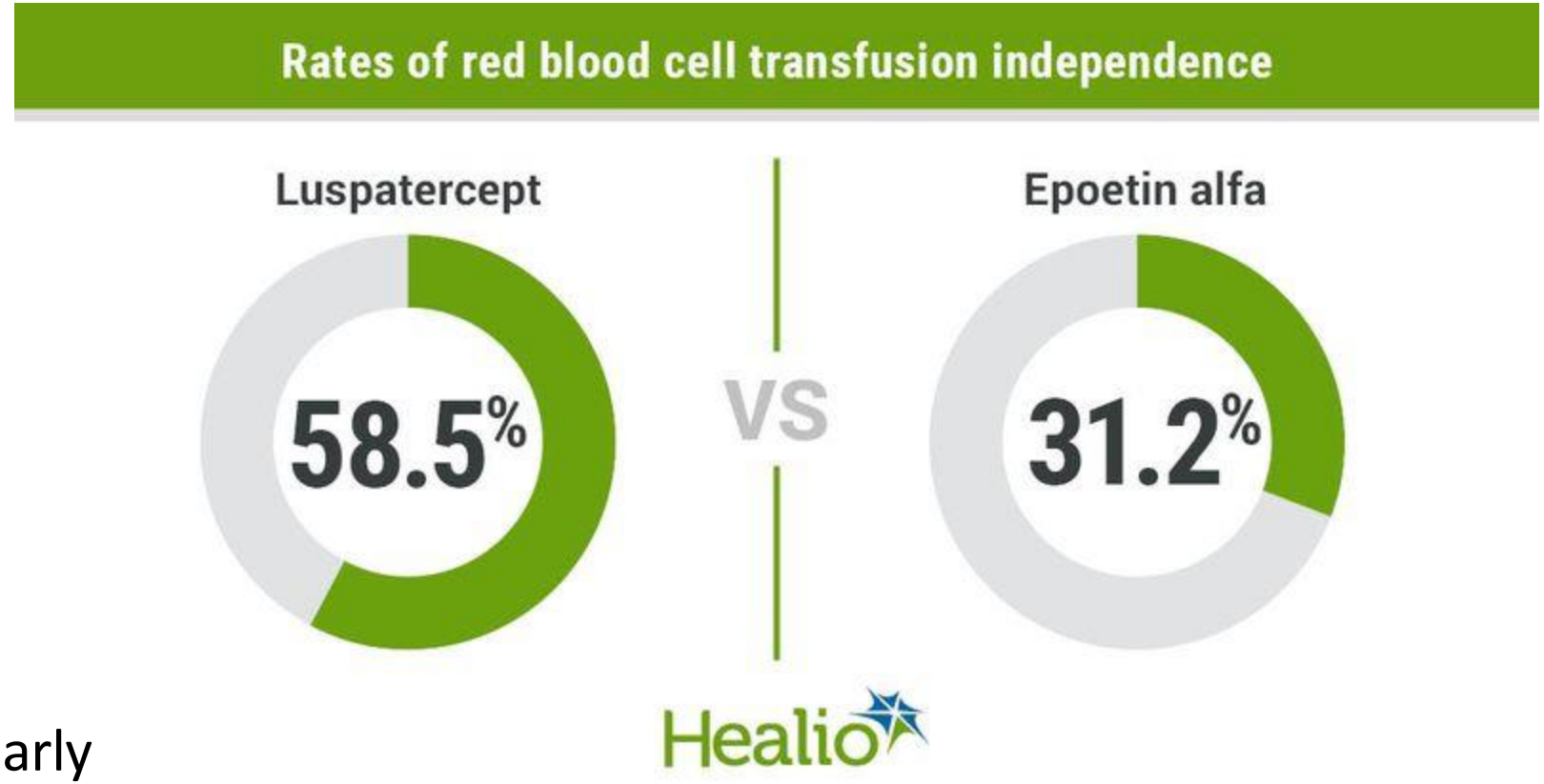
CURRENT TREATMENT ALGORITHM NOT GENE-SPECIFIC



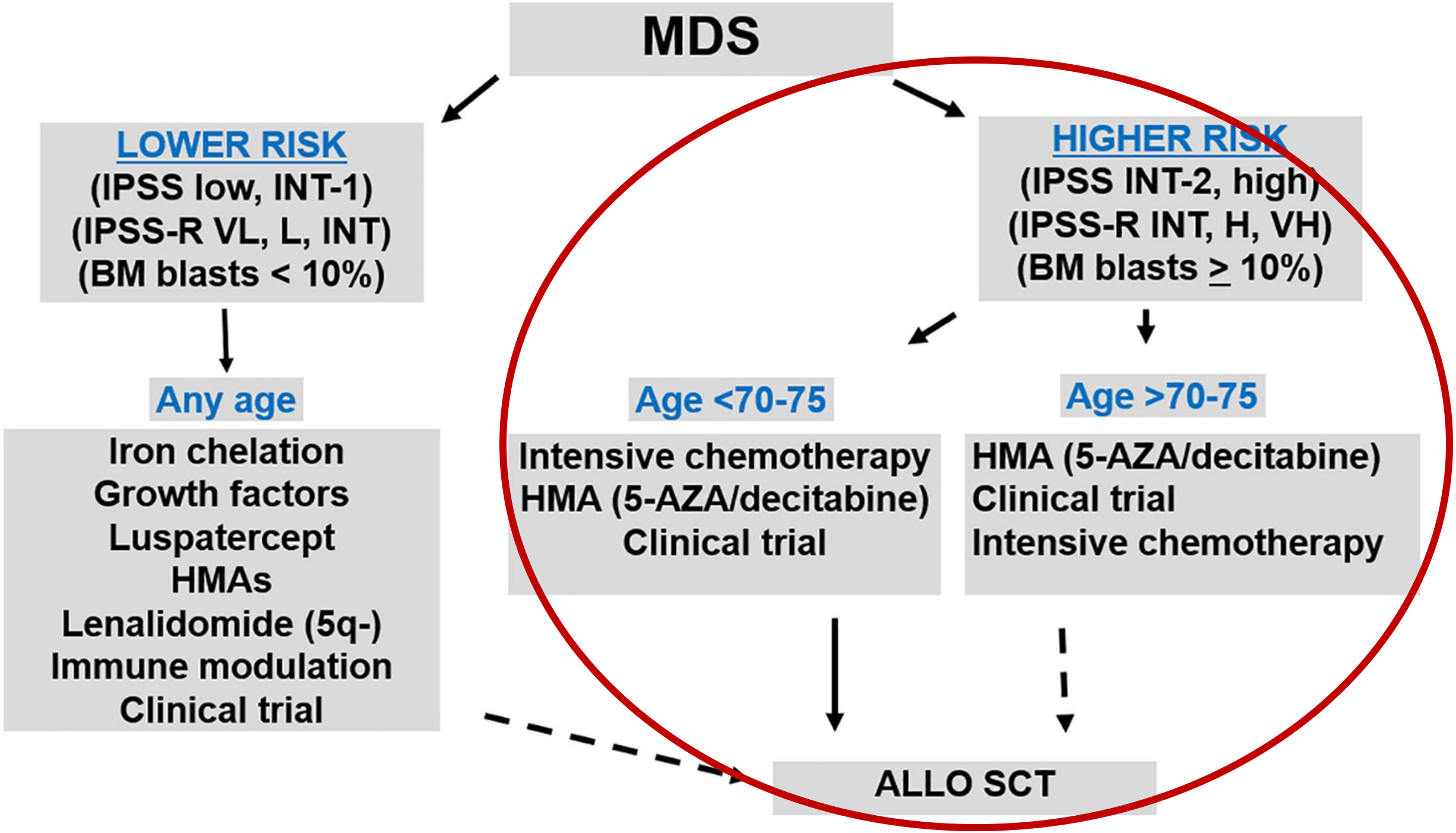
Lenalidomide Significantly Reduces Transfusion Requirements in MDS with "5q-minus Syndrome"



Luspatercept Significantly Improves Anemia in MDS with Sideroblastic Anemia



SF3B1 particularly Associated with Sideroblastic Anemia


MDS
LOWER RISK
(IPSS low, INT-1)
(IPSS-R VL, L, INT)
(BM blasts < 10%)
HIGHER RISK
(IPSS INT-2, high)
(IPSS-R INT, H, VH)
(BM blasts ≥ 10%)
Any age
Iron chelation
Growth factors
Luspatercept
HMAs
Lenalidomide (5q-)
Immune modulation
Clinical trial
Age <70-75
Intensive chemotherapy
HMA (5-AZA/decitabine)
Clinical trial
Age >70-75
HMA (5-AZA/decitabine)
Clinical trial
Intensive chemotherapy
ALLO SCT

Epigenetic Therapies Work Across Genetic Subtypes Because Myeloid Cancers Are Highly Methylated

2 FDA-approved Hypomethylating Agents:

- **VIDAZA (5-azacytidine)**
- **DACOGEN (decitabine)**

DNMT3A, TET2, Epigenetic Mutations May Respond Better

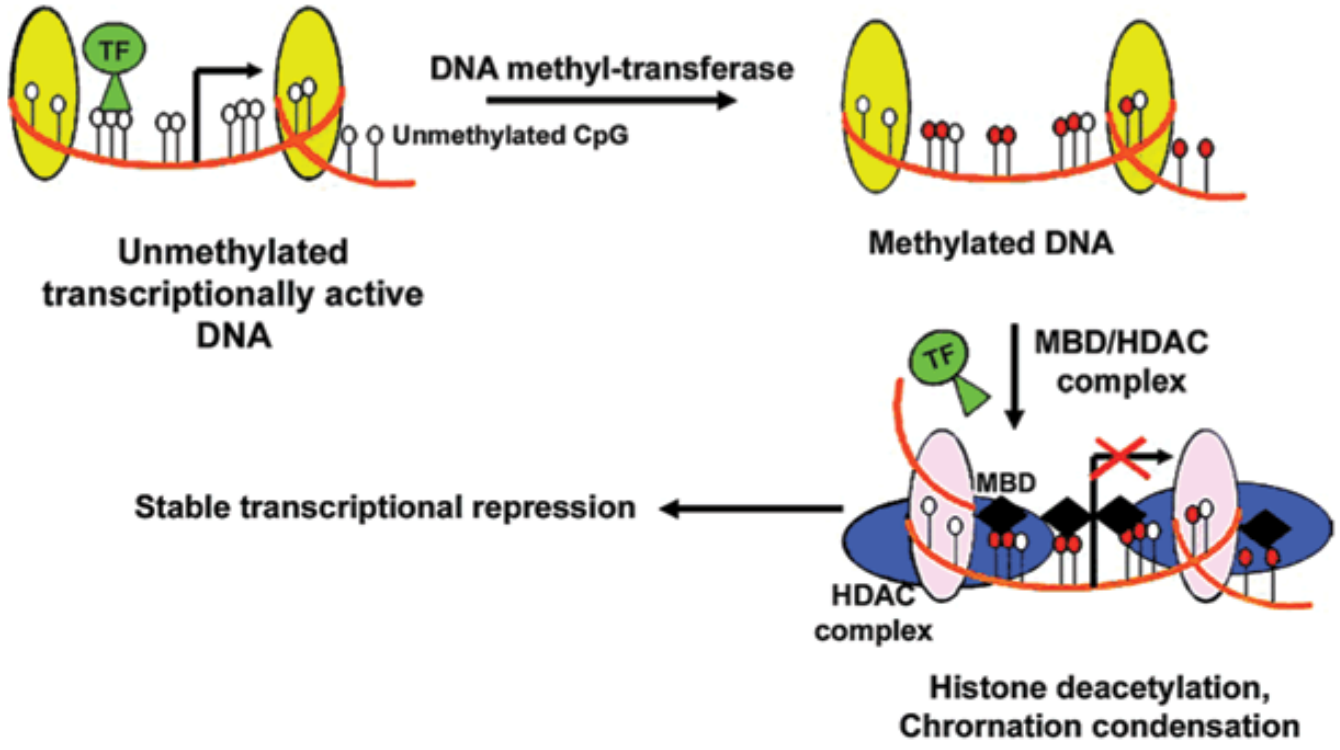
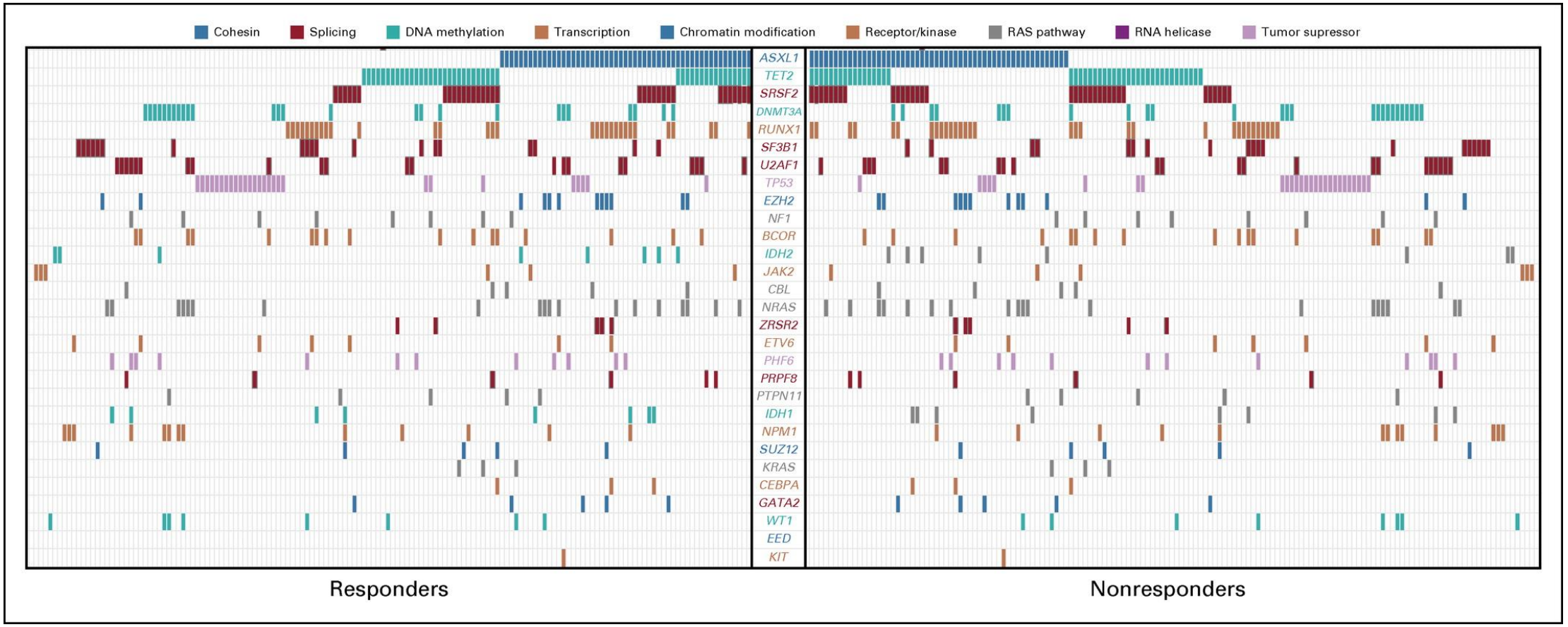


Figure 1 *– Epigenetic silencing of gene expression. DNA methyl-transferases carry out the methylation of CpG dinucleotides, which triggers the process of gene silencing by recruitment of methyl binding domain (MBD) and Histone deacetylases (HDAC) to bind to the methylated DNA. This results in histone deacetylation and chromatin condensation leading to loss of transcription factor binding and subsequent repression of transcription.*

Assessing Response Based on Genes is COMPLEX – Might AI Help US?



Aziz Nazha et al. JCO 2019

AI Program Identifies Mutations in MDS with Poor Response to Epigenetic Therapies

TABLE 3. Genomic Biomarkers Defined by the Recommender System

Association Rules for Resistance to HMAs
ASXL1, *NF1*
ASXL1, *EZH2*, *TET2*
ASXL1, *EZH2*, *RUNX1*
EZH2, *SRSF2*, *TET2*
ASXL1, *EZH2*, *SRSF2*
ASXL1, *RUNX1*, *SRSF2*
ASXL1, *TET2*, *SRSF2*
ASXL1, *BCOR*, *RUNX1*

ASXL1 (frameshift) Mutations Adverse in MDS

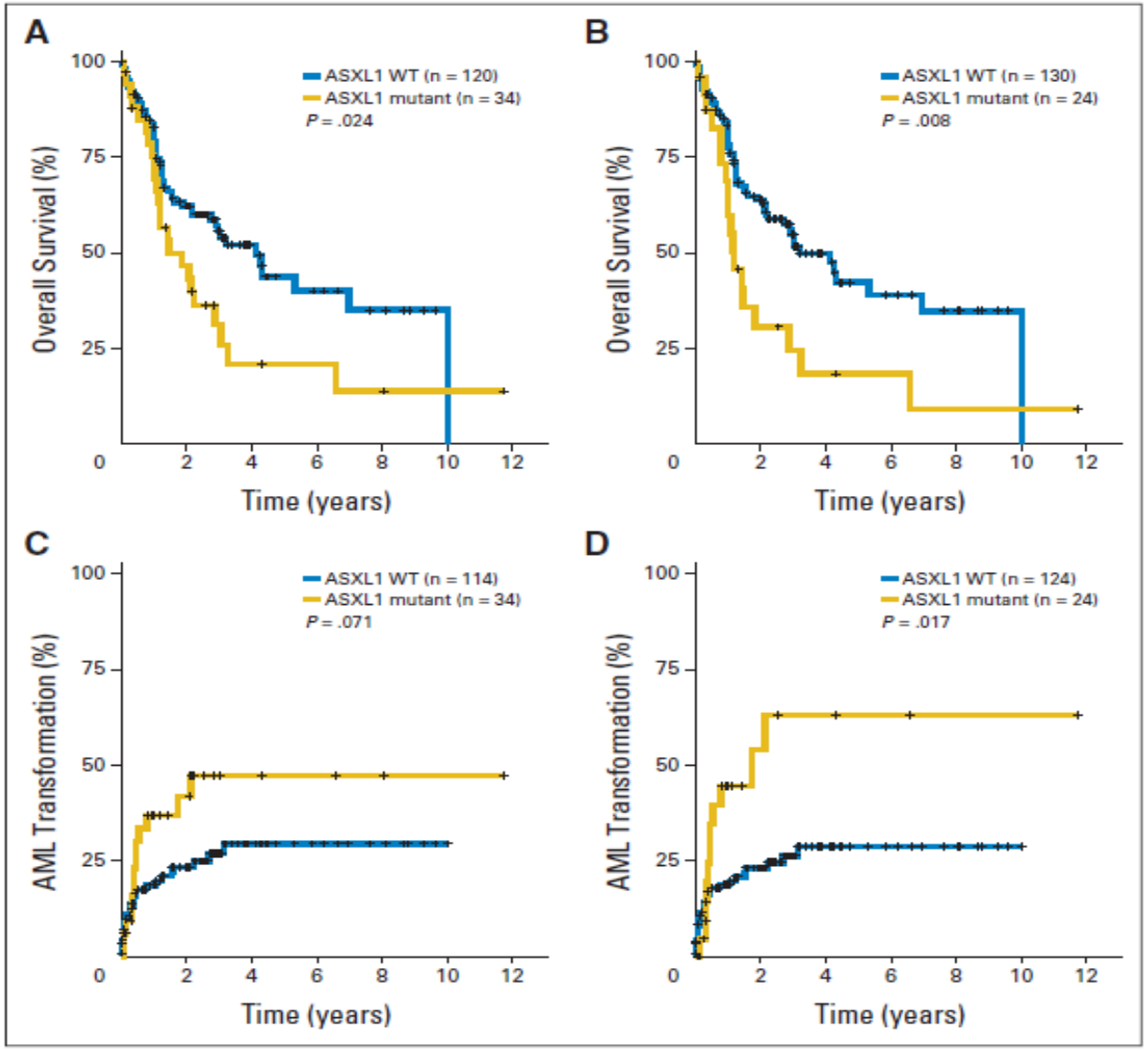


Fig 1. Kaplan-Meier curves for overall survival (OS) and time to acute myeloid leukemia (AML) transformation. (A) Point and frameshift mutations considered: OS in patients with myelodysplastic syndrome (MDS) with mutated (n = 34) and unmutated *ASXL1* (n = 120). (B) Only frameshift mutations considered: OS in patients with MDS with mutated (n = 24) and unmutated (n = 130) *ASXL1*. Genes in patients with *ASXL1* point mutations are considered wild type (WT) in this analysis. (C) Point and frameshift mutations considered: time to AML transformation in patients with MDS mutated (n = 34) and unmutated *ASXL1* (n = 114). (D) Only frameshift mutations considered: time to AML transformation for patients with MDS with mutated (n = 24) and unmutated *ASXL1* (n = 124). Genes in patients with *ASXL1* point mutations are considered WT in this analysis.

Thol et al. JCO 2011;39:2499-2506.

Hypothesis: AZA Resistance Due to T cell Silencing (via PD-1) in MDS

	All	*PD-1* demethylation	No *PD-1* demethylation	*p* value
Time (months) from diagnosis until 5-aza-start median (range)	4 (0-37)	6.5 (0-11)	3 (0-37)	.83
Median no. of cycles of 5-aza	5 (2-14)	4 (2-13)	5 (3-14)	.40
IWG 2006 Response				
CR	3 (11%)	1 (8%)	2 (13%)	1
Overall response	10 (37%)	1 (8%)	9 (60%)	.014
Overall survival (months) (median)	10.2	7.1	11.0	.11 (log rank)

Ørskov et al., Oncotarget, 2015

VARI/SU2C Phase I/II Multicenter Trial: Guadecitabine + Atezolizumab in R/R MDS

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VAN ANDEL RESEARCH INSTITUTE

STAND UP TO CANCER

AACR

USC Norris Comprehen Cancer Center
Keck Medicine of USC

Van Andel Research Institute–Stand Up To Cancer Epigenetics Dream Team

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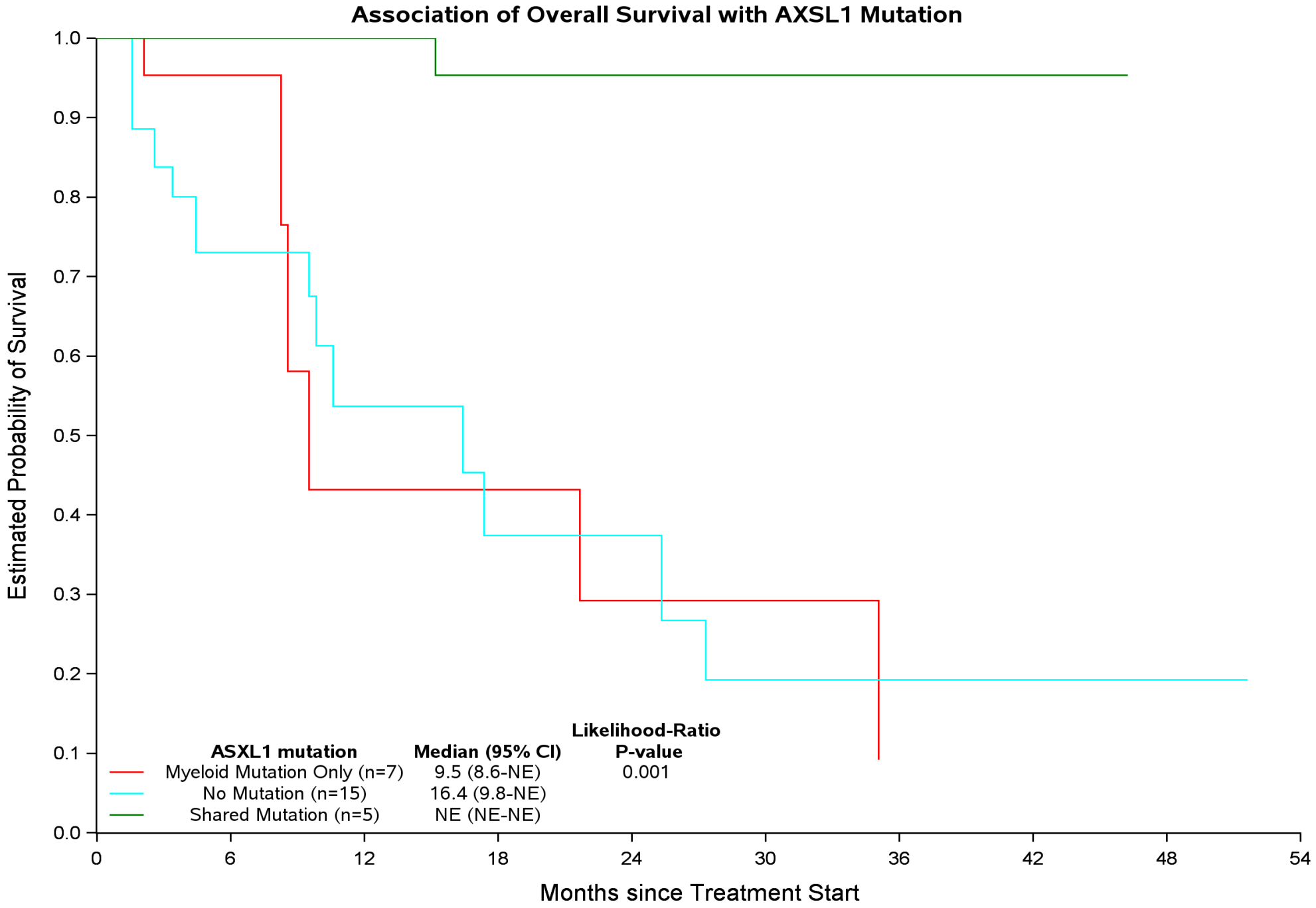
UNIVERSITY OF COPENHAGEN

CANCER & HEMATOLOGY CENTERS OF WESTERN MICHIGAN

FOX CHASE CANCER CENTER
TEMPLE HEALTH


Association of Overall Survival with AXSL1 Mutation
Estimated Probability of Survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
6
12
18
24
30
36
42
48
54
Months since Treatment Start
ASXL1 mutation
Median (95% CI)
Likelihood-Ratio P-value
Myeloid Mutation Only (n=7)
9.5 (8.6-NE)
0.001
No Mutation (n=15)
16.4 (9.8-NE)
Shared Mutation (n=5)
NE (NE-NE)

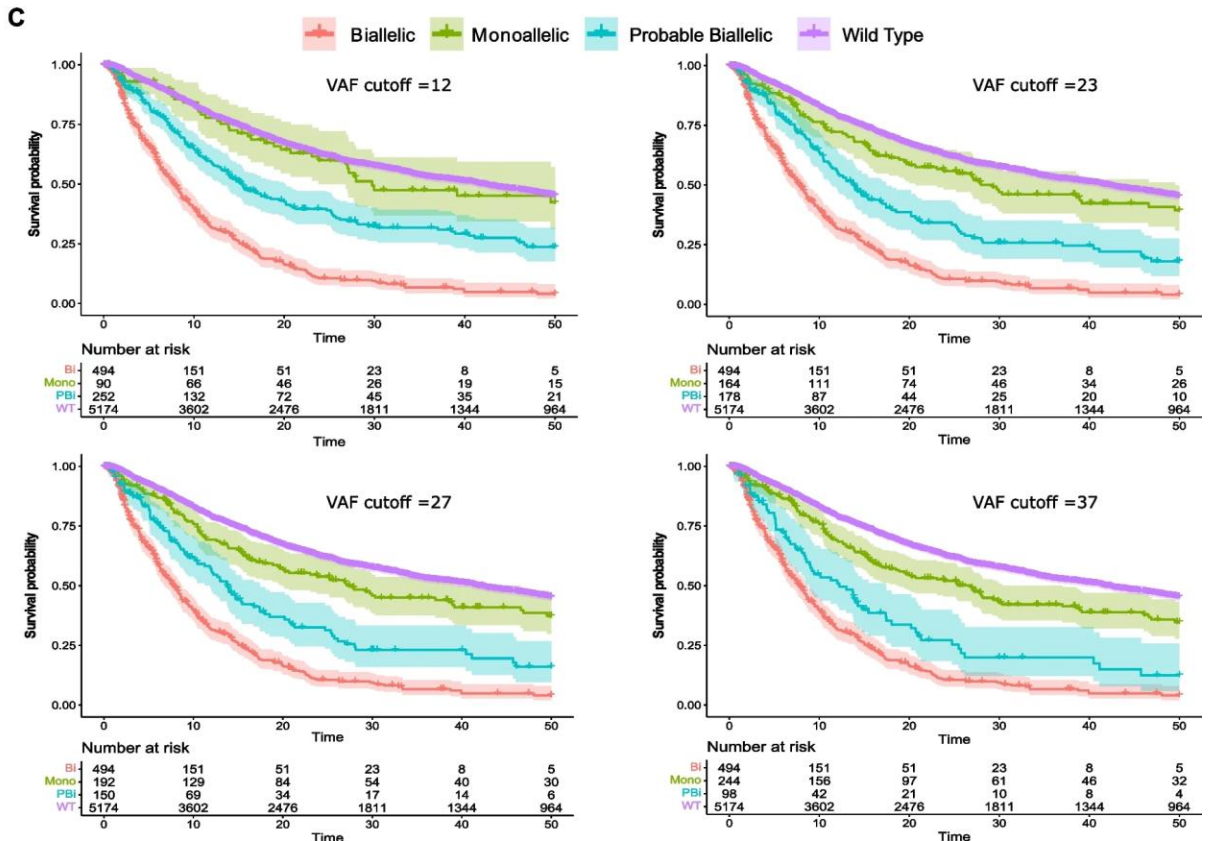
New Targeted Strategies in MDS

- HMA + **IDH1/2 Inhibitor**
  - Already approved in AML
  - FDA to fast track review of IDH1 inhibitor Ivosidenib (8/28/23)
  - Enasidenib for IDH2-mutant MDS effective in relapsed/refractory setting in combination with HMA or alone

  - Splicing Factor Targeted Therapy in Clinical Trials

What About P53 Mutations in MDS?

HAVING 2 COPIES OF P53 MUTATION SIGNIFICANTLY IMPACTS SURVIVAL BUT 1 COPY OF THE MUTATION DOES NOT

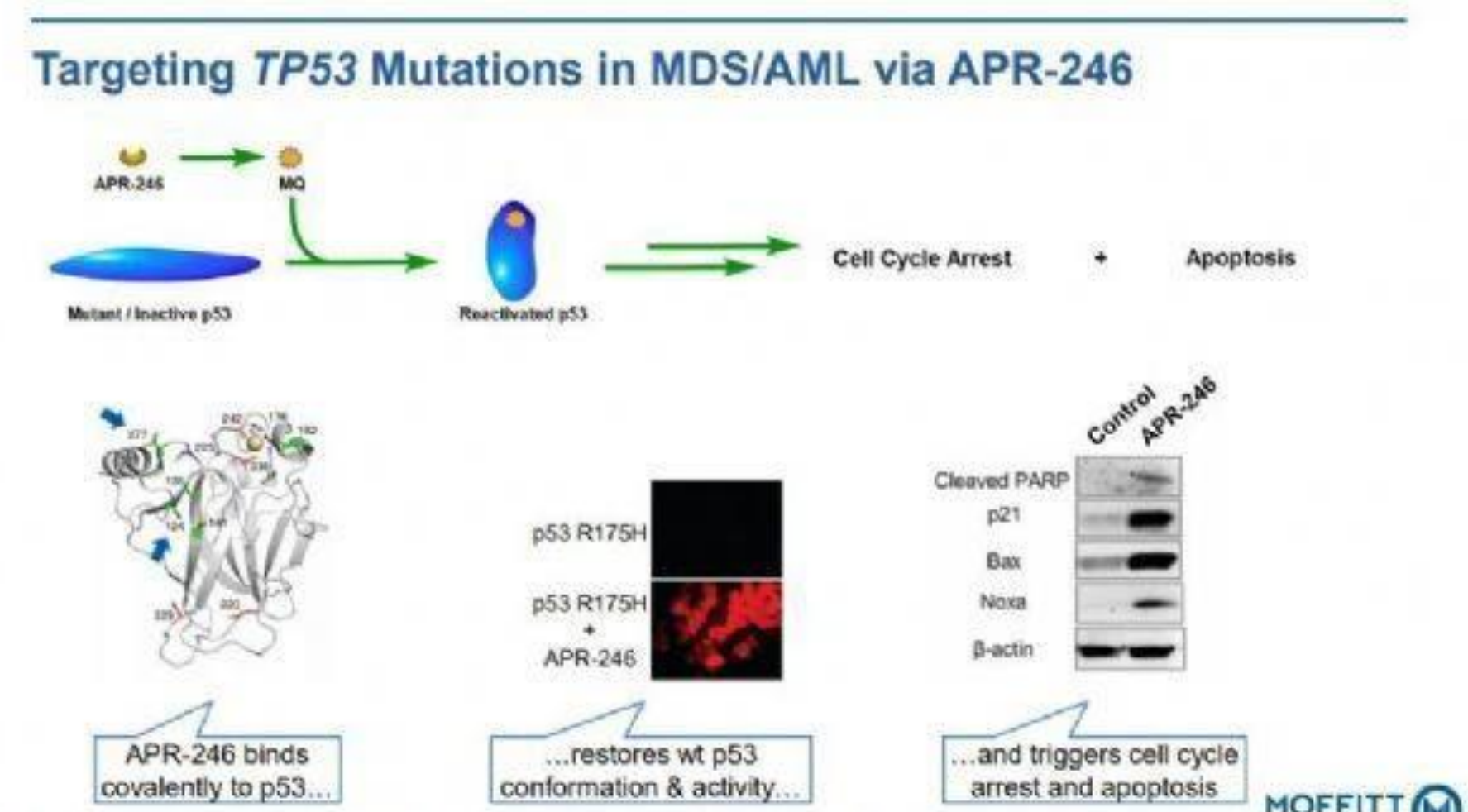


Azacitidine is Effective in P53-Mutated MDS

N	p53 negative patients	p53 positive patients	P
5-Aza cycles Median (range)	4 (1-29)	5 (1-22)	0.926‡
All ORR (CR, PR, SD with HI) SD without HI	n=65 16 (25%) 23/32 (72%)	n=35 16 (46%) 9/32 (28%)	 0.033* 0.020*
MDS ORR (CR, PR, SD with HI)	n=29 4 (14%)	n=24 11 (46%)	 0.008*
sAML ORR (CR, PR, SD with HI)	n=30 11 (37%)	n=9 3 (33%)	
Very poor cytogenetics ORR (CR, PR, SD with HI) + abnormal Chr. 5 + monosomal KT	 3/9 (33%) 2/6 (33%) 1/4 (25%)	 7/21 (33%) 7/17 (41%) 5/14 (36%)	

For comparison between patients with and without TP53 mutations a Fisher's exact (*), χ^2 (+) or Mann-Whitney-U test (‡) was used. ORR: overall response rate; CR: complete remission; PR: partial remission; SD: stable disease; HI: hematologic improvement.

APR-246 Not Successful in Targeting P53



Conclusions

- Genetic Mutations are Ubiquitous in MDS
- Mutations are Usually Acquired in MDS
- Analyzing the impact of mutations is a complex task as they are rarely found alone
- Targeting the mutations is also complex, must hit the DRIVER to stop the disease
- Enormous research efforts focused on this area
- Clinical trials and dedicated analysis required to personalize MDS care