CBC大海嘯來襲

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Review : Criteria for automated CBC & WBC diff analysis

Laboratory Hematology 11:83-90

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The International Consensus Group for Hematology Review: Suggested Criteria for Action Following Automated CBC and WBC Differential Analysis

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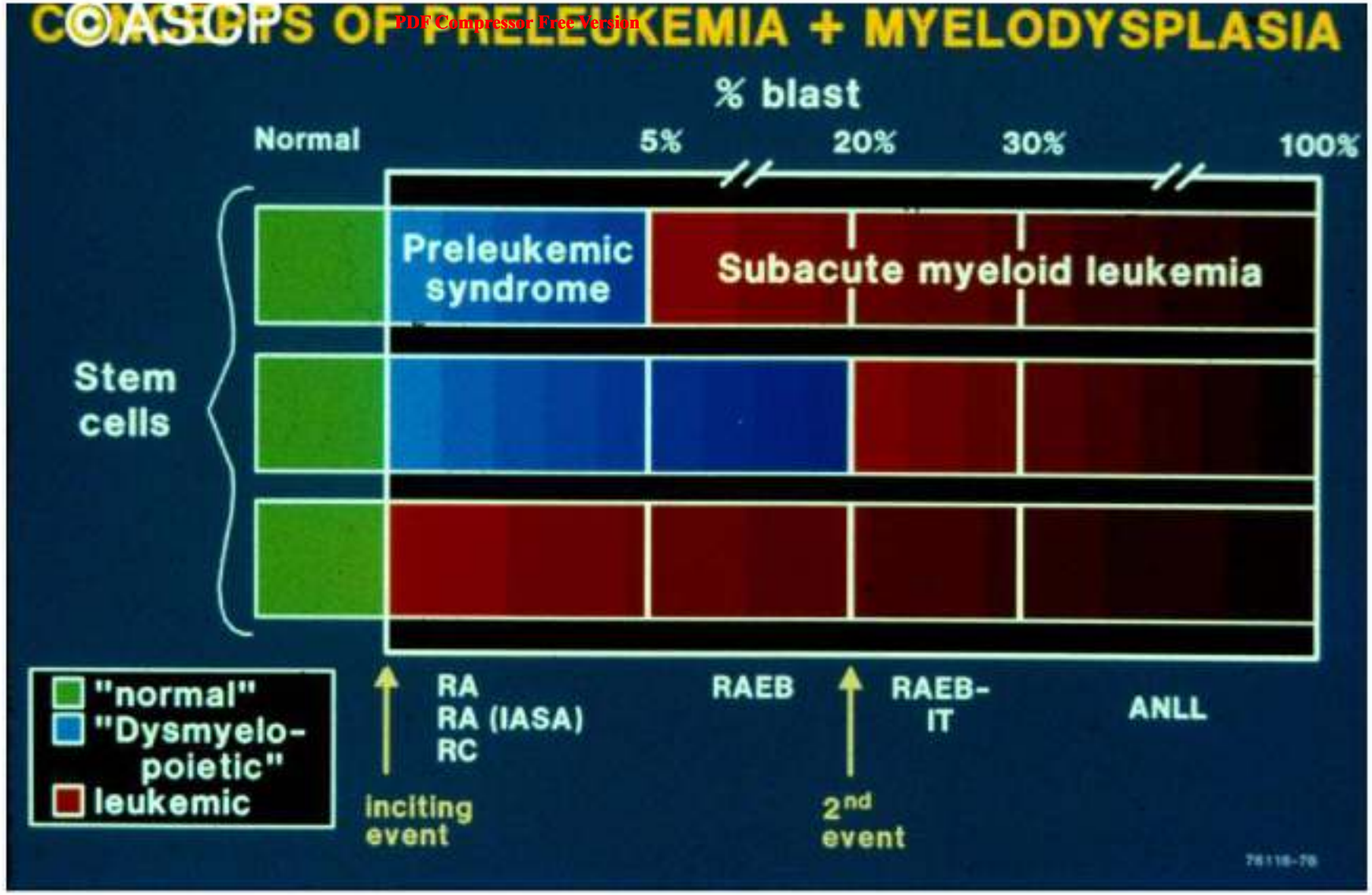
Criteria for a positive smear

Morphology

- Red cell 2+/moderate/>
- Platelet morph (giant forms) at 2+/>
- Platelet clumps at > rare
- Toxic granulation at 2+/>
- Vacuoles at 2+/>

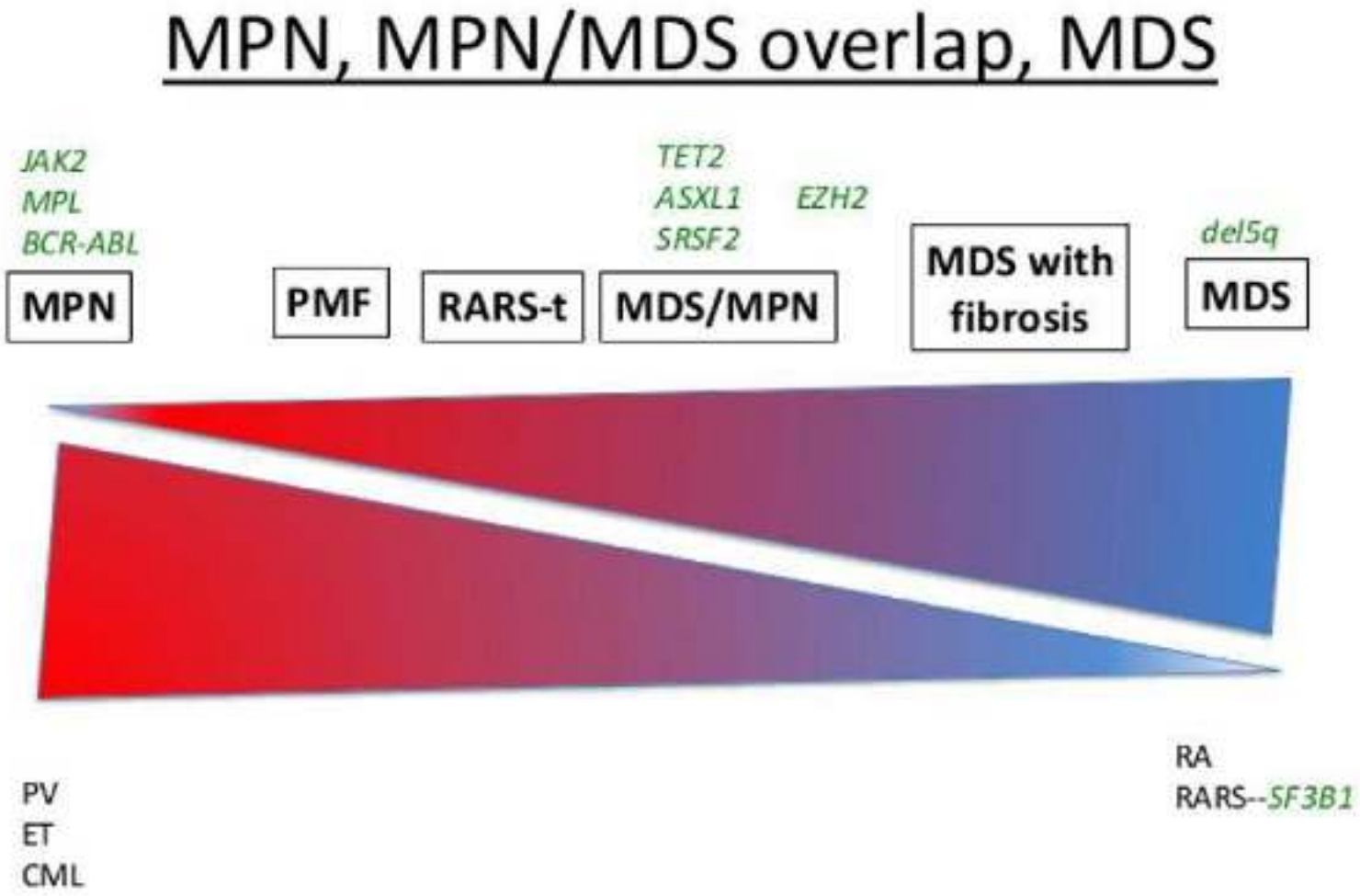
Abnormal cell types

- Blast>/=1
- Meta>2
- Myelo/promyelo>/=2
- Atypical lymph>5
- NRBC>/=1
- Plasma cells >/=1



Preleukemia

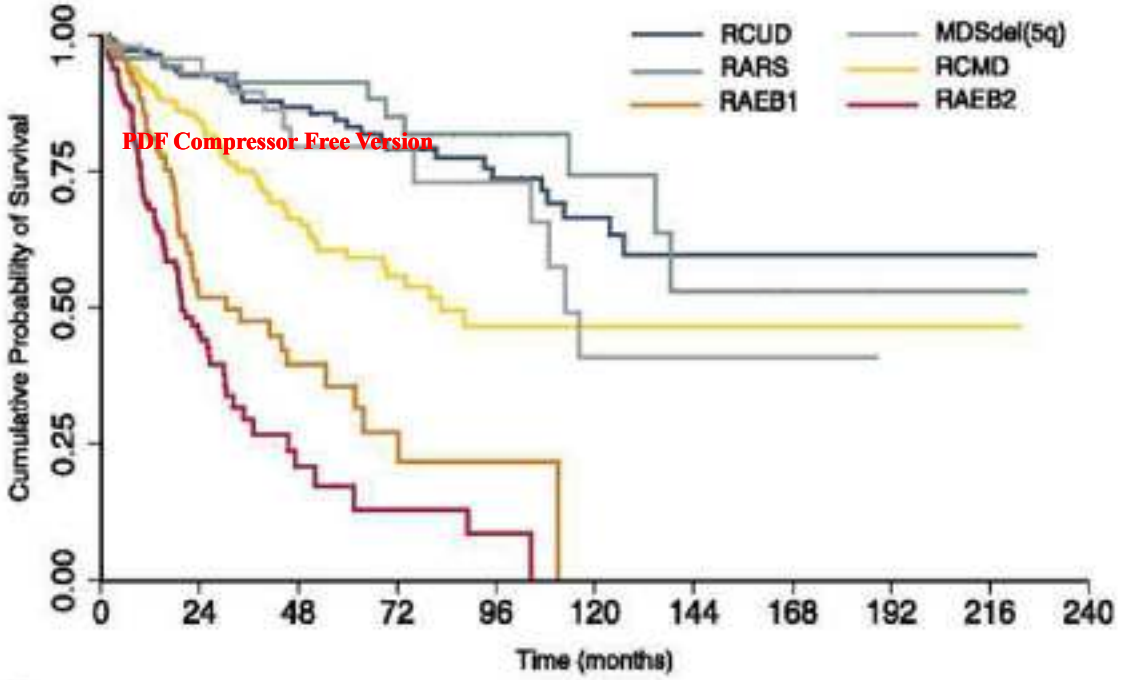
- MDS
- MPN
- MDS/MPN

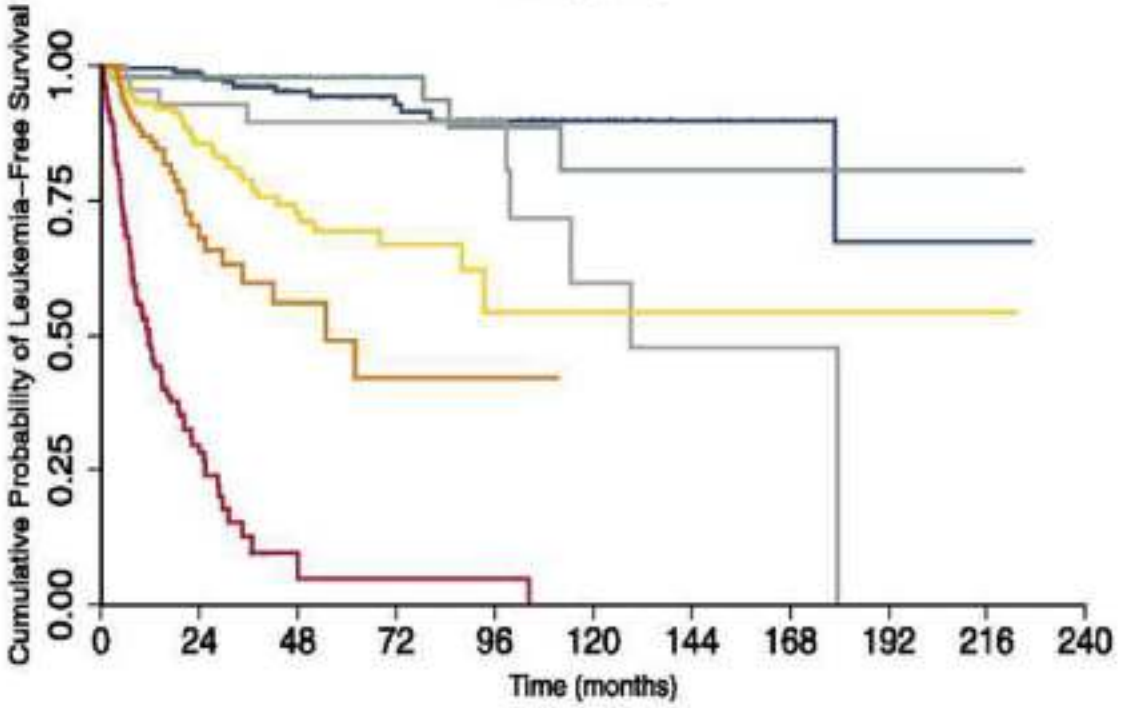


WHO Classification of MDS

- WHO 2008: Combination of morphology, immunophenotype, genetics, and clinical features
- The WHO classification system distinguishes six general entities with the following estimated percentages:
  1. Refractory cytopenia with unilineage dysplasia (refractory anemia, refractory neutropenia, or refractory thrombocytopenia) – <5 percent
  2. Refractory anemia with ring sideroblasts – <5 percent
  3. Refractory cytopenia with multilineage dysplasia – 70 percent
  4. Refractory anemia with excess blasts – 25 percent
  5. MDS with isolated del(5q) – 5 percent
  6. MDS, unclassified – <5 percent

- Childhood MDS –half of childhood MDS, the most common subtype in this setting





事情有些不對勁.....

1. Low risk MDS佔不到5%
2. High risk MDS佔95%

- MDS如何診斷的?
  - CBC/DC: 形態學、細胞計數、細胞分類
    - Cell dysplasia
    - Cytopenia
    - Premature cells in peripheral blood
  - False negative
  - False positive

Diagnostic criteria for MDS and common findings

Peripheral blood findings	Bone marrow findings	Chromosomal abnormalities considered presumptive evidence of disease
One or more of the following:	And one or more of the following:	Translocations:
Hemoglobin <11 g/dL	≥10 % dysplasia in the granulocytic, erythroid, or megakaryocytic lineage	t(11;16)(q23;p13.3) t(2;11)(p21;q23)
Absolute neutrophil count <1500/μl (1.5 x 10^9/L)	Myeloblasts comprise 5–19 % of total cellularity	inv(3)(q21q26.2) t(3;21)(q26.2;q22.1)
Platelet count <100,000/μl (100 x 10^9/L)	Presence of an acquired chromosomal abnormality specific for MDS	t(1;3)(p36.3;q21.2) t(6;9)(p23;q34)
Commonly observed features:	Commonly observed features:	Abnormal copy number:
Neutrophil hypogranularity	Hypercellularity	−7 or del(7q)
Hypolobulated neutrophil nuclei (e.g., pseudo Pelger-Huët cells)	Nuclear-cytoplasmic asynchrony Karyorrhexis	−5 or del(5q) i(17q) or t(17p)
Monocytosis (in CMML)	Irregular nuclear contours	−13 or del(13q)
Immature leukocytes	Ring sideroblasts	del(12p) or t(12p)
Macrocytosis	Hypolobated megakaryocytes	del(9q)
Anisopoikilocytosis	Micromegakaryocytes	del(11q)
Hypochromic erythrocytes	Abnormal leukocyte granulation	idic(X)(q13)
Large or hypogranular platelets	Abnormal localization of immature precursors Ectopic antigen expression by flow cytometry	Complex karyotype (3 or more abnormalities)
	Mild to moderate reticulin fibrosis	

Curr Hematol Malig Rep. 2015; 10(3): 282–291.

IPSS-R

Parameter	Categories and Associated Scores				
Cytogenetic risk group	Very good	Good	Intermediate	Poor	Very Poor
	0	1	2	3	4
Marrow blast proportion	≤2%	>2 - <5%	5 - 10%	>10%	
	0	1	2	3	
Hemoglobin	≥10 g/dL	8 - <10 g/dL	<8 g/dL		
	0	1	1.5		
Absolute neutrophil count	≥0.8 x 10^9/L	<0.8 x 10^9/L			
	0	0.5			
Platelet count	≥100 x 10^9/L	50 - 100 x 10^9/L	<50 x 10^9/L		
	0	0.5	1		

Possible range of summed scores: 0-10

Greenberg P et al *Blood* 2012 Sep 20;120(12):2454-65.

Prognostication of MDS in daily practice

Results from the Dutch PHAROS MDS registry

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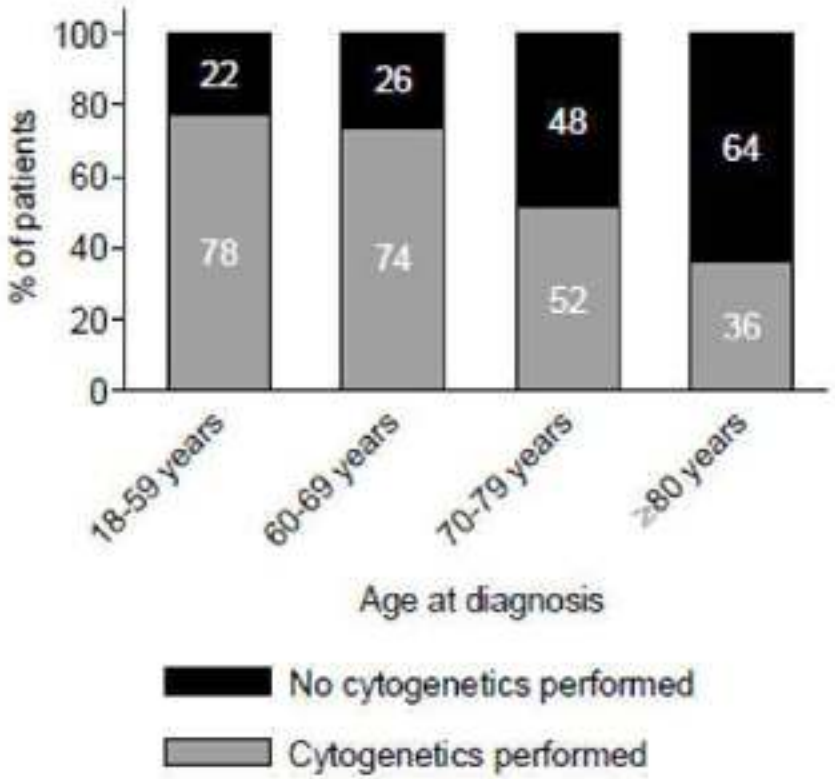
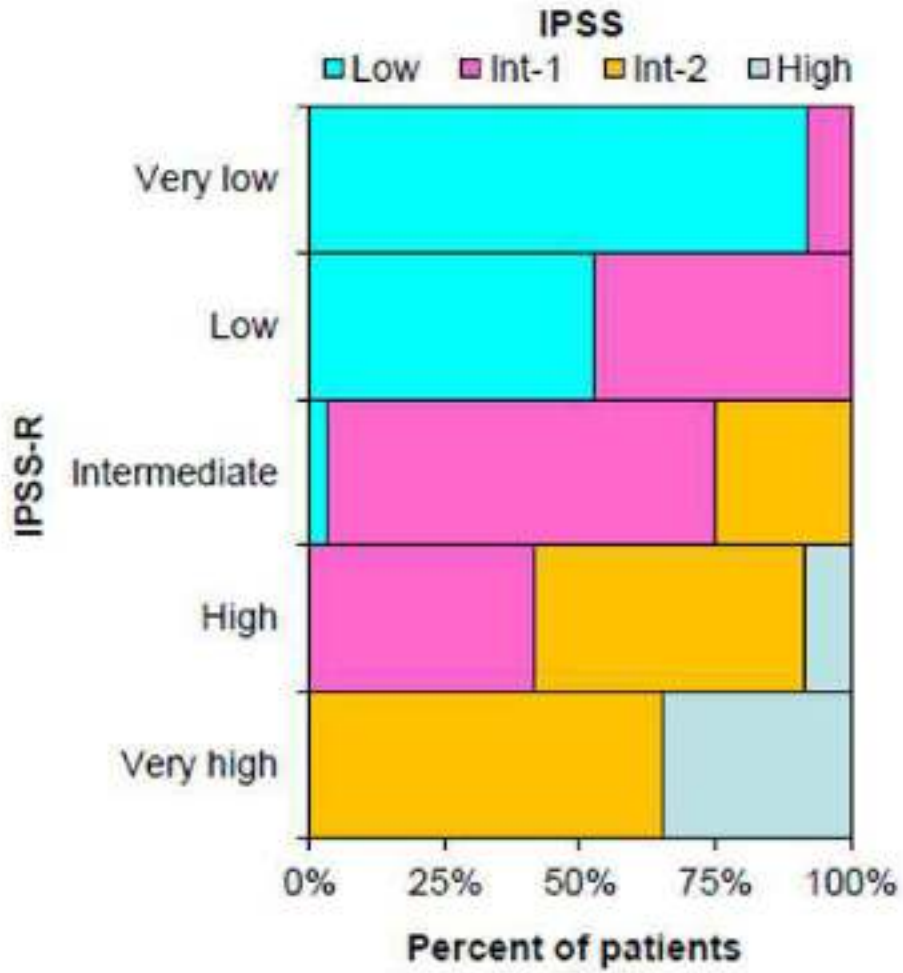
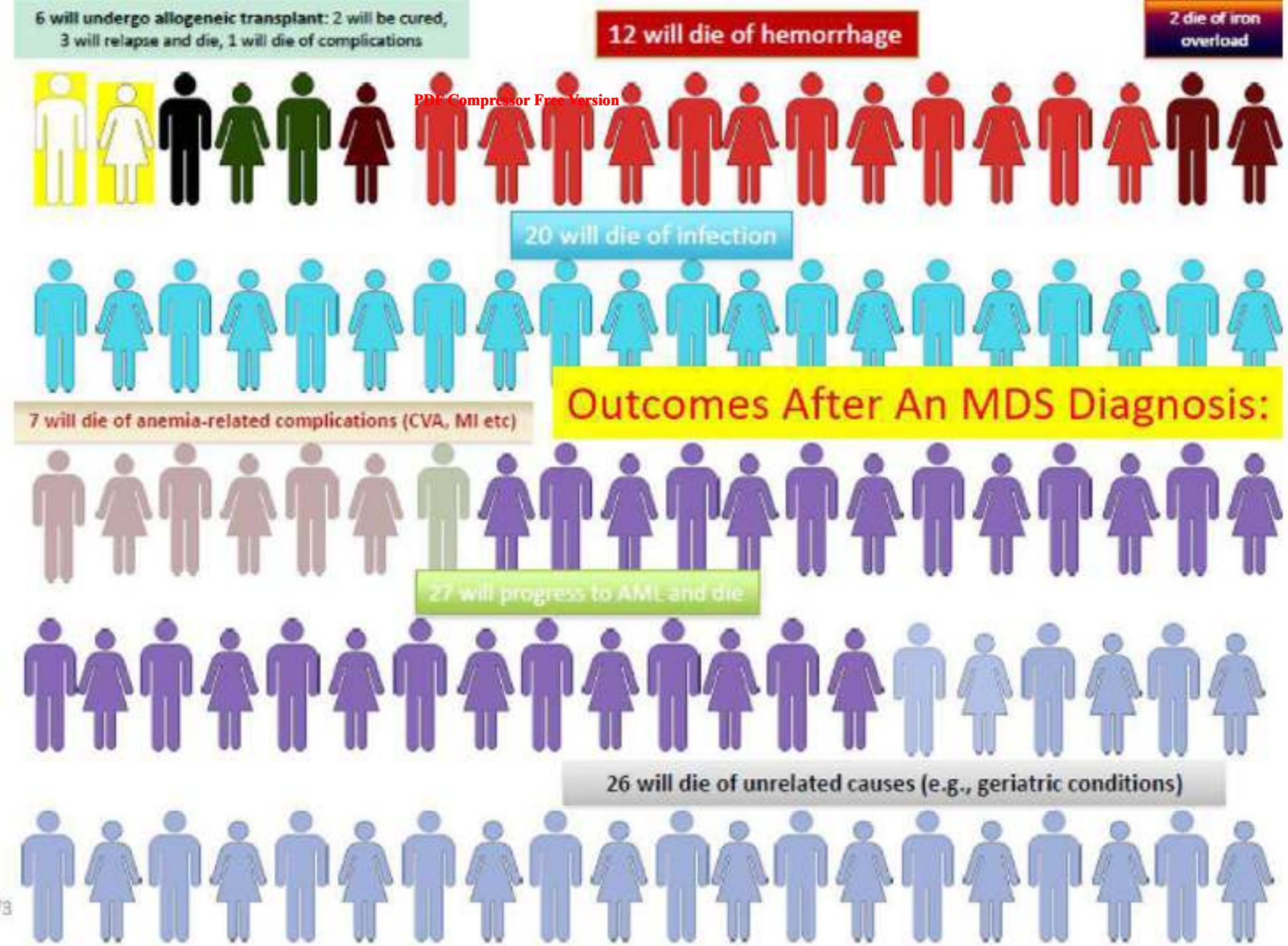




Dinmohamed AG, et al., 2016 (submitted)



Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence.

N Engl J Med. 2014 Dec 25;371(26):2477-87.

Clonal hematopoiesis with somatic mutations was observed in 10% of persons older than 65 years of age but in only 1% of those younger than 50 years of age. Detectable clonal expansions most frequently involved somatic mutations in three genes (*DNMT3A*, *ASXL1*, and *TET2*) that have previously been implicated in hematologic cancers. Clonal hematopoiesis was a strong risk factor for subsequent hematologic cancer (hazard ratio, 12.9; 95% confidence interval, 5.8 to 28.7). Approximately 42% of hematologic cancers in this cohort arose in persons who had clonality at the time of DNA sampling, more than 6 months before a first diagnosis of cancer. Analysis of bone marrow–biopsy specimens obtained from two patients at the time of diagnosis of acute myeloid leukemia revealed that their cancers arose from the earlier clones.

Blood. 2015 Apr 30. pii: blood-2015-03-631747. [Epub ahead of print]

Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes.

Steensma DP[1], Bejar R[2], Jaiswal S[3], Lindsley RC[3], Sekeres MA[4], Hasserjian RP[5], Ebert BL[3].

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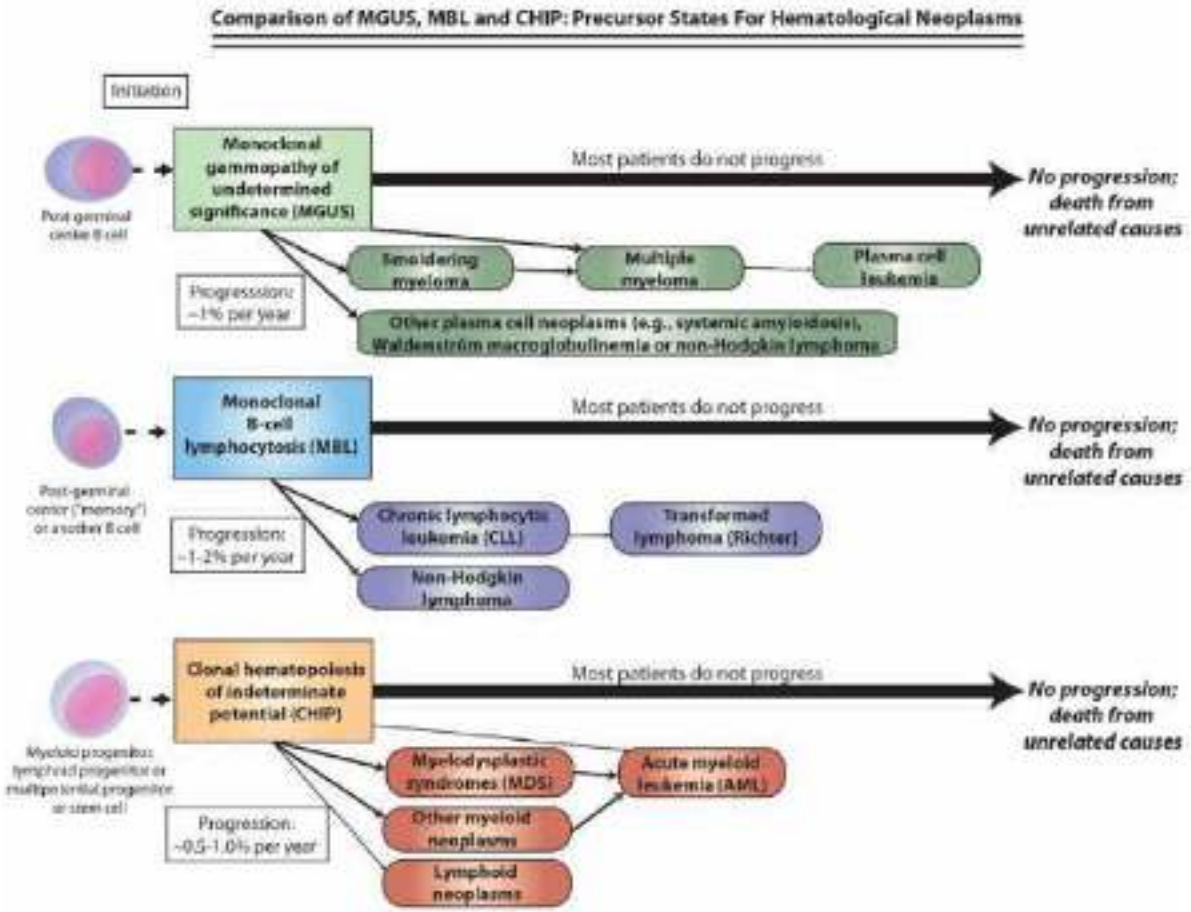
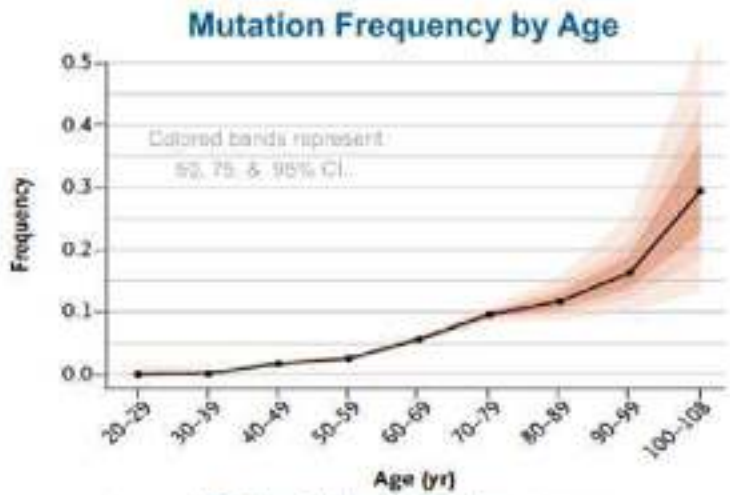
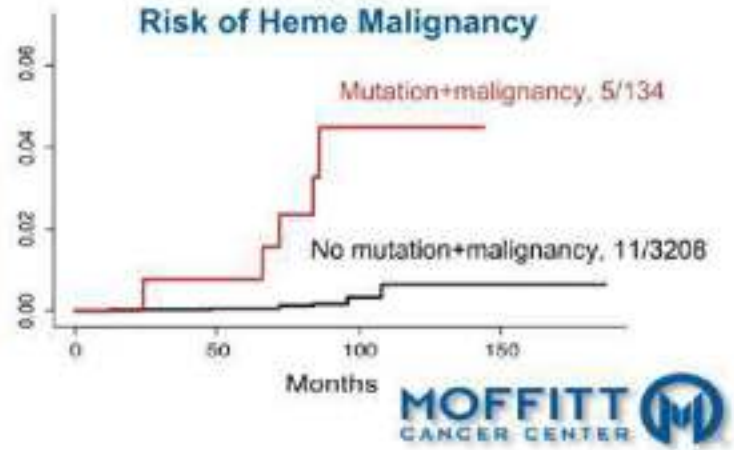


Age-Related Clonal Hematopoiesis (CHIP) is Linked to Risk of Inflammatory Co-morbidities

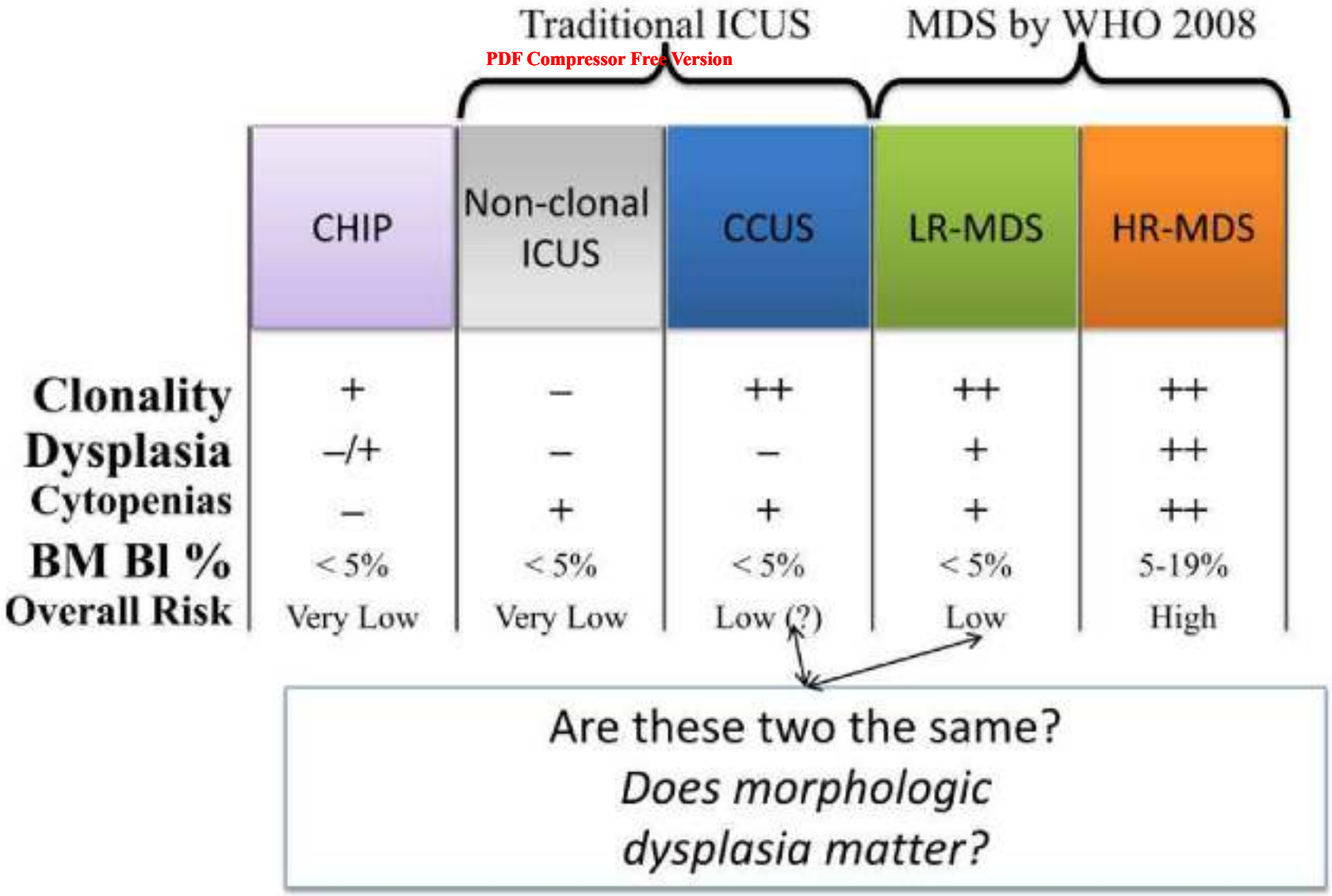
- Whole exome NGS on PB of 17,182 persons; median f/u 8 years
- 805 somatic mutations found in 73 genes from 746 (4.3%) individuals
- Majority involved 1 mutation: ***DNMT3A*** (n=403), ***TET2*** (72), & ***ASXL1*** (62)
- Median VAF was 0.09, ~18% of WBC
- Risk of myeloid malignancy was markedly increased in mutation carriers [HR 11, 95% CI 3.9-33] & higher VAF
- CHIP was associated with greater risk for **inflammatory morbidities**: Type 2 DM [OR1.3, 95% CI 1.1-1.5], CAD [HR 2.0, 95% CI 1.2-3.4] or stroke [HR 2.6, 95% CI1.4-4.8]





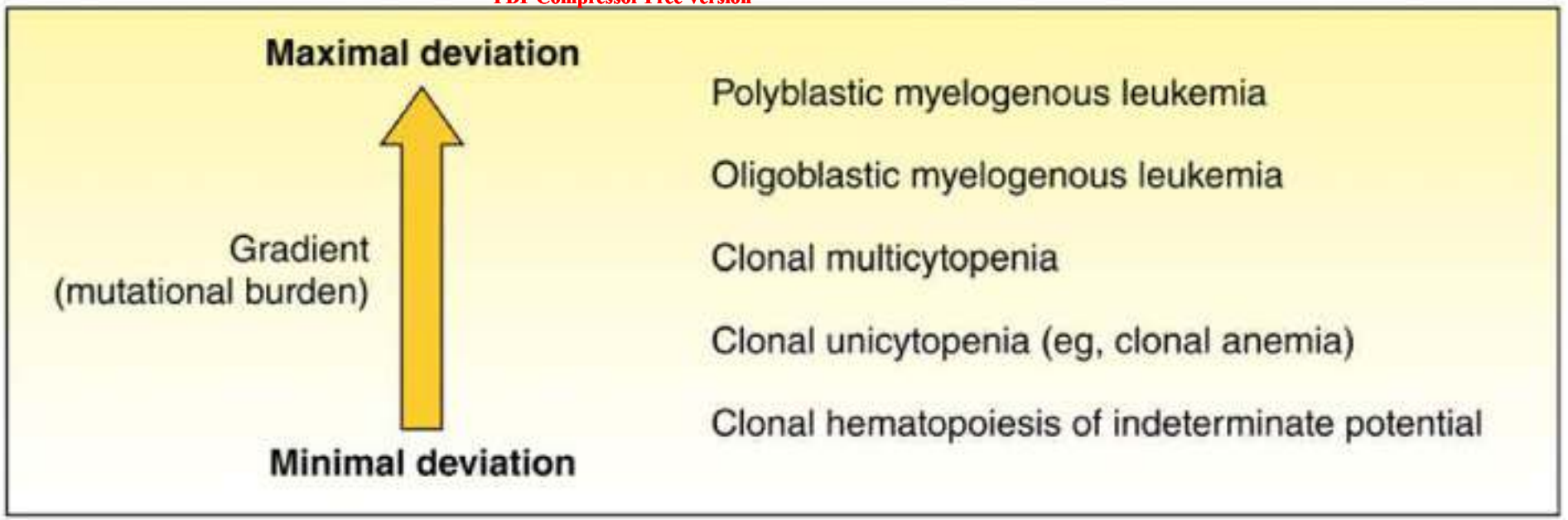
Jaiswal S, et. al. NEJM 2014; 371:2488; ASH 2014; 840a.

MOFFITT CANCER CENTER



CCUS = clonal cytopenias of undetermined significance; ICUS = idiopathic cytopenias of undetermined significance; CHIP = clonal hematopoiesis of indeterminate potential; LR = lower risk, HR = higher risk

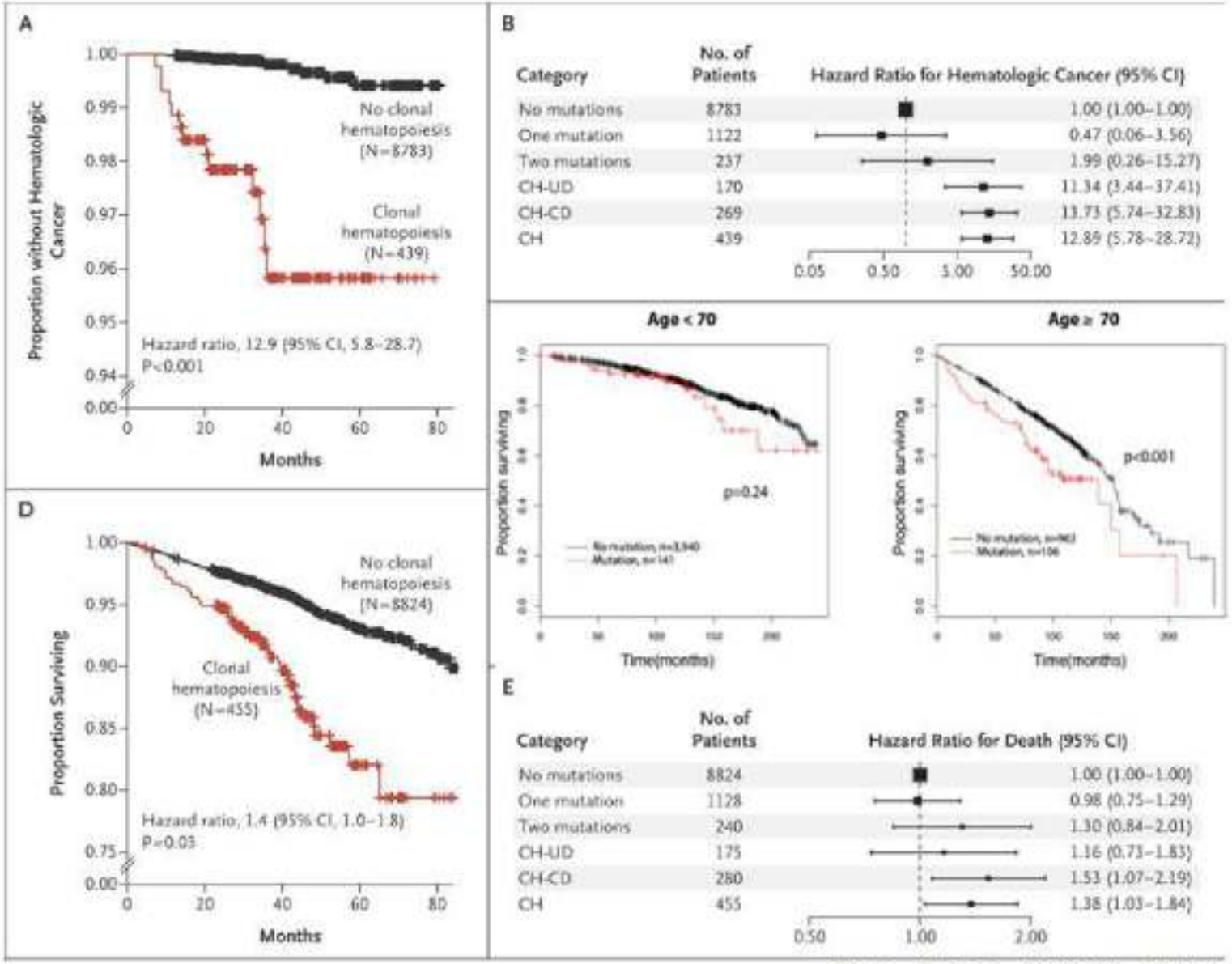
Steensma, Bejar, Jaiswal et al *Blood* 2015



A schematic relationship among the disorders that fall under the rubric of myelodysplastic neoplasms. Myelodysplastic disorders are less deviated forms of acute myelogenous leukemia.[7] Here, deviation is considered in terms of loss of regulated processes of proliferation, differentiation, and maturation compared with normal polyclonal hematopoiesis. Mutational burden considers qualitative as well as quantitative oncogenetic contributions to neoplasia. Professional illustration by Patrick Lane, ScEYEnce Studios.

BLOOD, 2 JULY 2015 • VOLUME 126, NUMBER 1

Risk of Hematologic Cancer for Participants with Clonal Hematopoiesis



N Engl J Med 2014; 371:2477-2487

Cytopenic and clonal states and their relationship to MDS

State	Key features	Comment
Normal hematopoiesis	No cytopenias, no clonal mutation. Mild cellular dysplasia may be present, especially in older persons.	Healthy state.
ICUS	Cytopenias are present. Dysplasia may be present, but is minimal (<10% of cells per lineage). By definition, a clonal mutation is not known to be present, either because testing was not performed or because testing was unrevealing.	Heterogeneous cluster of pathophysiologically unrelated disorders. May resolve with time, or a diagnosis may become clearer. Some patients with ICUS have MDS but do not meet current diagnostic criteria.
CHIP	Cytopenias are not necessarily present. Dysplasia may be present, but is minimal (<10% of cells per lineage). By definition, patients with CHIP do not meet WHO criteria for a hematological neoplasm. A clonal mutation is present.	Common in the healthy aging population. Confers a 0.5-1.0% per year risk of progression to MDS, AML or another neoplasm. Most patients have just 1 mutation detectable. Certain mutations may confer a higher risk of progression, but this is not yet clear.
CCUS	Required: both cytopenia(s) and clonal mutation(s) in a gene or genes associated with myeloid neoplasia. By definition, patients with CCUS do not meet WHO criteria for MDS or another hematological neoplasm.	Could be considered a subset of CHIP, probably with a higher risk of progression to hematological neoplasia. Some patients with CCUS may have clonal mutations that are not actually responsible for the cytopenia, therefore having CHIP plus a reactive, non-clonal cause of cytopenias. In other patents, the clone contributes to ineffective hematopoiesis. Patients often have 2 or more mutations detectable.
MDS without blast increase	Cytopenias are required for diagnosis. Clonal disorder. Usually, extensive dysplasia is seen; using WHO criteria, diagnosis currently can be made in the absence of dysplasia if certain karyotypic markers are present (e.g., monosomy 7 or del(5q)).	In the future, specific mutations or combinations of mutations may define MDS, even in the absence of dysplasia.
MDS with blast increase	Clonal hematopoietic neoplasm in which myeloid cell differentiation is impaired and blast cells accumulate in the marrow or blood. The WHO calls this "refractory anemia with excess blasts" (RAEB), but anemia is not necessarily present (though cytopenias are typical), nor is dysplasia always present.	Biologically similar to AML. AML is currently defined by the WHO as requiring ≥20% marrow or blood blasts (or else one of a short list of AML-defining karyotypes such as t(8;21), regardless of blast proportion), but MDS with excess blasts can be considered an oligoblastic form of AML.

Blood 2015 126:2349-2351

Proposed criteria for CCUS

Peripheral blood findings	Bone marrow findings	Genetic findings
1 or more of the following:	None of the following:	1 or more of the following:
Hemoglobin, <11 g/dL	≥10% dysplasia in the granulocytic, erythroid, or megakaryocytic lineage	An acquired chromosomal abnormality not diagnostic of a heme malignancy
ANC <1500/μL, 1.5×10^9/L	Myeloblasts comprise ≥5% of total cellularity	Presence of a somatic mutation with a VAF ≥2% in a heme malignancy–associated gene in the peripheral blood or bone marrow
Platelet count <100 000/μL, 100×10^9/L	An acquired chromosomal abnormality specific for MDS/AML	

Additional criteria: No other likely cause of cytopenias or evidence of another hematologic disorder.

Diagnostic approach to MDS 2016

Dutch/EU guidelines

VU medisch centrum

Diagnostic tool	Diagnostic value	Priority
Peripheral blood smear	• Evaluation of dysplasia in one or more cell lines • Enumeration of blasts	Mandatory
Bone marrow aspirate	• Evaluation of dysplasia in one or more myeloid cell lines • Enumeration of blasts • Enumeration of ring sideroblasts	Mandatory
Bone marrow biopsy	• Assessment of cellularity, CD34+ cells, and fibrosis	Mandatory
Cytogenetic analysis	• Detection of acquired clonal chromosomal abnormalities that can allow a conclusive diagnosis and also prognostic assessment	Mandatory
FISH	• Detection of targeted chromosomal abnormalities in interphase nuclei following failure of standard G-banding	Recommended
Flow cytometry immunophenotype	**• Detection of abnormalities in erythroid, immature myeloid, maturing granulocytes, monocytes, immature lymphoid compartments**	***Recommended**** **if according to ELN guidelines**
SNP-array	• Detection of chromosomal defects at a high resolution in combination with metaphase cytogenetics	Suggested (likely to become a diagnostic tool in the near future)
Mutation analysis of candidate genes	**• Detection of somatic mutations that can allow a conclusive diagnosis and also reliable prognostic evaluation**	***Suggested*** **(likely to become a diagnostic tool in the near future)**

Malcovati L, et al., ELN guidelines. Blood 2013;122:2943-64; Greenberg P et al., J Nat Compr Netw Canc 2013;11:838-74; *Westers TM, et al., Leukemia 2012;26:1730-41

MDS mutation landscape

The future of CBC/DC diagnosis and Therapeutic Strategy

- CBC/DC: hematology analyzer
  - Cell morphology, cell counting, cell differentiation
    - Electronic impedance
    - Light scattered
    - Image analysis + AI?
    - Manual microscopy
- Genetic mutation
  - Aged (>65 y/o)
  - MDS associated genes mutation
- CTC: flow cytometry, MEMS
- Correct chronic inflame:
  - Gut microbiota correction
  - Immune system strengthen

謝謝