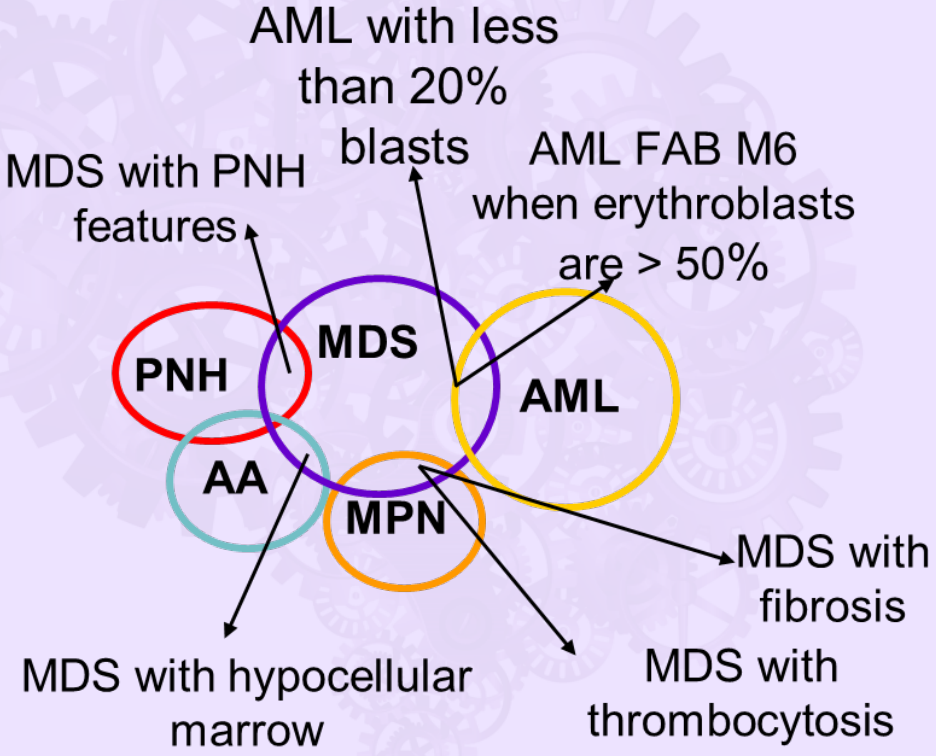
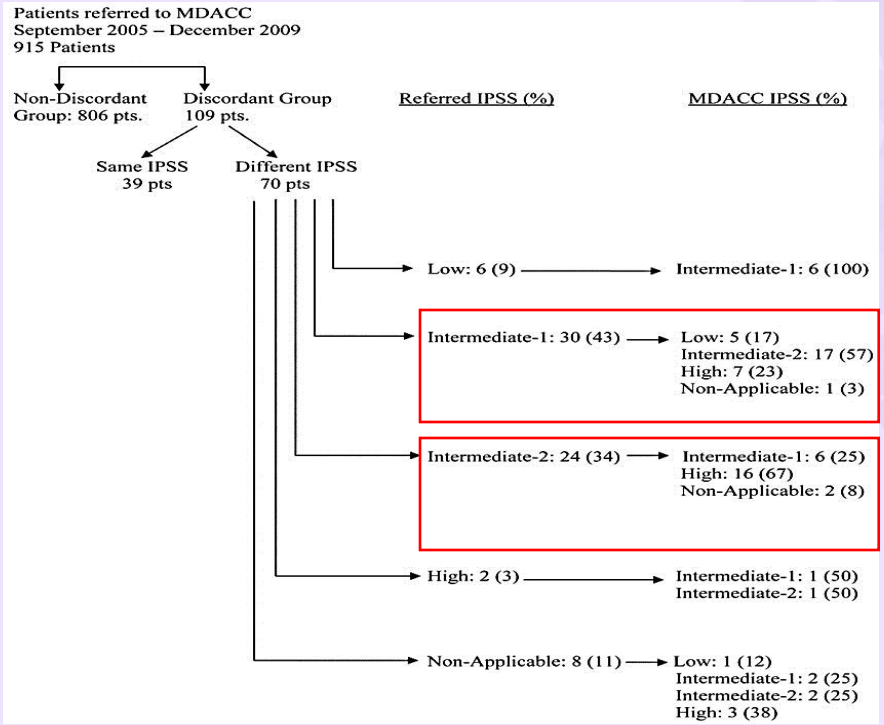
Myelodysplastic Syndromes (MDS)

- Clonal neoplasms of marrow stem/progenitors
- Cytopenias
- Dysplasia w/ineffective hematopoiesis
- Increased risk of blastic transformation



Implications of discrepancy in morphologic diagnosis of MDS between referral and tertiary care centers



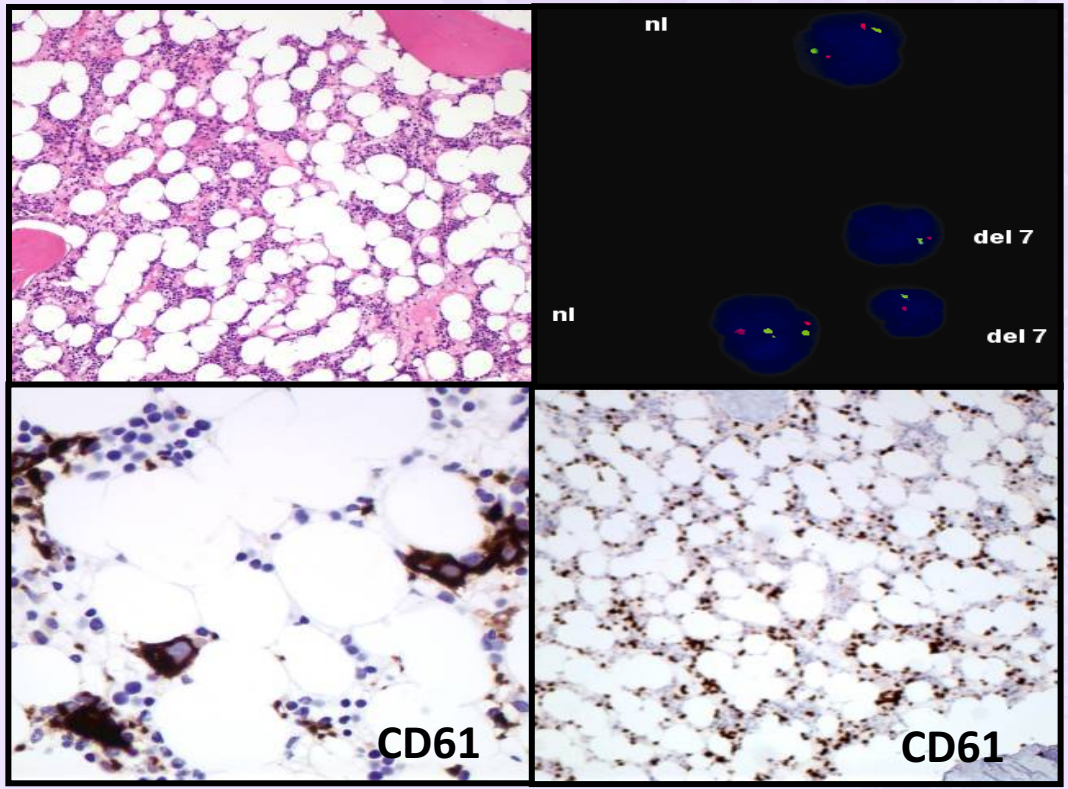
Discordance in the diagnosis was documented in 109 (12%) patients, with a majority reclassified as having higher-risk disease

Kiran Naqvi , et al. *Blood*. 2011;118(17):4690-4693

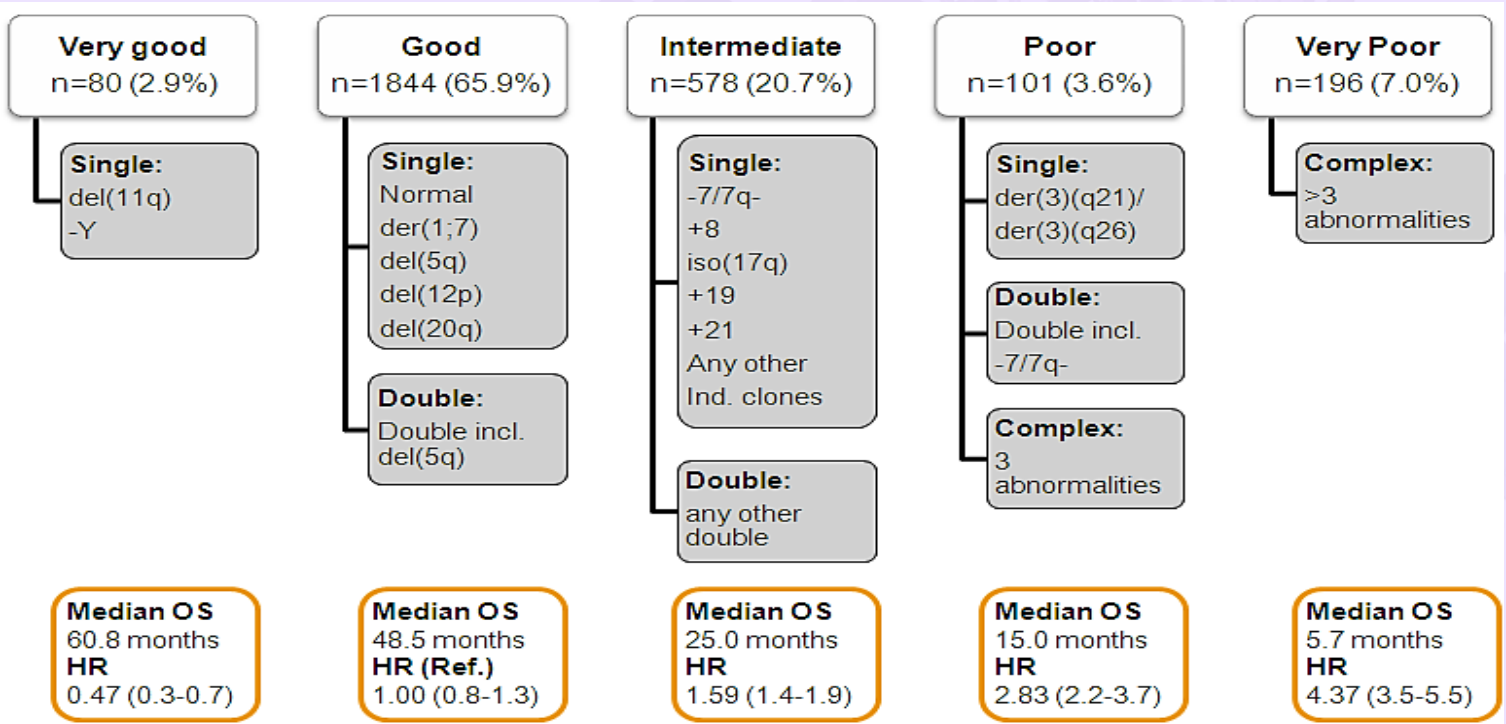
Clinical History

- 47 year-old man with refractory anemia and hypocellular bone marrow with dysmegakaryopoiesis and IPSS score of 0
- 46,XY[5]
- Over the next 12 months he developed worsening cytopenias and died

Hypoplastic MDS



Cytogenetic Scoring System

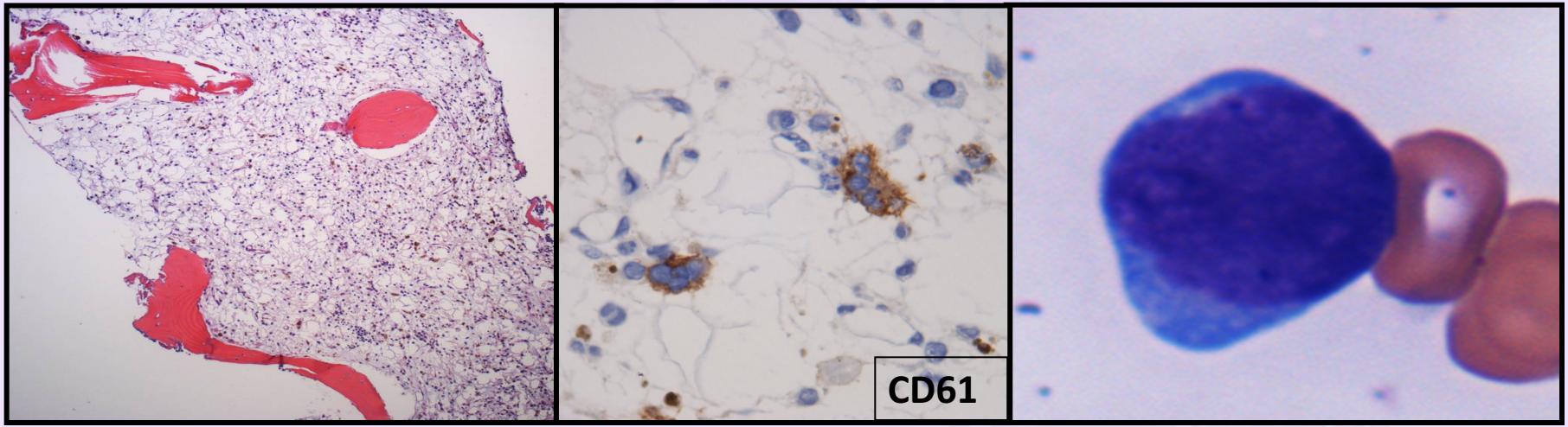


J Clin Oncol. 2012 Mar 10;30(8):820-9.

Clinical History

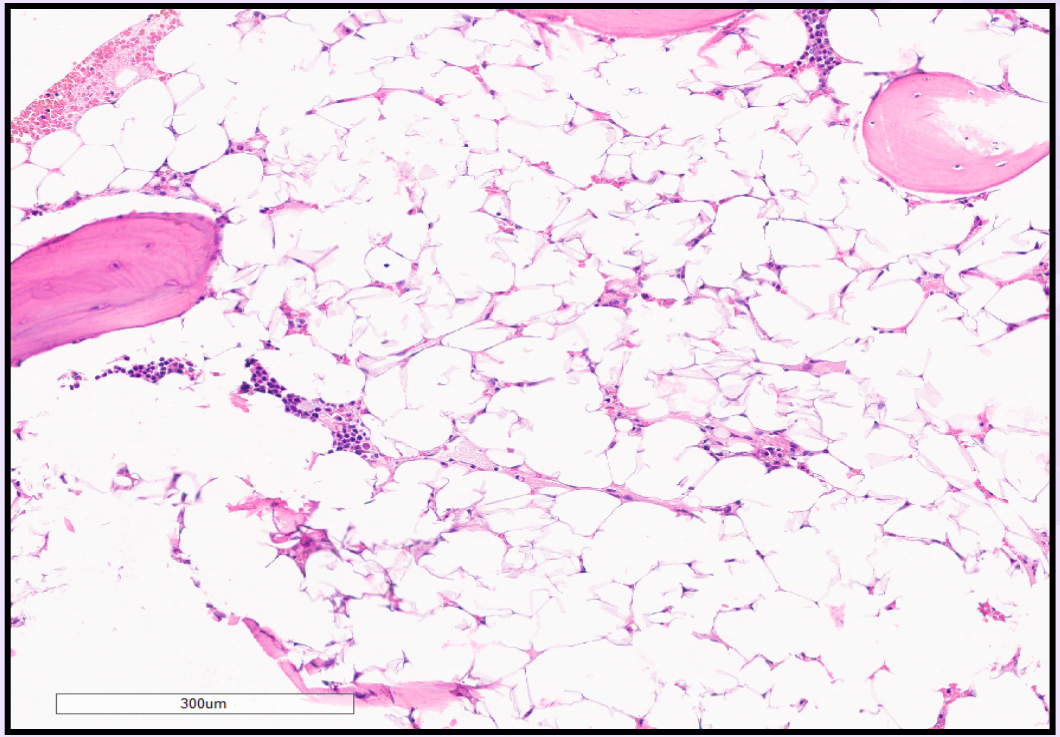
- 50 year-old woman with an 11-month history of acquired aplastic anemia
- Antithymocyte globulin, corticosteroid, cyclosporine
- CBC: WBC (1.0 K/uL), hemoglobin (8 g/dL) and platelet count (12 K/uL), 45% neutrophils, 1% blast

MDS Arising in a Patient With Aplastic Anemia



- 10% blasts in touch imprint
- 46,XX,t(3;21)(q26.2;q22),del(7)(q22q34)[9]

Bone Marrow Referral July 2014



- Aparticulate clot and smear
- Hypocellular bone marrow (10%) with myeloid and megakaryocytic hypoplasia

Marker	
CD42b	Rare, no hypolobated forms
MPO	Myeloid cells decreased
Glycophorin	Erythroid cells predominate
CD34 or CD117	0% blasts

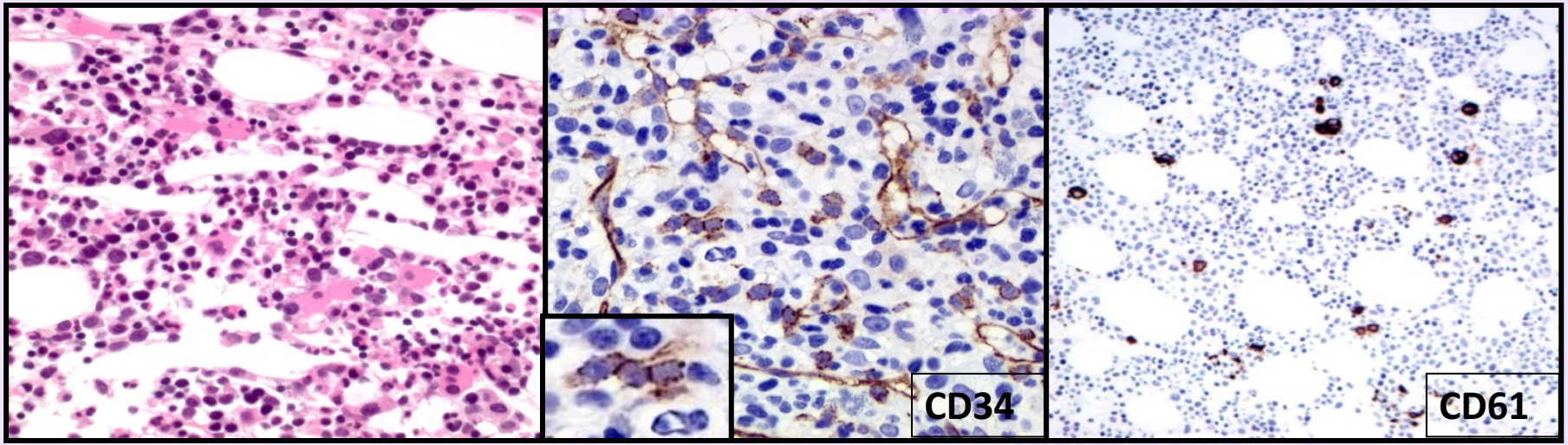
Histologic Features of MDS

Trephine biopsy

- Cellularity (*hypoplastic MDS*)
- Myelofibrosis (reticulin, MDS with fibrosis)
- Report % CD34+ blasts & cluster
- Dysmegakaryopoiesis (CD61)

Della Porta MG, et al. *Leukemia* 2014, 1-10

Morphological & Immunohistochemical Features of BM Biopsy in MDS



Recommended IHC Markers in MDS

	Markers	Cell type(s)
Minimal panel	CD34	Blast cells, progenitors, endothelial cells
	CD31 or CD42 or CD62	Megakaryocytes
	Tryptase	Mast cells, basophils, myeloid
Extended panel – according to the cell lineage to be examined	CD3	T cells
	CD15	Monocytes, granulocytes
	CD20	B cells
	CD25	T and B cell subset, atypical mast cells
	CD138	Plasma cells
	CD68	Monocytes, macrophages, myeloid cells
	Lysozyme	Monocytes, macrophages
	CD117	Progenitor cells, mast cells

Exclude hematopoietic and non-hematopoietic disorders as reason for cytopenia/dysplasia:

- Reticulocyte counts
- Chemistries
- Transaminases
- Bilirubin
- Hepatitis serologies
- PNH aerolysin assay
- Medications
- Exposures
- Transfusions
- Splenomegaly
- Hepatomegaly
- Lymphadenopathy
- Family history

Minimal Diagnostic Criteria in MDS

Prerequisite criteria

- Constant cytopenia in one or more of the following cell lineages erythroid (WHO hemoglobin <10 g dL)
- Neutrophilic (ANC <1800 µL) or megakaryocytic (platelets <100,000 µL)

Greenberg PL, et al. *J Natl Compr Canc Netw* 2017;15(1):60-87

Minimal Diagnostic Criteria in MDS

MDS-related (decisive) criteria

- Dysplasia in at least 10% of all cells in one of the following lineages in the bone marrow smear; erythroid; neutrophilic; or megakaryocytic or > 15% ringed sideroblasts
- 5-19% Blast of all nucleated cells in bone marrow MDS-associated karyotype

Greenberg PL, et al. *J Natl Compr Canc Netw* 2017;15(1):60-87
Valent P, et al. *Leukemia Research* 2007:727-736

Minimal Diagnostic Criteria in MDS

Co-criteria:

- Typical clinical features, macrocytic transfusion-dependent anemia
- Abnormal phenotype by flow cytometry of BM cells indicative of a monoclonal population
- Abnormal BM histology & IHC (abnormal CD34, fibrosis, dysplastic megs, abnormal localization of immature progenitors)
- Molecular: Monoclonal myeloid population

Greenberg PL, et al. J Natl Compr Canc Netw 2017;15(1):60-87
Valent P, et al. Leukemia Research 2007:727-736

MDS-related cytogenetic abnormalities

Unbalanced

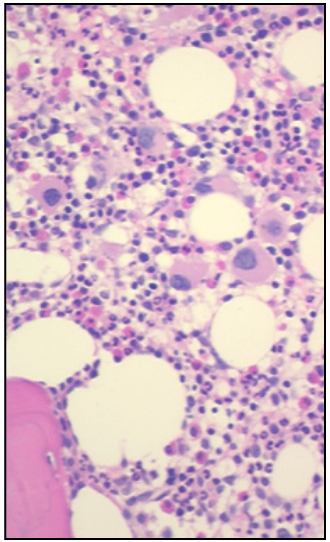
+8*
-7 or del(7q)
-5 or del(5q)
del(20q)*
-Y*
i(17q) or t(17p)
-13 or del(13q)
del(11q)
del(12p) or t(12p)
del(9q)
idic(X)(q13)

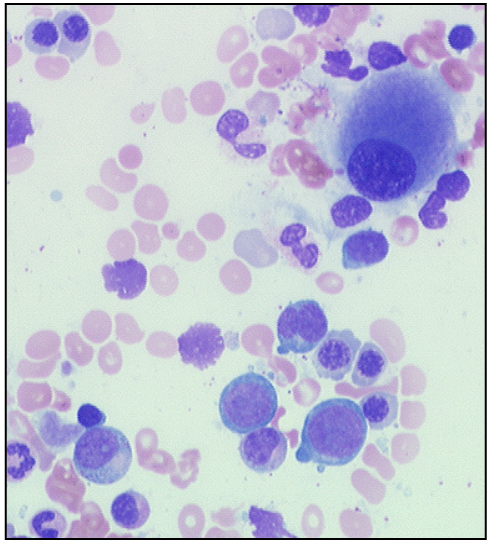
Balanced

t(11;16)(q23;p13.3)
t(3;21)(q26.2;q22.1)
t(1;3)(p36.3;q21.2)
t(2;11)(p21;q23)
inv(3)(q21q26.2)
t(6;9)(p23;q34)

-Y, trisomy 8 and del(20q) not disease defining

MDS with Deletion of Chromosome 5q:Persistent Malignant Stem cells in Remission





NEJM 2010;363:1025

Proposal: Provisional entity

Myelodysplastic/ Myeloproliferative Neoplasm with isolated isochromosome 17q

Carlos E. Bueso-Ramos

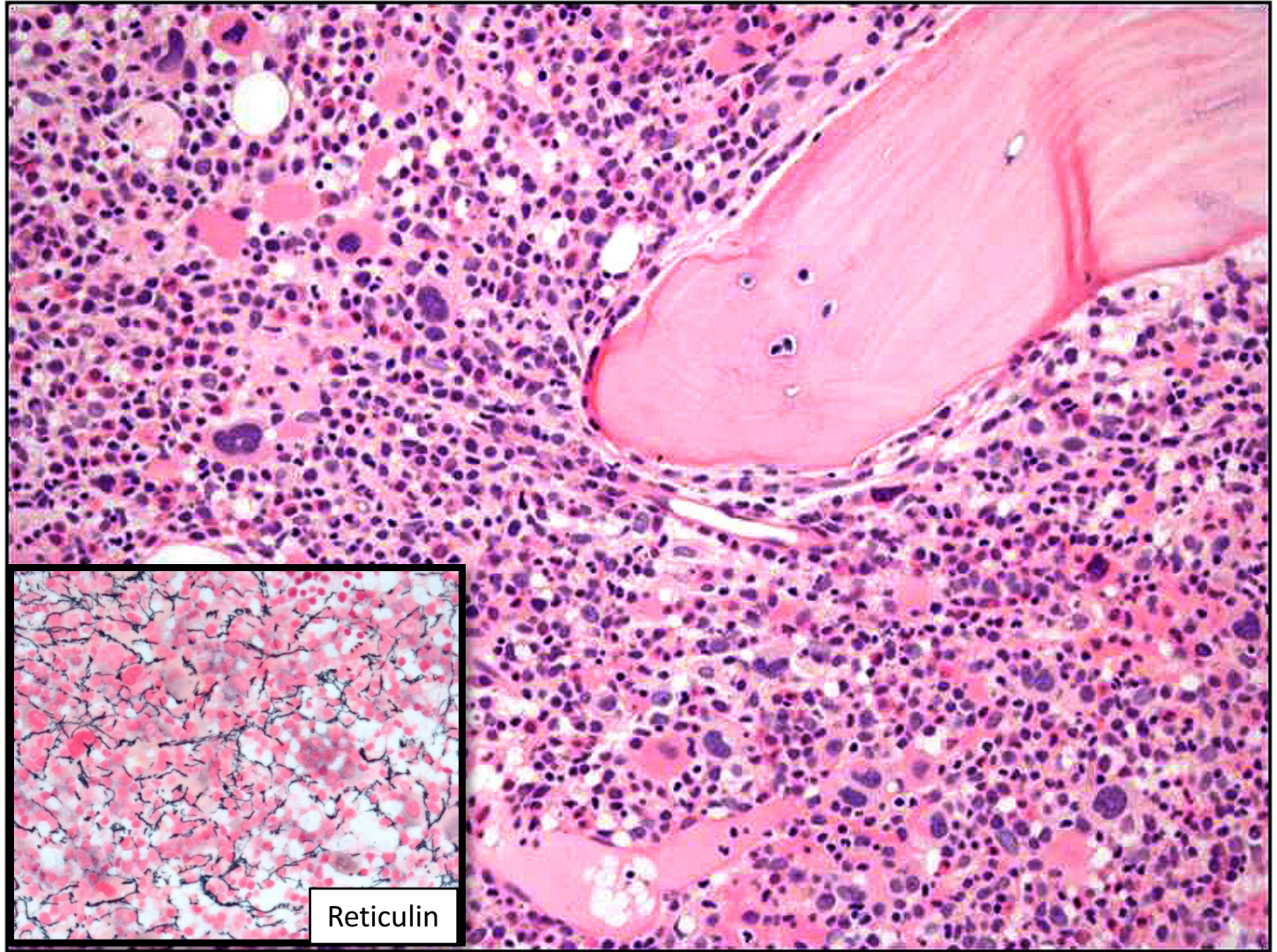
The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

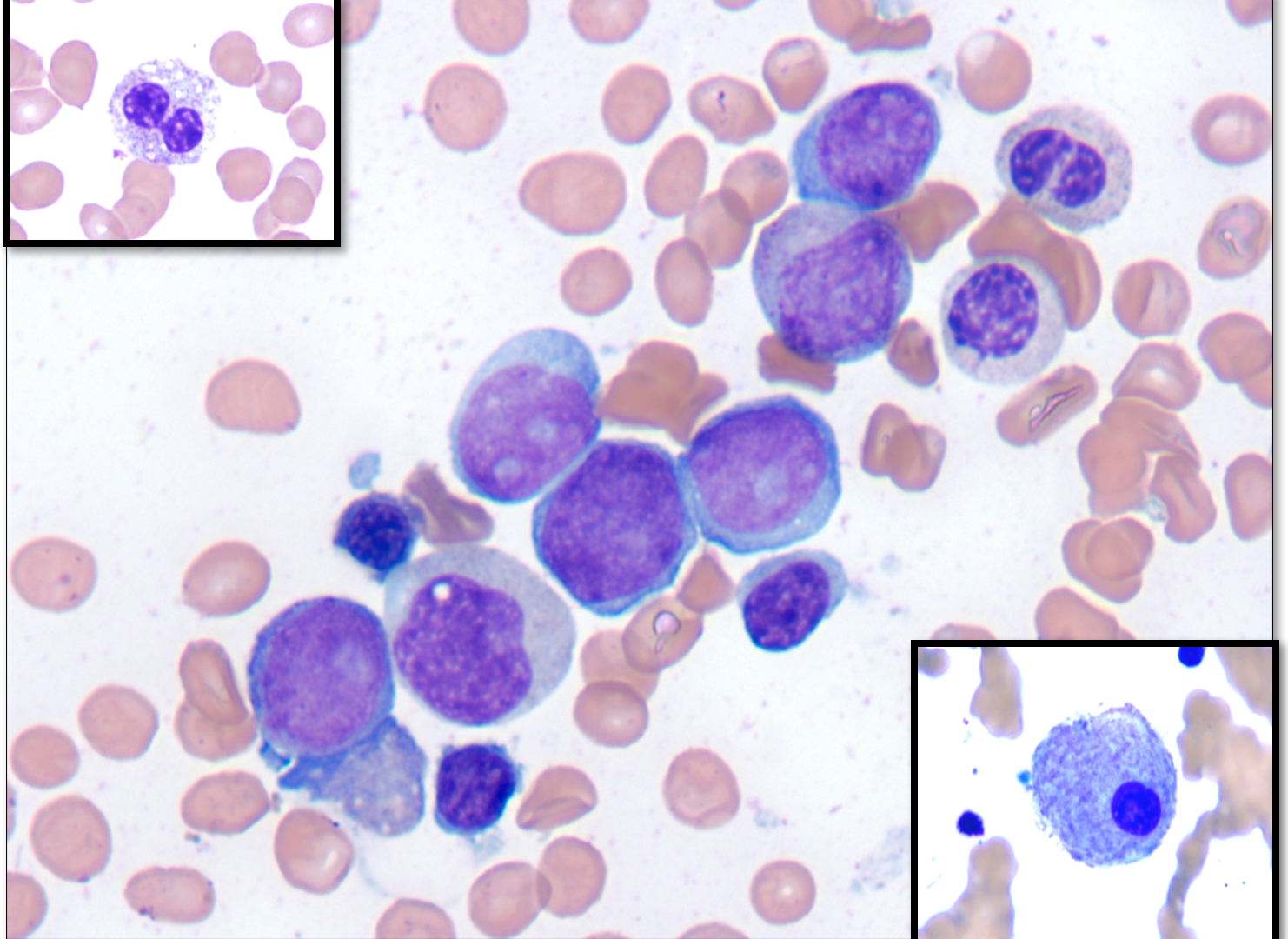
Key clinicopathologic features

- Splenomegaly
- Leukocytosis, monocytosis, basophilia
- Fibrosis
- Granulocytic dysplasia
- Megakaryocytic dysplasia
- Features of aCML, CMML (2008 WHO)

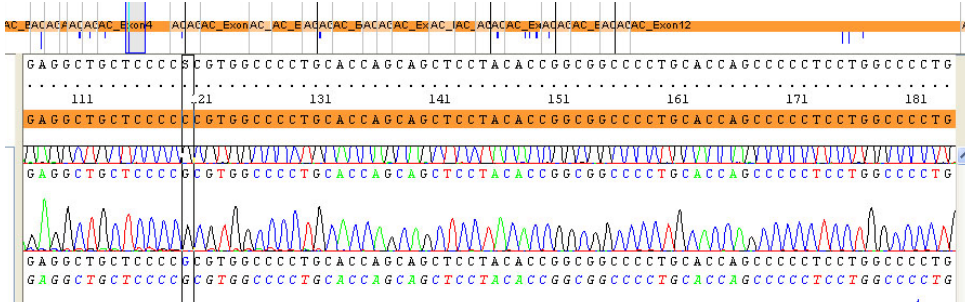
Br J Haematol. 1999;106(2):445-54

Cancer. 2012;118(11):2879-88


Reticulin



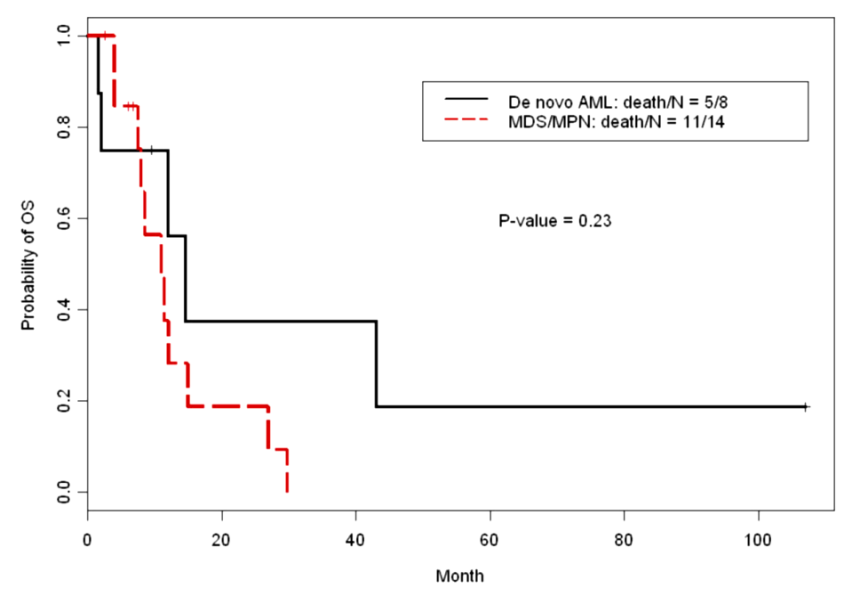
TP53 mutations absent in myeloid neoplasms with isolated i(17q)



- Sequencing of the entire coding region including 5’ and 3’ UTR
  - Wild type in 16/16 cases (6 AML; 10 MDS/MPN)

Cancer. 2012;118(11):2879-88

Clinical outcome



- Aggressive clinical course irrespective of the blast count or WHO classification

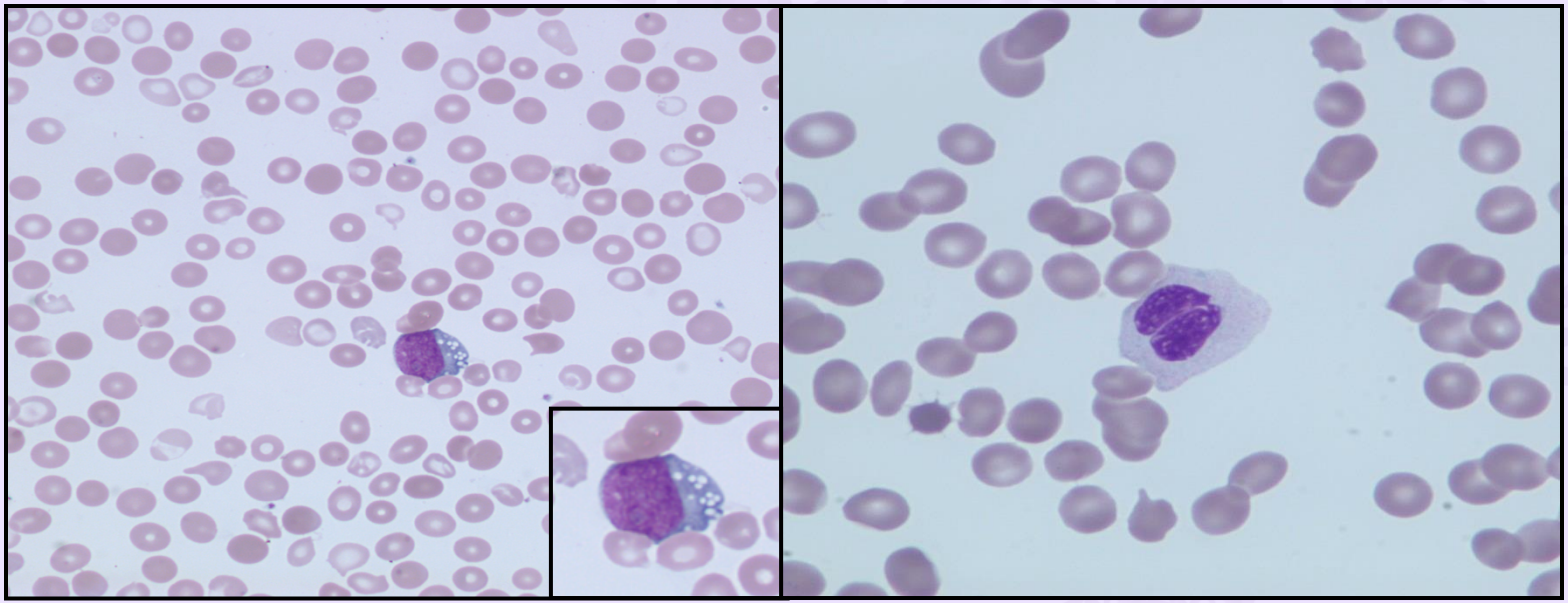
Cancer. 2012;118(11):2879-88

Summary

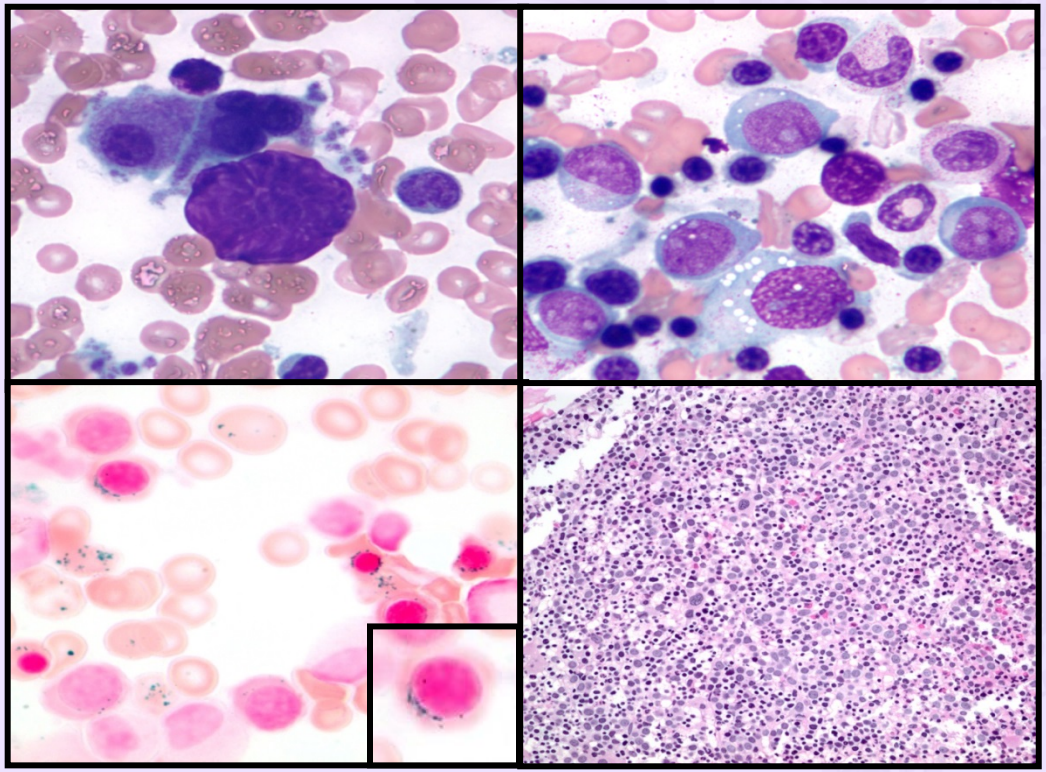
- Suspected in any myeloid neoplasm associated with MDS/MPN features
- A high risk for leukemic progression
- *SETBP1* mutations in ~54% cases and Co-occurred frequently with *ASXL1* mut and isolated i(17)(q10)
- No *P53* mutations
- Primary MDS with i(17q): Intermediate prognostic group

Leuk Res. 2013;37(7):769-76

Morphological Features of PB in MDS



Features of MDS in BM



Morphologic Features of MDS

ORIGINAL ARTICLE

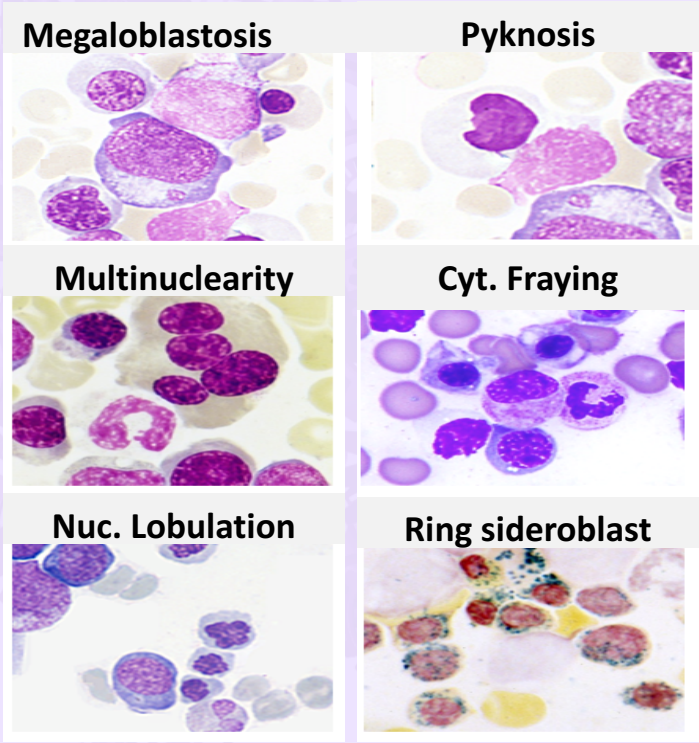
Minimal morphological criteria for defining bone marrow dysplasia: a basis for clinical implementation of WHO classification of myelodysplastic syndromes

MG Della Porta[1,2,13], E Travaglino[1,13], E Boveri[3,13], M Ponzoni[4], L Malcovati[1,5], E Papaemmanuil[6], GM Rigolin[7], C Pascutto[1], G Croci[3,5], U Gianelli[8], R Milani[4], I Ambaglio[1], C Elena[1], M Ubezio[1,5], MC Da Via'[1,5], E Bono[1,5], D Pietra[1], F Quaglia[2], R Bastia[2], V Ferretti[1], A Cuneo[7], E Morra[9], PJ Campbell[6,10,11], A Orazi[12], R Invernizzi[2,14] and M Cazzola[1,5,14] on behalf of Rete Ematologica Lombarda (REL) clinical network

Della Porta MG, et al. *Leukemia* 2015

Erythoid lineage

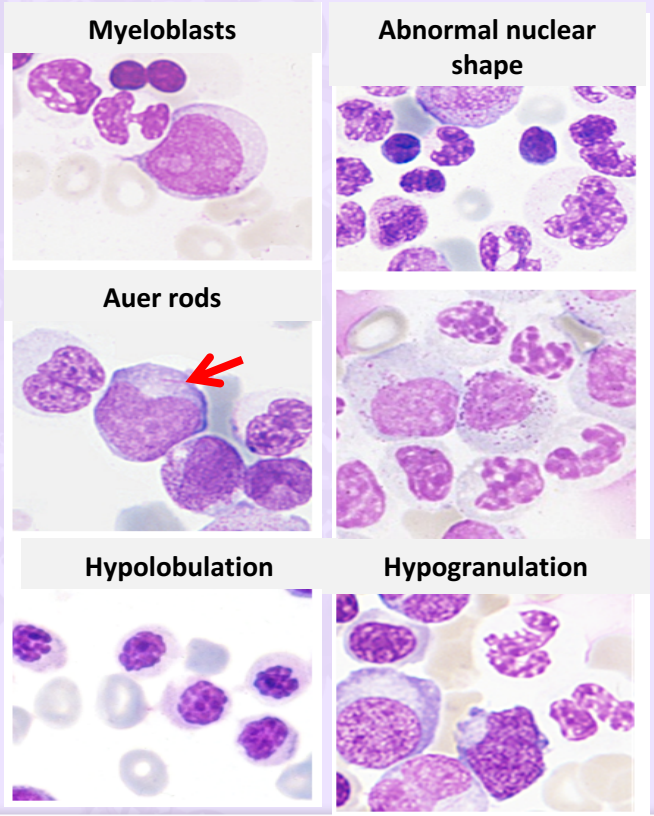
Morphological abnormalities	Cutoff values	Variable weighted score
Megaloblastoid changes	> 5%	2
Bi- or multinuclearity	> 3%	1
	> 5%	2
Nuclear lobulation or irregular contours	> 3%	1
Pyknosis	> 5%	1
Cytoplasmic fraying	≥ 7%	1
Ring sideroblasts	> 5%	2
	≥ 15%	3
Ferritin sideroblasts	≥ 30%	1



Della Porta MG, et al. *Leukemia* 2015

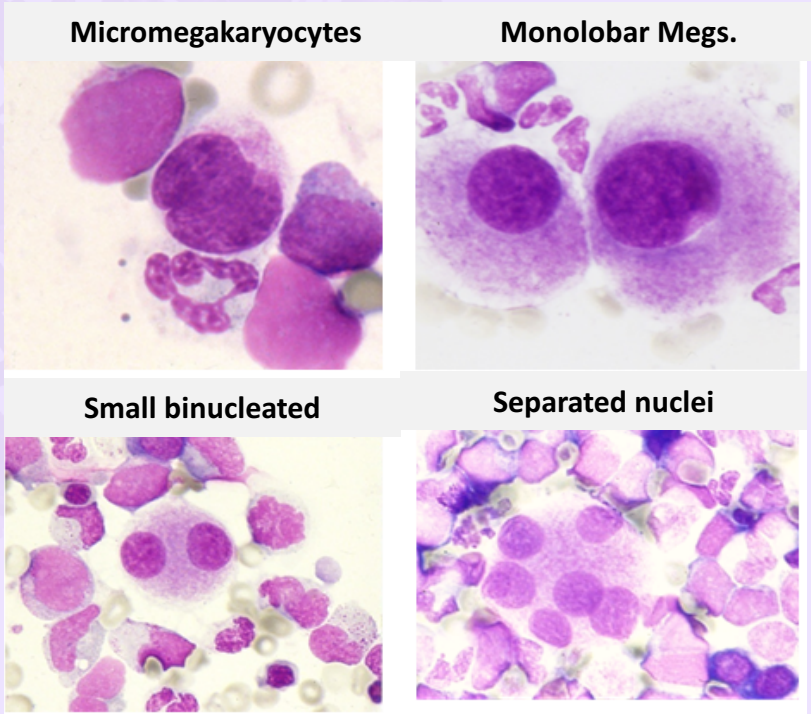
Granulocytic lineage

Morphological abnormalities	Cutoff values	Variable weighted score
Myeloblasts	> 3%	1
	> 5%	3
Auer rods	≥ 1%	3
Pseudo Pelger–Hüet anomaly	> 3%	1
	> 5%	2
Abnormal nuclear shape	≥ 7%	1
Neutrophil hypogranulation	> 3%	1
	> 5%	2



Megakaryocytic lineage

Morphological abnormalities	Cutoff values	Variable weighted score
Micromegakaryocytes	> 5%	3
Small binucleated megakaryocytes	> 5%	1
Megakaryocytes with multiple separated nuclei	> 5%	2
Hypolobated or monolobar megakaryocytes	> 5%	2



Della Porta MG, et al. *Leukemia* 2015

FCI Positive for MDS

- Increase in CD34+ blasts with aberrancies
- Expression of lymphoid antigens CD2, CD5, CD7, CD56, CD10, CD19
- Lack of Hematogones
- Discrete population
- Alteration of CD45, CD117, CD123, CD38, CD33/CD13
- Hypogranulation
- Myelomonocytic maturation alterations

Hypoplastic MDS and Aplastic Anemia

Bone marrow Examination	Aplastic Anemia	Hypoplastic MDS
Erythroid	Lack, or small erythroid clusters (<10 cells/cluster), mild dyserythropiesis. Low corrected reticulocyte count	Patchy distribution of erythroids, left-shifted. Moderate Dyserythropoiesis
Megakaryocytes	Often absent, or too few to assess	Reduced, dysplastic hypolobulated, multinucleated
Myeloid cells	Decreased, dysgranulopoiesis is mild	Decreased, more pronounced dysplasia
CD34 blasts	Decreased to absent (<1%)	More discernible CD34+, in clusters
Architecture	Normal	Altered
Reticulin fibrosis	Absent	Present in 20% cases

Hypoplastic MDS and Aplastic Anemia

Bone marrow Examination	Aplastic Anemia	Hypoplastic MDS
Flow Cytometry Immunophenotyping	Blasts with normal phenotype Hematogones are present PNH clones GPI-AP-deficient in 25% (0.1%-15% GPI-AP- cells) No hemolysis nor thrombosis Clonal evolution?	Blasts show aberrancies Hematogones reduced PNH clones seen in 9% cases, smaller GPI-AP- cells. No hemolysis, nor thrombosis
Cytogenetics	Often normal A few can have cytogenetic abnormality	40-50% abnormal Fatty marrows may lead to cytogenetic failures

Aplastic Anemia and Hypoplastic MDS

Clinical Data and Classification of Myelodysplasia						
Age, y	**AA-MDS, months**	**RS, %**	**Classification**	**IPSS**	**Follow-up, mo**	**Survival**
52	8	6	RCMD	0.5	96	Alive
53	6	20	RARS	0.5	39	Alive
50	11	12	RCMD	0.5	130	Alive
65	43		RCMD	1.5	82	Dead
72	16		RCMD	1.5	24	Alive
79	2		RAEB-1	2.0	37	Dead
51	7		RA	1.5	45	Alive
70	17	4	RA	1.5	33	Dead
65	2		RCMD	1.0	85	Alive
26	2		RCMD	1.0	11	Alive
45	9	3	RA	1.5	18	Alive
47	36	5	RAEB-1	2.0	50	Dead

Cancer. 2007;110(7):1520-1526

Comparison of Cytogenetics and FISH for Monosomy 7 at Diagnosis of AA and MDS

At AA diagnosis		At MDS diagnosis	
Cytogenetics	**FISH, %**	**Cytogenetics**	**FISH, %**
46,XX	—	46,XX [20]	—
46,XX	—	46,XX [20]	—
46,XX	—	46,XX [20]	8
46,XX	17	45,XX,−7 [19]/46XX [1]	—
46,XY	22	45,XY,−7 [14]/46XY [6]	47
46,XX	—	45,XX,−7 [12]	71
46,XX	—	45,XX,−7 [19]	—
46,XY	4	45,XY,−7,8q+[22]	29
46,XX	—	47,XX,+15 [3]/46,XX [17]	27
46,XX	—	47,XX,+8 [3]/46XX [17]	—
46,XX	—	50,XX,+X,+13,+19,+21[5]	28
46,XY	—	46,XY,der(1)t(1;12)(p13;q13)del(12) (q21q24.3), der(9) (1;9)(p13;q13)ins(1;12)(p22;q21q24.3), der(12)t(9;12)(q31;q11)[20]	22

Cancer. 2007;110(7):1520-1526

Comprehensive Approach to Diagnose Low Risk MDS

A multidisciplinary evaluation and follow up

- Clinical history
- Morphology: dysplasia, blasts
- Cytogenetics, SNP array, NGS
- Immunophenotype: flow cytometry, IHC

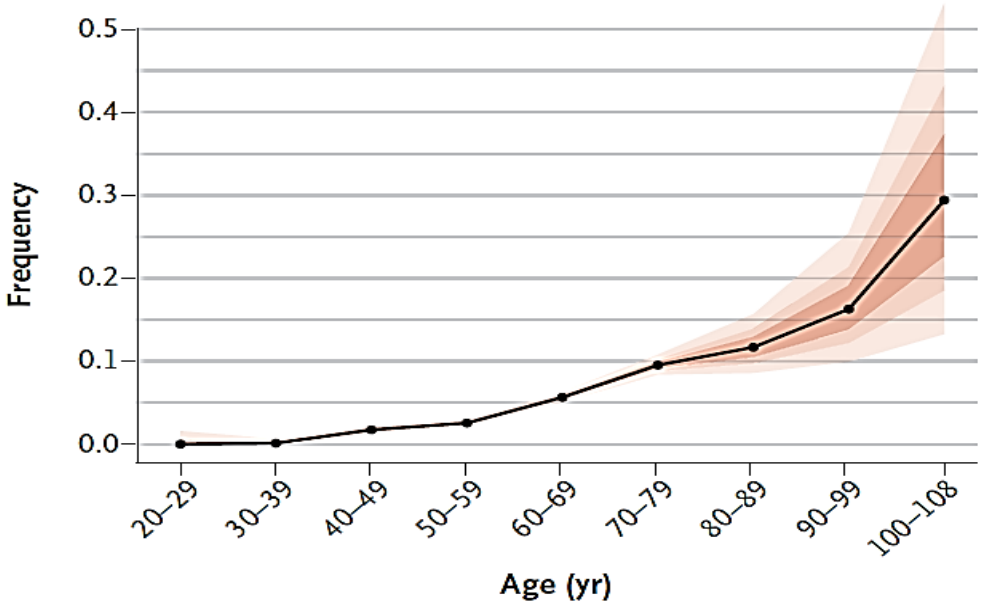
Monitor the patient over time, repeat blood, marrow examinations and other studies as dictated by clinical circumstances

ICUS vs. IDUS

ICUS: Idiopathic Cytopenia	IDUS: Idiopathic Dysplasia
• Cytopenia (Hgb<11, neutrophils <1.5K, PLT <100K) persistent for at least 6 months	• Normal CBC
• Does not meet minimal Dx criteria for MDS: – >10% dysplastic cells or – 5-19% blasts or – Abnormal karyotype typical for MDS	• Not meet minimal criteria for MDS
• Other causes of cytopenia ruled out; carefully monitor • Does not require evidence of clonal population	• Dysplastic changes: – Pseudo-Pelger-Huet cells – Megaloblastoid changes in normoblasts

Greenberg PL, et al. J Natl Compr Canc Netw 2017; 15(1): 60–87
Greenberg PL, et al. Blood 2016;128(16):2096-2097
Valent P, et al. Leukemia Research 2007:727-736

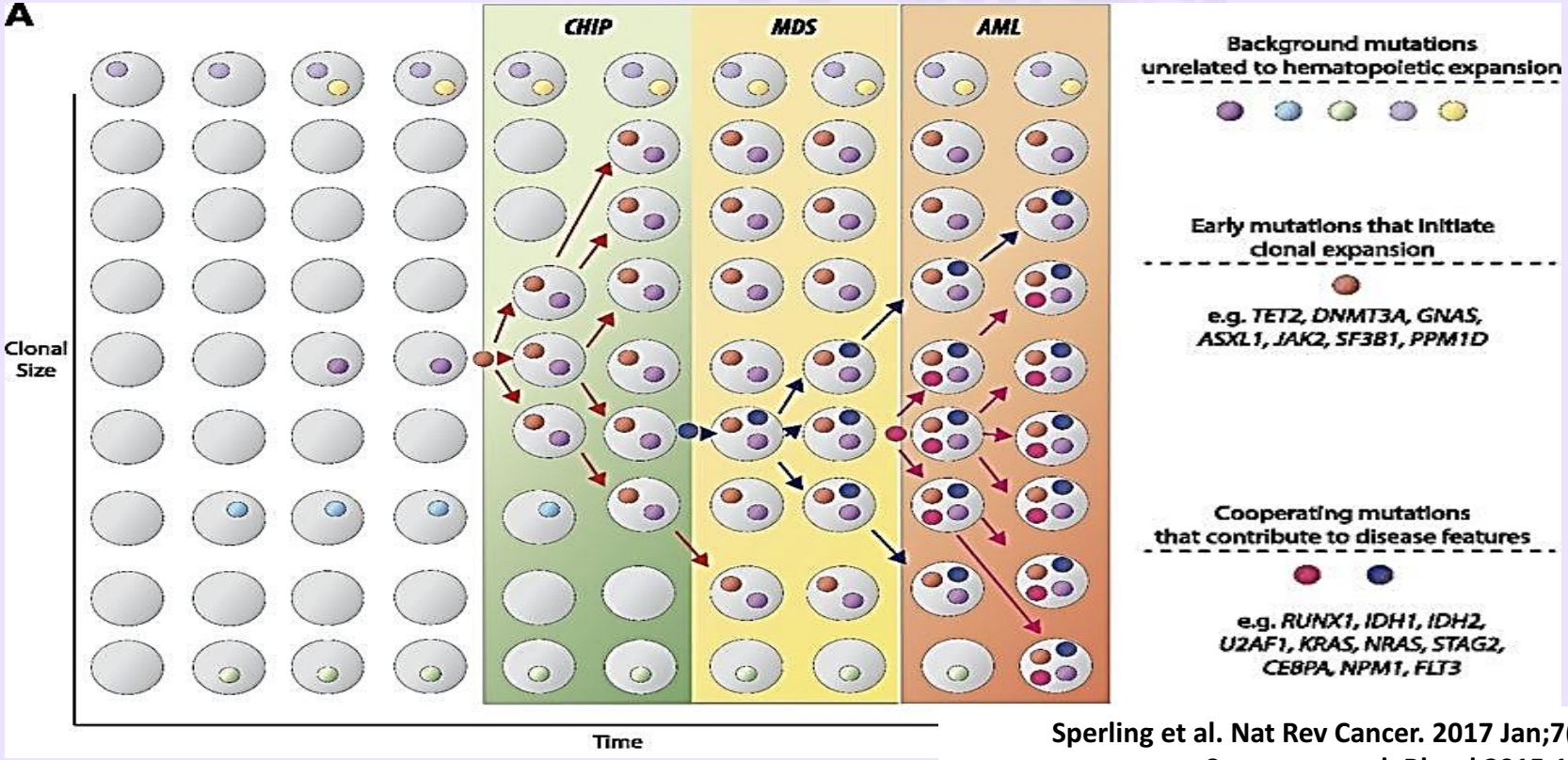
Age-related clonal hematopoiesis



No. with Mutation	0	1	50	138	282	219	37	14	5
Total	240	855	2894	5441	5002	2300	317	86	17

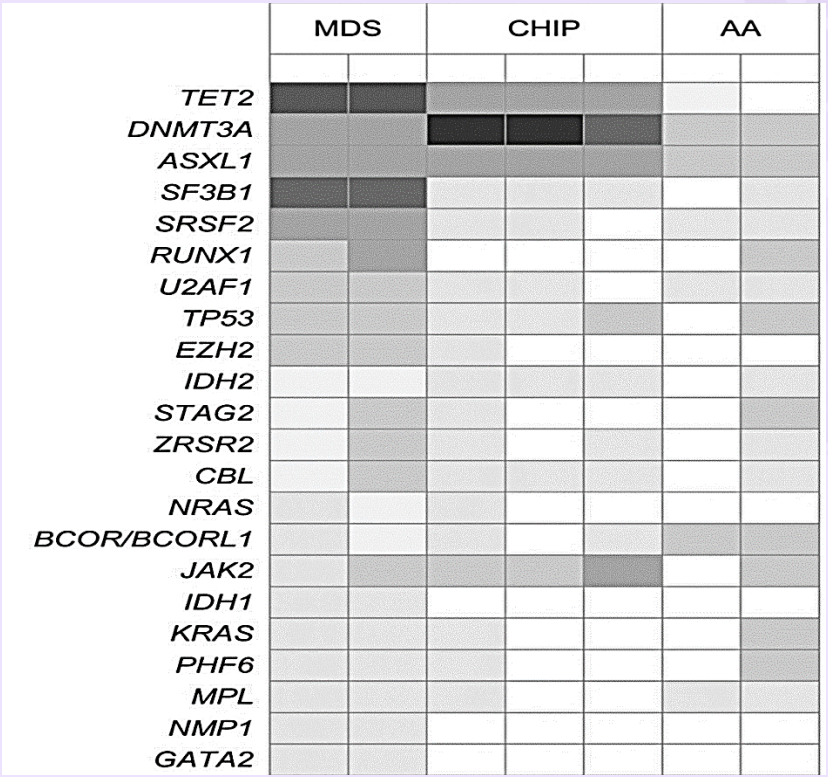
Genovese et al. N Engl J Med 2014;371:2477-87
Jaiswal S. N Engl J Med. 2014;371:2488

CHIP: precursor for hematological neoplasms



Sperling et al. Nat Rev Cancer. 2017 Jan;7(1):5-19
Steensma et al. Blood 2015;126:9-16

Frequency of Somatic Mutations in MDS, CHIP and AA



Mutation Prevalence	≥50	20-49	10-19	5-9	1-4

Luca Malcovati and Mario Cazzola Hematology 2015:299-307

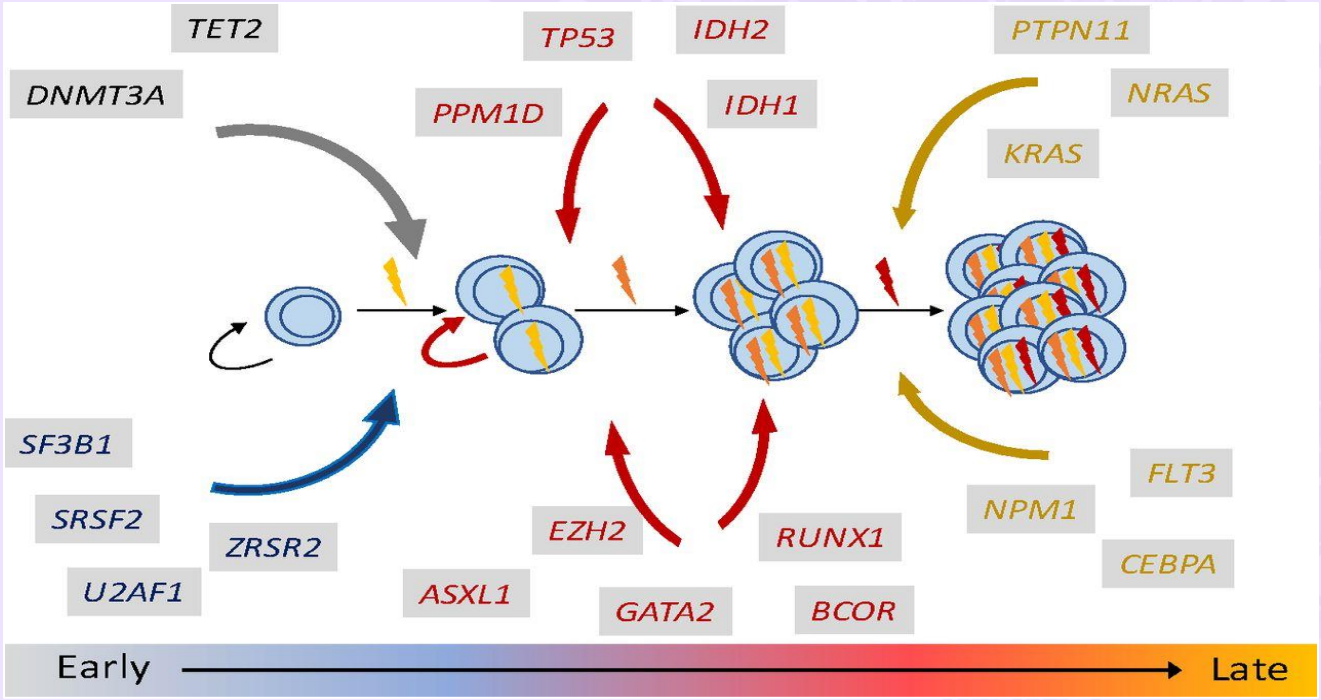
Spectrum of Indolent Myeloid Hematopoietic Disorders

Feature	ICUS	IDUS	CHIP	CCUS	MDS
Somatic mutation	-	-	+/-	+/-	+/-
Clonal karyotypic abnormality	-	-	+/-	+/-	+/-
Marrow dysplasia	-	+	-	-	+
Cytopenia	+	-	-	+	+

- Regular monitoring (every 6 mo.)
- Clonality: either has karyotypic abnormality (≥2 metaphases) and/or a somatic mutation (>2% variant allele frequency)
- NGS should include at lease the 21 most frequently mutated MDS-related genes
- Rarely mutated genes can also provide evidence for CHIP or CCUS

Greenberg PL, et al. J Natl Compr Canc Netw 2017;15(1):60–87

Gene mutations have stereotyped positions in the MDS clonal hierarchy



R. Coleman Lindsley Hematology 2017;2017:447-452

Outcome implications of gene mutations

Gene	Pathway	Frequency	Prognostic impact
SF3B1	RNA splicing	20-30%	Favourable
TET2	DNA methylation	20-30%	Unknown
ASXL1	Histone modification	15-20%	Adverse
SRSF2a	RNA splicing	15%	Adverse
DNMT3Aa	DNA methylation	10%	Adverse
RUNX1	Transcription	10%	Adverse
U2AF1	RNA splicing	5-10%	Adverse
TP53	Tumour suppressor	5-10%	Adverse
EZH2	Histone modification	5-10%	Adverse
ZRSR2	RNA splicing	5-10%	Unknown
STAG2	Cohesin complex	5-7%	Adverse
IDH1/IDH2	DNA methylation	5%	Adverse
CBL	Signalling	5%	Adverse
NRAS	Transcription	5%	Adverse
BCOR	Transcription	5%	Adverse

2016 WHO

Distinctive clinicopathologic associations

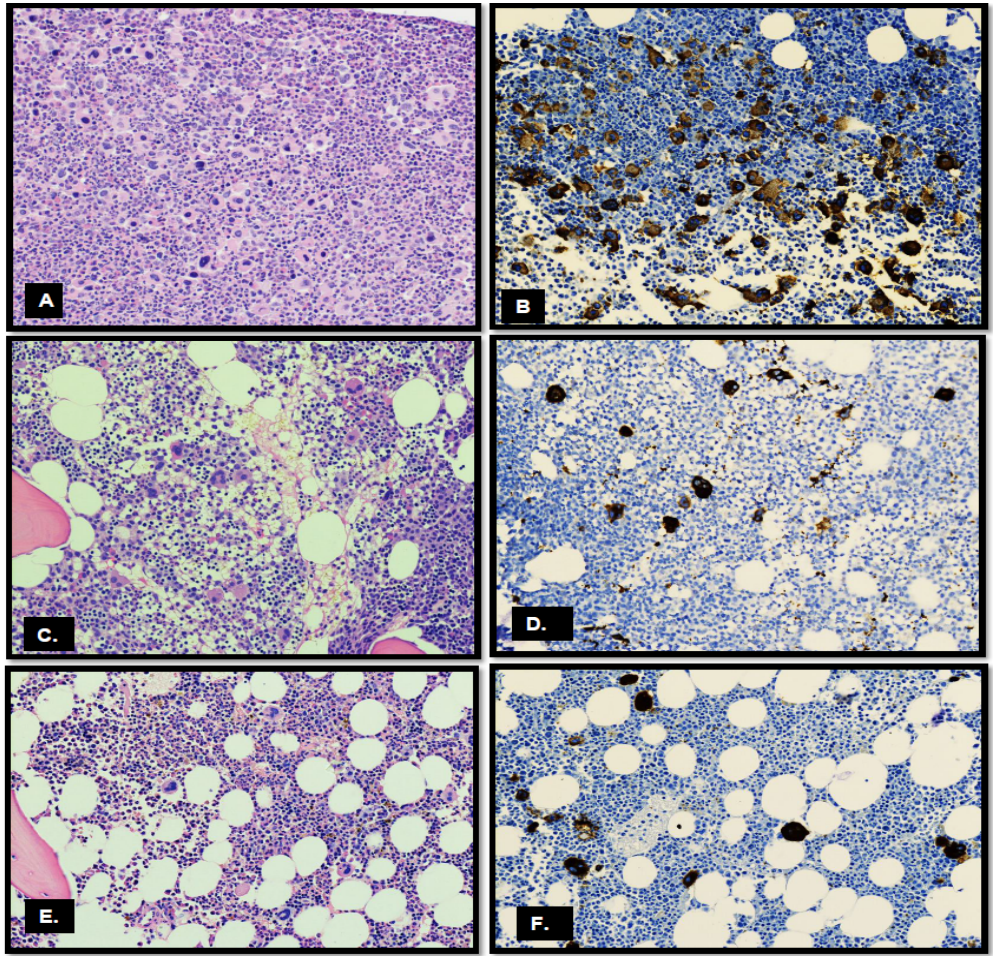
Del 5q	Female predominance; non-lobated MKs; Response to lenalidomide
Del 17p	PPH neutrophils; *TP53* mutation
Isochromosome 17q	MDS/MPN with fibrosis; PPH neutrophils; *ASXL1, SRSF2, SETBP1* mutation; aggressive behavior
Del 20q	Thrombocytopenia; Dysmegakaryopoiesis
Inv(3)	Thrombocytosis, abnormal MKs

Table 1. Pre-CML Cases: Indications for Bone Marrow Study andInitial Peripheral Blood Counts

Pre-CML Patients	Age (Years)	Gender	Indications for bone marrow biopsy	WBC (10^9/L)	Hemoglobin (g/dL)	Platelets (10^9/L)	Neutrophils* (%)	Eosinophils* (%)	Basophils* (%)	Blasts* (%)
1	62	M	Anemia and thrombocytopenia	6.2	10.5	197	61	1	5	0
2	67	F	Persistent leukocytosis and BCR-ABL1 fusion gene discovered in peripheral blood	14.3	13.5	316	69	5	4	0
3	44	F	Leukopenia and neutropenia	3.6	11.4	225	50	0	2	0
4	70	M	Follicular lymphoma bone marrow staging	12.8	13.6	144	73	2	2	0
5	70	M	Mild leukocytosis and thrombocytopenia	14.7	13.4	89	59	1	0	0

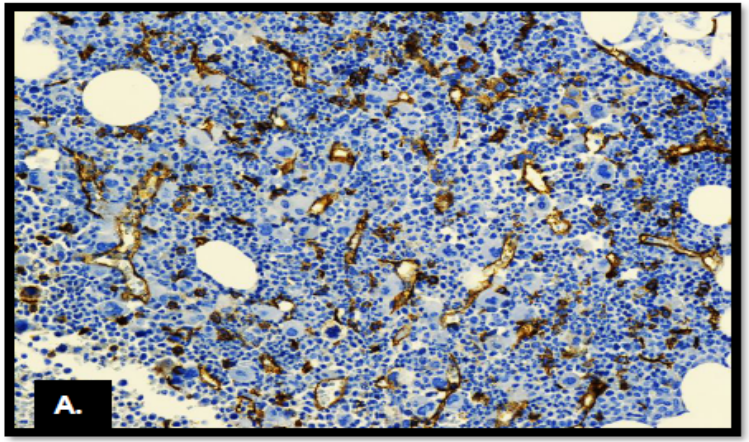
*Based on 100 cells differential count

A. Megakaryocytes in CML-CP are mostly small and hypolobated.

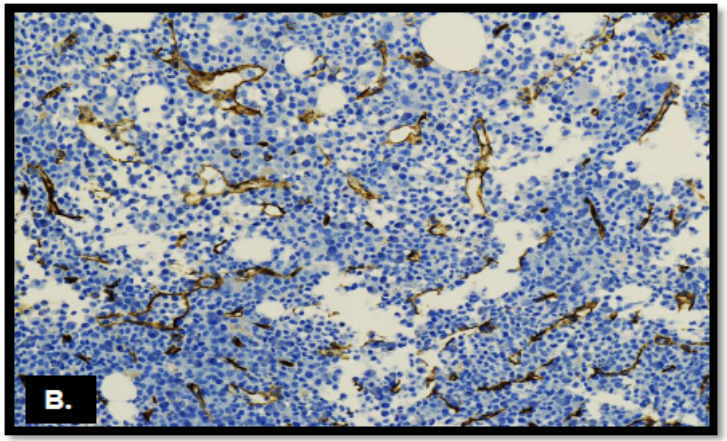


B. Megakaryocytes in pre-CML are an admixture of normal appearing cells, and small, hypolobated forms.

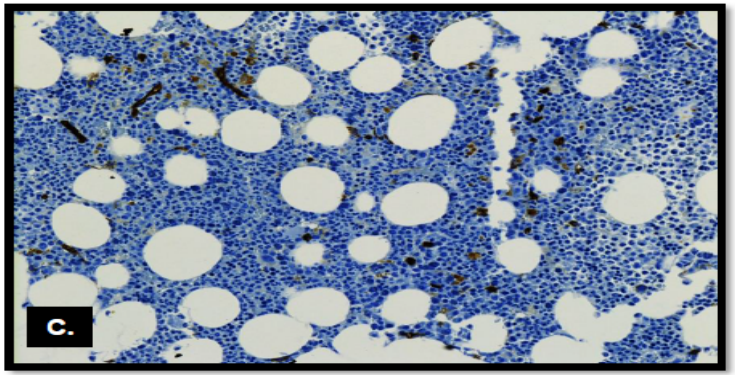
C. Megakaryocytes in leukemoid reaction have a normal appearance.



A. Microvessels in CML-CP are tortuous with abnormal branching.



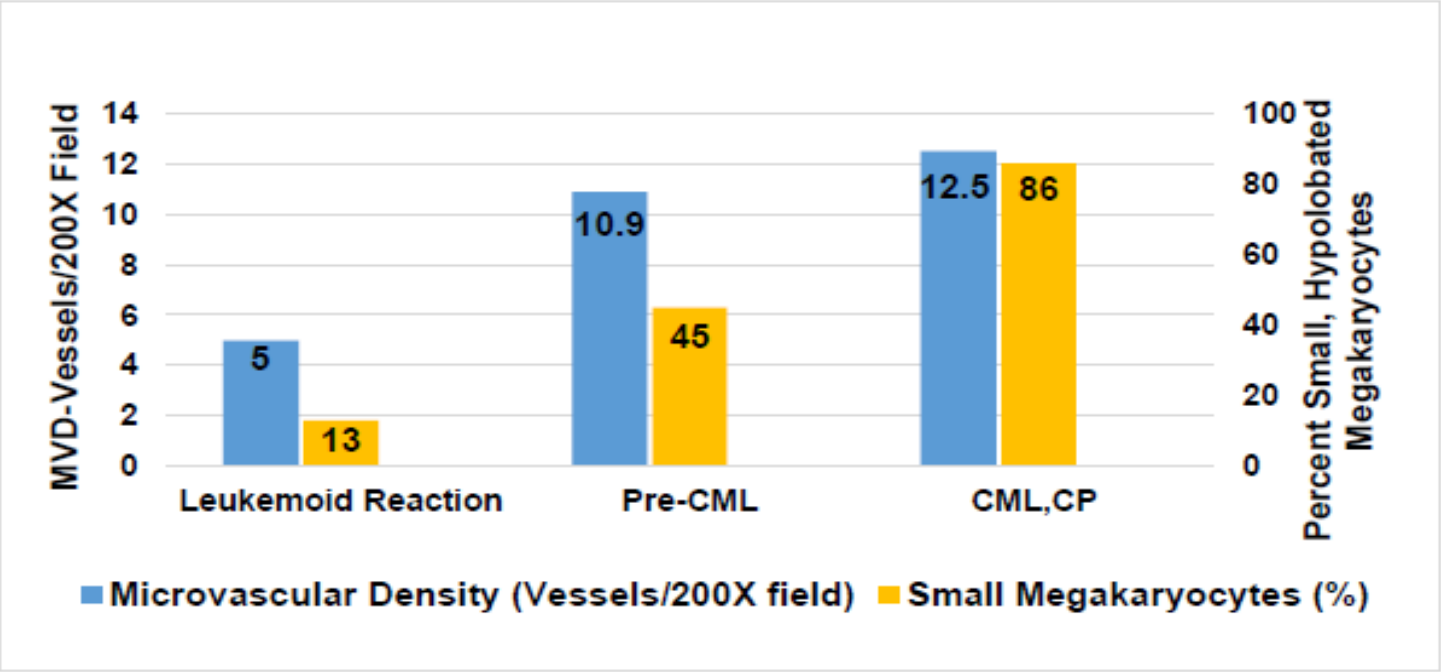
B. The vascular branching and tortuosity of microvessels are less pronounced in Pre-CML.



C. Microvessels in leukemoid reaction are few in number and generally straight. CD34 immunostains; x200.

Comparison of microvascular density and percentage of small megakaryocytes

Figure 1.



Summary of MDS genetics

MDS pathogenesis involves dysfunction of 8 cellular pathways

- MicroRNA: let-7a, miR-16, miR-144/451
- Telomere dysfunction
- Epigenetic regulators: *TET2*, *ASXL1*, *EZH2*
- RNA splicing: *SF3B1*, *SRSF2*, *U2AF1*
- Cohesin complex: *STAG2, RAD21, SMC3*
- DNA damage response: *TP53*
- Transcription factors: *RUNX1*, *ETV6*
- Tyrosine kinase signaling: *JAK2*, *NRAS*, *KRAS*, *BRAF*
- Ribosome: haploinsufficiency for *RPS14*

Mutations are powerfully associated with clinical features

- Mutations in 5 genes are independent predictors of overall survival
  - *TP53*, *EZH2*, *ASXL1*, *RUNX1*, *ASXL1*
- Individual lesions associated with a specific clinical phenotype
  - Del(5q): 5q- syndrome
  - *TP53*: complex karyotype
  - *SF3B1*: MDS-RS
  - i(17)(q10): wild-type *TP53* and mutated *SETBP1* (54%)

Conclusion

Classification: Defining more homogeneous MDS subtypes

MDS with characteristic genetic events:

- del(5q): haploinsufficiency of *RPS14, SPARC*
- MDS-RS: perturbation of genes involved in mitochondrial metabolism, mut SF3B1
- trisomy 8: genes involved in inflammatory and immune responses