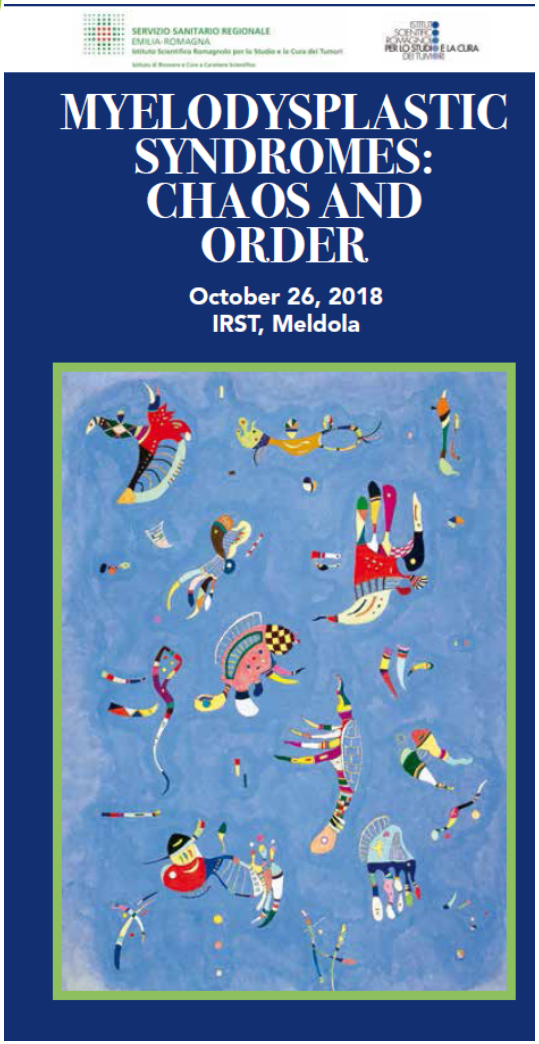


MDS classificazione WHO 2017

8.15 **Welcome by IRST Scientific Director**
G. Martinelli (Meldola-FC)

SESSION I

BIOLOGY OF MDS:
From the genes to stem cells and microenvironment
Chairmen: *A. Lucchesi (Meldola-FC)*
G. Musuraca (Meldola-FC)

8.30 **Genetic alterations in MDS**
G. Martinelli (Meldola-FC)

9.00 **Hematopoietic stem cells and Mesenchymal stem cells in MDS**
M. Krampera (Verona)

9.30 **Dysregulation of the immune system and inflammation in MDS**
M. Bonafé (Bologna)

10.00 Coffe break

SESSION II

DIAGNOSIS AND PROGNOSIS
Chairman: *A.L. Molinari (Rimini)*

10.20 **WHO Classification**
E. Sabattini (Bologna)

10.50 **Prognostic factors in MDS**
E.N. Oliva (Reggio Calabria)

11.20 **CMML**
R. Itzykson (Paris-FR)

11.50 **Aplastic anemia/hypoplastic MDS**
A. Risitano (Napoli)

12.20 Lunch

Neoplasms with proliferation and apoptosis of hameatopoietic stem cells

Defined by
(1) cytopenia
(2) dysplasia (>10%) of one or more of myeloid lineage
(3) ineffective haemopoiesis
(4) recurrent genetic abnormalities
(5) higher risk of developing acute leukaemia

- WHO approach: **combination** of morphology, immunophenotype and genetic features to define distinct clinicopathologic disease entities, **independently** from the underlying causes that are often unknown
  - refinement in morphologic interpretation assessing the rapidly accumulating genetic information useful for diagnosis and prognosis

(1) cytopenia

WHO values:
Hb <10g/dL, PLT <100x10^9L, neutrophil <1.8x10^9L
IPSS-R (2012): absolute neutrophil count <0.8x10^9L

*laboratory variability
*ethnies with lower neutrophil count
*caution in interpreting neutropenia if only cytopenia

*possible milder levels of cytopenia (Hb <13g/dL men or 12g/dL women, PLT <150x10^9L) allow MDS diagnosis if at least one cytopenia is present and/or morphologic and cytogenetic findings are present

Greenberg et al. Blood 1997-1998; Greenberg et al. Blood 2012;

Although cytopenia is a «sine qua non» condition for MDS, classification mostly relies on degree of dysplasia and blast percentage while the **type of cytopenia with minor impact on classification**
Type of cytopenias is captured by IPSS-R

*Type of cytopenia **does not always correspond** to lineage dysplasia nella RCUD
*Type and severity of dysplasia cannot predict peripheral count abnormalities

Not referred to type of cytopenia but generally «myelodysplasia»

New terminology

MDS with Single Lineage Dysplasia (MDS-SLD)	RCUD
MDS with Single Lineage Dysplasia (MDS-SLD) with Ring Sideroblasts	RARS
MDS with Multi Lineage Dysplasia (MDS-MLD)	RCMD
MDS with Multi Lineage Dysplasia (MDS-MLD) with Ring Sideroblasts	RCMD-RS
MDS with isolated del(5q)	
MDS with Excess Blasts	
type 1 (MDS-EB1)	RAEB-1
type 2 (MDS-EB2)	RAEB-2
MDS, unclassifiable (MDS-U)	

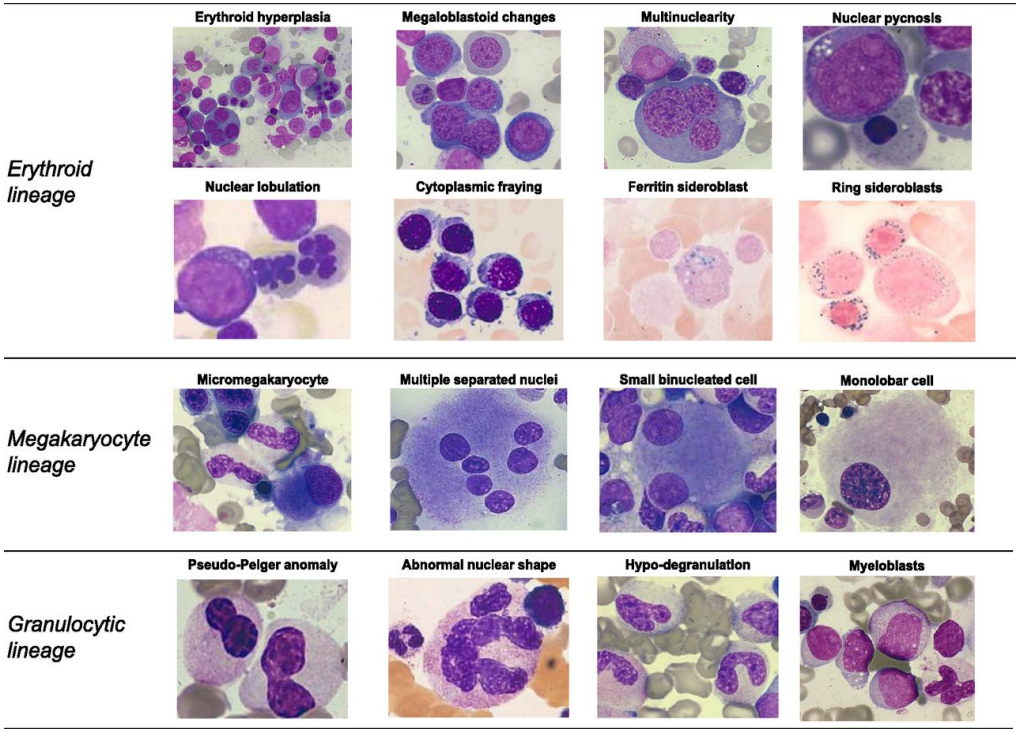
Remove reference to anaemia/cytopenia from names

"Myelodysplastic syndrome, consistent with refractory cytopenia with unilineage dysplasia"

Call it MDS directly

"Myelodysplastic syndrome, with single lineage dysplasia"

(2) dysplasia
Specified Qualifiers of MDS: *(1) Single/Multilineage*



Mario Cazzola et al. Blood 2013;122:4021-4034

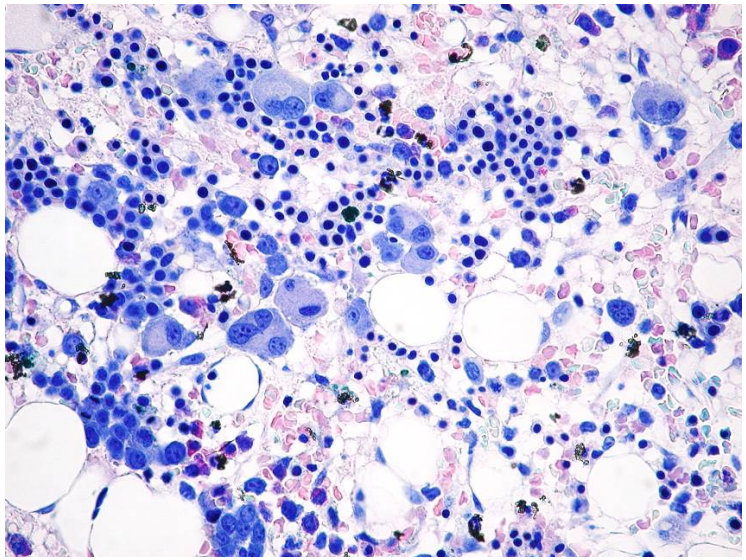
WHO 2017 – Table 6 pag 102

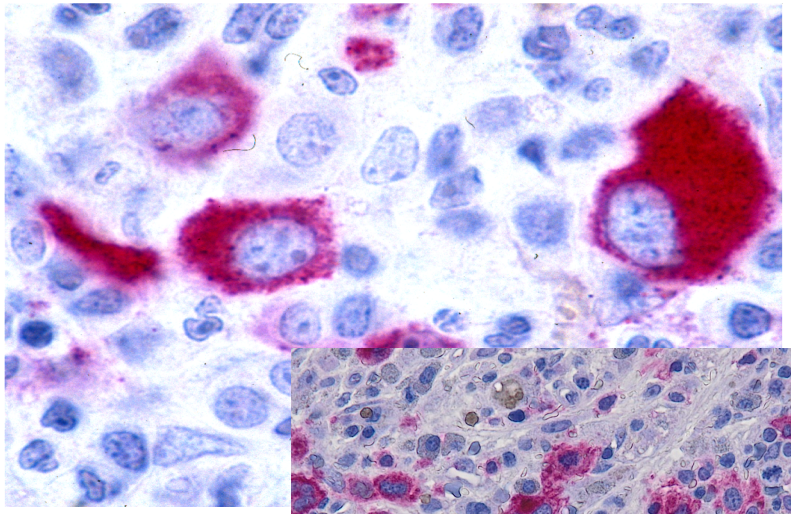
Nuclear: nuclear budding, internuclear bridging, karyorexis, multinuclearity, megaloblastoid changes

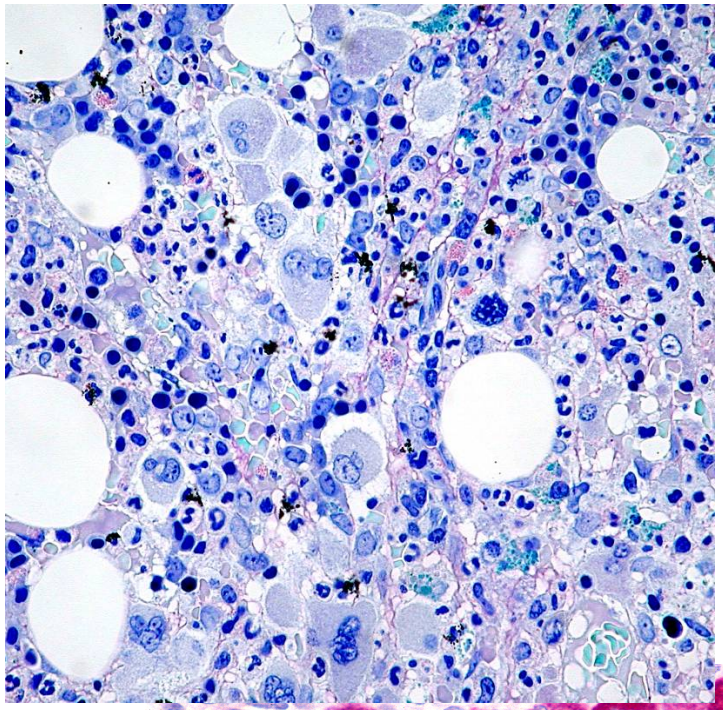
Cytoplasmic: ring sideroblasts, vacuolization, PAS positivity

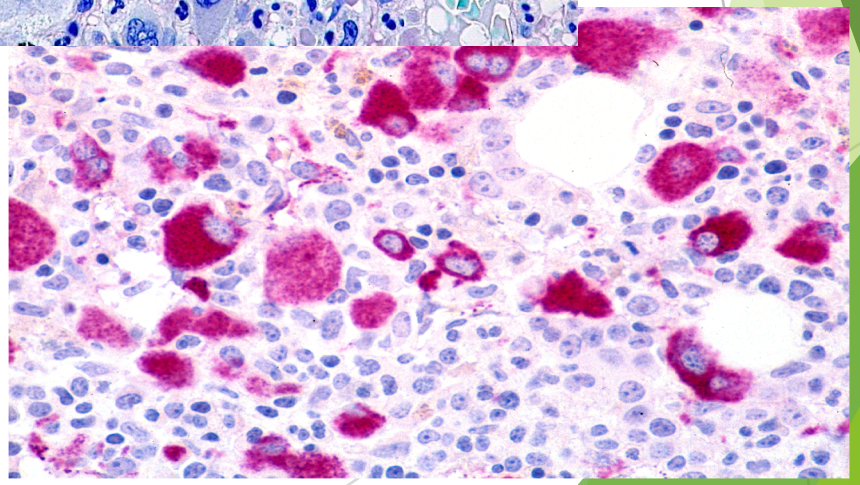
microMKC, nuclear hypolobation, multinucleation (no multilobation)

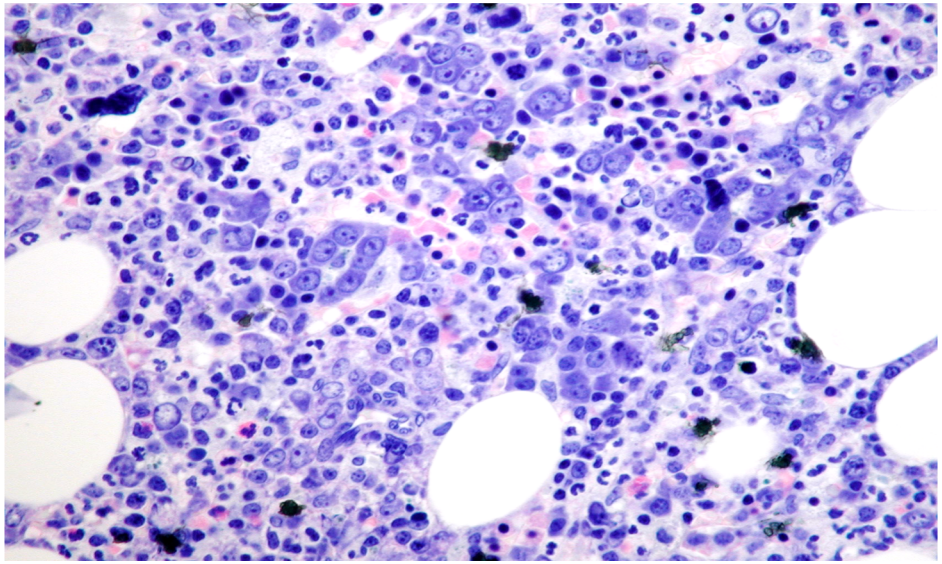
Nuclear: small/unusually large size, hyposegmentation (pseudoPelger-Huet), hypersegmentation
Cytoplasmic: decreased/agranularity, pseudo Chediak-Higashi, Dohle bodies, Auer rods

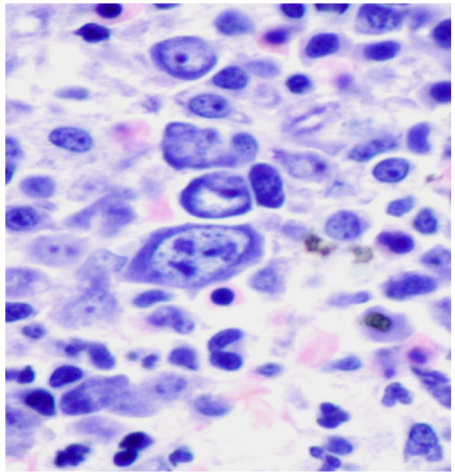


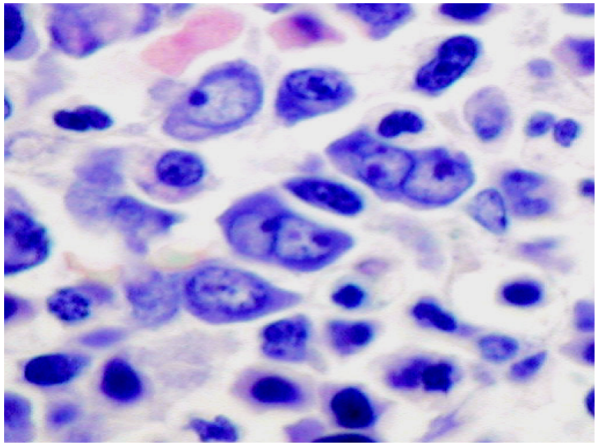


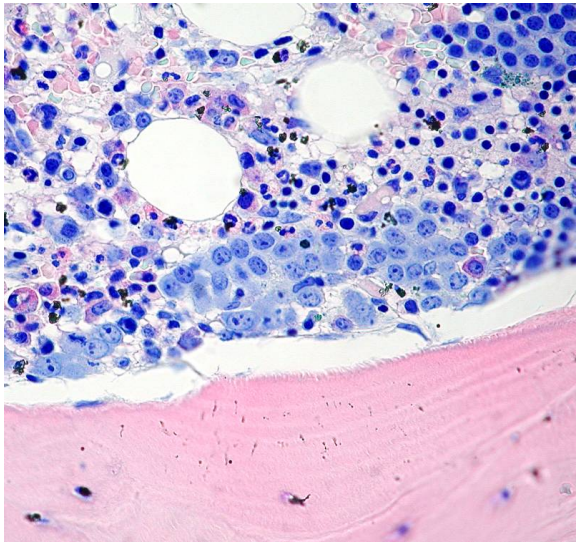


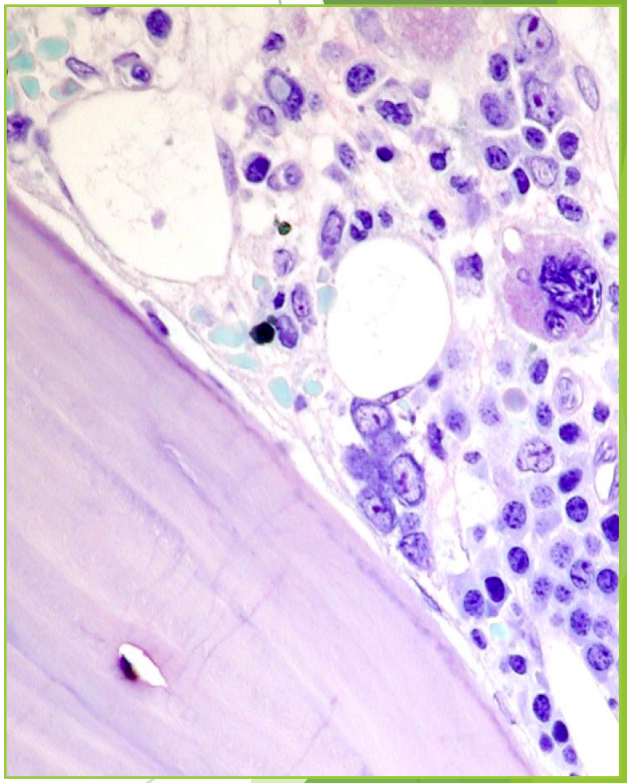


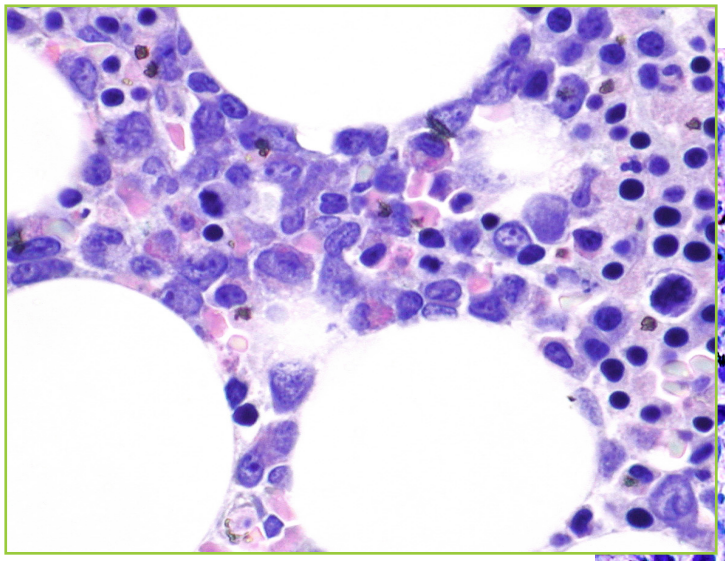


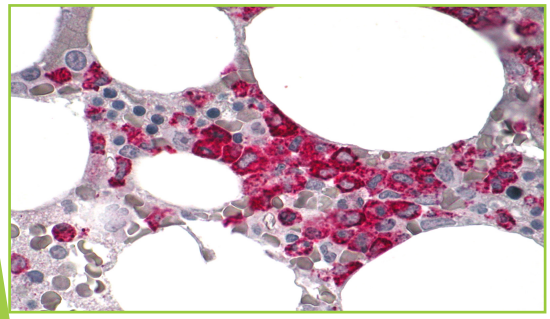


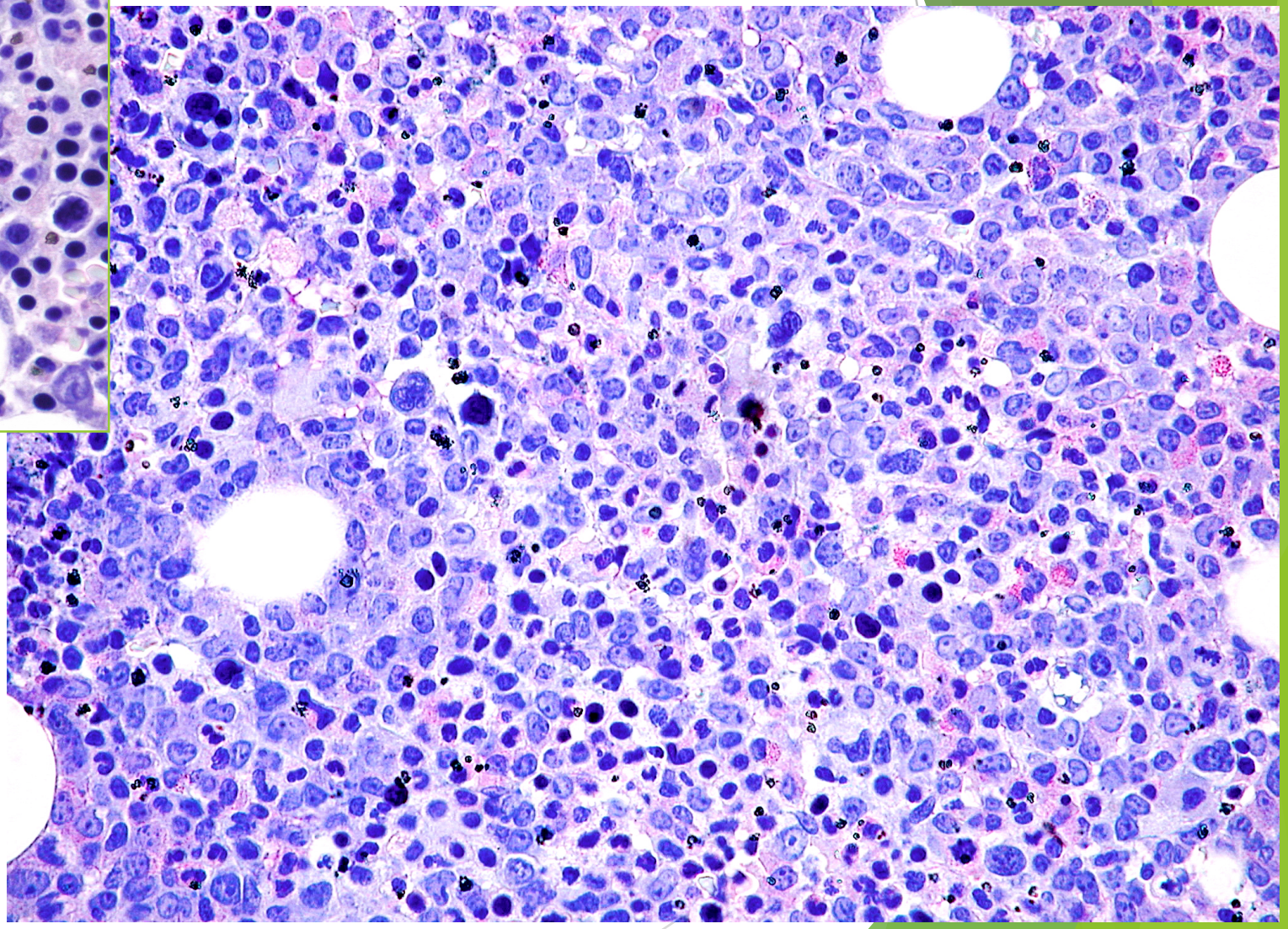


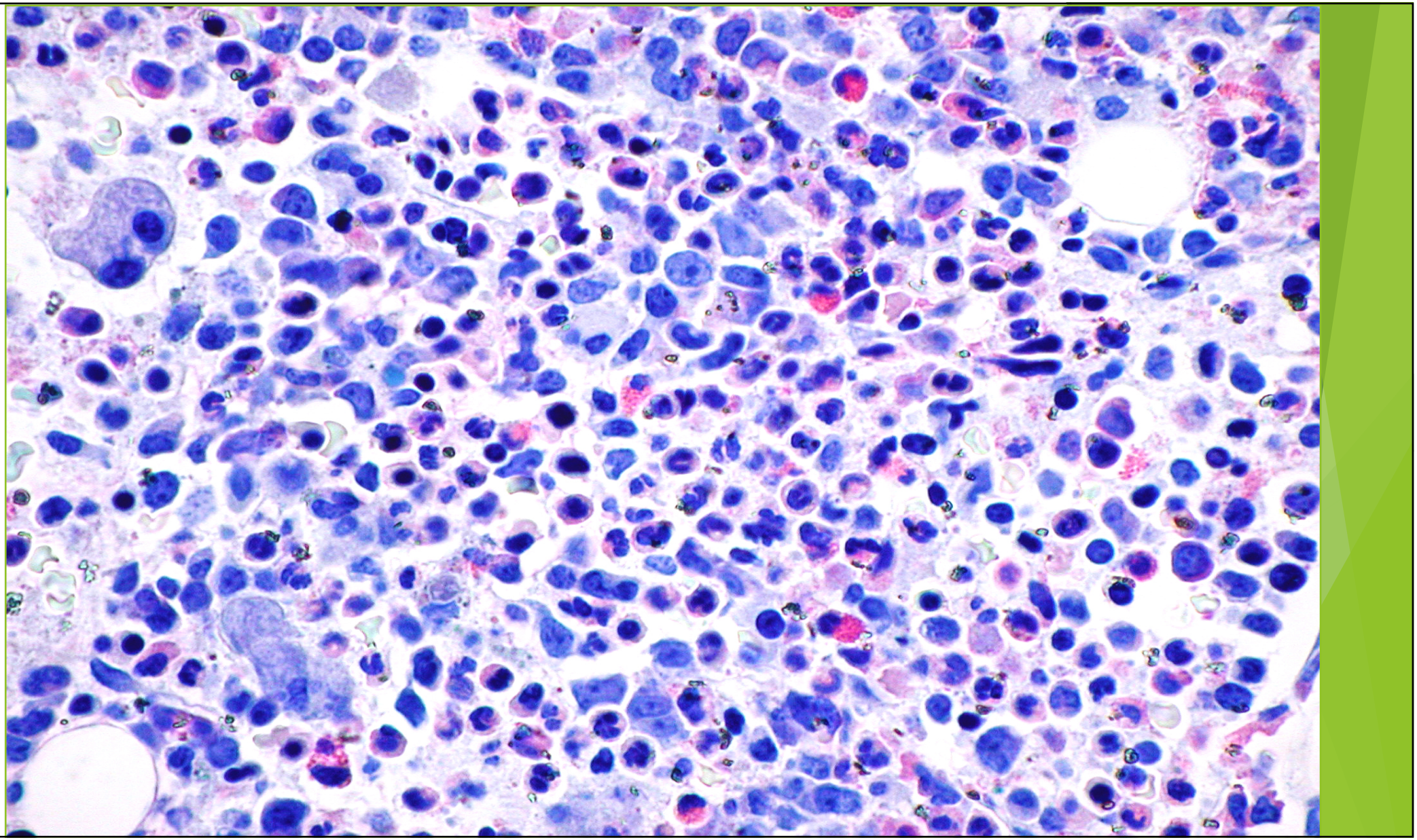












(2) dysplasia
Specified Qualifiers of MDS: *(1) Single/Multilineage*

•thresholds to define as significant the percentage of dysplastic cells, are admittedly arbitrary and limitations have emerged in the adopted criteria since their first proposal in 2001

•possible impact of interobserver variability in defining dysplasia
higher in low grade MDS where it is near the 10% requisite;

•possible >10% dysplasia in non cytopenic pts and in non clonal disorders;
•need for optimal slide preparations and application of strict criteria for dysplasia

single: >erythroid lineage;
E/G ≥10%;
M ≥10% on 30 MKCs (some studies reach 30-40% for higher specificity);

Much effort has been made to standardize morphologic parameters, and to define minimal diagnostic criteria.

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 43), pp: 73483-73500

Priority Review

Proposed minimal diagnostic criteria for myelodysplastic syndromes (MDS) and potential pre-MDS conditions

Peter Valent[1,2], Attilio Orazi[3], David P. Steensma[4], Benjamin L. Ebert[5], Detlef Haase[6], Luca Malcovati[7], Arjan A. van de Loosdrecht[8], Torsten Haferlach[9], Theresia M. Westers[8], Denise A. Wells[10], Aristoteles Giagounidis[11], Michael Loken[10], Alberto Orfao[12], Michael Lübbert[13], Arnold Ganser[14], Wolf-Karsten Hofmann[15], Kiyoyuki Ogata[16], Julie Schanz[6], Marie C. Béné[17], Gregor Hoermann[18], Wolfgang R. Sperr[1,2], Karl Sotlar[19], Peter Bettelheim[20], Reinhard Stauder[21], Michael Pfeilstöcker[22], Hans-Peter Horny[23], Ulrich Germing[24], Peter Greenberg[25] and John M. Bennett[26]

Leukemia (2015) **29**, 66–75

www.nature.com/leu

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

Table 1: Proposed minimal diagnostic criteria of MDS*

A. Prerequisite Criteria (both must be fulfilled)

- Persistent (4 months) peripheral blood cytopenia** in one or more of the following lineages: erythroid cells, neutrophils, platelets (exception: in the presence of a blast cell excess and MDS-related cytogenetic abnormalities the diagnosis of MDS can be established without delay)

- Exclusion of all other hematopoietic or non-hematopoietic disorders as primary reason for cytopenia/dysplasia***

B. MDS-Related (Major) Criteria (at least one must be fulfilled)

- Dysplasia in at least 10% of all cells in one of the following lineages in the bone marrow smear: erythroid; neutrophilic; megakaryocytic****

- ≥15% ring sideroblasts (iron stain) or ≥5% ring sideroblasts (iron stain) in the presence of *SF3B1* mutation

- 5-19% myeloblasts on bone marrow smears (or 2-19% myeloblasts on blood smears)

- Typical chromosome abnormality(ies) by conventional karyotyping or FISH*****

C. Co-Criteria (for patients fulfilling A but not B, and otherwise show typical clinical features, e.g. macrocytic transfusion-dependent anemia; two or more of these co-criteria must be fulfilled for considering a provisional diagnosis of MDS)

- Abnormal findings in histologic and/or immunohistochemical studies of bone marrow biopsy sections supporting the diagnosis of MDS****

- Abnormal immunophenotype of bone marrow cells by flow cytometry, with multiple MDS-associated phenotypic aberrancies indicating the presence of a monoclonal population of erythroid and/or myeloid cells

- Evidence of a clonal population of myeloid cells determined by molecular (sequencing) studies revealing MDS-related mutations******

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 43), pp: 73483-73500

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*The diagnosis of MDS can be established when both prerequisite criteria (´A´) and at least one major criterion (´B´) are fulfilled. If no major criterion is fulfilled, but the patient is likely to suffer from a clonal myeloid disease, co-criteria (´C´) should be applied and may help in reaching the conclusion that the patient has a myloid neoplasm resembling MDS or will develop MDS. In this diagnostic setting, repeated bone marrow investigations during follow-up may be required to arrive at a final diagnosis of MDS.

**Cytopenia defined by local institutional reference values.

***As more and more patients with two co-existing bone marrow neoplasms are diagnosed, it is important to state that in rare cases, MDS can be diagnosed even if another co-existing disease potentially causing cytopenia is also detected.

****Examples: clusters of abnormally localized immature precursors (ALIP); clusters of CD34+ blast cells; dysplastic micromegakaryocytes detected by immunohistochemistry (≥10% dysplastic megakaryocytes).

*****Typical chromosome abnormalities are those recurrently and typically found in MDS patients (e.g. 5q-, -7) and considered as indicative of MDS by the WHO even in the absence of morphologic criteria of MDS.

******Detection of multiple mutations typically seen in MDS (e.g. SF3B1) increases the likelihood that the patient suffers from MDS or will develop MDS.

Table 2. Calculation of the morphological score for the definition of bone marrow dysplasia

Morphological abnormalities[a]	*Cutoff values*[b]	*AUC*	*Cohen's K-coefficient (inter-observer agreement)*[c]	*Variable weighted score*[d]
Erythroid lineage				
Megaloblastoid changes	> 5%	0.814, $P<0.001$	0.83	2
Bi- or multinuclearity	> 3%	0.679, $P<0.001$	0.87	1
	> 5%	0.698, $P<0.001$		2
Nuclear lobulation or irregular contours	> 3%	0.674, $P<0.001$	0.84	1
Pyknosis	> 5%	0.677, $P<0.001$	0.81	1
Cytoplasmic fraying	⩾7%	0.602, $P<0.001$	0.82	1
Ring sideroblasts	> 5%	0.650, $P<0.001$	0.95	2
	⩾15%	0.719, $P<0.001$		3
Ferritin sideroblasts	⩾30%	0.670, $P<0.001$	0.92	1
Granulocytic lineage				
Myeloblasts	> 3%	0.777, $P<0.001$	0.92	1
	> 5%	0.723, $P<0.001$		3
Auer rods	⩾1%	0.524, $P=0.001$	0.90	3
Pseudo Pelger–Hüet anomaly	> 3%	0.714, $P<0.001$	0.87	1
	> 5%	0.814, $P<0.001$		2
Abnormal nuclear shape	⩾7%	0.700, $P<0.001$	0.86	1
Neutrophil hypogranulation	> 3%	0.791, $P<0.001$	0.81	1
	> 5%	0.821, $P<0.001$		2
Megakaryocytic lineage				
Micromegakaryocytes	> 5%	0.916, $P<0.001$	0.88	3
Small binucleated megakaryocytes	> 5%	0.845, $P=0.001$	0.81	1
Megakaryocytes with multiple separated nuclei	> 5%	0.750, $P<0.001$	0.84	2
Hypolobated or monolobar megakaryocytes	> 5%	0.646, $P<0.001$	0.86	2

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*Morphological score : high sensitivity and specificity (>90%) even in patients with early-stage disease/wo specific markers of dysplasia and abnormal karyotype.

*The inter-observer reproducibility for the definition of each morphological variable associated with marrow dysplasia was satisfactory.

*None of the subjects without peripheral blood cytopenia (including healthy subjects) was incorrectly classified

*Morphological abnormalities involving 10% or more cells (mostly in erythroid lineage) were detected in a significant proportion of control patients affected with non-clonal cytopenia, and in some non-cytopenic controls

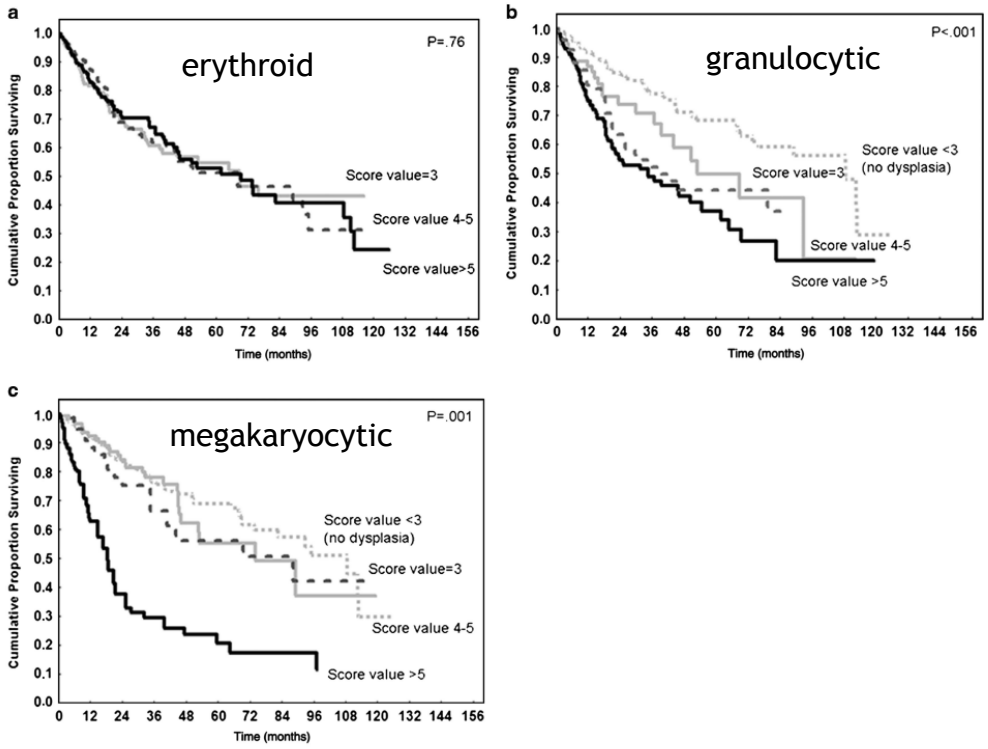


Figure 2. (**a**–**c**) Overall survival according to erythroid, granulocytic and megakaryocytic morphological score value; (**d**) overall survival according to the number of dysplastic hematopoietic lineages as defined by applying morphological scores.

*Granulocytic and megakaryocytic dysplasia significantly affected the probability of survival.

*The threshold of 10% of granulocytic dysplastic cells was the best cutoff to capture adverse prognosis

*Close relationship between RS and SF3B1 mutations Association between severe G dysplasia and mutations of ASXL1, RUNX1, TP53 and SRSF2 genes (reported to increase the risk of leukemic evolution)

*The threshold of 30% of dysplastic megakaryocytes appeared more appropriate to detect patients with reduced survival.

Cytomorphology review of 100 newly diagnosed lower-risk MDS patients in the European LeukemiaNet MDS (EUMDS) registry reveals a high inter-observer concordance.

de Swart L, Smith A, MacKenzie M, Symeonidis A, Neukirchen J, Mikulenková D, Vallespí T, Zini G, Paszkowska-Kowalewska M, Kruger A, **Saft L**, Fenaux P, Bowen D, Hellström-Lindberg E, Čermák J, Stauder R, Tatic A, Holm MS, Malcovati L, Mądry K, Droste J, Blijlevens N, de Witte T, Germing U.

Ann Hematol. 2017

IPSS low or intermediate-1 category, newly diagnosed by local cytologists.
blood and bone marrow slides of 10% of the first 1000 patients were reviewed by an 11-person panel of cytomorphologists. All slides were rated by at least 3 panel members (median 8 panel members; range 3–9). Marrow slides from 98 out of 105 patients were of good quality and therefore could be rated properly according to the WHO 2001 classification, including assessment of dysplastic lineages. The agreement between the reviewers whether the diagnosis was MDS or non-MDS was strong with an intra-class correlation coefficient (ICC) of 0.85. Six cases were detected not to fit the entry criteria of the registry, because they were diagnosed uniformly as CMML or AML by the panel members. The agreement by WHO 2001 classification was strong as well (ICC = 0.83). The concordance of the assessment of dysplastic lineages was substantial for megakaryopoiesis and myelopoiesis and moderate for erythropoiesis. Our data show that in general, the inter-observer agreement was high and a very low percentage of misdiagnosed cases had been entered into the EUMDS registry. Further studies including histomorphology are warranted.

Specified Qualifiers of MDS: Excess blasts (EB): BM 5-19%, PB 2-19%, w/o Auer rods

*blast percentage is critical in WHO risk-category definition
defined on marrow smears or trephine imprint on 500 cells; on 200 leukocyte differential in pb; less defined on bmb

blast percentage defined as percentage of *all nucleated marrow cells (including nucleated erythroid cells)

Achieve consistency of blast counting acrosso all myeloid neoplasms

Small changes in blast percentage can change diagnosis with major clinical impact

MAJOR CHANGE in myeloid neoplasm with ≥50% erythroid precursors
Erythoids may fluctuate due to therapy, metabolic deficiencies or EPO effects changing diagnosis

Avoid abrupt changes when erythroids reach 50%

erythroid/myeloid subtype of erythroid leukaemia now classified as MDS-EB

- Cases with ≥20% blasts and ≥50% erythroids will still be classified as AML (most are AML-MRC)
- Pure erythroleukemia will remain in AML

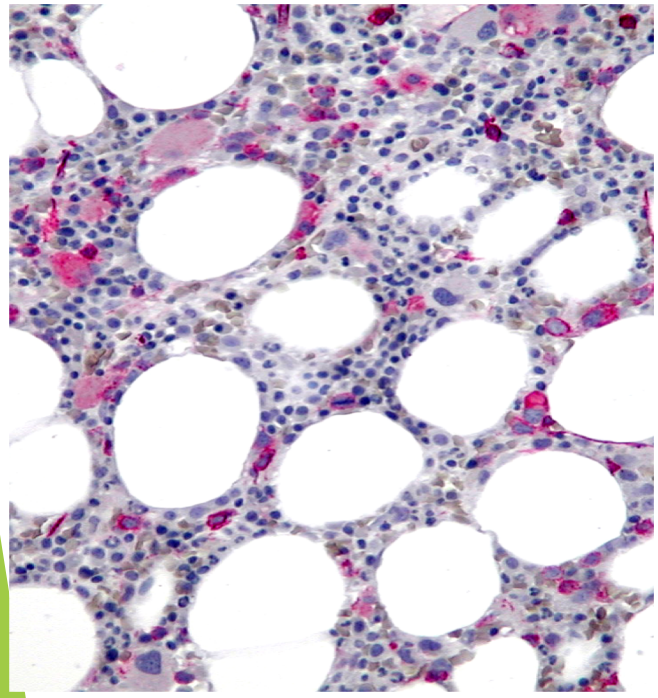
	N.Dysplastic lineages	N.cytopenias	RS (% erythoid cells)	PB-blasts M-blasts	Conventional Karyotyping
MDS-SLD	1	1-2	<15%, <5%	PB <1% M <5% no AuerRods	Any (no isolated 5q-)
MDS-MLD	2-3	1-3RS	<15%, <5%	PB <1% M <5% no AuerRods	Any (no isolated 5q-)
MDS-EB EB1 EB2	1-3	1-3	none	 PB 2-4% M 5-9% no AuerRods PB 5-19% M 10-19% or AuerRods	 Any Any

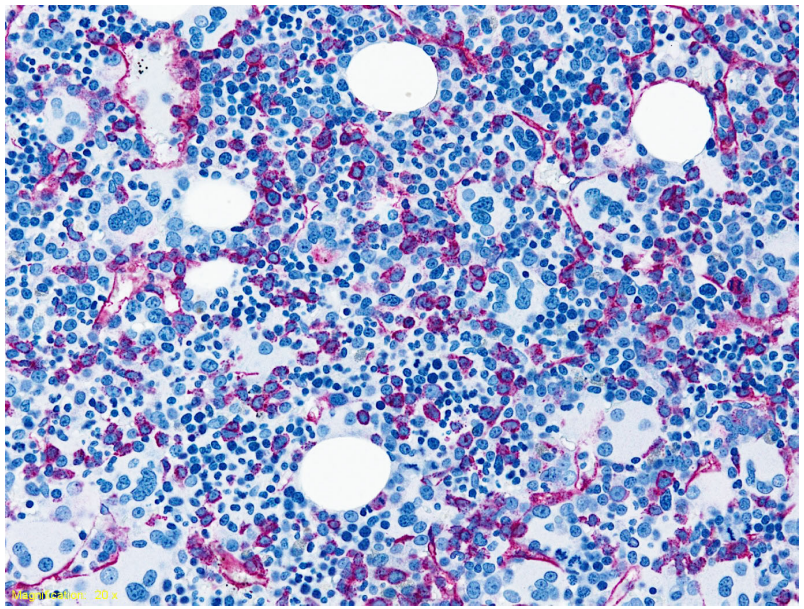
Blast count in bmb

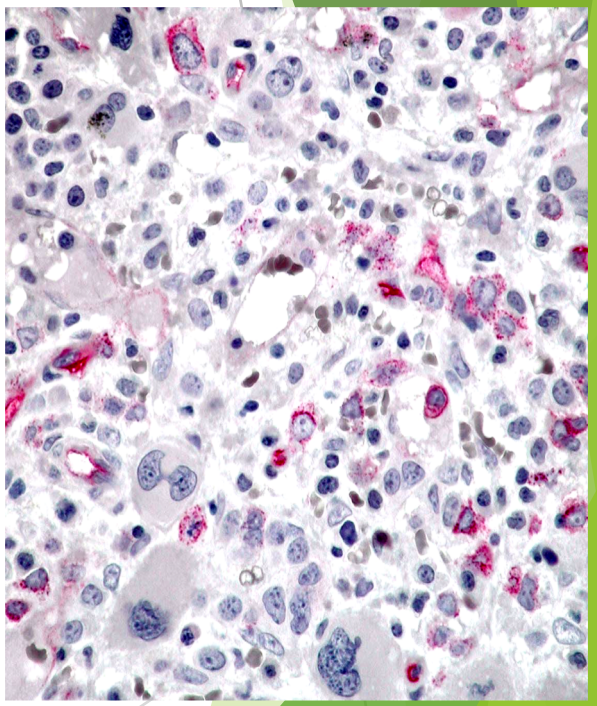
On CD34 positive cells; CD117 helpful in CD34 negative blasts

MINIMAL MORPHOLOGICAL CRITERIA FOR DEFINING BONE MARROW DYSPLASIA: A BASIS FOR CLINICAL IMPLEMENTATION OF WHO CLASSIFICATION OF MDS

Della Porta et al. Leukemia 2014





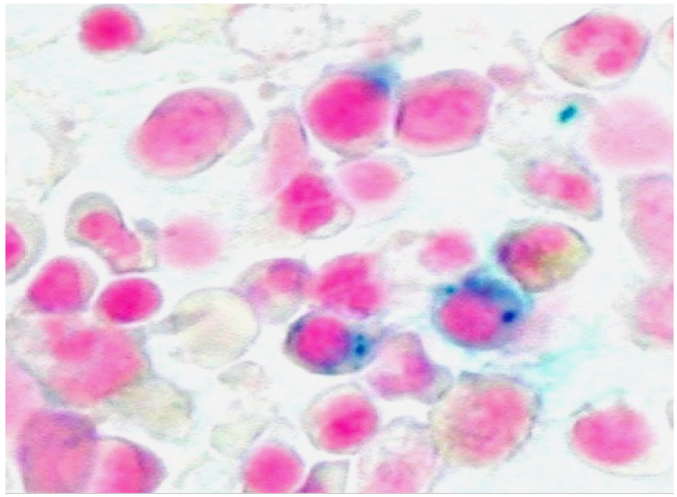


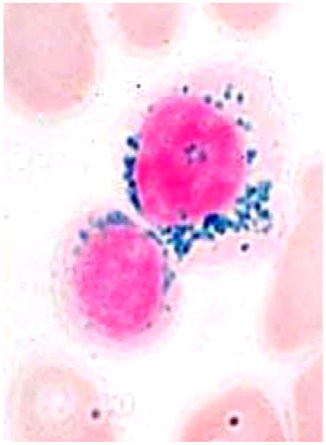
Working Group

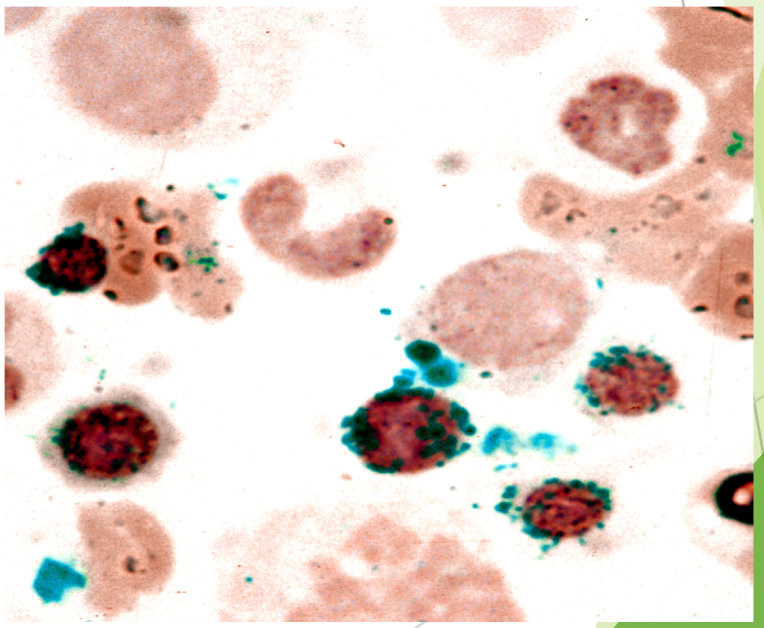
E.Torlakovic (CAN), Chair
A.Porwit (SWE), Co-Chair
E.Hyjek (USA)
C.Calvo (USA)
T.George (USA)
S.H.Lee (AUS)
E.Sabattini (ITA)
L.Saft (SWE)
A.Tzankov (CHE)
X.Zhou (CHN)

Specified Qualifiers of MDS: Ring sideroblasts (RS)

Not demonstrable in bmb
Usually ≥15% erythroid precursors
Secondary causes of RS need to be excluded
*heterozygous recurrent point mutations in splicesome gene SF3B1 strongly correlate (80-90% cases MDS-RS-sl; 30-70% MDS-RS-ml) with MDS-RS
*altered splicing of mitochondrial genes that encode for iron transporter proteins – affection of E differentiation
*driver abnormality
*n. of RS tend to correlate with allele burden







Specified Qualifiers of MDS: Ring sideroblasts (RS)

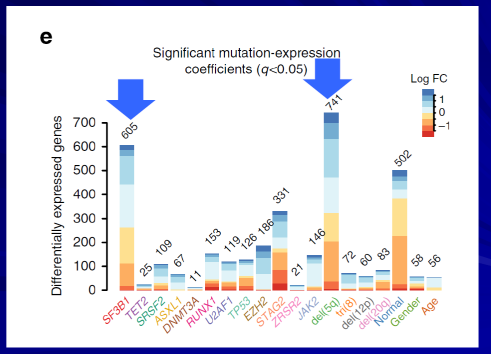
Since %RS is not prognostically relevant: RS at least ≥5% if SF3B1 mutation is present; at least 15% if mutation is not demonstrable;

specific/different GEP (Gerstung M et al. Nat Comm 2015);

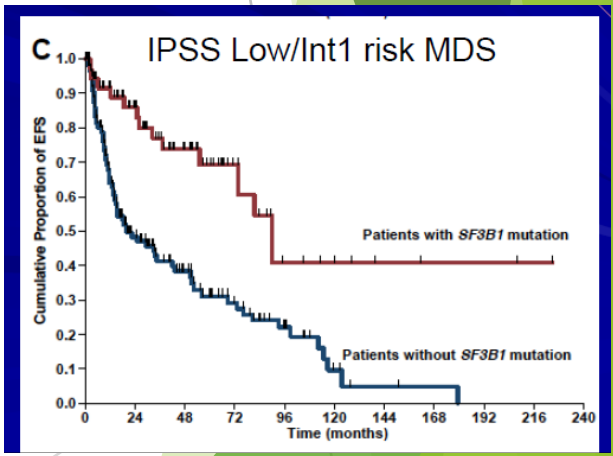
*SF3B1 mutation conveys favourable prognosis in low grade / int1 MDS uncertainties on SF3B1 mutation porgnostic influence is independent of multilineage dysplasia;

**MDS-RS-sl only dysplasia on E*
MDS-RS-ml

_SF3B1_ mutation is associated with highly differential gene expression

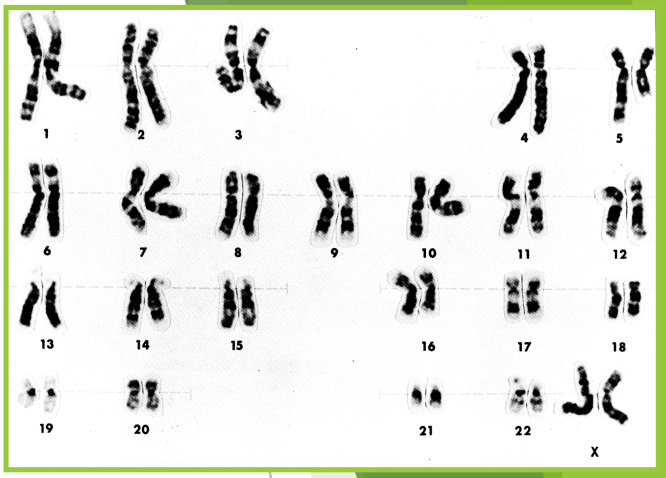


Gerstung M Nature Comm 2015;6:5901



Papaemmanuil E NEJM 2011;365:1384, Patniak MM Blood 2012;119:5674, Bejar R JCO 2012;30:3376, Malcovati L Blood 2011;118:6239, Cazzola M Blood 2013;121:260, Visconte V Blood 2012;120:3173

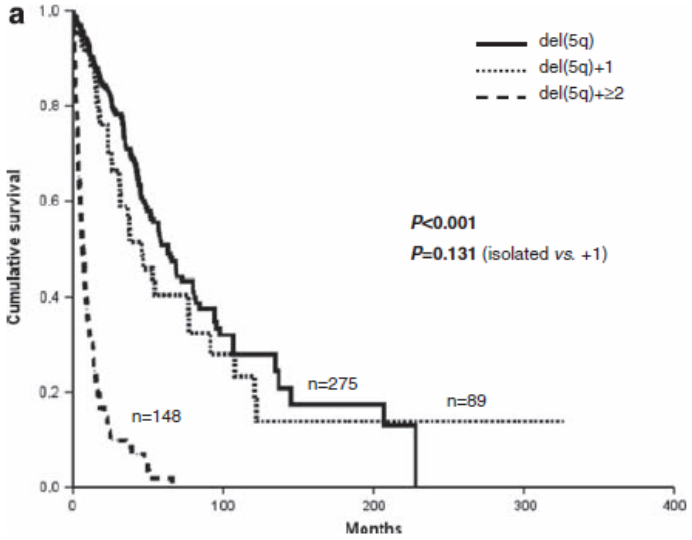
Specified Qualifiers of MDS: del 5q (as only cytogenetic abnormality)

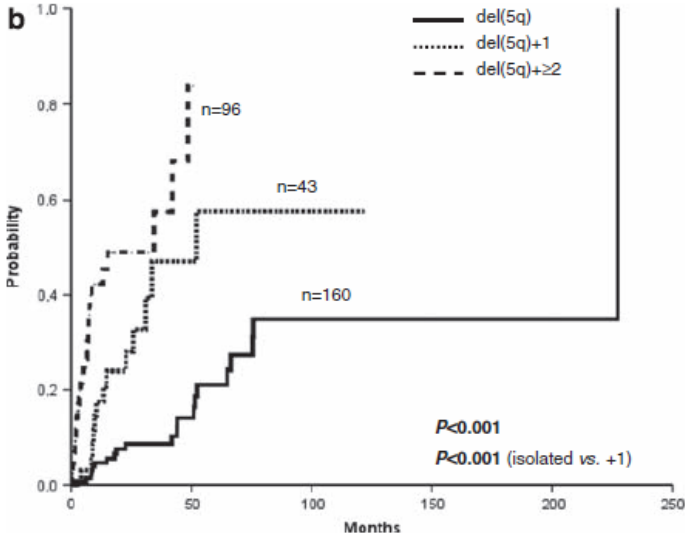


only genetic abnormality defining a category
despite the increasing knowledge of the prognostic impact of genetic findings (IPSS-R); however karyotyping is mandatory in all MDS
new diagnosis
*MDS-defining cytogenetic abnormalities also w/o morphologic dysplasia (need for conventional karyotyping; no FISH or seq)
*span of deletion variable but q31-33 invariably deleted

*Clinical features likley related to deleted genes (haploinsufficiency of RPS14/through p53 pathway activation, miR-145-miR146a/MKC morphology, CaseinKinase1A1/WNT-beta catenin pathway, APC and EGR1/WNT-beta catenin pathway)

*only pre MDS with thrombocytosis (30%)
*benefit from lenalidomide





Leukemia (2011) 25, 110–120

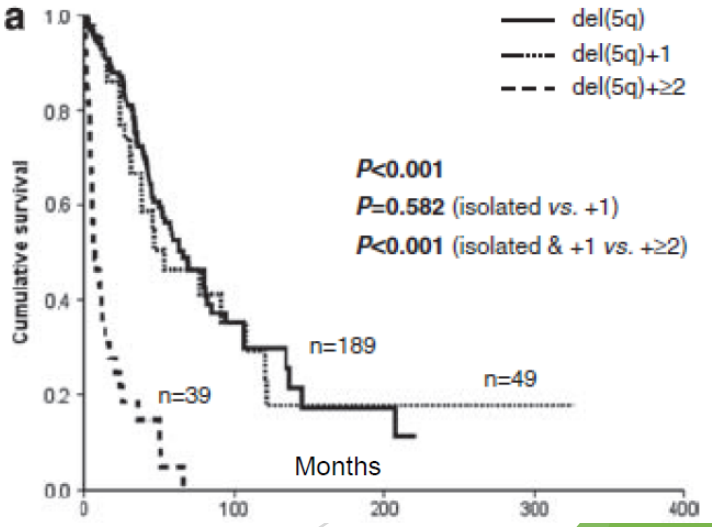
www.nature.com/leu

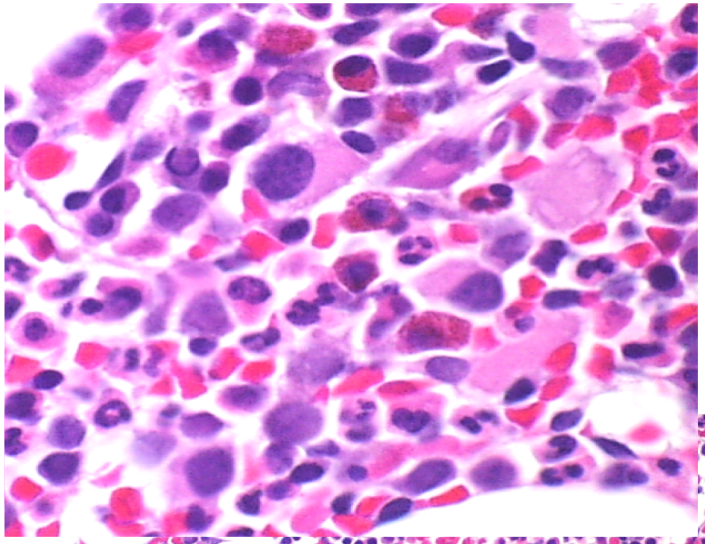
ORIGINAL ARTICLE

Impact of adjunct cytogenetic abnormalities for prognostic stratification in patients with myelodysplastic syndrome and deletion 5q

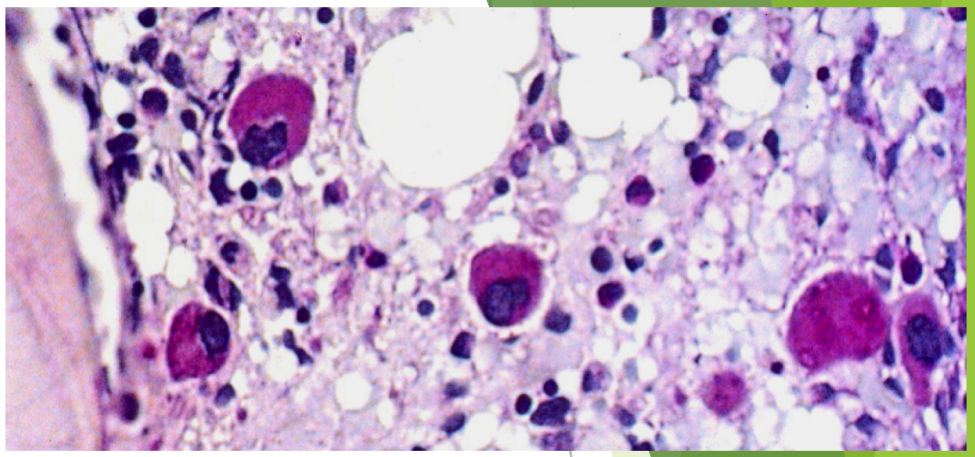
M Mallo[1,2,3,12], J Cervera[1,3,12], J Schanz[4], E Such[1,3], G García-Manero[5], E Luño[1,3], C Steidl[4], B Espinet[1], T Vallespí[1,3,6], U Germing[4], S Blum[7], K Ohyashiki[8,9], J Grau[1,3], M Pfeilstöcker[4], JM Hernández[1,3], T Noesslinger[4], A Giagounidis[4], C Aul[4], MJ Calasanz[1,3], ML Martín[1,3], P Valent[10], R Collado[1,3], C Haferlach[9,11], C Fonatsch[4], M Lübbert[4], R Stauder[4], B Hildebrandt[4], O Krieger[4], C Pedro[3], L Arenillas[3], MÁ Sanz[3], A Valencia[1,3], L Florensa[3], GF Sanz[3,13], D Haase[4,9,13] and F Solé[1,2,3,9,13]

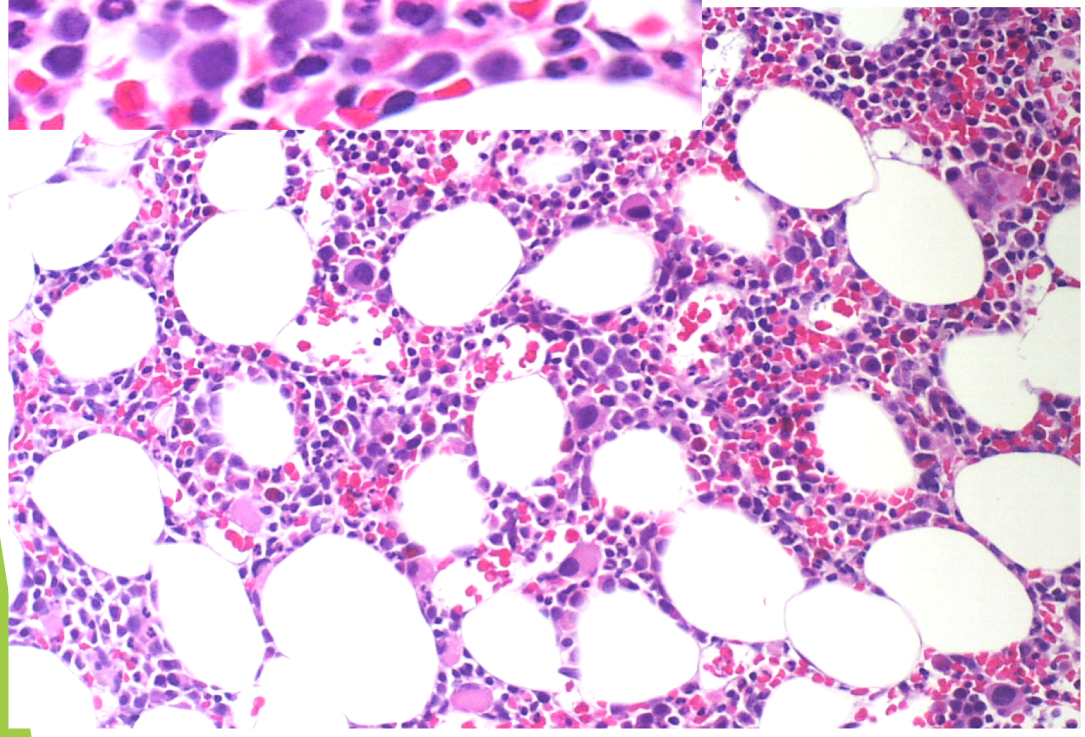
*no impact of one additional chromosomal abnormality (monosomy 7/del(7q) as high risk aberrancy excluded)

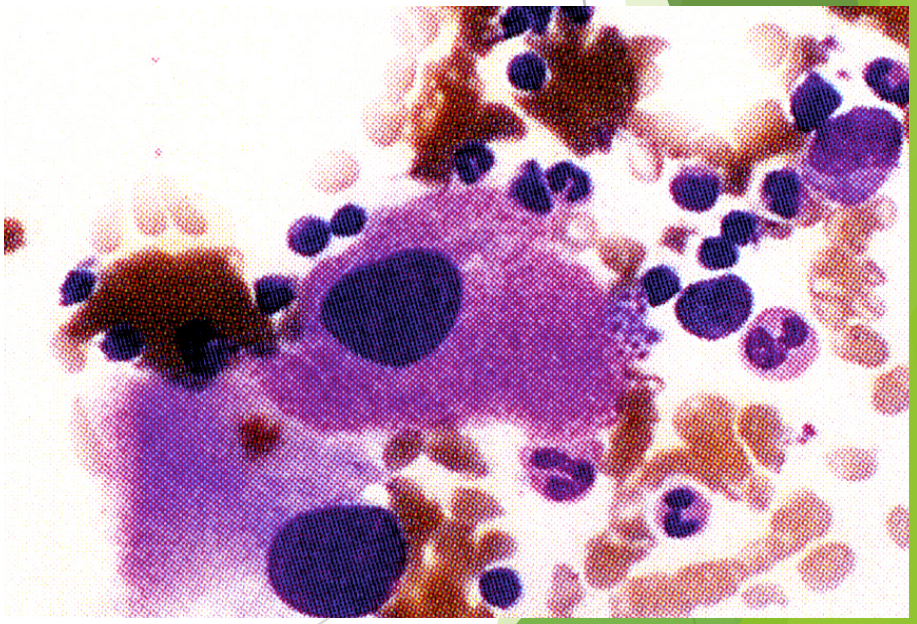




Mild G dysplasia
E dysplasia present
but not pronounced





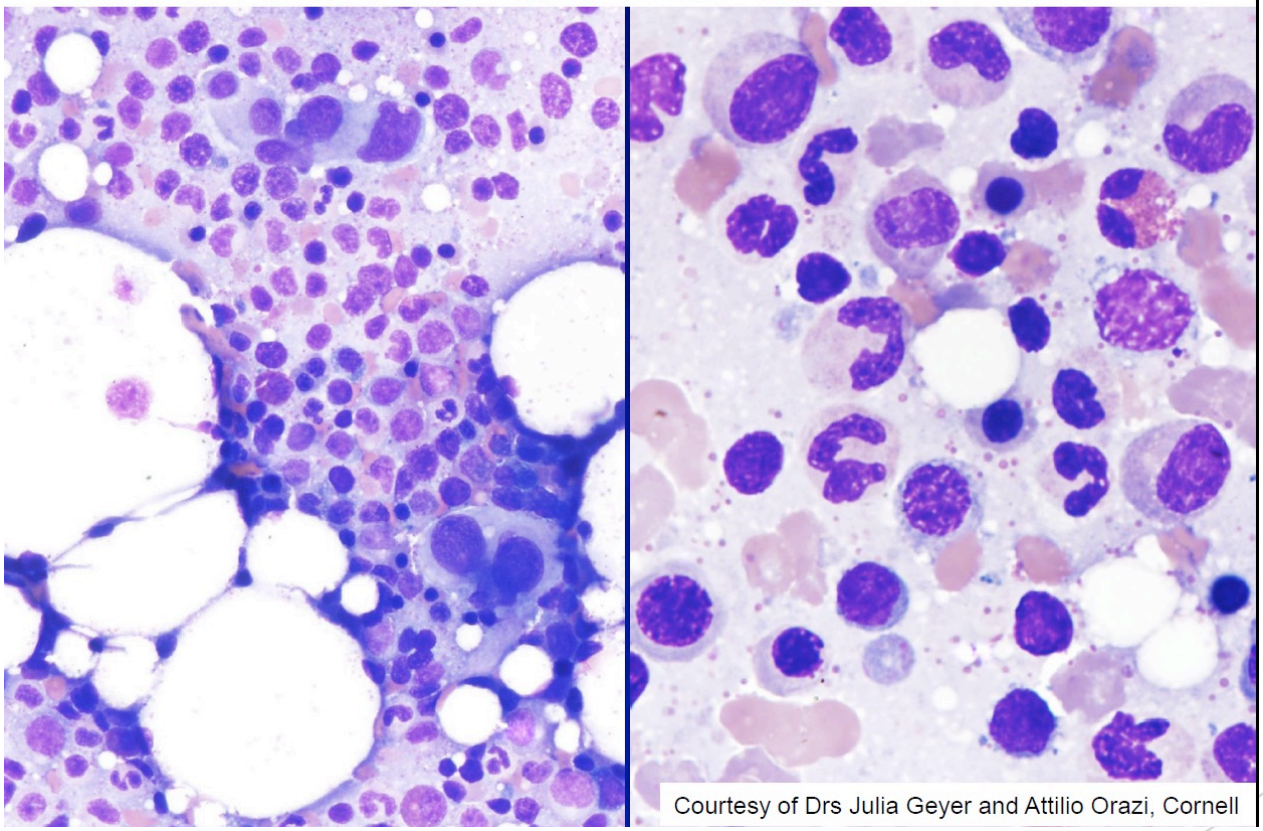


	N.Dysplastic lineages	N.cytopenias	RS (% erythoid cells)	PB-blasts M-blasts	Conventional Karyotyping
MDS-RS-SLD	1	1-2	≥15%, ≥ 5%	PB <1% M <5% no AuerRods	Any (no isolated 5q-)
MDS-RS-MLD	2-3	1-3	≥ 15%, ≥ 5%	PB <1% M <5% no AuerRods	Any (no isolated 5q-)
MDS with isolated del5q*	1-3	1-2	none	PB <1% M <5% no AuerRods	del/(5q) alone or 1 additonal abnormality (except -7 or del(7q)

- Presence of RS will not affect the diagnosis of RAEB or isolated del5
- *G dysplasia and/or increased PB or M blasts

G dysplasia and/or increased PB or M blasts

Cytogenetics shows isolated del(5q). . .but excluded from MDS with isolated del(5q) due to granulocytic dysplasia (RCMD)

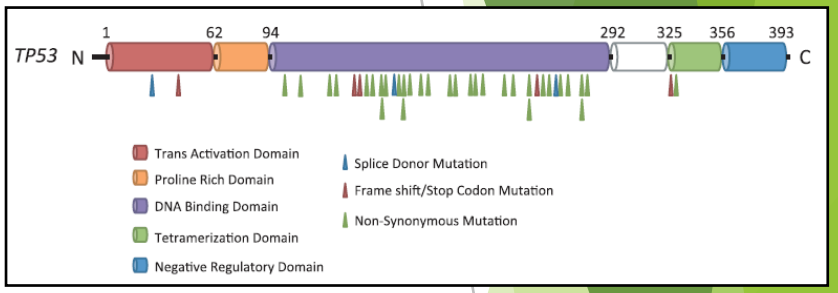


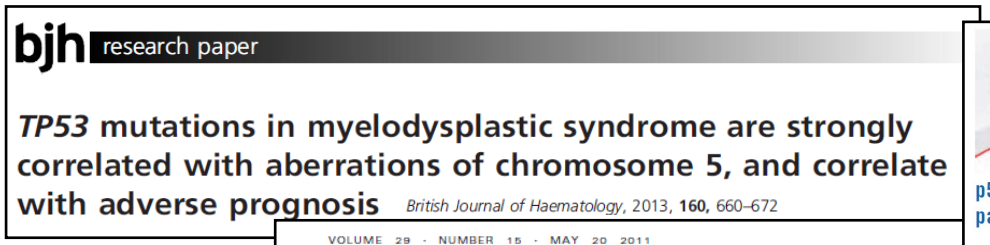
Courtesy of Drs Julia Geyer and Attilio Orazi, Cornell

*<u>***TP53 mutations are often associated with loss of heterozygosity of the TP53 locus and cytogenetic defects such as 5/del(5q) and 17p-.**</u>

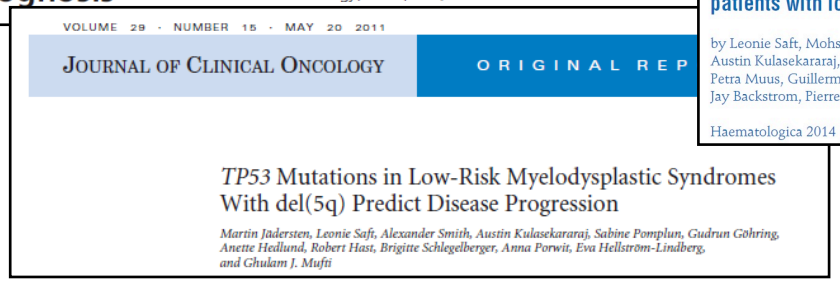
*TP53 mutations is associated with increased risk of leukaemic evolution and lower response to lenalidomide
*reccomended search

Complete assessment of TP53 mutation requires sequencing of all exons, because mutations often occur outside of the most commonly recognized hot spots; highly sensitive assay best required because mutation may only be present in a subset of cells.
*>mutations are missense, frame shift, splice site; rare nonsense




bjh research paper

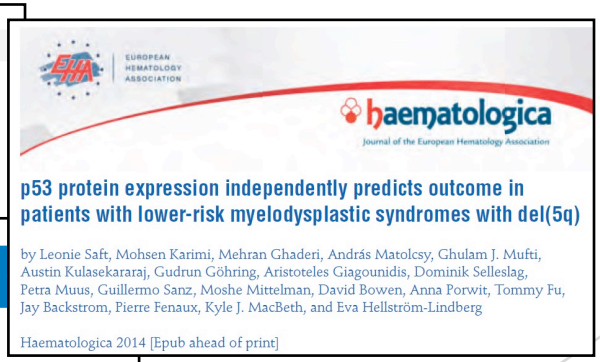
***TP53* mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis** *British Journal of Haematology*, 2013, **160**, 660–672


VOLUME 29 · NUMBER 15 · MAY 20 2011

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REP

TP53 Mutations in Low-Risk Myelodysplastic Syndromes With del(5q) Predict Disease Progression

Martin Jädersten, Leonie Saft, Alexander Smith, Austin Kulasekararaj, Sabine Pomplun, Gudrun Göhring, Anette Hedlund, Robert Hast, Brigitte Schlegelberger, Anna Porwit, Eva Hellström-Lindberg, and Ghulam J. Mufti


EUROPEAN HEMATOLOGY ASSOCIATION

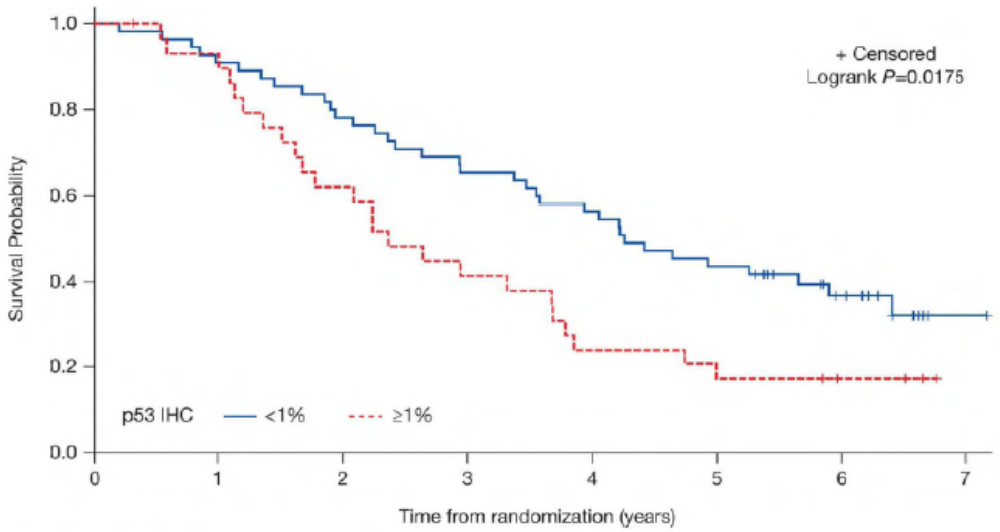
haematologica

Journal of the European Hematology Association

p53 protein expression independently predicts outcome in patients with lower-risk myelodysplastic syndromes with del(5q)

by Leonie Saft, Mohsen Karimi, Mehran Ghaderi, András Matolcsy, Ghulam J. Mufti, Austin Kulasekararaj, Gudrun Göhring, Aristoteles Giagounidis, Dominik Selleslag, Petra Muus, Guillermo Sanz, Moshe Mittelman, David Bowen, Anna Porwit, Tommy Fu, Jay Backstrom, Pierre Fenaux, Kyle J. MacBeth, and Eva Hellström-Lindberg

Haematologica 2014 [Epub ahead of print]



TP53 mutation causes overexpression of the mutated protein
(also in solid tumors and lymphomas)

EUROPEAN HEMATOLOGY ASSOCIATION

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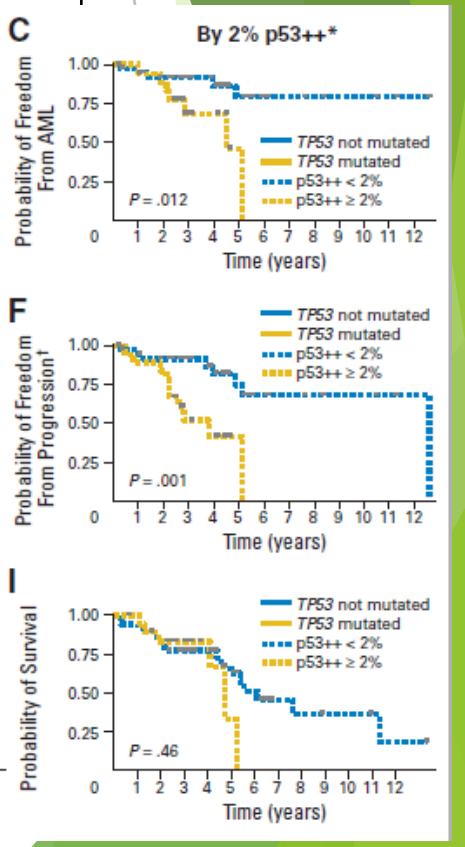

VOLUME 29 · NUMBER 15 · MAY 20 2011

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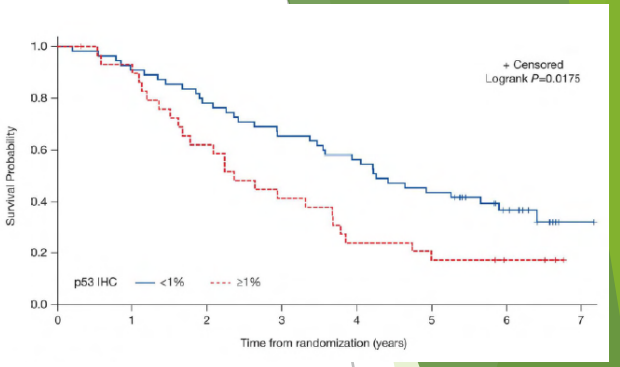
bjh research paper

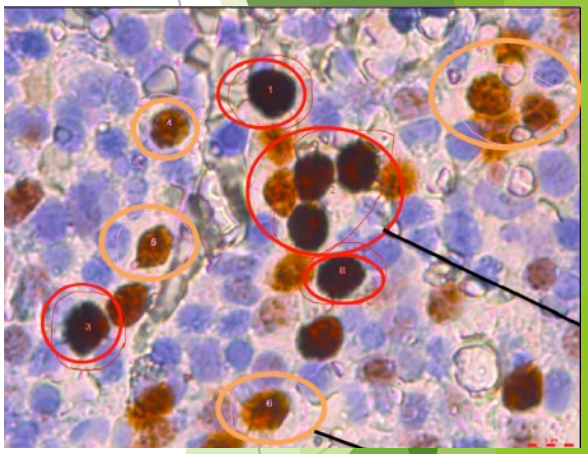
_TP53_ mutations in myelodysplastic syndrome are strongly correlated with aberrations of chromosome 5, and correlate with adverse prognosis *British Journal of Haematology*, 2013, **160**, 660–672

***DO-1/DO-7 antibody**;
*recognize both wild-type and mutant p53 protein

***cutoff of ≥1% p53** strongest independent predictor of AML transformation and an independent predictor of OS
the outcome curves of pts with ≥1% p53-positive cells were identical to those with 2% cutoff (significantly different from <1% p53-pos cells)



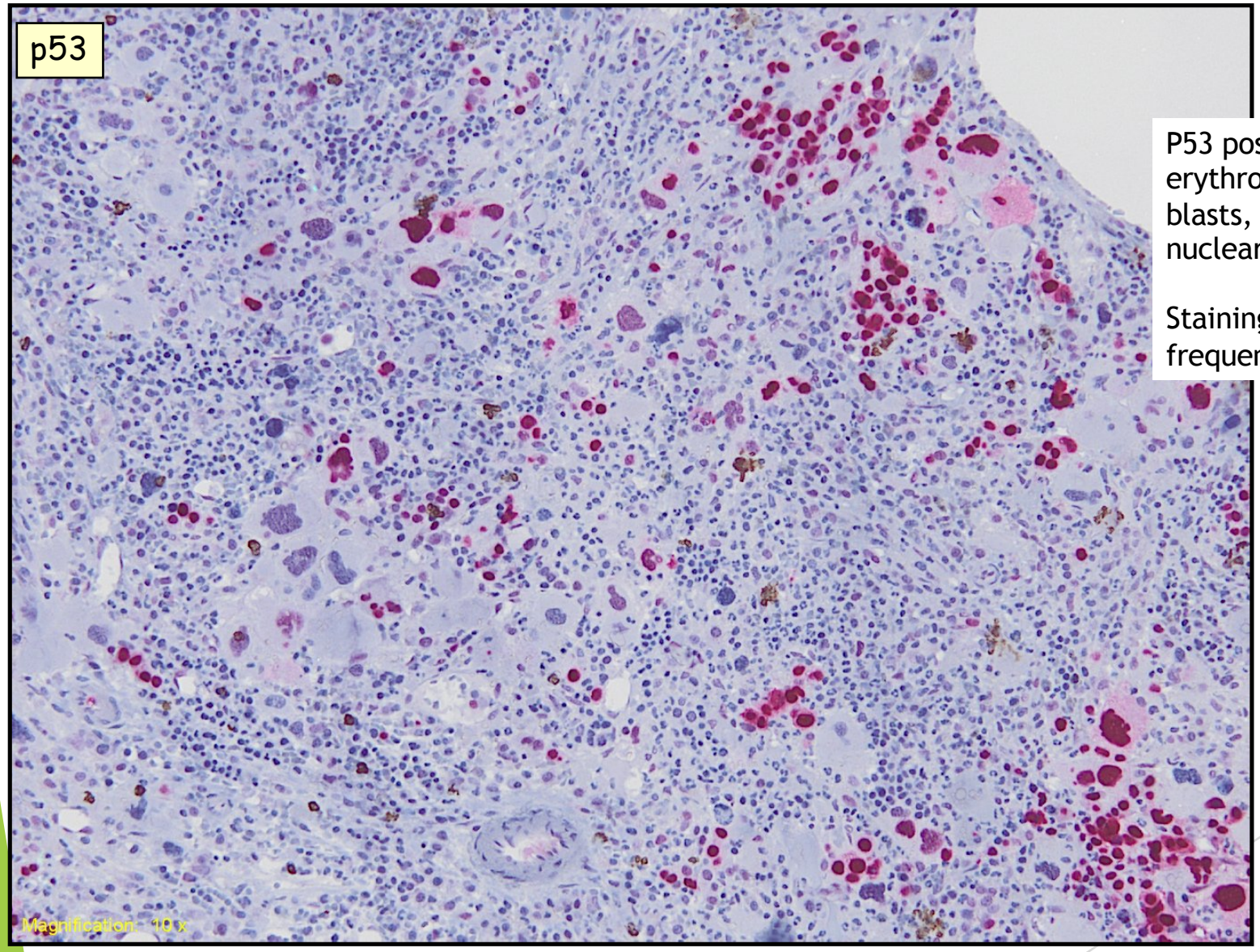
***3+ cells (intense staining)**
single-cell microdissection: weak to moderate p53+ cells lack TP53 mutation (avoid counting moderately or weakly stained cells)



*Correspondence between automated and manual counting;
excellent interobserver concordance at cutoff of ≥ 1%

*no correlation between the percentage of p53 3+ cells and the estimated mutant allele burden by sequence analysis

* None of the samples with secondary, non-MDS related cytopenia showed strong p53 staining.



P53 pos cells were mostly early erythroid forms,
blasts, and early myeloid cells (lacking nuclear segmentation

Staining on MKC variable likely more frequent in MDS-EB

Modern Pathology (2015) 28, 552–563

High p53 protein expression in therapy-related myeloid neoplasms is associated with adverse karyotype and poor outcome

Arjen HG Cleven[1], Valentina Nardi[2], Chi Young Ok[3], Maitrayee Goswami[3], Paola Dal Cin[4], Zongli Zheng[2], A John Iafrate[2], Myrurgia A Abdul Hamid[5], Sa A Wang[3] and Robert P Hasserjian[2]

MOLECULAR AND CLINICAL ONCOLOGY 6: 876-880, 2017

p53 protein expression in patients with myelodysplasia treated with allogeneic bone marrow transplantation

ACHILLE PICH[1], LAURA GODIO[2] and LAURA DAVICO BONINO[2]

Modern Pathology (2017) 30, 382–392

Immunohistochemistry for p53 is a useful tool to identify cases of acute myeloid leukemia with myelodysplasia-related changes that are *TP53* mutated, have complex karyotype, and have poor prognosis

Sebastian Fernandez-Pol, Lisa Ma, Robert S Ohgami and Daniel A Arber

TP53

point mutations of the tumor suppressor gene TP53 represent the most frequent single genetic abnormality
in therapy related myeloid neoplasms: 20- 40%
de novo acute myeloid leukemia and myelodysplastic syndrome: 5-20%

TP53 mutations in therapy related myeloid neoplasms predicted an inferior clinical outcome compared with wild-type TP53

LOW RISK MDS

Possible false IHC negative (~12-15% cases):

nonsense mutations (give rise to a stop codon);
constitute <10% of high-risk myeloid malignancies and are uncommon in lower-risk del(5q) MDS.

negative IHC status, in spite of an underlying mutation, is likely to be infrequent in this population.

THERAPY-RELATED MDS

p53 immunohistochemistry-positive with no TP53 mutation :
p53 IHC borderline positive (1.1% for both)

p53 immunohistochemistry-negative with TP53 mutation (12%) :

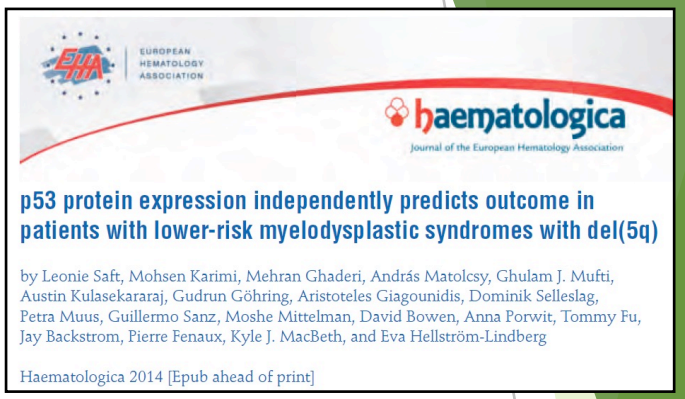
Two nonsense mutations (stop codon resulting in truncated protein undetectable by IHC; two single missense mutations (c.529C4T/p.Pro177Ser and c.393C4

Overall, p53 immunohistochemistry positivity was strongly associated with the presence of TP53 mutation (Po0.0001).

excellent interobserver concordance at cutoff of ≥ 1%

some lack of interobserver concordance in cases with p53 immunohistochemistry scores of 1– 2%

p53 immunohistochemistry scores of 1–2% interpret with caution (may be less reliable in predicting a TP53 mutation than p53 IHC >2%)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Haematologica 2014 [Epub ahead of print]


MODERN PATHOLOGY (2015) 28, 552–563

552

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High p53 protein expression in therapy-related myeloid neoplasms is associated with adverse karyotype and poor outcome

Arjen HG Cleven[1], Valentina Nardi[2], Chi Young Ok[3], Maitrayee Goswami[3], Paola Dal Cin[4], Zongli Zheng[2], A John Iafrate[2], Myrurgia A Abdul Hamid[5], Sa A Wang[3] and Robert P Hasserjian[2]

MDS-U (all BM blasts <5%, no Auer Rods, PB =1/<1%)

1) MDS with exactly 1% pb-blasts and dysplasia (1-3 lineages) and cytopenia (1-3 lineages)
<5% marrow-blasts
No Auer rods
No RS, Normal karyotype (conventional)
Need for at least 2 separate blast counting

2) MDS single lineage dysplasia and pancytopenia (all levels below thresholds)
Also possible isolated del5q (but pancytopenia not 1-2 cytopenia as in classical del5q

3) MDS with cytopenia and MDS definig cytogenetic abnormality
No dysplasia
RS<15%

New terminology

MDS NOMENCLATURE

MDS with Single Lineage Dysplasia (MDS-SLD)	**RCUD**
MDS with Single Lineage Dysplasia (MDS-SLD) with Ring Sideroblasts	RARS
MDS with Multi Lineage Dysplasia (MDS-MLD)	**RCMD**
MDS with Multi Lineage Dysplasia (MDS-MLD) with Ring Sideroblasts	RCMD-RS

→ either with 15% RS or any RS and SF3B1 mutation

MDS with isolated del(5q) →

- One additional (non high risk) anormality allowed
- Reccomend TP53 testing
- Exclude cases with G dysplasia

MDS with Excess Blasts	
type 1 (MDS-EB1)	**RAEB-1**
type 2 (MDS-EB2)	**RAEB-2**

- Will include most cases pously classified as E/M acute leukaemia

MDS, unclassifiable (MDS-U)

Additional tools for diagnosis

- Flow cytometry
- Molecular biology (mutations)

  10% healthy individuals>65yrs harbor somatic MDS-type mutations in haematopoietic cells
  - mostly DNMT3, TET2, ASXL1, TP53, JAK2, SB3B1
  - 10-20% allele burden in PB
  - increased risk of subsequent hematologic malignancy

increase the accuracy:
sensitivity in identifying MDS from 60% to 98%, with a specificity of 93% to 100%.

in cases not fulfilling criteria for the diagnosis of MDS at first evaluation: flow cytometry has up to 76% sensitivity in patients receiving a final diagnosis of MDS, as well as a high negative predictive value

Table 6: Recurrent immunophenotypic abnormalities detected by flow cytometry in MDS

$CD34^+$ progenitor cells
- increase in $CD34^+$ cells*

Monocytes
- lack of or abnormal expression of CD13, CD14, CD16, or CD33
- abnormal expression of CD11b or HLA-DR
- overexpression of CD56
- abnormal granularity or distribution of immature and mature subsets

Erythroid precursor cells
- decreased or heterogeneous expression of CD36 and CD71
- abnormal frequency of $CD117^+$ erythroid precursors
- abnormal frequency of $CD105^+$ erythroid precursors
- abnormal CD105 fluorescence intensity

*An increase in $CD45^-/CD34^+$ cells by flow cytometry can assist in the quantification of the compartment in MDS. In cases where blast cells lack CD34, CD117/KIT can be employed as alternati (blast) cells. MDS, myelodysplastic syndromes

Will still be considered as "supportive" of MDS and will not alone be sufficient for making a primary MDS diagnosis

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 43), pp: 73483-73500

Priority Review

Proposed minimal diagnostic criteria for myelodysplastic syndromes (MDS) and potential pre-MDS conditions

Peter Valent[1,2], Attilio Orazi[3], David P. Steensma[4], Benjamin L. Ebert[5], Detlef Haase[6], Luca Malcovati[7], Arjan A. van de Loosdrecht[8], Torsten Haferlach[9], Theresia M. Westers[8], Denise A. Wells[10], Aristoteles Giagounidis[11], Michael Loken[10], Alberto Orfao[12], Michael Lübbert[13], Arnold Ganser[14], Wolf-Karsten Hofmann[15], Kiyoyuki Ogata[16], Julie Schanz[6], Marie C. Béné[17], Gregor Hoermann[18], Wolfgang R. Sperr[1,2], Karl Sotlar[19], Peter Bettelheim[20], Reinhard Stauder[21], Michael Pfeilstöcker[22], Hans-Peter Horny[23], Ulrich Germing[24], Peter Greenberg[25] and John M. Bennett[26]

plasia
er other causes of cytopenia if
is normal
gs correlate with prognosis in
wn MDS cases

ing guidelines for integration of flow ry results in the WHO classification of myelodysplastic syndromes-proposal from the International/Europena LeukemiaNet Working Group for Flow Cytometry in MDS
Porwit A et al. Leukemia. 2014

Pitfalls and Abnormal conditions

- *Reactive changes*
- *"Gray-zone" dysmyelopoiesis: when MDS is possible but not provable*

Leukemia Research 36 (2012) 1–5

Contents lists available at SciVerse ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Invited review

Idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of uncertain significance (IDUS), and their distinction from low risk MDS

Peter Valent[a,b,*], Barbara J. Bain[c], John M. Bennett[d], Friedrich Wimazal[a,e], Wolfgang R. Sperr[a,b], Ghulam Mufti[f], Hans-Peter Horny[g]

The shadowlands of MDS: idiopathic cytopenias of undetermined significance (ICUS) and clonal hematopoiesis of indeterminate potential (CHIP)

Luca Malcovati[1,2] and Mario Cazzola[1,2]

Hematology 2015

Grazie per la attenzione

Idiopathic cytopenia of uncertain significance—ICUS

*as for MDS, cytopenia must be substantial and recorded over a time period of at least 6 months to count as 'diagnostic'for ICUS
*Sometimes, clonal hematopoiesis is detectable by molecular studies or FISH
*These clones are often small at diagnosis or are only transiently detectable. In other cases, no karyotypic abnormality and no gene defects are present
*some patients with ICUS, flow cytometry reveals an aberrant phenotype of myeloid cells suggesting clonality
*role of microenvironment: inadequate production of cytokines ? suboptimal response in erythropoietin?

Table 3
Proposed classification of idiopathic cytopenia of uncertain significance (ICUS).

Proposed term	Suggested abbreviation	Definition (ICUS criteria already met[a])
Idiopathic anemia of uncertain significance	ICUS-A	Hb <110 g/L PLT ≥100 × 10^9/L ANC ≥1.0 × 10^9/L
Idiopathic neutropenia of uncertain significance	ICUS-N	Hb ≥110 g/L PLT ≥100 × 10^9/L ANC <1.0 × 10^9/L
Idiopathic thrombocytopenia of uncertain significance	ICUS-T	Hb ≥110 g/L PLT <100 × 10^9/L ANC ≥1.0 × 10^9/L
Idiopathic bi/pancytopenia of uncertain significance	ICUS-BI/PAN	Hb <110 g/L and/or[b] PLT <100 × 10^9/L and/or[b] ANC <1.0 × 10^9/L

[a] ICUS criteria have already been fulfilled: the patient has constant cytopenia for at least 6 months, MDS criteria are not met, and no other reason/underlying disease that could explain cytopenia has been found.
[b] Two or three cytopenias required.

Idiopathic dysplasia of uncertain significance—IDUS

*can only be diagnosed when blood counts remain stable (above diagnostic cut off levels) over at least 6 months.
*patients with IDUS may also progress to AML, MPN, or an MDS/MPN
*latency period for evolution variable (sometimes stable clonal myelopoiesis recorded over many years or decades, in other cases relatively fast evolution to MDS and AML is seen).
*adequate EPO levels and BFU-E levels measurable at normal or relatively high levels (not the case in overt MDS)
*the exact mechanisms and triggering pathogenetic factors remain at present unknown (few cases may show JAK2 V617F mutation – 20% of cases abnormal karyotpe (is it still IDUS?)

clonal hematopoiesis of indeterminate potential (CHIP)

*has been introduced to define the condition characterized by the presence of a somatic mutation associated with hematologic malignancy in the absence of definitive diagnostic criteria for neoplasm

*hematologic cancer gene mutations present in up to 10% of persons 70-79 years of age, and 20% of persons 90 years of age or older = expansion of cells harboring an initiating driver mutation (Clonal Hematopoiesis) is an aspect of aging hematopoietic system

considering that 1) approximately 90% of MDS patients carry one or more oncogenic mutations and that 2)
For WHO 2008-2017 selected abnormalities are recognized as presumptive evidence of MDS, even in the absence of definitive morphologic features (few cases)
proof of concept that MDS may not be a required criterion for the diagnosis of MDS

*>subjects only have one mutation (>in the DNMT3A, TET2, ASXL1)
Mutated at a lower rate: JAK2, SF3B1, SRSF2, TP53
few clonal hematopoiesis without clear candidate driver mutations

*clonal hematopoiesis brings a risk of developing hematologic cancers elevated by a factor up to 13 than subjects without any detectable putative somatic mutations
In evolving cases N. of driver mutations per person and the mutant allele burden are increasing from CHIP to MDS (confirm potential to progress to myeloid neoplasm or are **small malignant clones at a preclinical stage (pre-malignant stage)**.

mechanisms sustaining the transformation of the hematopoietic cell and role of factors external (environment) need to be clarified.