Erytroïde uitrijping in MDS

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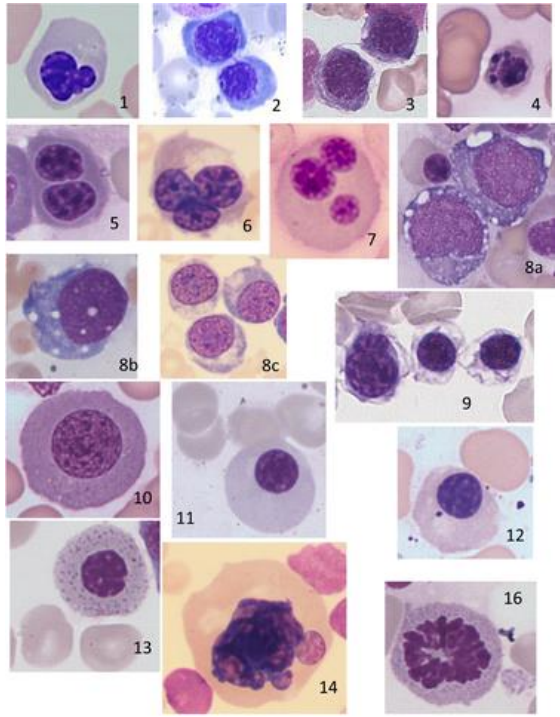
Dyserythropoiesis in the diagnosis of MDS

Myelodysplastic syndromes (MDS) characterized by:

- Cytopenias, frequently anemia
- Dysplastic features such as erythroid dysplasia
- Varying percentage of blast cells



Morphology: gold standard for evaluation of dysplasia

Flow cytometry: additional diagnostic tool in evaluation of dysplasia

Valent, et al., Oncotarget. 2017;8(43):73483-500
Porwit et al., Leukemia. 2014;28(9):1793-8
Goasguen, et al., Br J Haematol. 2018;182(4):526-33

Dyserythropoiesis – Erythroid dysplasia

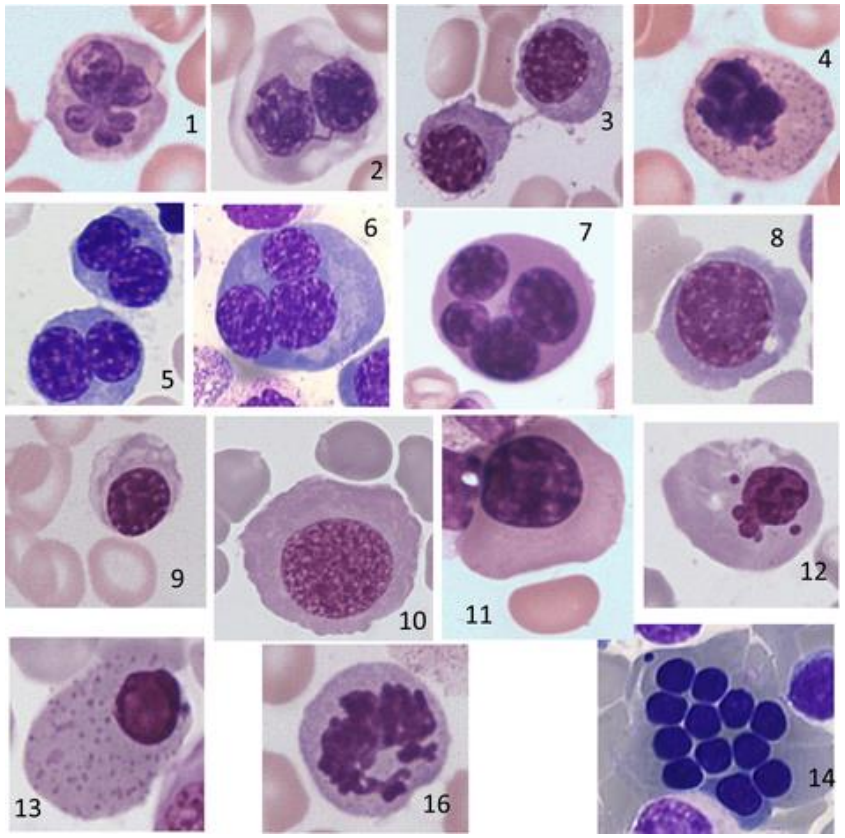
Characteristics of erythroid dysplasia

- Megaloblastic changes
- Nuclear alterations

  budding, internuclear bridging, karyorrhexis, multinuclearity
- Cytoplasmic features:

  ring sideroblasts, vacuolization,

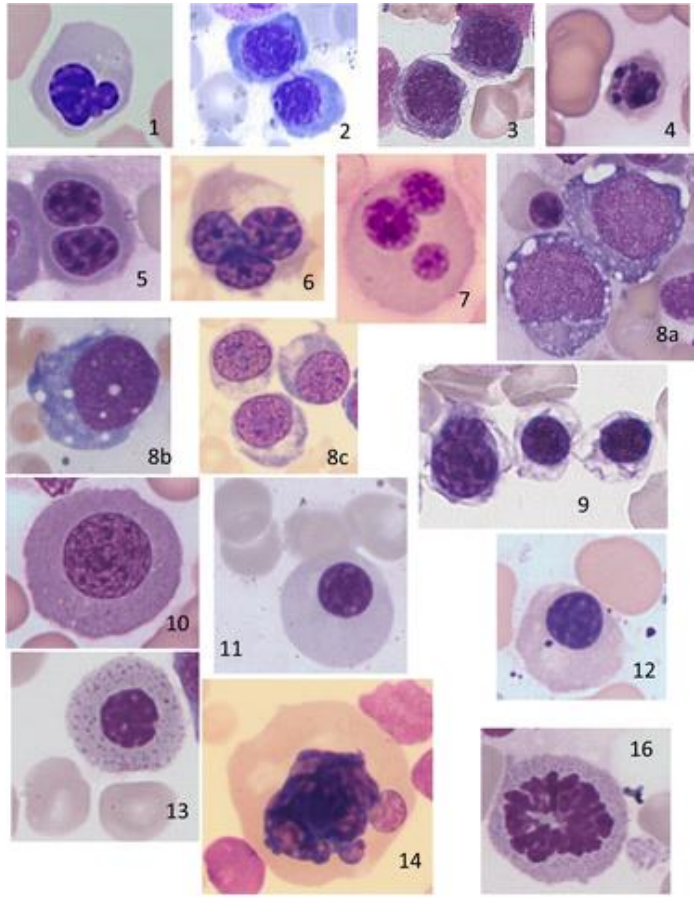
  aberrant periodic Acid-Schiff positivity (granular or diffuse)

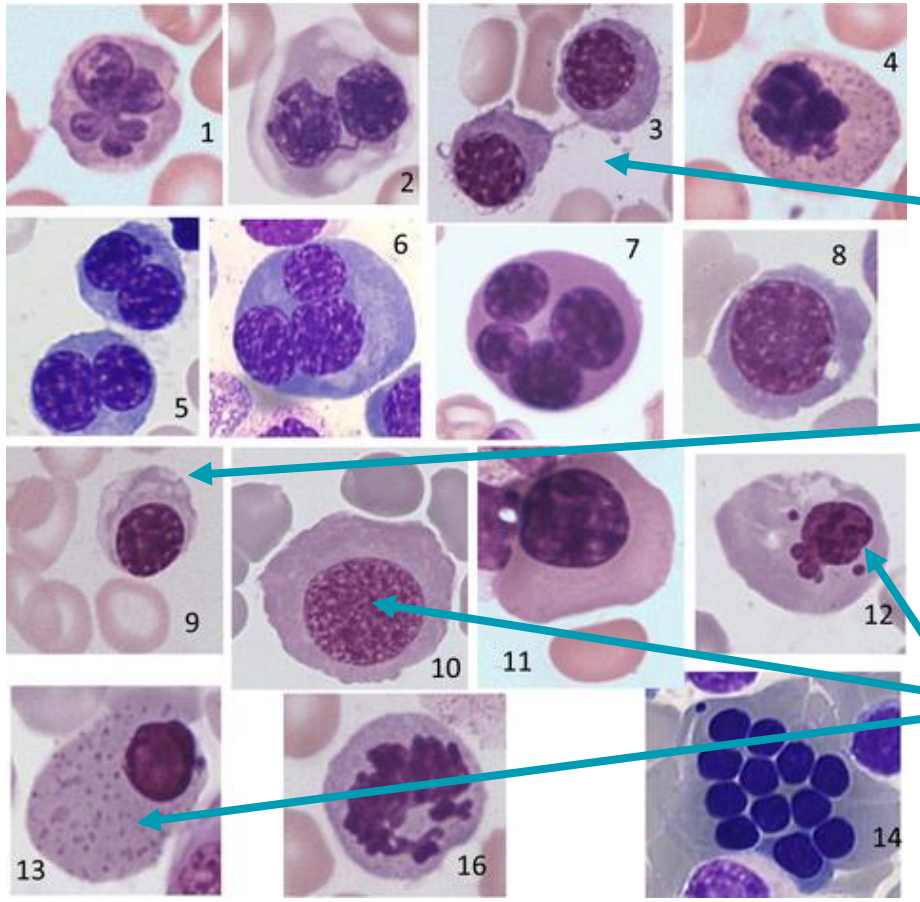


Swerdlow, et al. WHO 2016, p.100; Goasguen, et al., Br J Haematol. 2018;182(4):526-33

Dyserythropoiesis in the diagnosis of MDS

MDS
and
AML with
MDS-related
changes





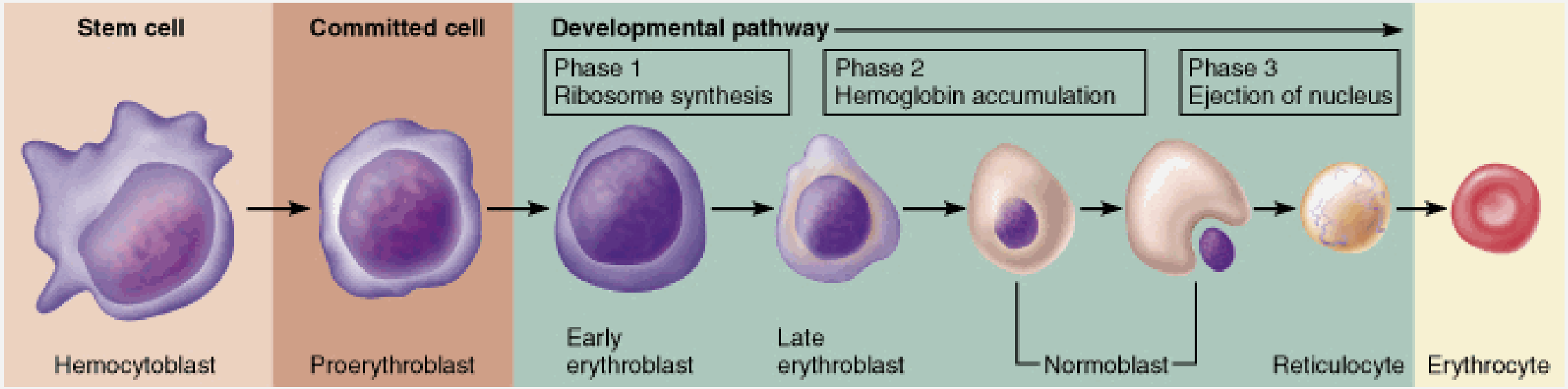
healthy volunteer (3)

iron deficiency (9)

vit.B12 deficiency (10,12,13)

Goasguen, et al., Br J Haematol. 2018;182(4):526-33

Normal erythropoiesis by cytomorphology

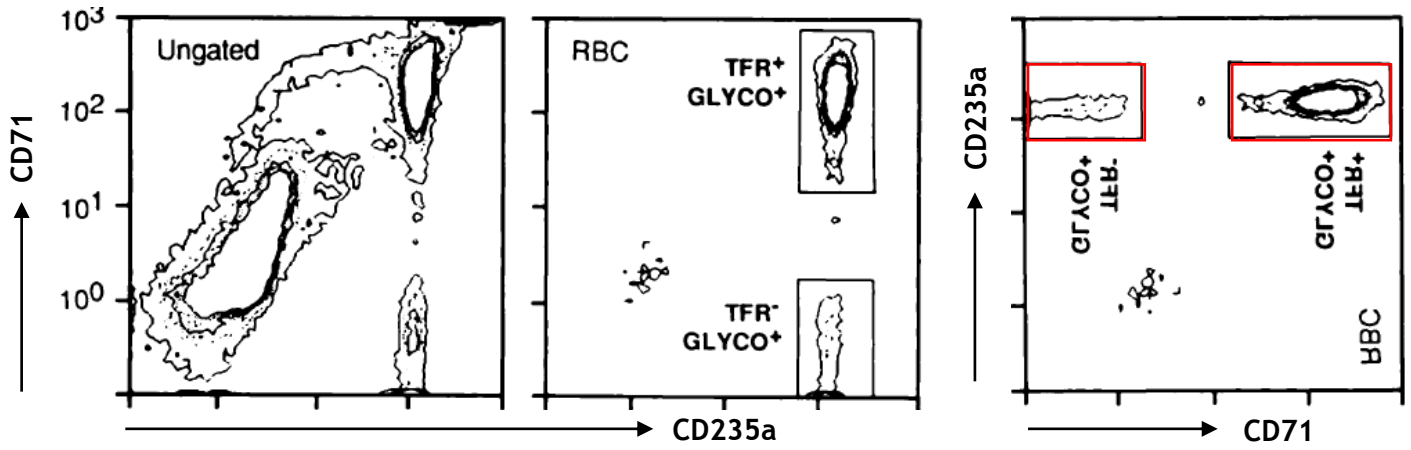


Normal erythropoiesis by flow cytometry

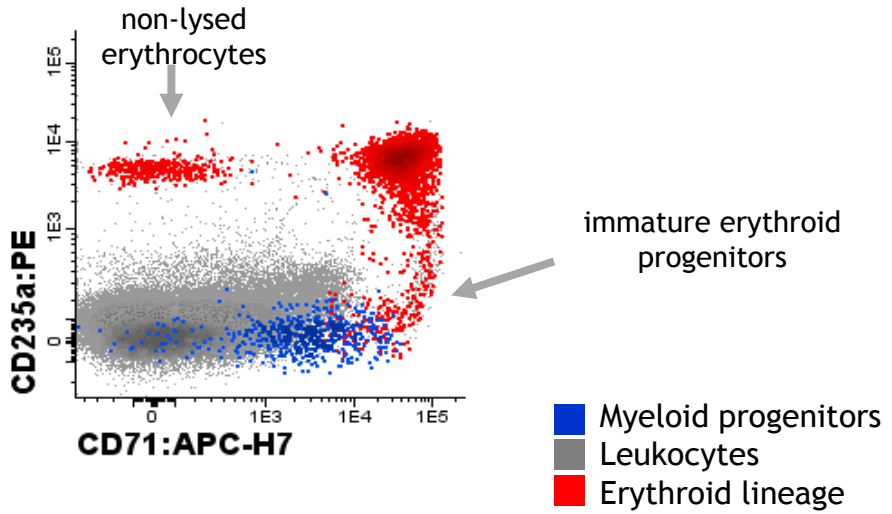
Most well-known markers for studying the erythroid lineage:

- CD235a, glycophorin A
- CD71, transferrin receptor

1987



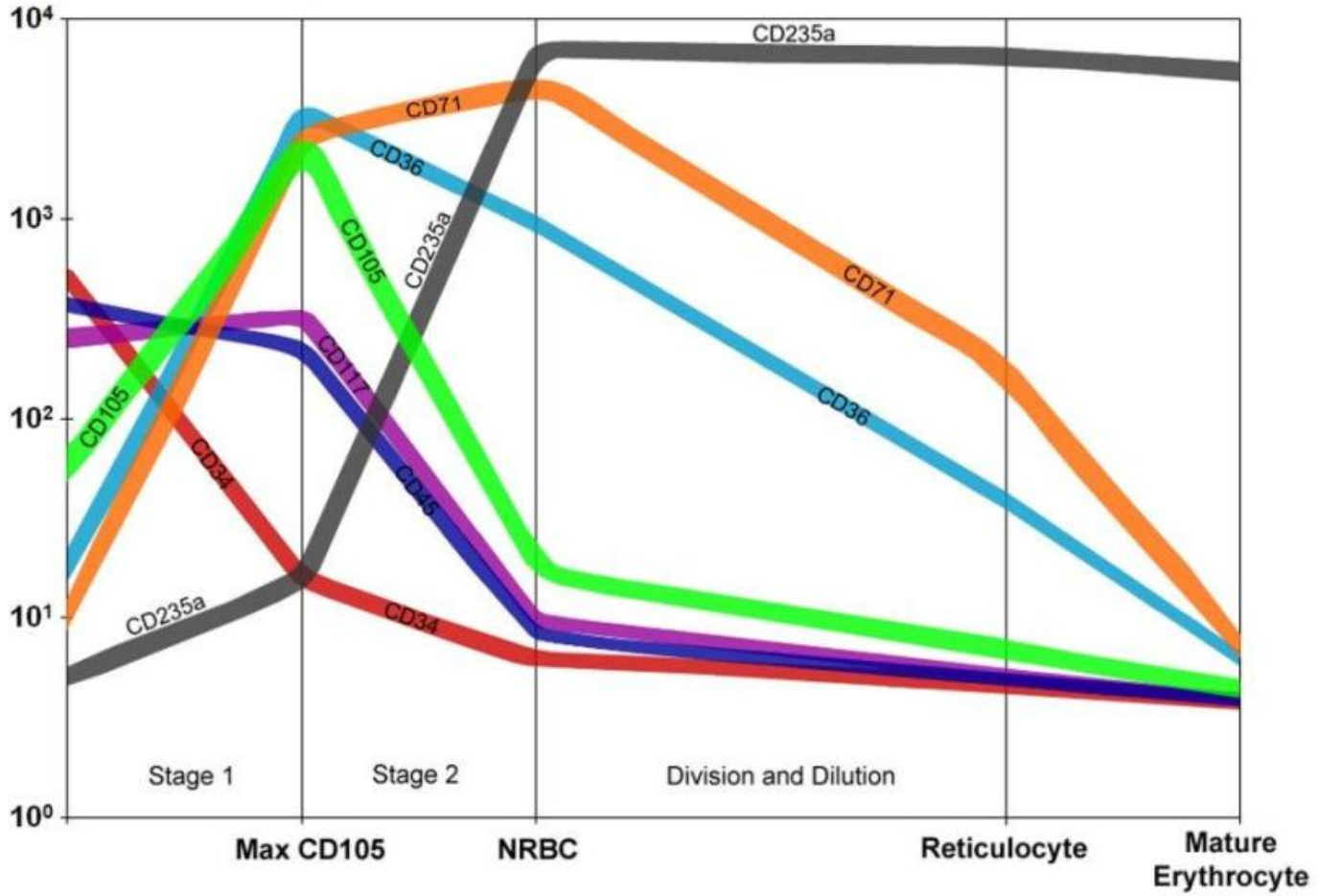
2017



Loken et al. Blood 1987;69(1): 255-63;
Westers et al., Haematologica 2017;102(2):308-19

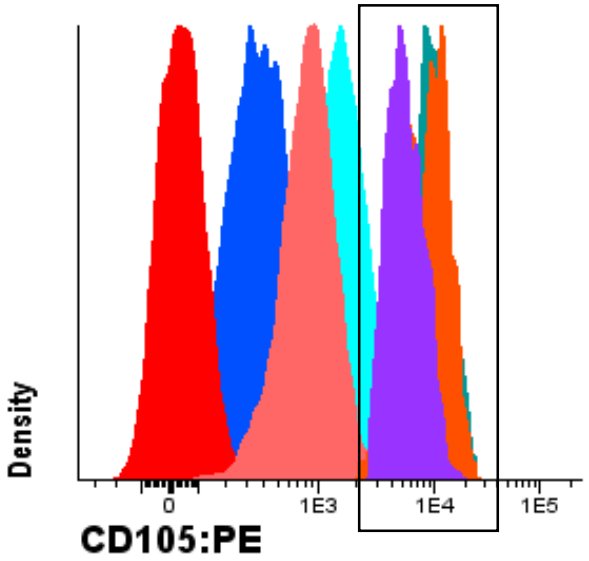
Normal erythropoiesis by flow cytometry

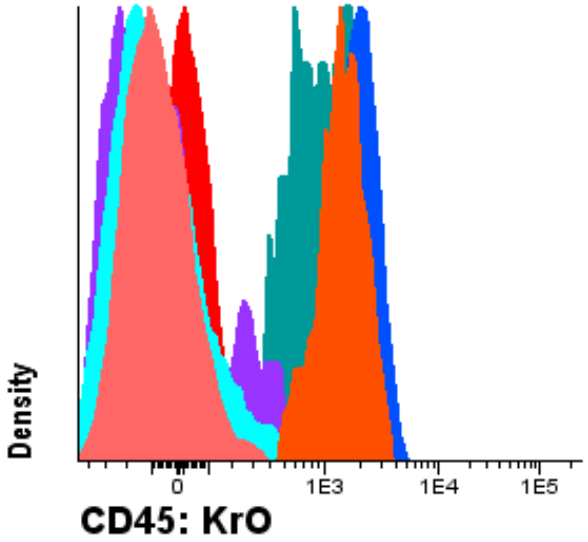
CD45 | CD34 | CD117 | CD105 | CD36 | CD71 | CD235a

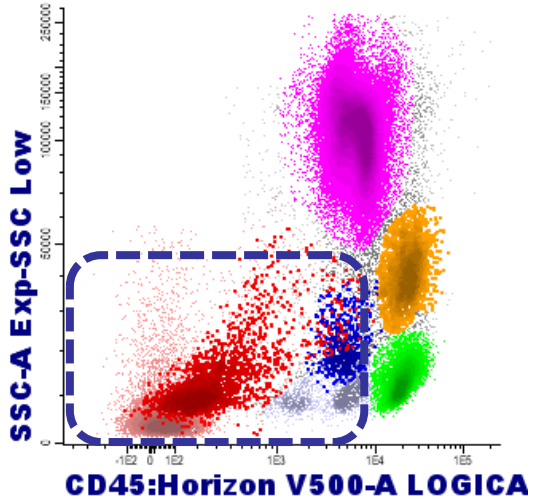


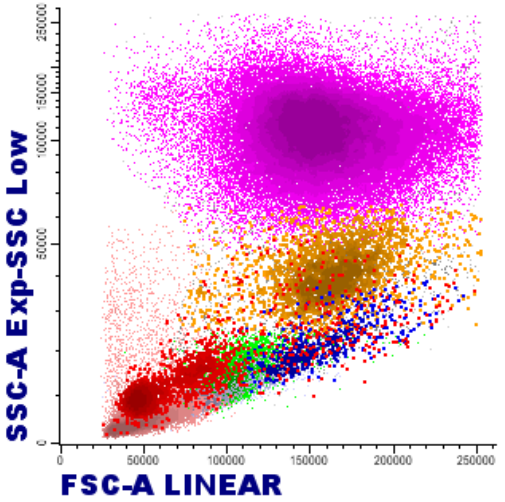
Wangen et al, Int J Lab Hem. 2014;36(2):184-96; Eidenschink Brodersen et al., Cytometry B Clin Cytom. 2015;88(2):125-35
Machherndl-Spandl, et al., Blood Cancer J. 2013;3(1): e100; Fajtova et al., Leuk Lymphoma. 2013;54(11):2523-30

Proper gating strategy regarding the erythroid lineage









- Myeloid progenitor
- Proerythroblast
- Erythroblast
- Basophilic erythroblast
- Polychromatic erythroblast
- Orthochromatic erythroblast
- Mature erythrocyte

Erythroid gate should be CD45$^{dim\text{-}to\text{-}neg}$ to include early erythroid progenitors

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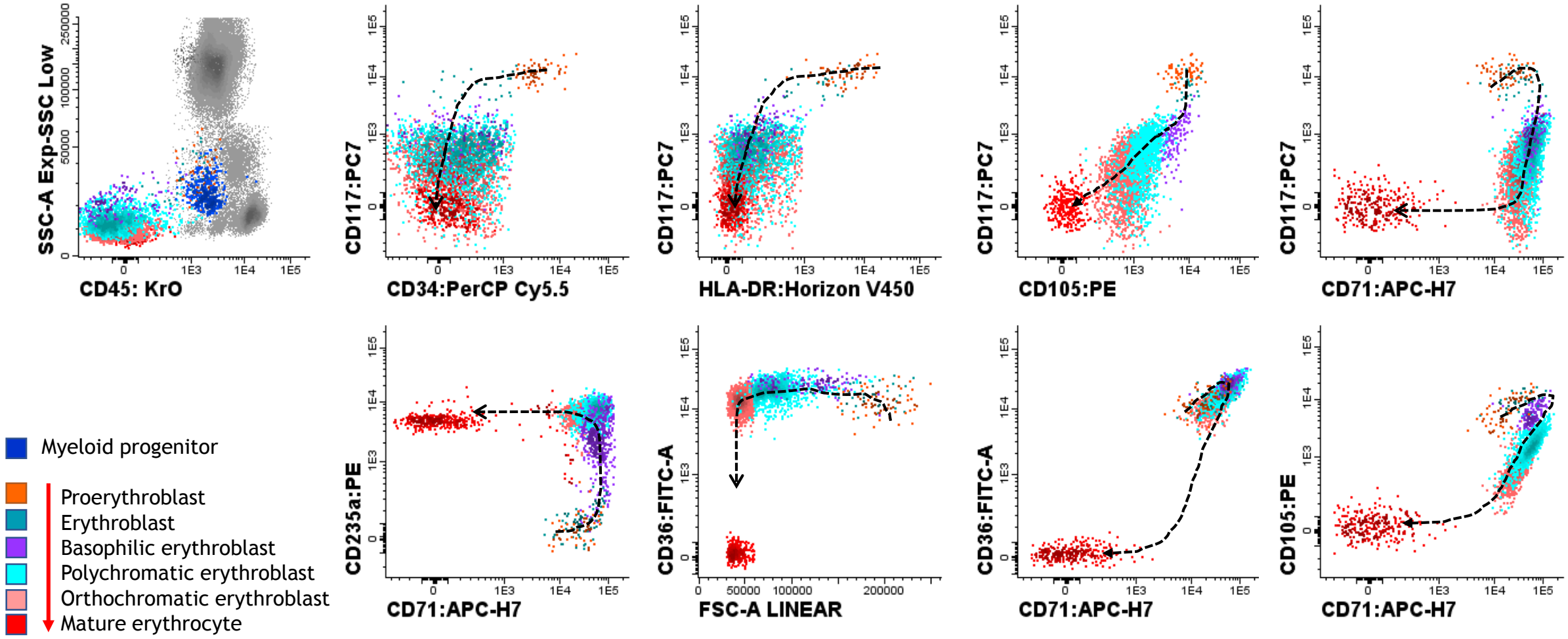
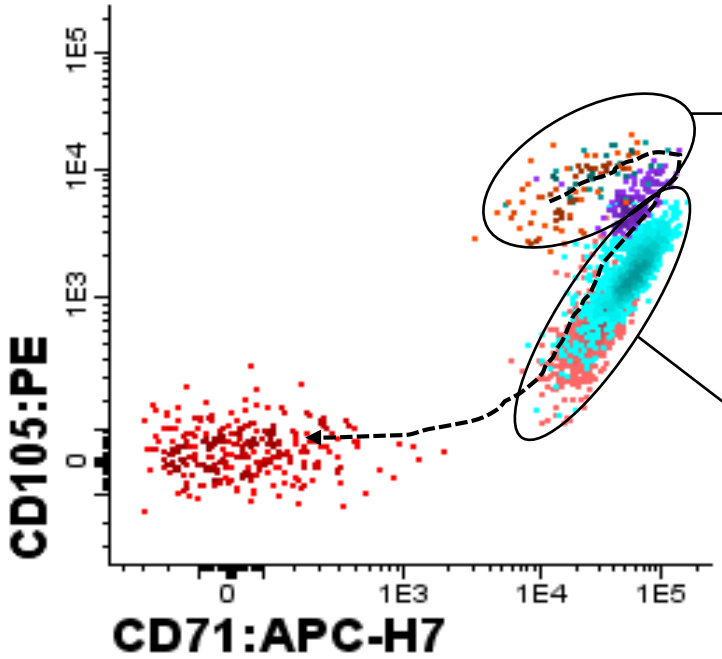
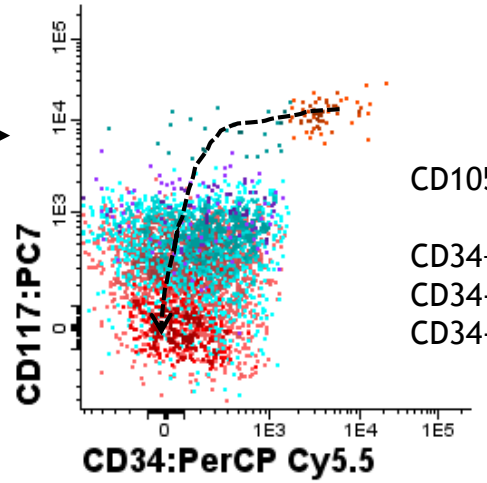
Normal erythropoiesis by flow cytometry



Westers, et al., Haematologica 2017;102(2):308-19

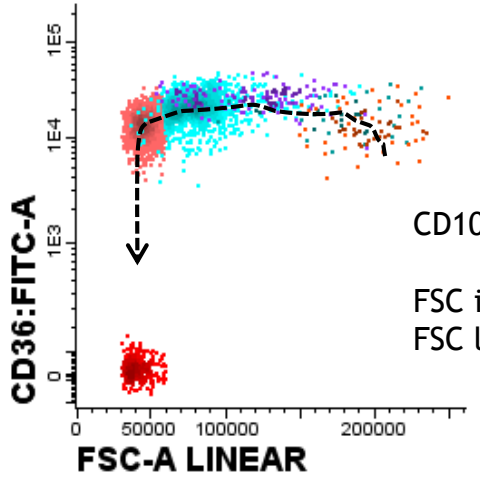
Normal erythropoiesis by flow cytometry





CD105+:

CD34+ CD117+
CD34- CD117+
CD34- CD117-



CD105-:

FSC int
FSC low

- Proerythroblast
- Erythroblast
- Basophilic erythroblast
- Polychromatic erythroblast
- Orthochromatic erythroblast
- Mature erythrocyte

Westers, et al., Haematologica 2017;102(2):308-19

Dyserythropoiesis in MDS by flow cytometry

- Analyzed features differ from morphology
- Immunophenotypic features must be able to separate MDS from normal and non-clonal cytopenic controls (NCCC)

EuroFlow

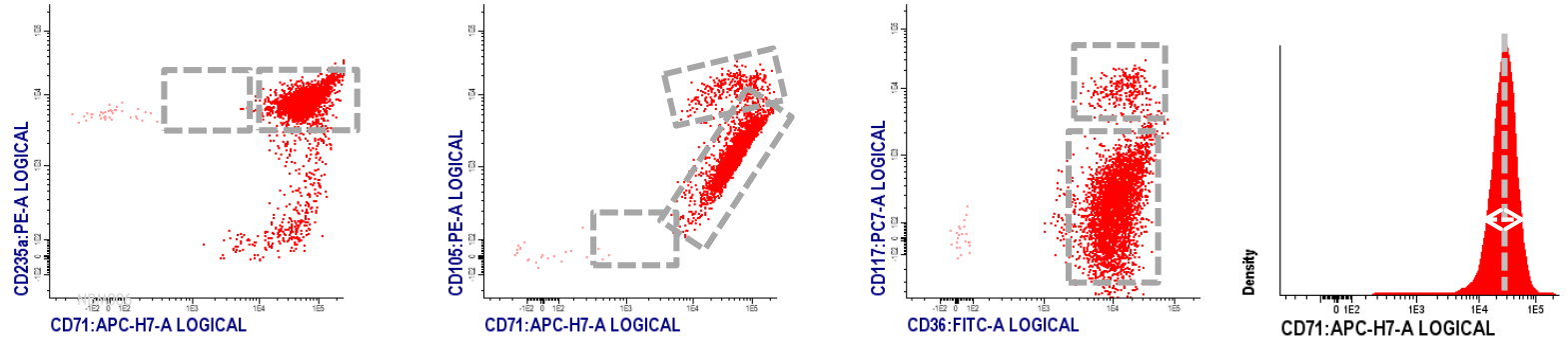
Proposed aberrancies in maturing erythroid cells to study dysplasia

Optional analyses	Aberrancy
% of nucleated erythroid cells (NEC)	increased
relationship CD71 *vs.* CD235a	altered pattern
expression of CD71	decreased
expression of CD36	decreased
coefficient of variation (CV) of CD71	increased
coefficient of variation (CV) of CD36	increased
% of CD117-positive precursors	increased/decreased
% of CD105-positive precursors	increased/decreased
expression of CD105	increased/decreased

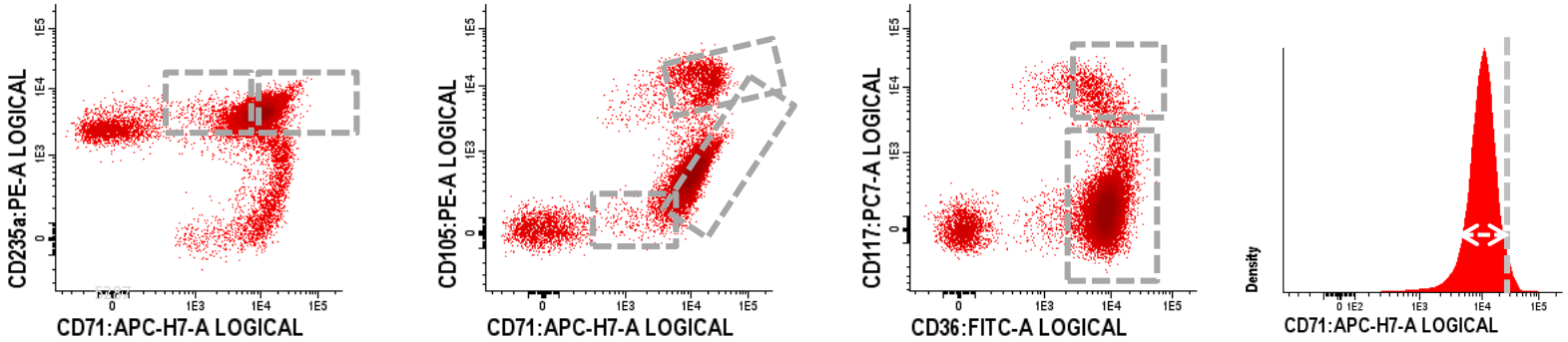
Van de Loosdrecht et al., Haematologica 2009;94(8):1124-34 ; Westers et al., Leukemia 2012;26(7)36:422-30; Porwit et al., Leukemia. 2014;28(9):1793-8; Westers et al., Haematologica 2017;102(2):308-19

Dysplastic erythroid immunophenotypes associated with MDS

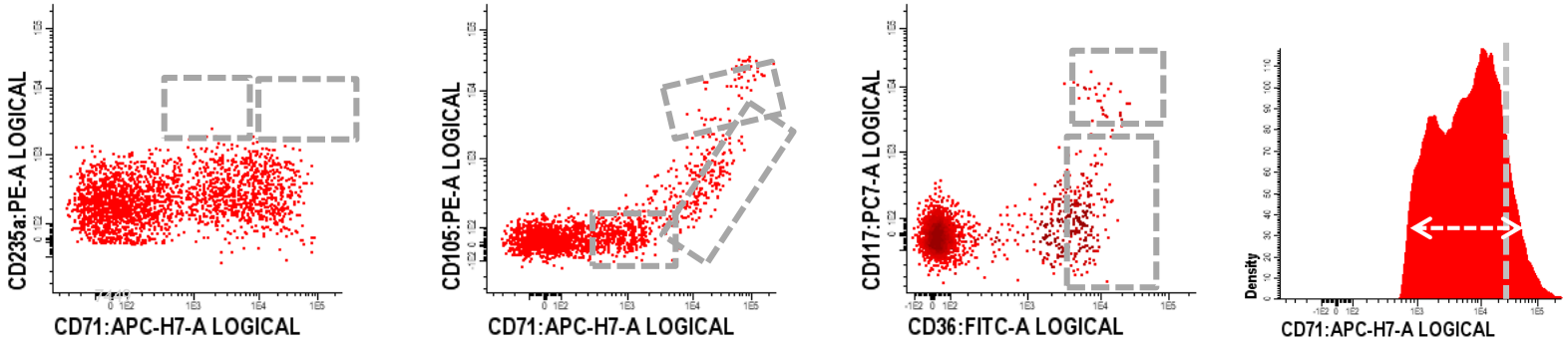
normal



MDS-MLD



MDS-RS-MLD



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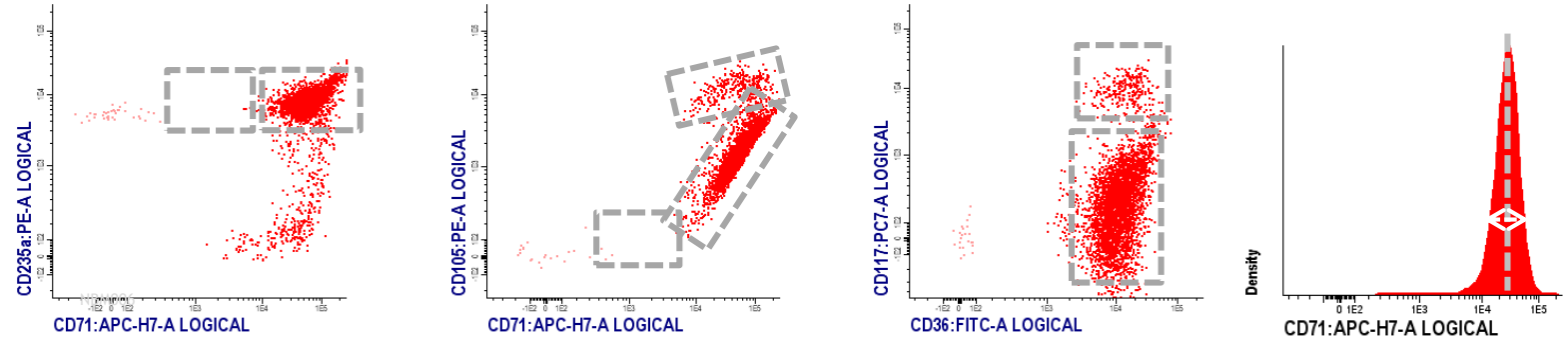
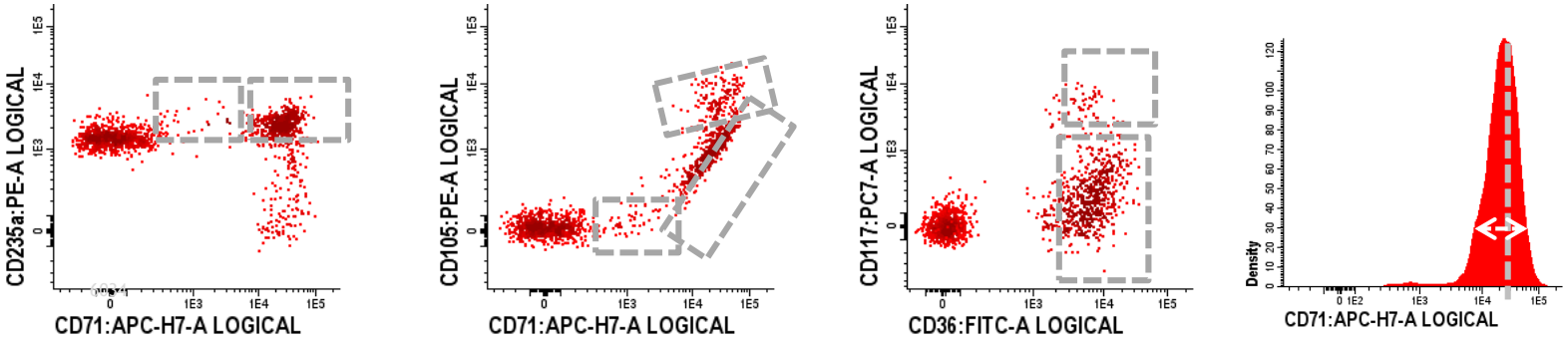
Dysplastic erythroid immunophenotypes associated with MDS

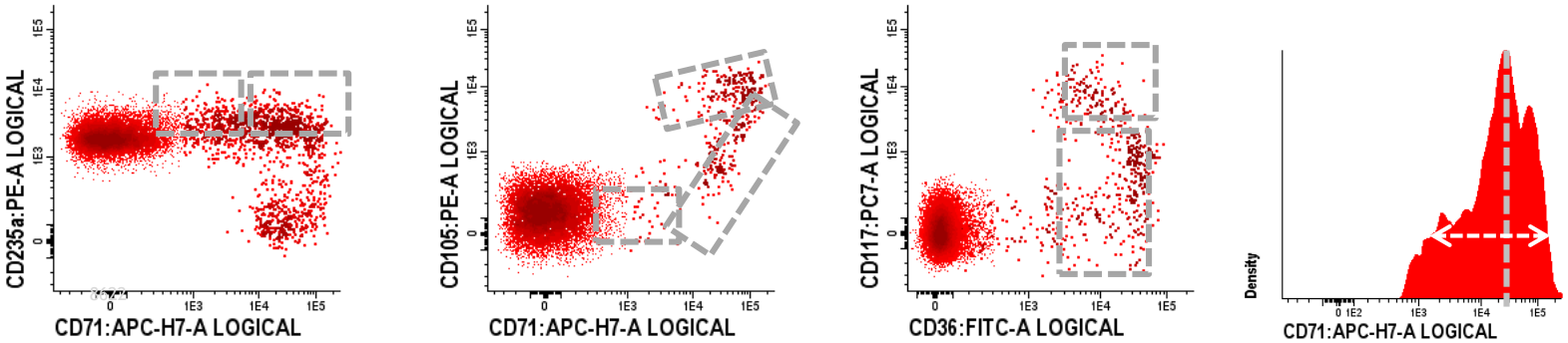
normal



Fe-def.



Vit.B12-def.



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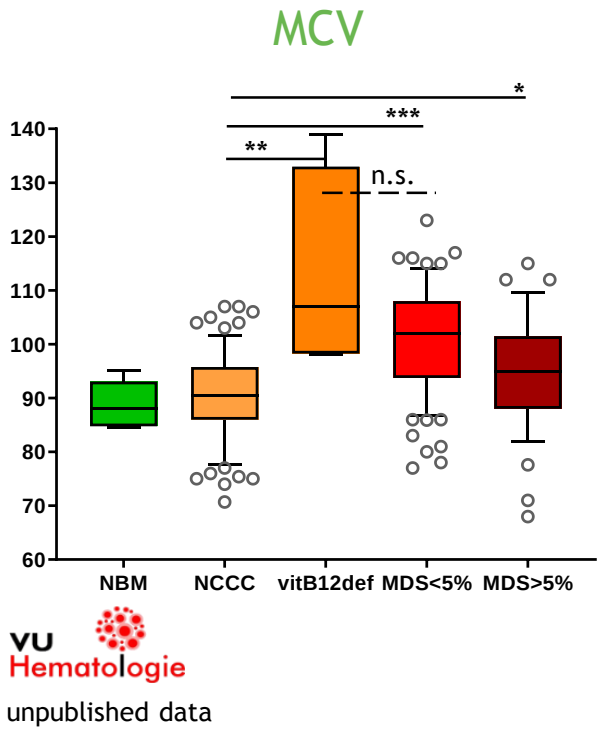
MDS vs. megaloblastic anemia

Megaloblastic anemia cases (increased MCV, e.g. due to vitamin B12 and folate deficiencies) may show severe erythroid dyplasia as assessed by morphology

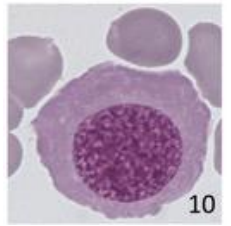


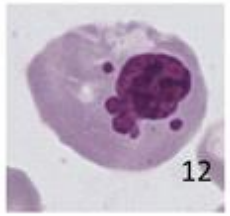
unpublished data

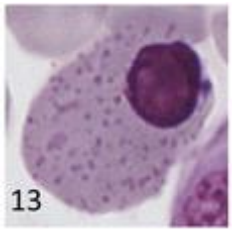
MCV: mean cell volume

immunophenotypic aberrancies similar as seen in MDS

Heterogeneous CD36 and CD71
Block in differentiation
Increased FSC and SSC





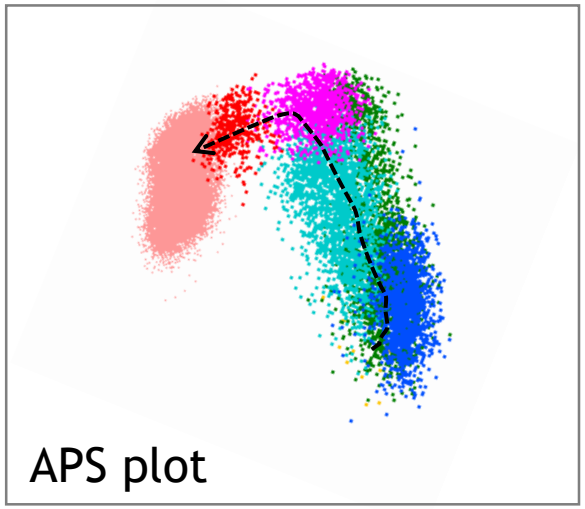


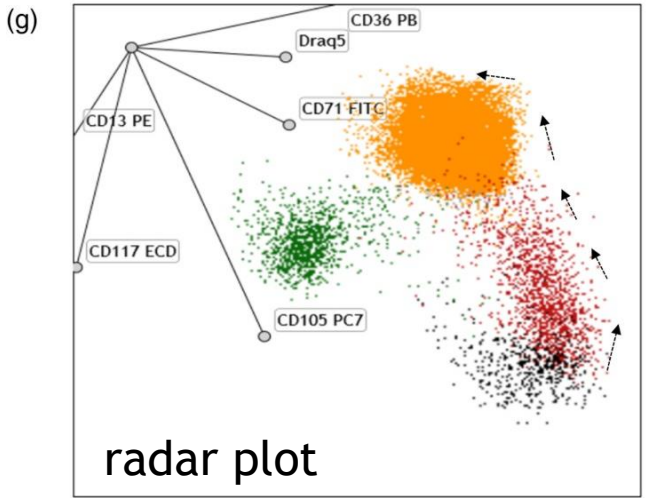
interpretation of ‘erythroid’ results requires knowledge of other clinical features (integrated approach) !

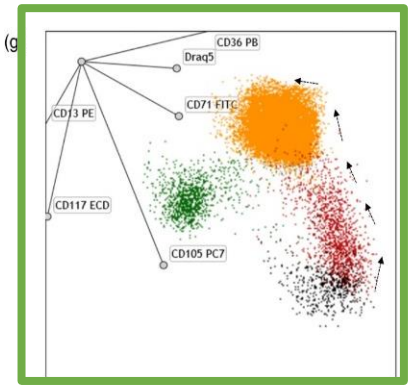
Sandes et al., Abstract HEM-07. ESCCA Valencia 2018
Porwit and Bild et al., Abstract HEM-25. ESCCA Valencia 2018
Goasguen, et al., Br J Haematol. 2018;182(4):526-33

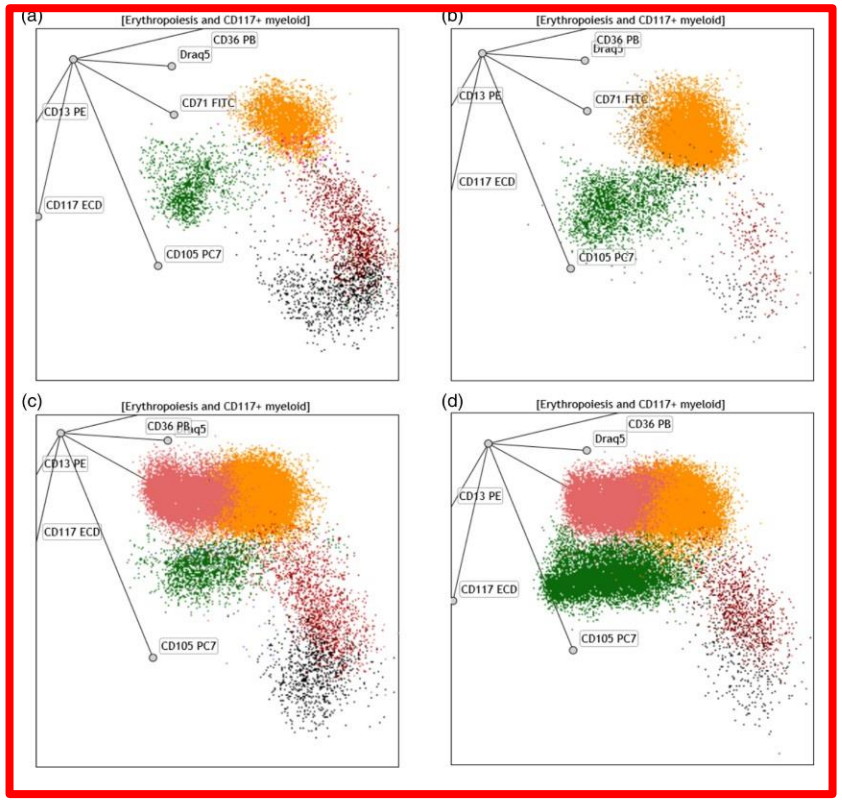
Beyond histograms and 2D plots

- Erythroid maturation in radar plots:
  - mapping of cell clusters on multidimensional radar plots
  - provide insight into BM cell composition and maturation
  - may serve as reference map to assess abnormal hematopoiesis in MDS









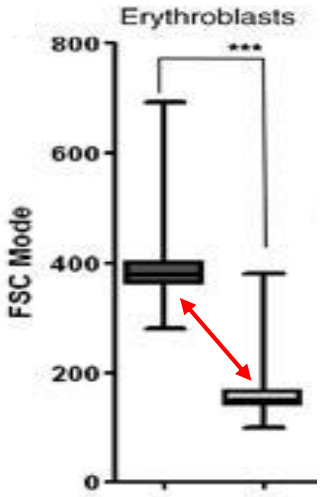
Violidaki et al. Cytometry B Clin Cytom, 2020, 98:399-41

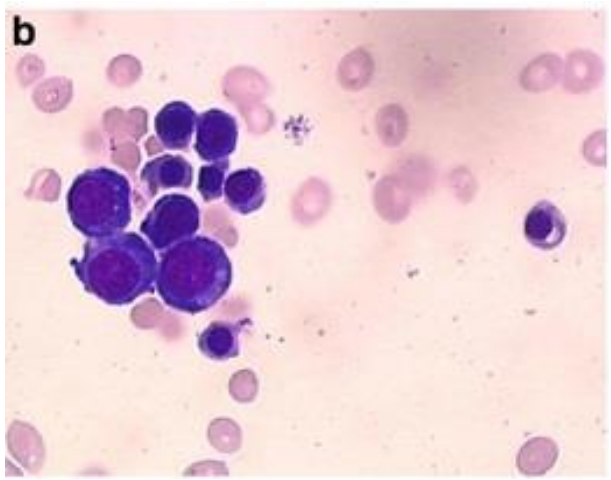
QC – ‘Lysis vs. no-lysis’

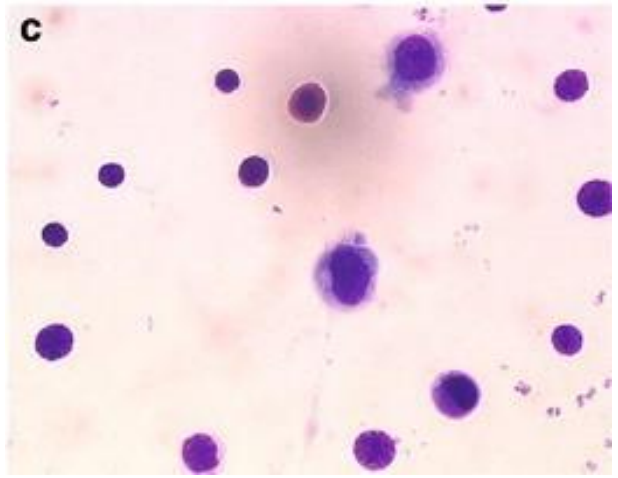
- Standard processing
  - anti coagulant, time-to-processing
  - lysis y/n; kind of lysing solution, duration, temperature
- Standard staining procedure
  - lyse-wash-stain-wash *vs*. stain-lyse-wash … Note! CD235a
- Standardized instrument settings

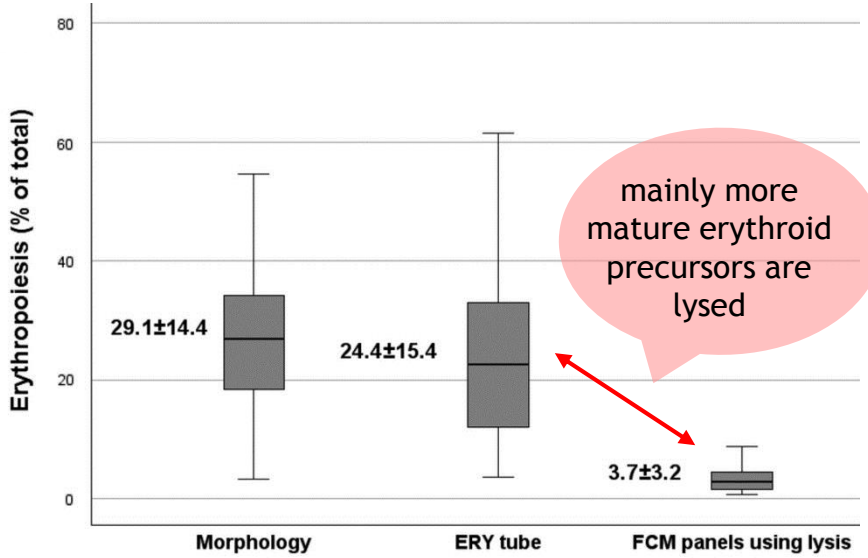
Wangen et al. Int J Lab Hem. 2014;36(2):184-96
Porwit et al., Leukemia. 2014;28(9):1793-8
Kárai B et al. Biochem Med 2018;28(2):020704

‘Lysis vs. no-lysis’

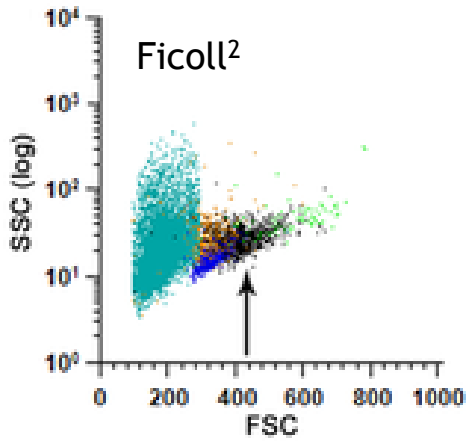


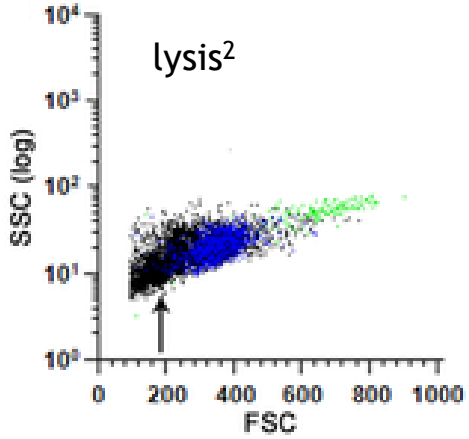






erythroid
CD105+
erythrocytes
lymphocytes



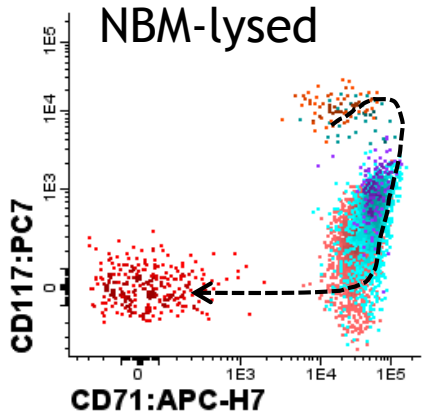


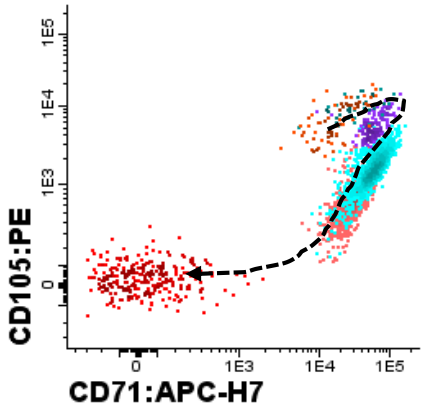
After lysis:

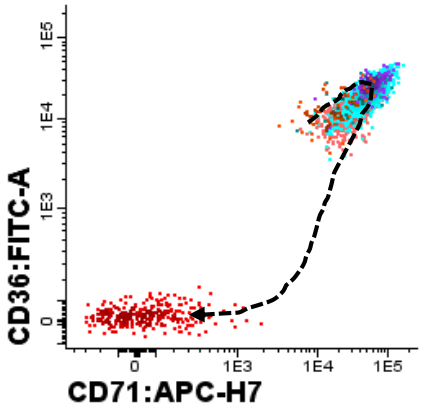
- Reduced number of erythroid cells
- Reduced erythroid FSC

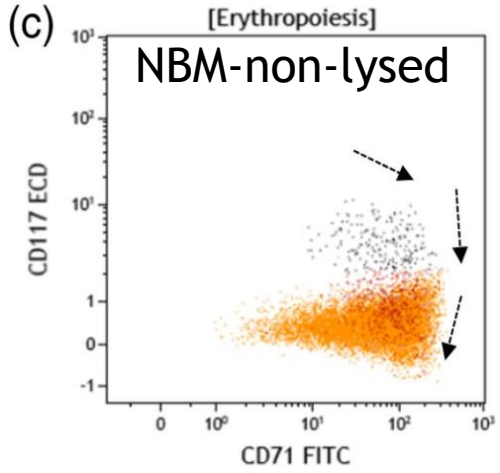
Mathis et al. Leukemia, 2013, 27, 1981–1987; [2]Wangen et al. Int J Lab Hem 2014;36(2):184-196
Violidaki et al. Cytometry B Clin Cytom, 2020, 98:399-41

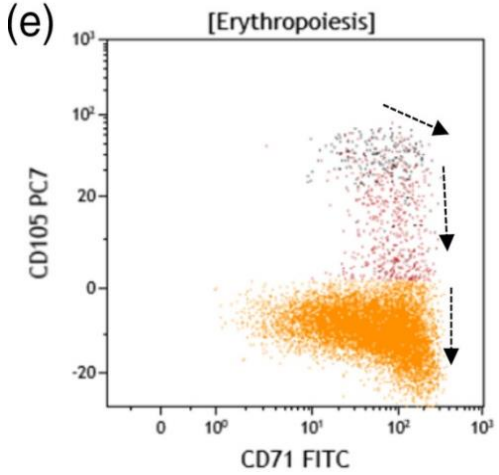
Comparison of patterns upon lysis vs. no-lysis

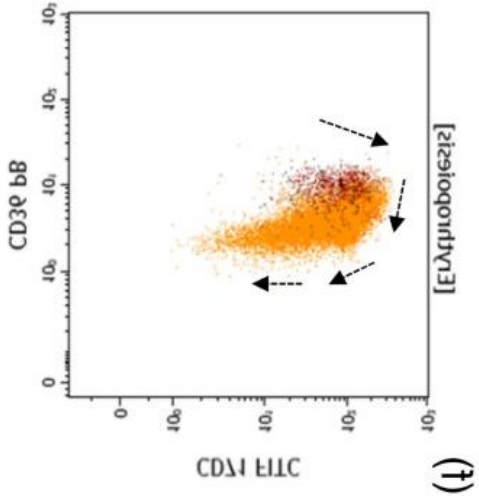








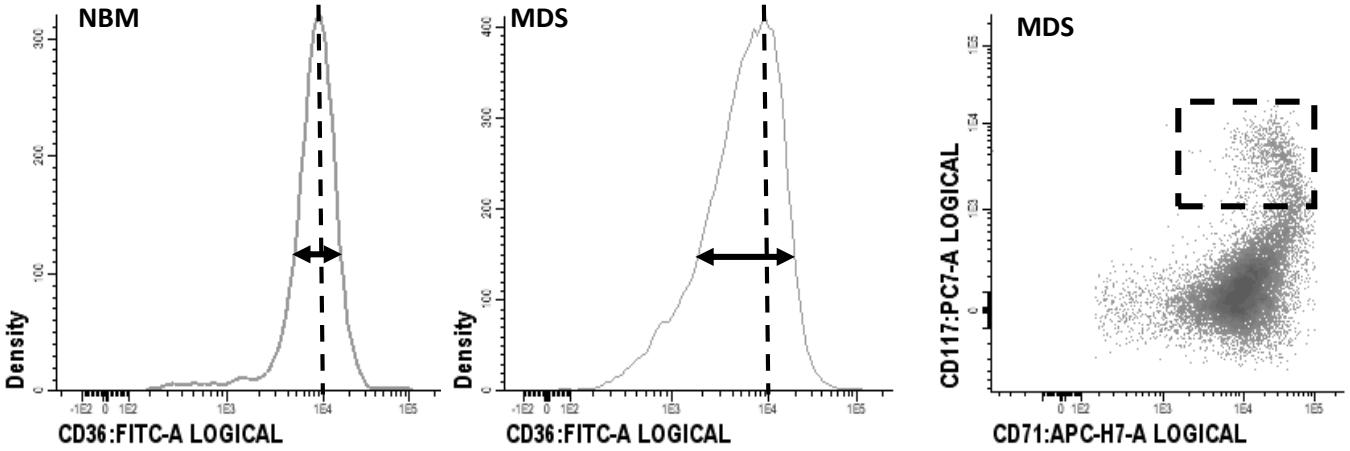




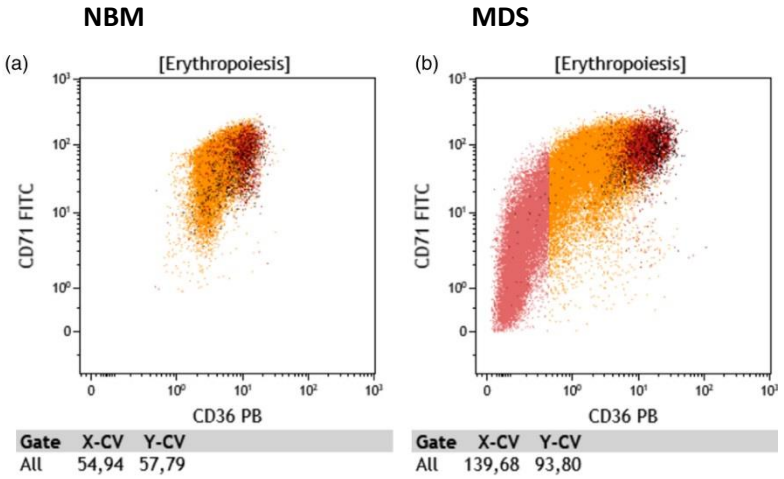
Westers, et al., Haematologica 2017;102(2):308-19
Violidaki et al. Cytometry B Clin Cytom, 2020, 98:399-41

Effect of lysis *vs*. no-lysis on expression patterns in MDS

analysis in
lysed bone marrow



analysis in
non-lysed bone marrow

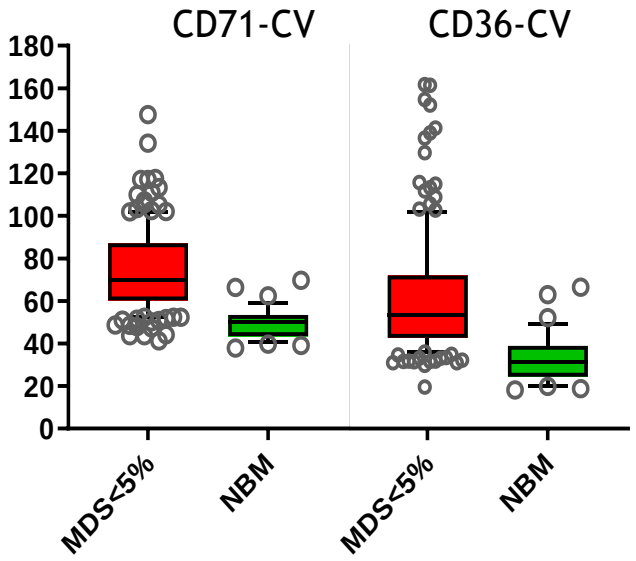


Westers et al., Haematologica, 2017, 102(2)

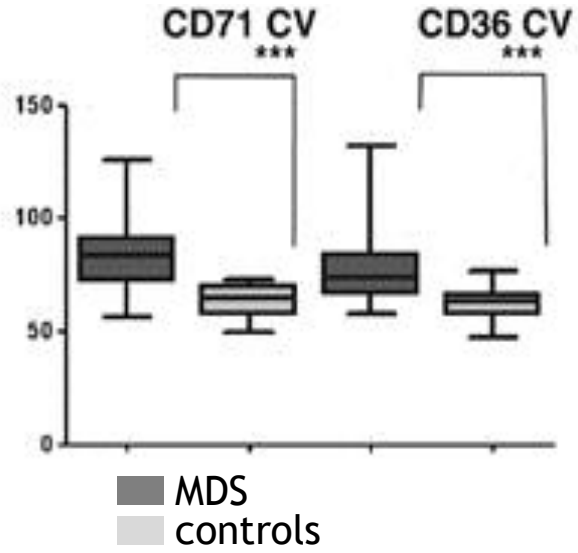
Violidaki et al. Cytometry B Clin Cytom, 2020, 98:399-41

Effect of lysis *vs*. no-lysis on CV values in MDS

lysis



no lysis, whole BM staining



Both preparation methods have shown to detect aberrancies in MDS, so:

standardize !!

and determine your own reference values

VU Hematologie
unpublished data

Mathis et al., Leukemia 2013;27(10):1981-98

Proposed aberrancies in maturing erythroid cells to study dysplasia

Optional analyses	Aberrancy
% of nucleated erythroid cells (NEC)	increased
relationship CD71 *vs.* CD235a	altered pattern
expression of CD71	decreased
expression of CD36	decreased
coefficient of variation (CV) of CD71	increased
coefficient of variation (CV) of CD36	increased
% of CD117-positive precursors	increased/decreased
% of CD105-positive precursors	increased/decreased
expression of CD105	increased/decreased

Van de Loosdrecht et al., Haematologica 2009;94(8):1124-34 ; Westers et al., Leukemia 2012;26(7)36:422-30; Porwit et al., Leukemia. 2014;28(9):1793-8; Westers et al., Haematologica 2017;102(2):308-19

Dysplastic erythroid immunophenotypes associated with MDS

Multicentric study revealed a combination of markers specific for MDS-associated erythroid dysplasia (19 centers, 1037 cases)

- Increase in CV of CD71 expression
- Increase in CV of CD36 expression
- Decrease in CD71 expression
- Decrease or increase in % of CD117+ erythroid progenitors

All compared to reference values in non-clonal cytopenic controls

Note! Analysis in lysed bone marrow samples
CD105 not included in the analysis

ELN European LeukemiaNet

Westers et al., Haematologica, 2017, 102(2):308-319

Dysplastic erythroid immunophenotypes associated with MDS

parameter	exp(B)	95% CI	p-value
CD36 CV increased	4	1.57 - 8.48	0.003
CD71 CV increased	3	1.61 – 6.37	0.001
CD71 MFI decreased	2	1.07 – 4.45	0.033
%CD117 EryProg decreased/increased	2	0.92 – 3.23	0.084

MDS-associated erythroid dysplasia:

ROC curve: cut-off ≥ 5; specificity 90%

parameter		95% CI	p-value
CD36 CV increased	1	1.57 - 8.48	0.003
CD71 CV increased	1	1.61 – 6.37	0.001
CD71 MFI decreased	1	1.07 – 4.45	0.033
%CD117 EryProg decreased/increased	1	0.92 – 3.23	0.084

Cut-off ≥ 2 aberrancies

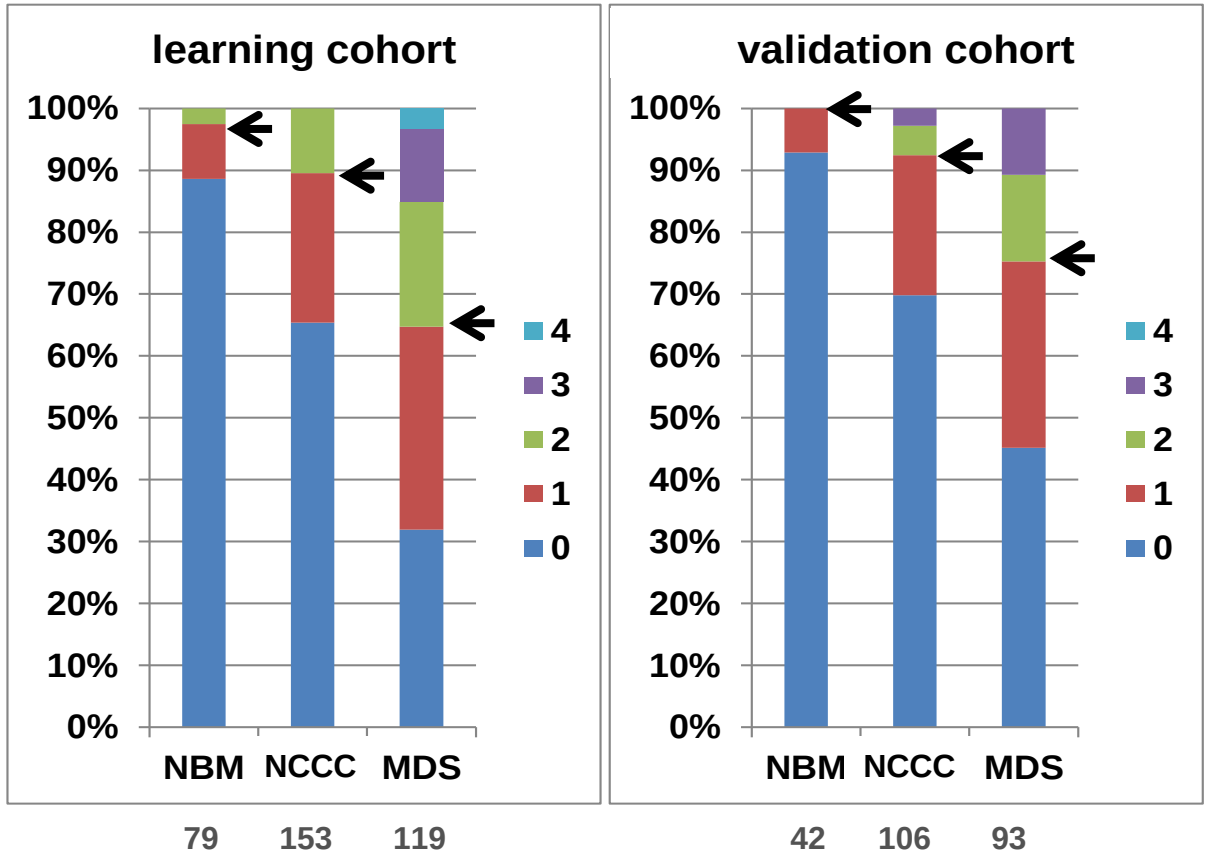
Note!
CD71MFI and %CD117 not enough to indicate MDS-associated erythroid dysplasia

CV: coefficient of variation;
MFI: mean fluorescence intensity

ELN European LeukemiaNet

Westers, et al., Haematologica 2017;102(2):308-19

Dysplastic erythroid immunophenotypes associated with MDS



Results when translated into a numerical model

Cut-off ≥ 2 aberrancies

Note!
This is not a diagnostic score

NCCC: non clonal cytopenic controls

ELN European LeukemiaNet

Westers, et al., Haematologica 2017;102(2):308-19

Dysplastic erythroid immunophenotypes associated with MDS

FCM parameter	MDS (106)	NCCC (61)
CD36 CV increased	34%	3%
CD71 CV increased	66%	23%
CD71 MFI decreased	21%	5%
%CD117 EryProg decreased/increased	60%	66%
≥ 2	**64%**	**11%**
	↕	↕
erythroid dysplasia by cytomorphology	**84%**	**10%**

Validation of ELNet proposal in
a <5% blast count MDS cohort vs. controls:

CD36-CV most specific

CD71-CV most sensitive

Cremers et al. Haematologica 2017;102(2):320-326

Summary: Aberrancies in maturing erythroid cells

Optional/recommended analyses	Aberrancy
✓ Percentage of nucleated erythroid cells	Increased
✓ Relationship of CD71 and CD235a	Altered Pattern
✓ Expression of CD71	Decreased
✓ Expression of CD36	Decreased
✓ CD71 CV	Increased
✓ CD36 CV	Increased
✓ Percentage of CD117-positive precursors	Increased/decreased
✓ Percentage CD105-positive precursors	Increased
✓ Expression of CD105	Increased/decreased

ELN European LeukemiaNet

Van de Loosdrecht et al., Haematologica 2009; 94(8):1124-34; Westers et al., Leukemia 2012;36:422-30
Mathis et al., Leukemia 2013;27:1981-198; Westers et al., Haematologica, 2017, 102(2):308-319

Conclusions

- Erythroid differentiation can be visualized using flow cytometry

- Immunophenotypic changes may point to dysplastic changes as seen in MDS

  thereby: supporting the diagnosis of MDS in cytopenic patients

  increasing the sensitivity of other flow scores in MDS

  Note:

  - standard processing and knowledge of patterns in controls is of utmost importance
  - reliable diagnosis cannot be made on erythroid analysis and/or FCM alone
  - interpretation requires knowledge of clinical pathological features (integrated approach)
  - immunophenotypic patterns in other conditions may be confused with MDS

    (e.g. iron/vitamin deficiencies, medication-induced, reactive marrow, etc.)

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