International Flow Cytometry Working Group (IMDSFlow)

# Flow Cytometry Testing and Reporting in Myelodysplastic Syndromes


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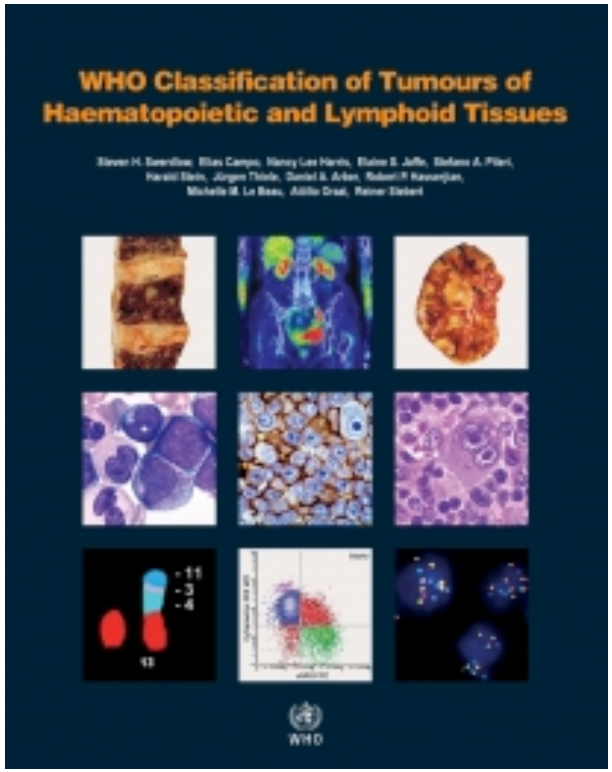
LUNDS UNIVERSITET

ELN European LeukemiaNet

# Myelodysplastic syndromes (MDS)

- **MDS are a group of clonal haematopoietic stem cell diseases characterised by dysplasia, ineffective haematopoiesis in one or more of the major myeloid cell lines, cytopenia(s), and increased risk of development of acute myeloid leukaemia (AML).**




2016 update of the WHO classification, IARC 2017

# Myelodysplastic Syndromes WHO 2008

- Refractory cytopenia with unilineage dysplasia (RCUD)
- [Anaemia (RA); Neutropenia (RN); Thrombocytopenia (RT)]
- Refractory Anemia with Ring Sideroblasts (RARS)
- Refractory Cytopenia with Multilineage Dysplasia (RCMD) ± RS
- Refractory Anemia with Excess Blasts-1 (RAEB-1)
- Refractory Anemia with Excess Blasts-2 (RAEB-2)
- Myelodysplastic Syndrome-Unclassified (MDS-u)
- 5q- Syndrome

*WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th* EDS: Swerdlow, S., Campo, E., Harris, N., Jaffe, E., Pileri, S.A., Stein, H., Thiele, J., Vardiman, J.W

# MDS and flow cytometry – WHO 2008

- **Multiple aberrant features (≥3) in maturation patterns of erythroid and myeloid lineage are highly specific for MDS, single aberrancies are not diagnostic**
- **Emerging pathological CD34**
- **and/or CD117 positive populations are suggestive of transformation**
- **THERE WAS A NEED FOR GUIDELINES**

# European Leukemia Net Workshops on Flow Cytometry Diagnostics in MDS:

Arjan A van de Loosdrecht, Amsterdam

- Amsterdam, March 27/28, 2008
- Munich, October 29/30, 2009
- London, November 5/6, 2010
- Pavia, November 4/5 2011
- Amsterdam, October 26/27, 2012
- Munich Nov1/2, 2013
- Vienna Oct 30-Nov1, 2014
- Athens  Oct 30-31/2015
- Paris  Oct. 29-30, 2016
- Lund Nov.3-5/2017
- Munich Nov.1-2/2018

The Netherlands
Austria
Australia
France
Germany
Greece
Italy
Japan
Spain
Sweden
Taiwan
UK
USA

# Publications


- van de Loosdrecht AA, et al Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. Haematologica. 2009 Aug;94(8):1124-34.
- Westers TM,et al. Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European LeukemiaNet Working Group. Leukemia. 2012 Jul;26(7):1730-41.
- van de Loosdrecht AA, et al. Rationale for the Clinical Application of Flow Cytometry in Patients with Myelodysplastic Syndromes. Leuk Lymphoma. 2013 Mar;54(3):472-5.
- Porwit A, et al. Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes-proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. Leukemia 2014 Jun 12;28:1793-8.
- Westers TM,et al; IMDSFlow Working Group. Immunophenotypic analysis of erythroid dysplasia in myelodysplastic syndromes. A report from the IMDSFlow working group. Haematologica. 2017 Feb;102(2):308-319

# FCM is a part of European Leukemia Net diagnostic approach to MDS

| Diagnostic tool | Diagnostic value | Priority |
|---|---|---|
| Peripheral blood smear | • Evaluation of dysplasia in one or more cell lines<br>• Enumeration of blasts | Mandatory |
| Bone marrow aspirate | • Evaluation of dysplasia in one or more myeloid cell lines<br>• Enumeration of blasts<br>• 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**

# Recommendation of WHO 2016

- **a standardized approach regarding not only processing but also documentation and reporting of bone marrow findings is emphasized**
- **it is assumed that this evaluation will be performed with full knowledge of the clinical history and pertinent laboratory data**
- **integrated approach including blood, bone marrow smears, bone marrow biopsy and ancillary studies (molecular, cytogenetics, flow cytometry, immunohistochemistry) is recommended for the final classification**

*(1) Lee SH, Erber WN, Porwit A, Tomonaga M, Peterson LC, ICSH guidelines for the standardization of bone marrow specimes and reports, Int J Lab Hematol, 2008, 30 (5), 349-64*

# Summary: WHO 2016 Revision of MDS classification

## No excess of blasts (<5%)

- MDS with single lineage dysplasia
- MDS with multilineage dysplasia
- MDS with ring sideroblasts
  - *and unilineage dysplasia*
  - *and multilineage dysplasia*
- MDS with isolated del(5q)
- MDS, unclassifiable (MDS-U)

## Excess blasts

- MDS with excess blasts
  - MDS with excess blasts-1 (5-9%)
  - MDS with excess blasts-2 (≥10%)

▼

- *Now will include most cases previously classified as acute erythroid leukemia,*
- *Diagnosis will be based on blast % of total marrow cells*

*Arber et al., The 2016 revision of the WHO classification of myeloid neoplasms… Blood, 2016, 127(20):2391-405*

# How flow cytometry aberrancies are applied in integrated diagnostics of MDS in 2016?

- Accumulating evidence suggests that abnormal flow cytometry patterns predict MDS with good sensitivity/specificity
- Specific panels should be carefully chosen and validated according to published guidelines
- Flow cytometry results should be integrated with the bone marrow morphology report
- Similar to molecular methods FCM will still only be considered as "supportive" of MDS and will not alone be sufficient for making a primary MDS diagnosis

Malcovati L et al. Blood 2013;122:2943, Porwit A et al. Leukemia 2014;28:1793, Arber et al Blood *2016, 127(20):2391-405*

# Recommended methods for cell sampling, handling and processing

- Bone marrow samples (blood insufficient)
- Heparin (EDTA less optimal)
- Processing within 24h, store at RT
- Lyse-stain-wash
- Bulk-lysis with ammonium chloride preferred
- $5x10^5$ cells per tube
- Incubation 15 min in the dark
- Wash, fix in 0.5%PFA/PBS
- Immediate acquisition

van de Loosdrecht AA, et al Haematologica. 2009 Aug;94(8):1124-34.

# Antibody panels

- **a minimum screening panel should include**

**CD19, CD34, CD45***

**Ogata score for new patients with cytopenia**

- **Second step:**

**a consensus panel of antibody combinations for detailed evaluation patients with clinical picture suspect for MDS**

***Della Porta MG, et al. Multicenter validation of a reproducible flow cytometric score for the diagnosis of low-grade myelodysplastic syndromes: results of a European LeukemiaNET study. Haematologica. 2012 Aug;97(8):1209-17**

# Flow cytometry in MDS: antigens and patterns to be tested

**Table 1.** Aberrant FCM features to be included in the diagnostic work-up of patients with myelodysplastic syndromes (modified from Westers *et al.*[28])

| *Markers* | *Progenitor myeloid* | *Neutrophils* | *Monocytes* | *Progenitor B* | *Erythroid* |
|---|---|---|---|---|---|
| **SSC** | Increased SSC | **Low ratio to lymphocytes** | Decreased SSC | | |
| **CD45** | Decreased expression | Decreased expression | Decreased expression | | |
| **CD117** | Decreased frequency | Increased expression | | | Increased frequency of positive precursors |
| **CD34** | **Increased frequency of $CD34^+/CD19^-$ (>2%) Increased proportion of $CD38^{-/dim}/CD34^+$** | **Asynchronous expression** | **Asynchronous expression** | $CD19^+/CD34^+$ ⩽5% of $CD34^+$ cells | |
| **HLA-DR** | Increased proportion of $HLA\text{-}DR^{-/dim}/CD34^+$ cells | Increased expression | Decreased expression | | |
| **CD11b** | Increased expression on $CD34^+$ cells | | Decreased expression | | |
| **HLA-DR/CD11b** | | Aberrant pattern | Aberrant pattern | | |
| **CD11b/CD16** | | **Aberrant pattern (most often due to low CD16)** | **Abnormal expression of CD16 on $CD11b^+$ monocytes** | | |
| **CD13/CD11b** | | Aberrant maturation pattern | | | |
| **CD13/CD16** | | Aberrant maturation pattern | | | |
| **CD13/CD33** | Increased number of $CD33^+/CD13^-$ or $CD33^-/CD13^+$ cells | Increased number of $CD33^+/CD13^-$ or $CD33^-/CD13^+$ cells | Increased number of $CD33^+/CD13^-$ or $CD33^-/CD13^+$ cells | | |
| **CD14** | | | Decreased expression | | |
| **CD15** | Asynchronous expression on progenitors | Asynchronous expression together with CD34 | | | |
| **CD15/CD10** | | Aberrant pattern Lack of CD10 on mature neutrophil granulocytes | | | |
| **CD19** | Decreased $CD34^+/CD19^+$ lymphoid progenitors | Abnormal expression | | | |
| **CD19/CD10** | | | | Decreased frequency | |
| **CD36** | | Increased expression | Abnormal Expression | | **Abnormal heterogeneous and/or low expression** |
| **CD36/CD14** | | | Aberrant pattern | | |
| **CD5** | **Abnormal expression on $CD34^+$ and/or $CD117^+$ cells** | **Abnormal expression** | **Abnormal expression** | | |
| **CD56** | **Abnormal expression on $CD34^+$ and/or $CD117^+$ cells** | **Abnormal expression** | **Abnormal expression** | | |
| **CD7** | **Abnormal expression on $CD34^+$ and/or $CD117^+$ cells** | **Abnormal expression** | **Abnormal expression** | | |
| **CD71** | | | | | **Abnormal heterogeneous and/or low expression** |
| **CD71/CD235** | | | | | Aberrant pattern |

Abbreviation: SSC, side scatter. Items in boldface have been reported to have strong value in supporting MDS diagnosis. Aberrant pattern indicates a difference from the pattern seen in normal bone marrow. Abnormal expression indicates that the relevant marker is not present on this cell type in normal bone marrow. Increased/decreased expression is to be considered in comparison to normal bone marrow counterparts.

**Important:**

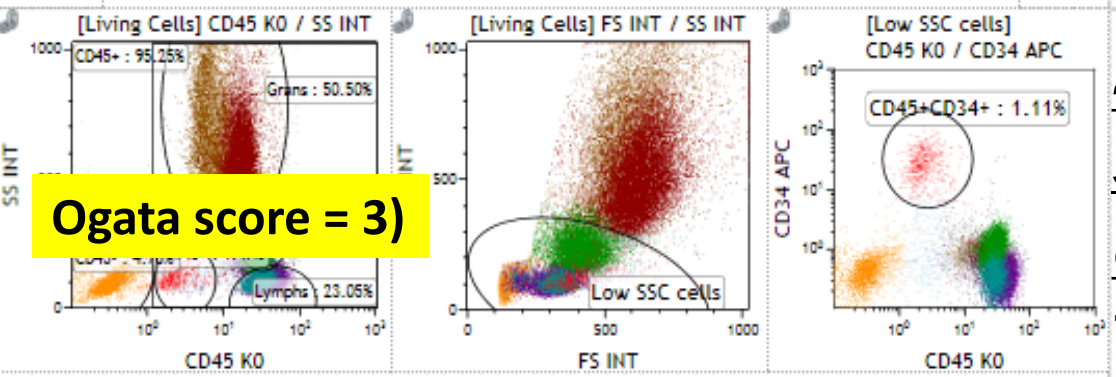
**Patterns to be assessed in comparison to normal bone marrow**


**Westers TM et al., Leukemia 2012;36:422-30**
**Van de Loosdrecht AA et al., J Natl Comp Canc Netw 2013;11:892-902**
**Porwit A, Loosdrecht AA van de, et al., Leukemia 2014;28:1793-98**

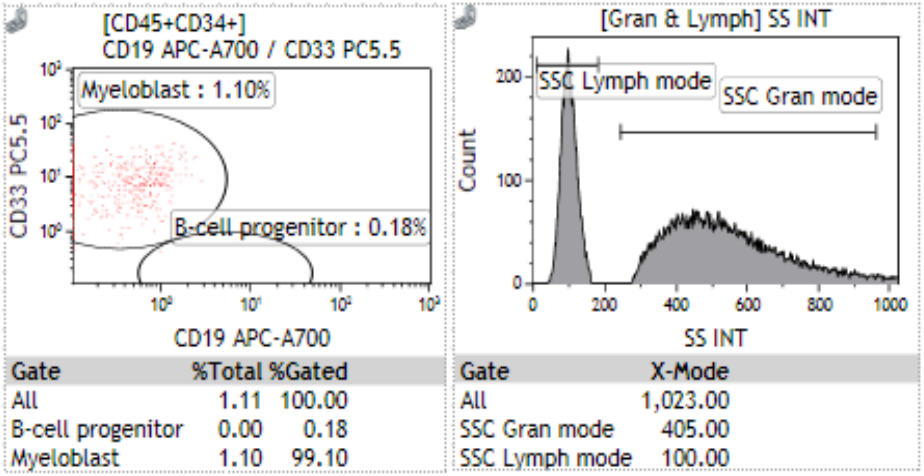
# Screening tube

| Fluorescence | Ab | clone | Titre (ul) |
|---|---|---|---|
| FITC | CD4 | 13B8.2 | 10 |
| | Kappa | Polyclonal | 10 |
| PE | CD8 | B9.11 | 10 |
| | Lambda | Polyclonal | 10 |
| ECD | CD3 | UCHT1 | 5 |
| | CD14 | RMO52 | 3 |
| PC5.5 | CD33 | D3HL60.251 | 3 |
| PC7 | CD20 | B9E9 | 5 |
| | CD56 | N901 | 10 |
| APC | CD34 | 581 | 5 |
| A700 | CD19 | J3-119 | 3 |
| A750 | CD10 | ALB1 | 5 |
| PB | CD5 | BL1a | 5 |
| KO | CD45 | J.33 | 5 |

Rajab A, Porwit A. Screening bone marrow samples for abnormal lymphoid populations and myelodysplasia-related features with one 10-color 14-antibody screening tube. Cytometry B Clin Cytom. 2015;88(4):253-60.



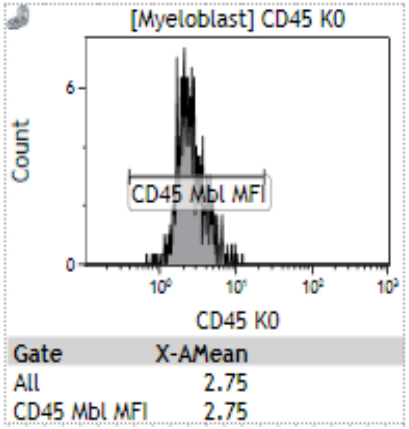

**Ogata score = 3)**



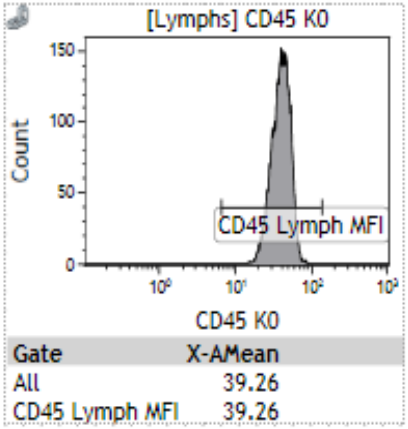

| Gate | %Total | %Gated |
|---|---|---|
| All | 1.11 | 100.00 |
| B-cell progenitor | 0.00 | 0.18 |
| Myeloblast | 1.10 | 99.10 |




| Gate | X-Mode |
|---|---|
| All | 1,023.00 |
| SSC Gran mode | 405.00 |
| SSC Lymph mode | 100.00 |




| Gate | X-AMean |
|---|---|
| All | 2.75 |
| CD45 Mbl MFI | 2.75 |




| Gate | X-AMean |
|---|---|
| All | 39.26 |
| CD45 Lymph MFI | 39.26 |

## ***4-parameter screening score consists of:***

**1. % CD34⁺ myeloid progenitor cells among all nucleated cells (<2%)**

**2.% CD34⁺ B cell precursors among all CD34⁺ cells (>5%)**

**1.3. SSC of granulocytes (ratio to lymphocytes >6)**

**4. CD45 expression of myeloid progenitor cells (ratio to lymphocytes 4-7.5)**


*Ogata et al., Blood 2006;108;1037-1044;*
*Ogata et al., Haematologica 2009;94:1066-74;*
*Della Porta MG, et al., Haematologica 2012;97:1209-17*
*Rajab & Porwit, Clin Cytometry, 2015 Feb.9*
*Bardet et al. Haematologica,* 2015 Apr;100(4):472-8

# Validation of FCM score

Table 1
*Diagnoses and MDS-score Distribution in 440 BMA Evaluated with the Screening Panel*

| | FCM-score | | | | | FCM-score >2 | | | FCM-score ≥2 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | Positive cases | Sensitivity | Specificity | Positive cases | Sensitivity | Specificity |
| Normal; $n=8$ | 5 | 3 | 0 | 0 | 0 | 0(0%) | | 100% | 0 (0%) | | 100% |
| Hospital controls[a]; $N=207$ | 105 | 80 | 20 | 2 | 0 | 2 (1%) | | 99% | 22 (14%) | | 89% |
| MDS; $n=92$ | 7 | 19 | 23 | 31 | 12 | 43 (47%) | 47% | | 66 (72%) | 72% | |
| MDS-MPN; $n=14$ | 2 | 4 | 1 | 5 | 2 | 7 (50%) | 50% | | 8 (57%) | 57% | |
| MPN; $n=47$ | 13 | 18 | 9 | 7 | 0 | 7(15%) | 15% | | 16 (34%) | 34% | |
| B-cell or plasma cell neoplasm; $n=18$ | 8 | 7 | 3 | 0 | 0 | 0 (0%) | | 100% | 3(17%) | | 83% |
| Post treatment for other malignancy; $n=33$ | 14 | 14 | 4 | 1 | 0 | 1 (3%) | | 97% | 5(15%) | | 85% |
| Post BM transplant; $n=21$ | 4 | 10 | 6 | 1 | 0 | 1 (5%) | | 95% | 7 (33%) | | 67% |

[a]Hospital controls (HC): Patients with cytopenia(s) with no evidence of underlying malignancy, BM samples with reactive inflammatory changes or lymphoma staging specimens without lymphoma involvement.

**Differences in scores between the control group and the MDS & MDS/MPN and the MPN groups were statistically significant ($p<0.001$).**
**Scores above 2 had high predictive value for MDS and MDS/MPN diagnosis**

**Rajab A, Porwit A. Cytometry B Clin Cytom. 2015;88(4):253-60**

# Importance of various parameters of the score

Table 3
*Frequency of Aberrant Findings in Various FCM Score Components in BMA Samples with Score 2*

| Score components | Gated myeloblasts in all nucleated cells | Gated B-cell progenitor in CD34+ cells | Granulocyte to lymphocyte SSC mode ratio | Lymphocyte to myeloblast CD45 MFI ratio |
|---|---|---|---|---|
| No myeloid malignancy[a]; $N = 33$ | 1 (3%) | 23 (69%) | 9 (27%) | 13 (39%) |
| Myeloid malignancy[b]; $N = 33$ | 6 (18%) | 26 (78%) | 17 (51%) | 7 (21%) |

[a]Includes: Normal, hospital controls, B-cell or plasma cell neoplasm, post treatment for other malignancy and post BM transplant.
[b]Includes: MDS, MDS-MPN and MPNs.

**Rajab A, Porwit A. Cytometry B Clin Cytom. 2015;88(4):253-60**

Leukemia Research 71 (2018) 75–81

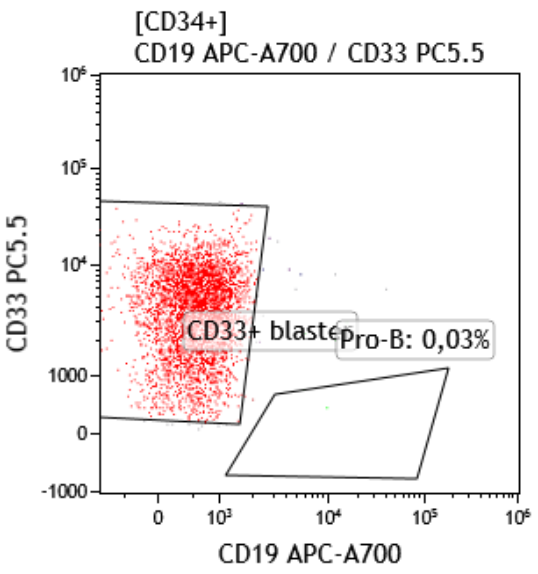
# New ”Ogata” score

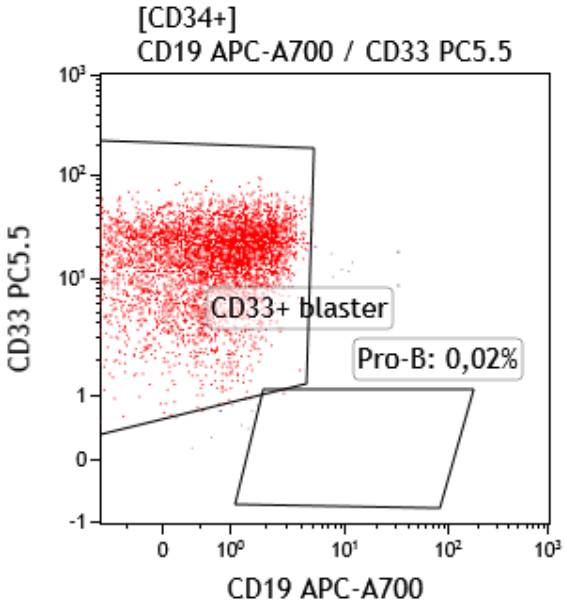
**Table 3**
Results of logistic regression analysis and development of scoring system for diagnosing low-grade MDS.

| FCM parameter | Reference range | Univariate analysis | | Multivariate analysis | | | Weighted score |
|---|---|---|---|---|---|---|---|
| | | Regression coefficient | P value | Regression coefficient | Odds ratio | P value | |
| Granulocyte/CD34 + cell CD33 ratio | > 2.5 | 2.10 | 0.0006 | 2.45 | 11.6 | 0.0019 | 1 |
| Myeloblast-related cluster size | < 2% | 2.48 | 0.023 | 3.15 | 23.3 | 0.0135 | 2 |
| B-progenitor-related cluster size | > 5% | 1.27 | 0.0239 | – * | – | – | |
| G/L SSC mode ratio | > 6.5 | 3.15 | 0.0035 | 2.70 | 14.9 | 0.0251 | 1 |
| Ly/Mbl CD45 ratio | > 4 | 0.54 | 0.664 | NA | NA | NA | |

Diagnostic power of abnormal FCM data (data outside the reference range) was analyzed by logistic regression analysis.
NA, not analyzed by multivariate analysis.

Normal CD33 in a control BM with low B-precursors

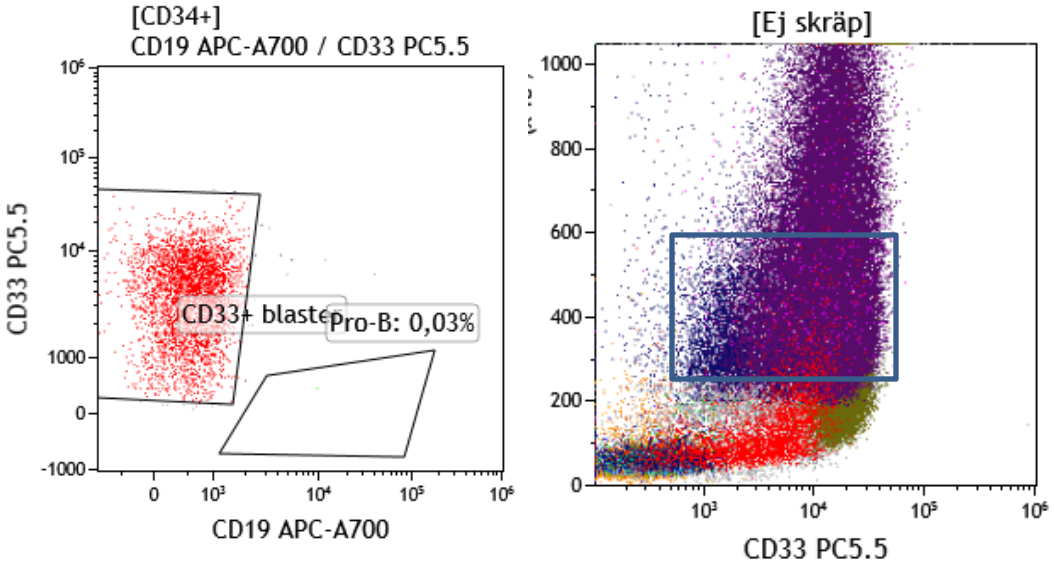



CD33 Overexpression in an MDS case

# Additional parameters in the screening tube

## CD33 on CD34 /Gran ratio




**MFI CD33 G/**
**MFI CD34 (M) ≥2.5**

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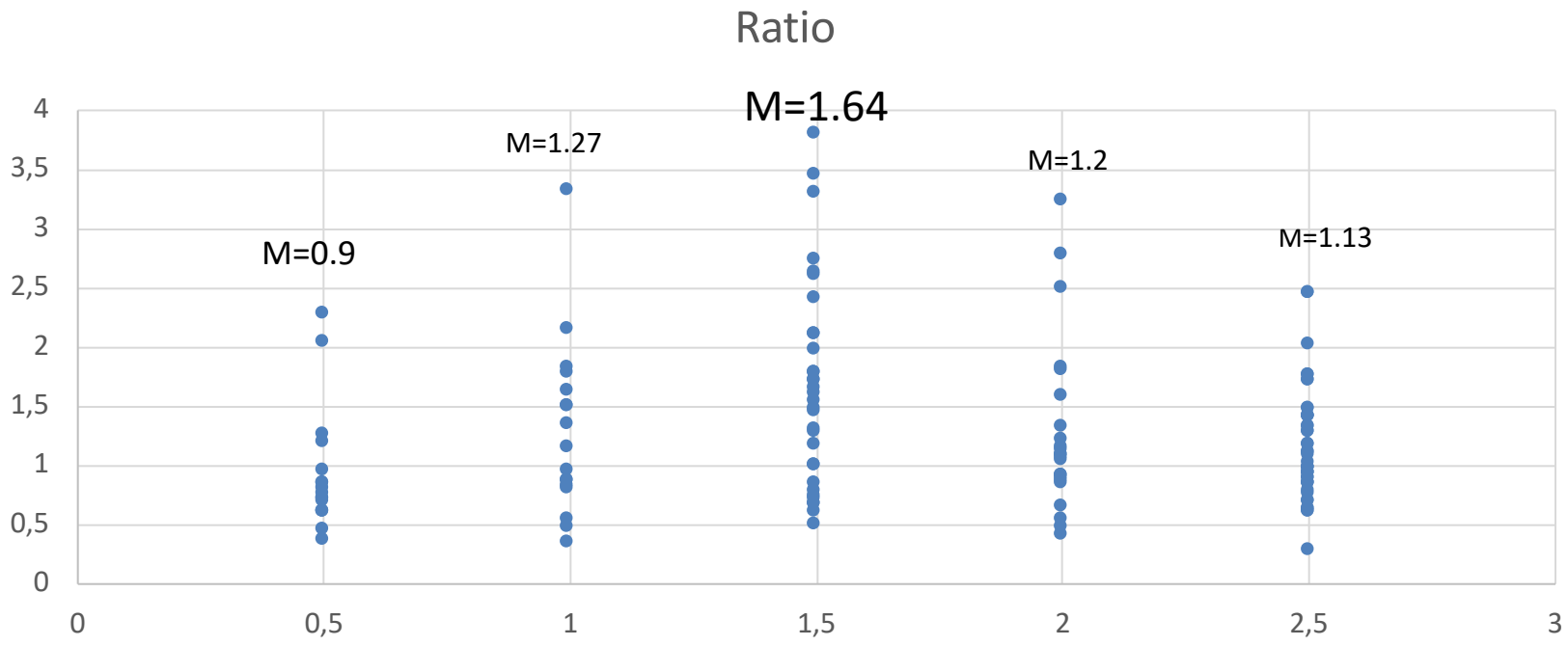
# CD33 MFI ratio between granulocytes and CD34+ cells




MDS score 0-1 N=18

MDS score 2 N=15

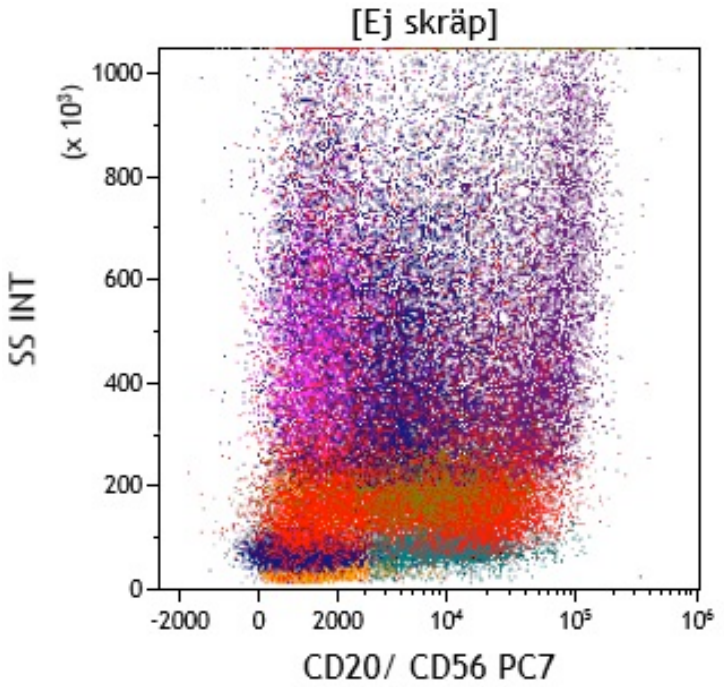
MDS score 3-4 N=31

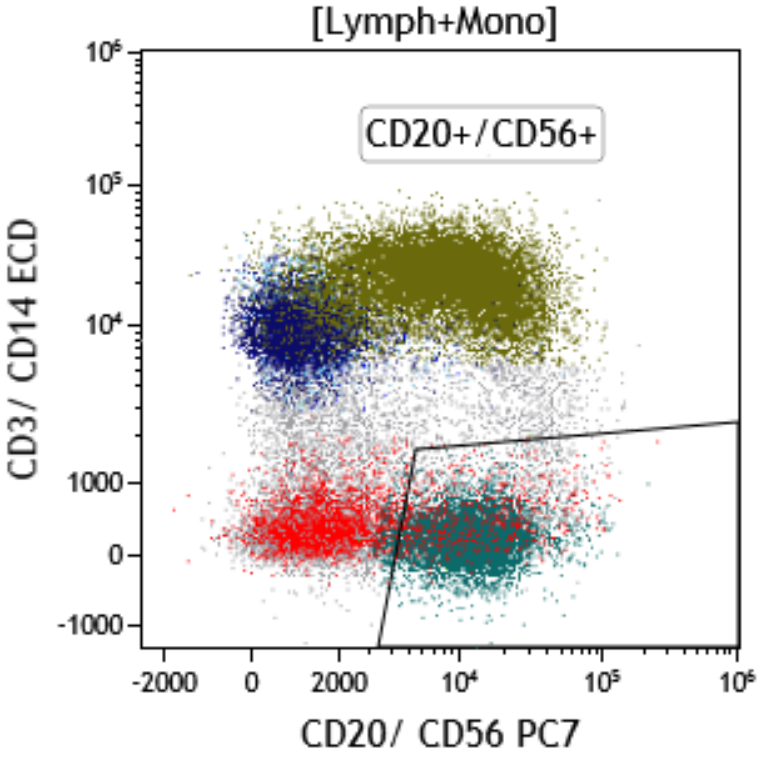
Control score 0-1 N=22

Control score 2 N=42

# Additional parameters in the screening tube

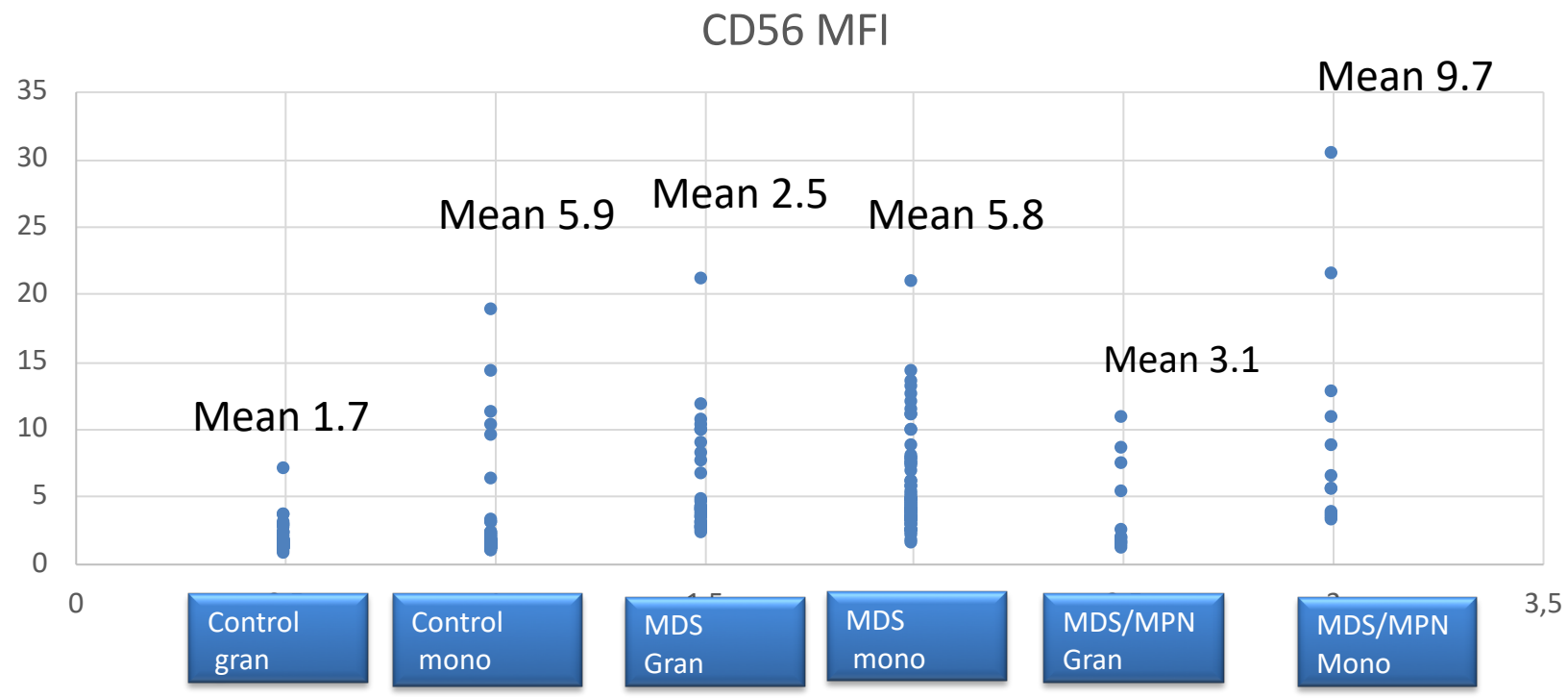
# CD56 aberrant expression



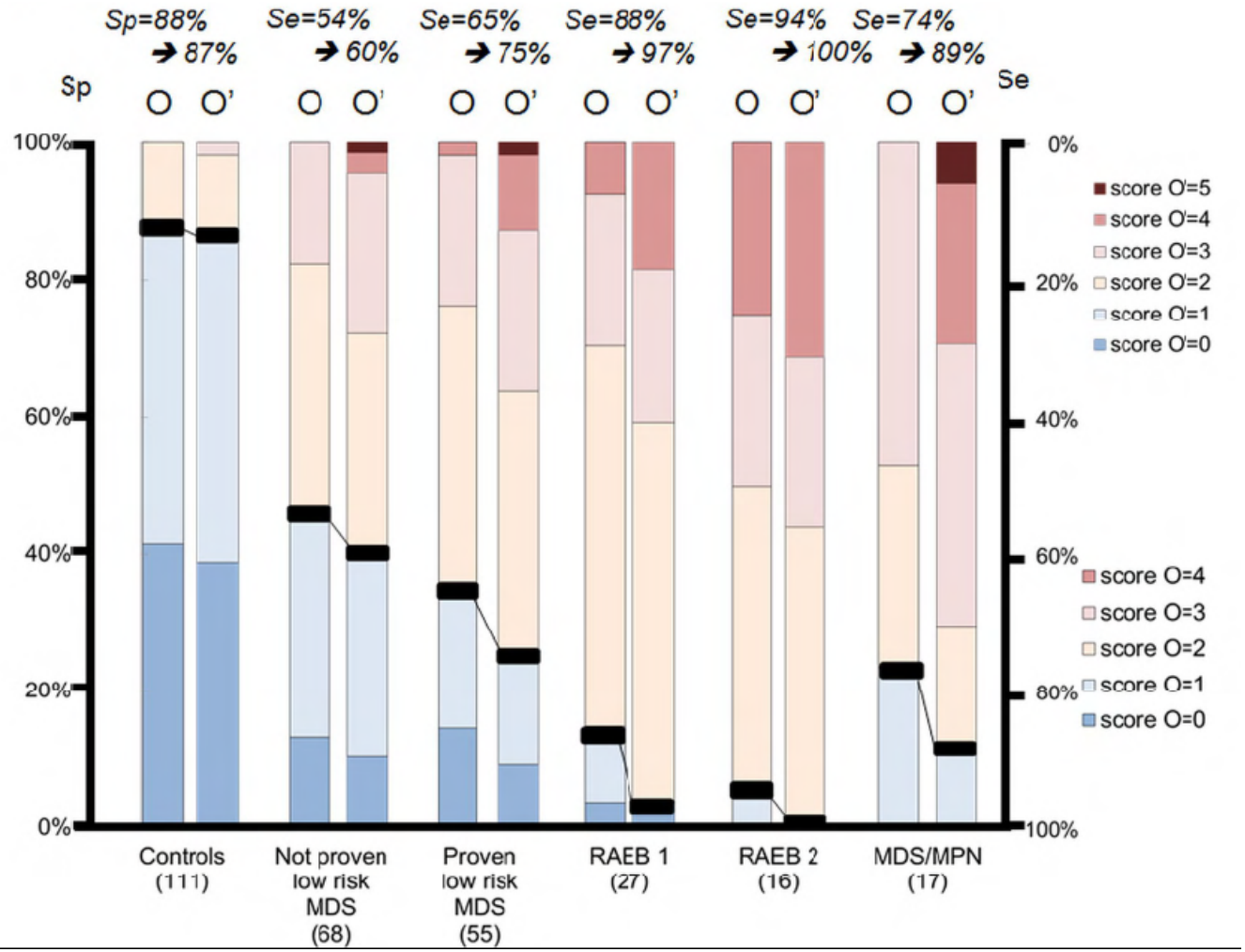

Aberrant expression of CD5, CD14, CD19 on myeloid cells as well as lower than normal CD10 expression on mature granulocytes can also be evaluated

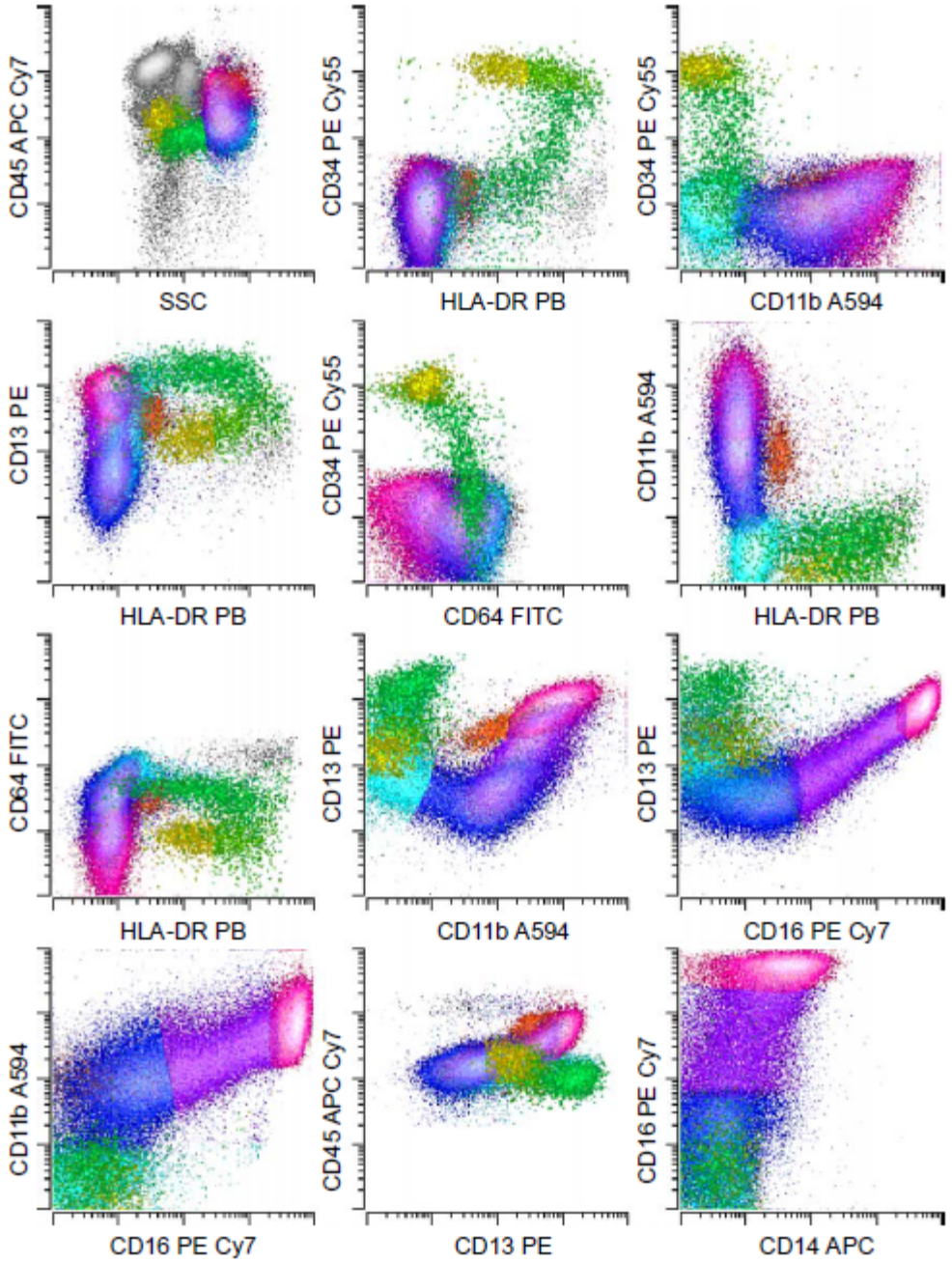
# CD56 expression i monocytes and granulocytes



# Multicentric study underlining the interest of adding CD5, CD7 and CD56 expression assessment to the flow cytometric Ogata score in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms

Valérie Bardet,[1] Orianne Wagner-Ballon,[2] Julien Guy,[3] Céline Morvan,[4] Camille Debord,[1] Franck Trimoreau,[4] Emmanuel Benayoun,[2] Nicolas Chapuis,[1] Nicolas Freynet,[2] Cédric Rossi,[3] Stéphanie Mathis,[1] Marie-Pierre Gourin,[5] Andréa Toma,[6] Marie C. Béné,[7] Jean Feuillard,[4] and Estelle Guérin;[4] on behalf of the Groupe Francophone des Myélodysplasies (GFM) and the Groupe d'Etude Immunologique des Leucémies (GEIL)






Multicolor Immunophenotyping: Human Immune System Hematopoiesis

**Brent Wood**
Department of Laboratory Medicine
University of Washington
Seattle, Washington 98195

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**Patterns of antigen expression**
**In normal bone marrow**

# Normal bone marrow FCM atlas:

- **by Marie-Christine Béné et al**

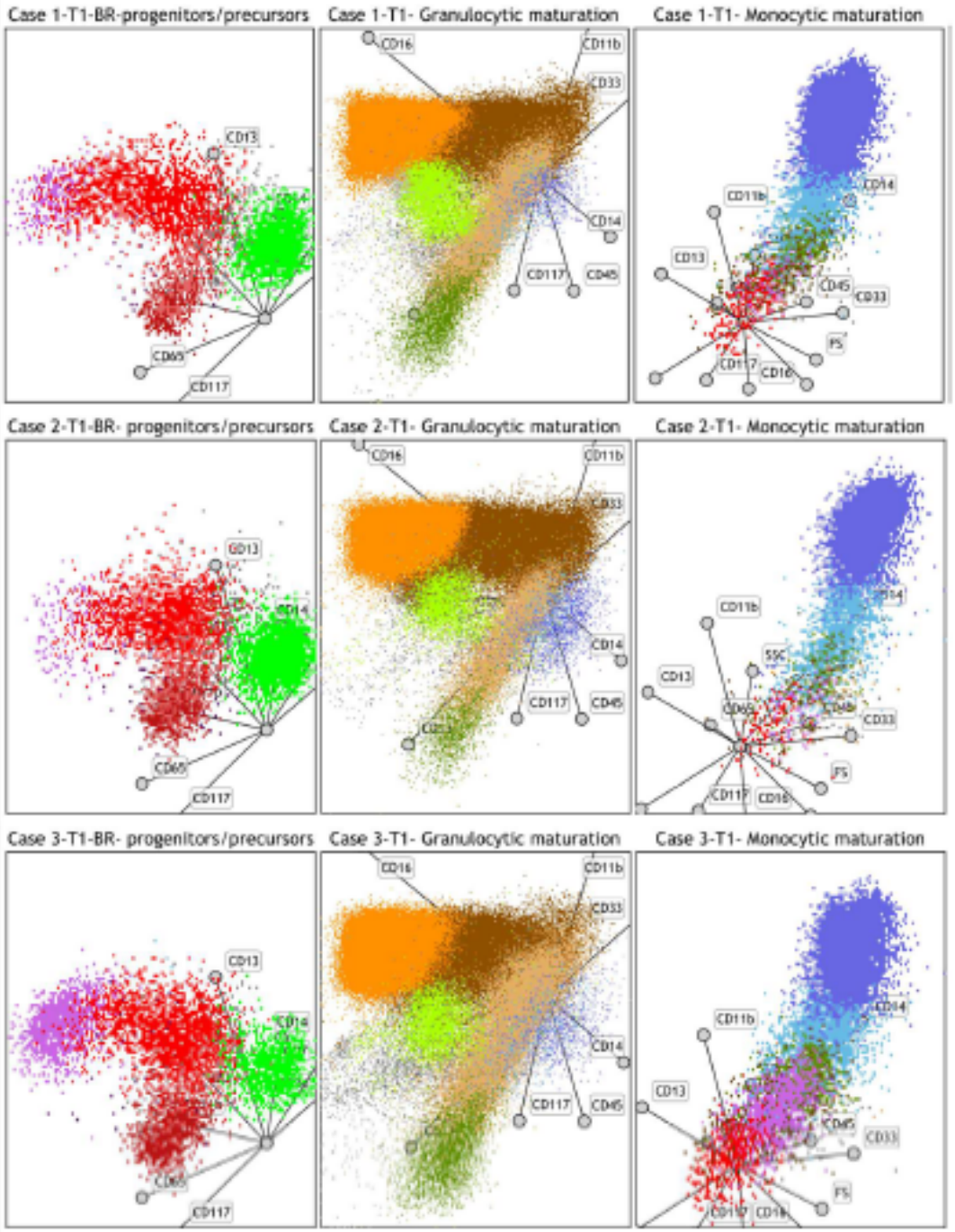
- **ELN Website**

**http://www.leukemia-net.org/ content/diagnostics/ diagnostics/ flow_cytometry_atlas/**

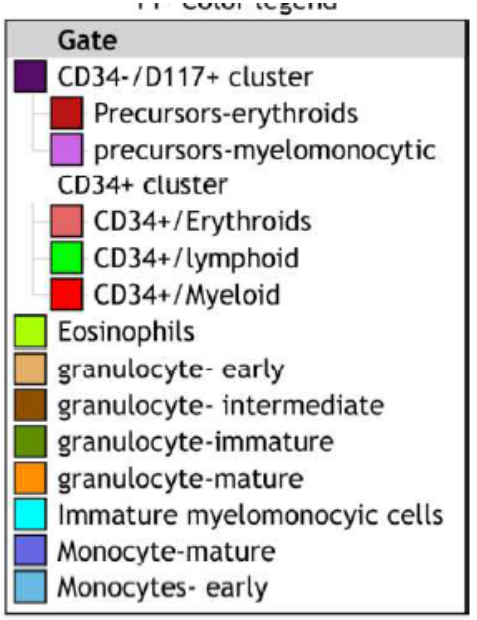
**Ref. Arnoulet C, Béné MC,et al. Four- and five-color flow cytometry analysis of leukocyte differentiation pathways in normal bone marrow: a reference document based on a systematic approach by the GTLLF and GEIL. Cytometry B Clin Cytom. 2010 Jan;78(1):4-10.**

# Example of 10 color acute leukemia/MDS panel

| | AML 1 | AML 2 | AML 3 |
|---|---|---|---|
| FITC | CD65 | CD36 | CD71 |
| PE | CD13 | CD64 | CD11c |
| ECD | CD14 | CD56 | CD4 |
| PC5.5 | CD33 | CD33 | CD33 |
| PC7 | CD34 | CD34 | CD34 |
| APC | CD117 | CD123 | CD2 |
| APC_AlexaF700 | CD7 | CD19 | CD10 |
| APC_AlexaF750 | CD11b | CD38 | CD235a |
| Pacific_BLUE | CD16 | HLA-DR | CD15 |
| Krome Orange | CD45 | CD45 | CD45 |

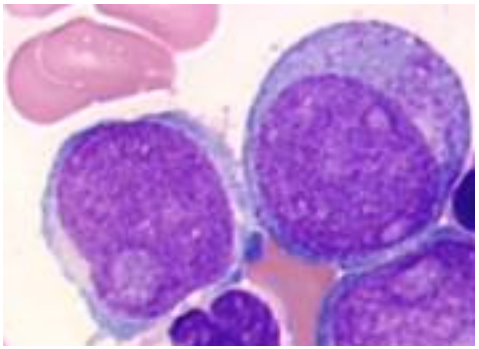



# Maturation patterns are preserved in normal and reactive bone marrows




Jafari K, Tierens A, Rajab A, Musani R, Schuh A, Porwit A. Visualization of Cell Composition and Maturation in the Bone Marrow Using 10-Color Flow Cytometry and Radar Plots. Cytometry B Clin Cytom. 2018 Mar;94(2):219-229

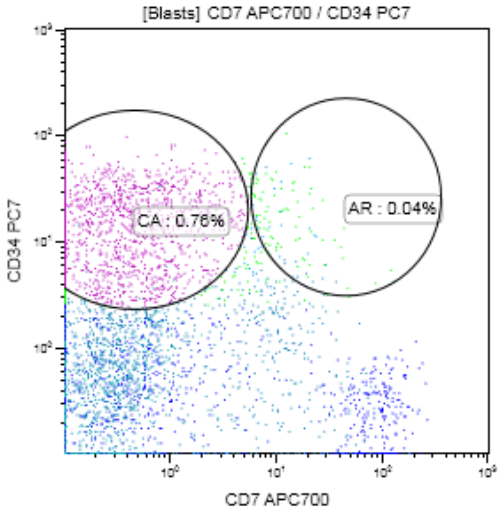
# Aberrancies to be assessed by FCM recommended by ELN Workshops

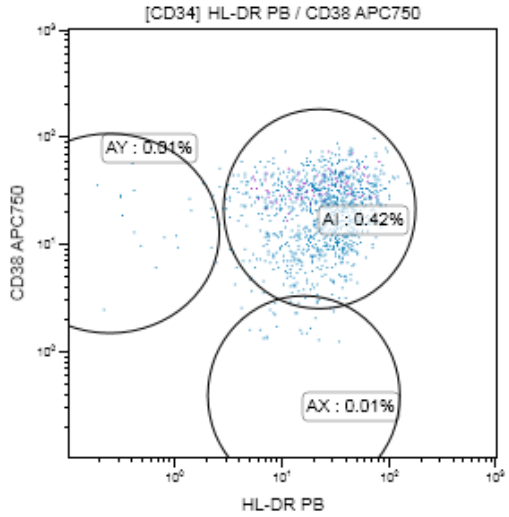


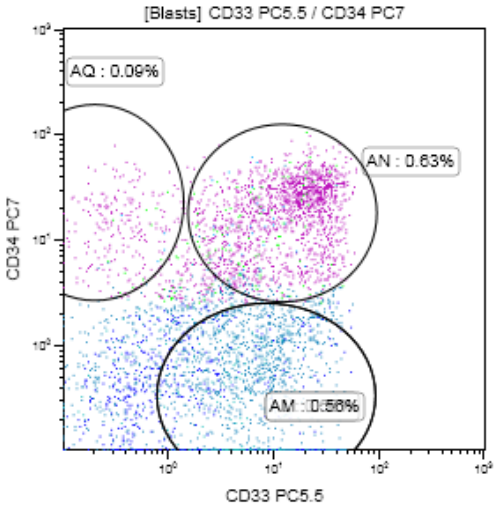
- **Abnormal Features of Progenitors:**
- Increased percentage
- Abnormal scatter and CD45 expression
- Abnormal expression of stem cell markers CD34 and CD117
- Abnormal expression of HLA-DR, CD11b, CD15
- Lineage infidelity markers CD7, CD2, CD5, CD56
- Abnormal expression of CD13, CD33, TdT, CD36, CD4

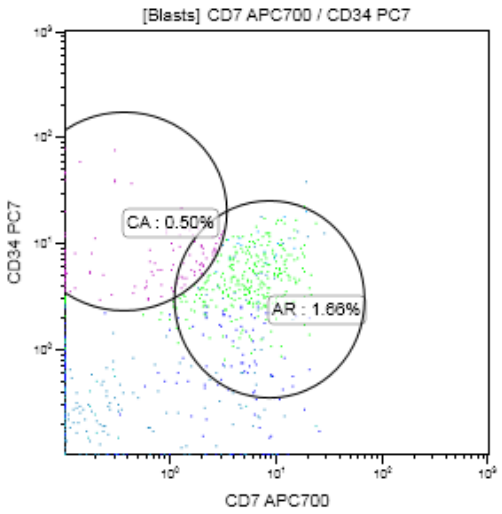
# Examples of aberrant findings in Blast/progenitor region

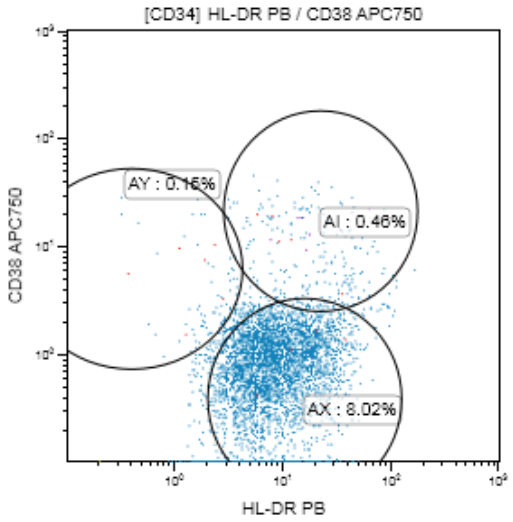
Normal

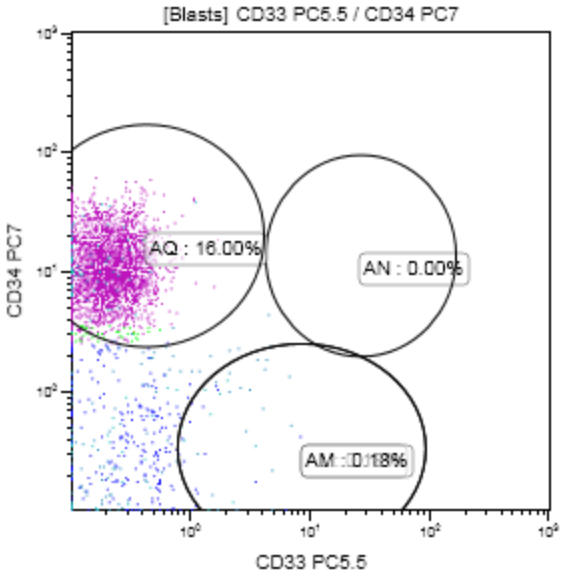


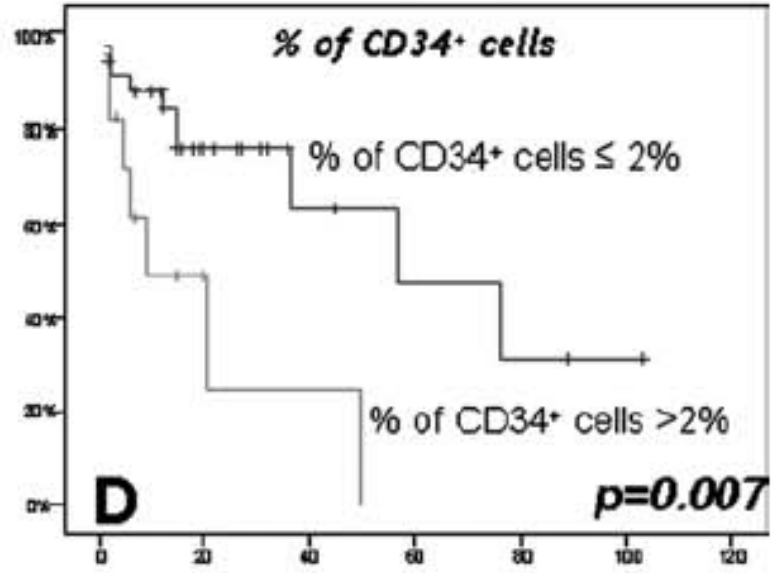



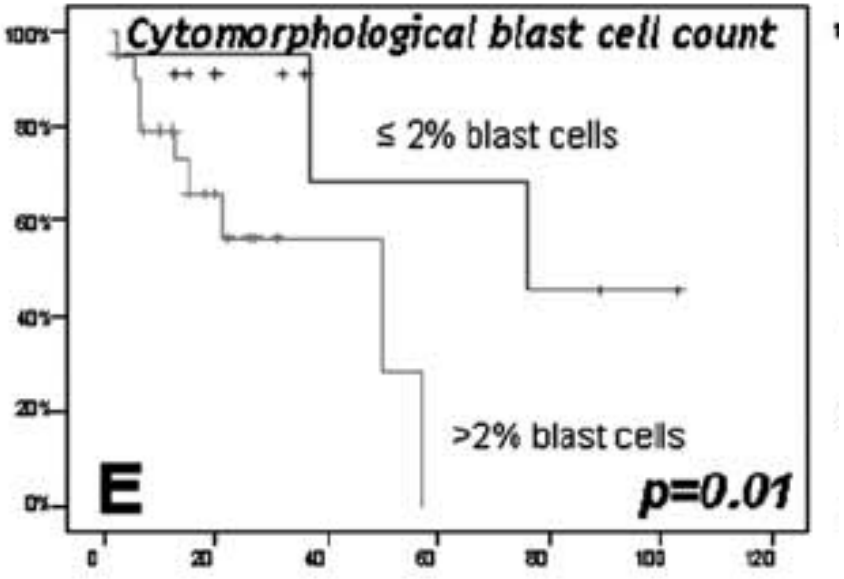




Aberrant

# CD34 levels and survival

OS

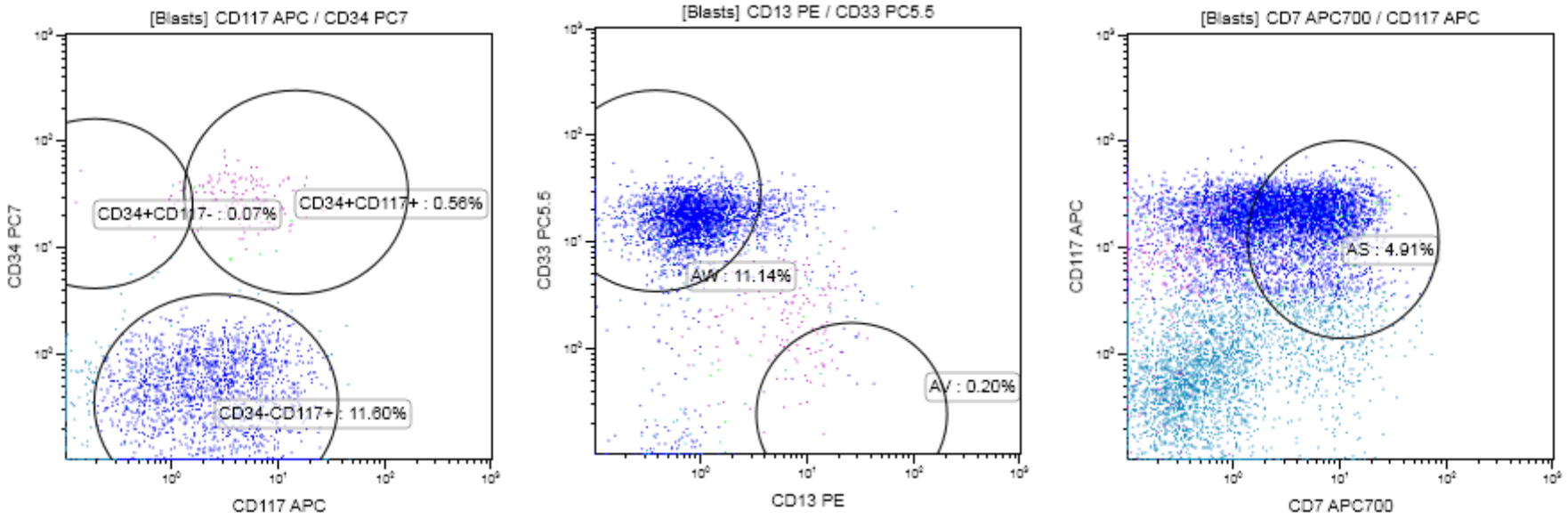



Matarraz et al

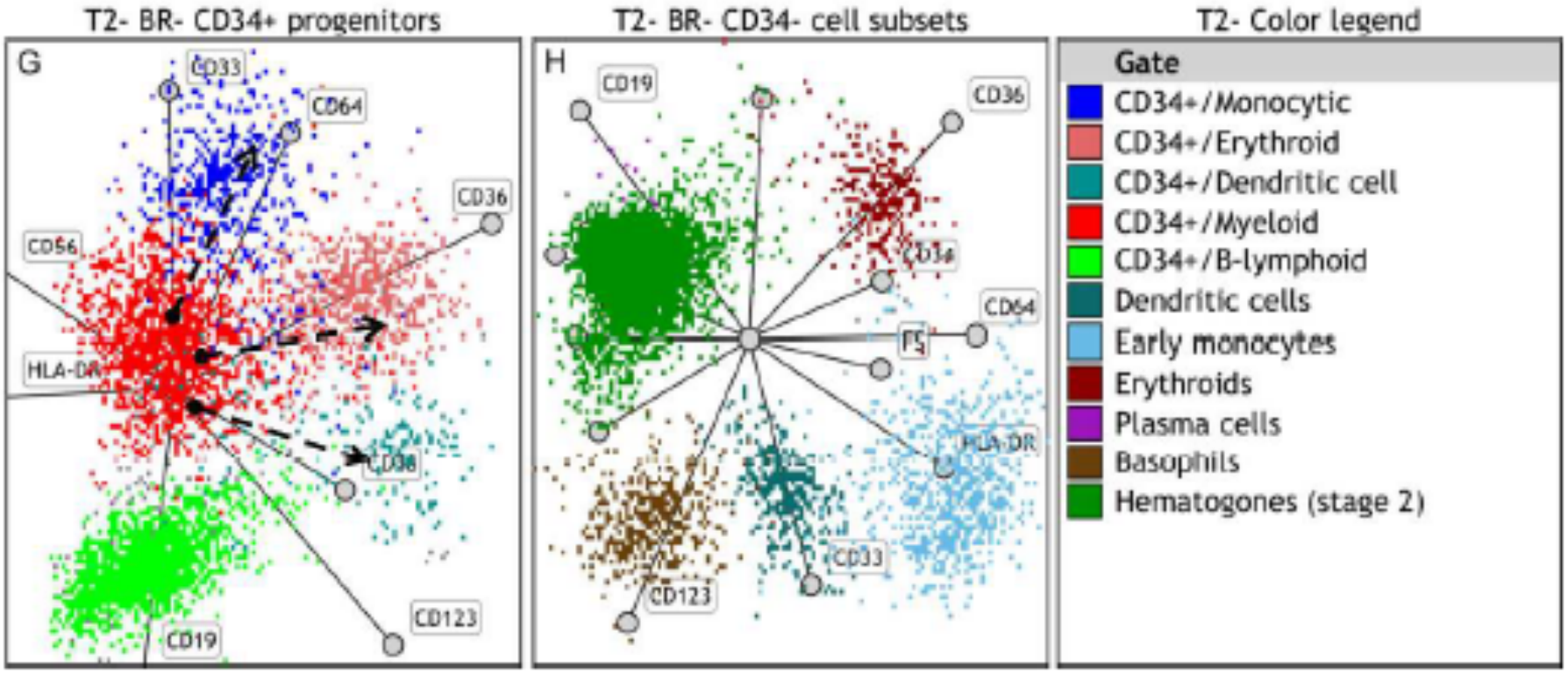
Cytometry Part B (Clinical Cytometry) 78B:154–168 (2010)

# Examples of aberrant findings in Blast/progenitor region



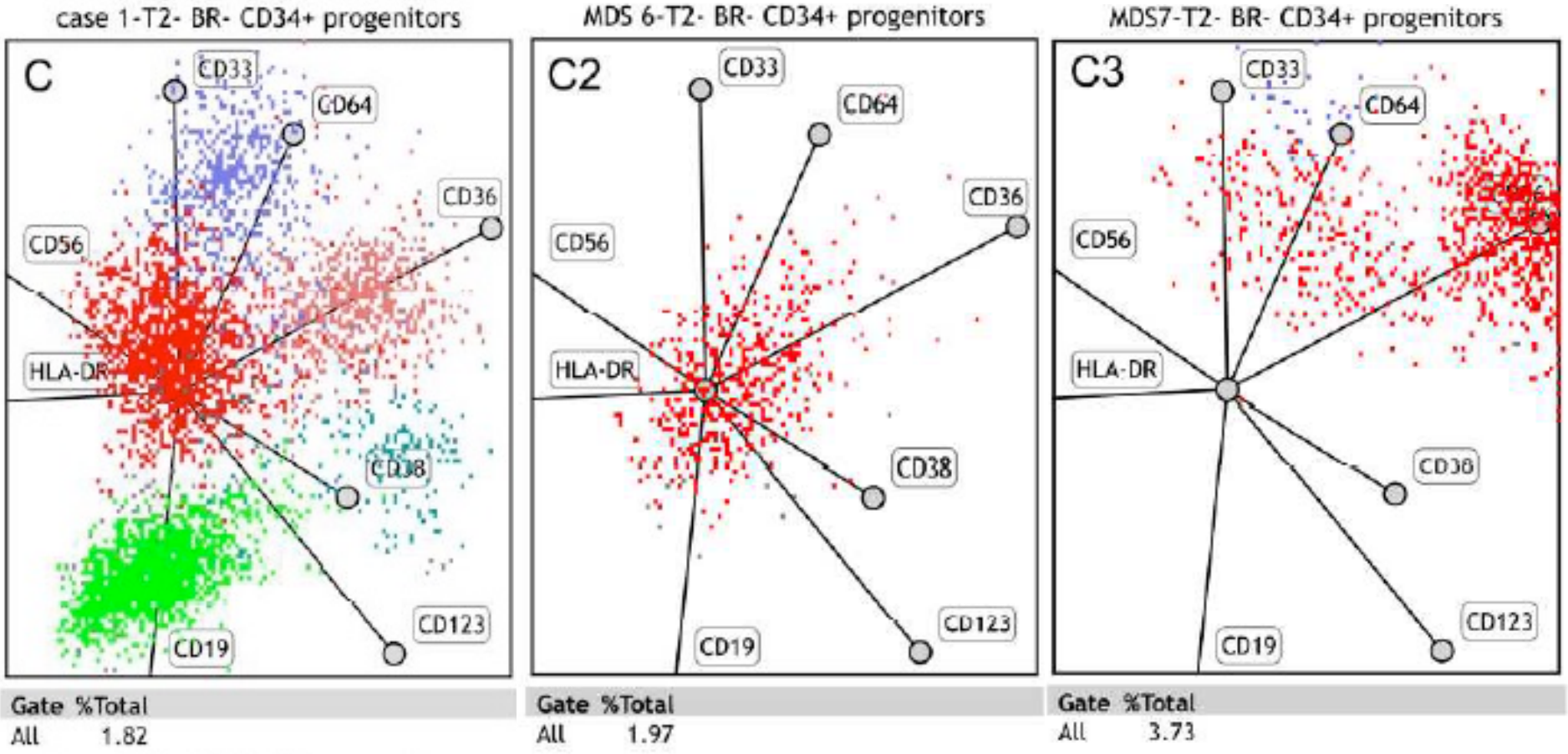

**In some patients most blasts are CD34 negative**

# RADAR plots of normal Blast/progenitor region




Jafari K,et al. Cytometry B Clin Cytom. 2018 Mar;94(2):219-229

# Aberrant patterns seen in Radar plots

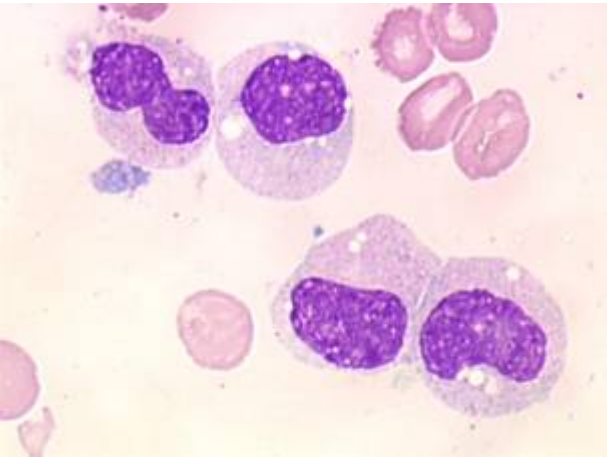



NORMAL

MDS CD34+ cells <5%

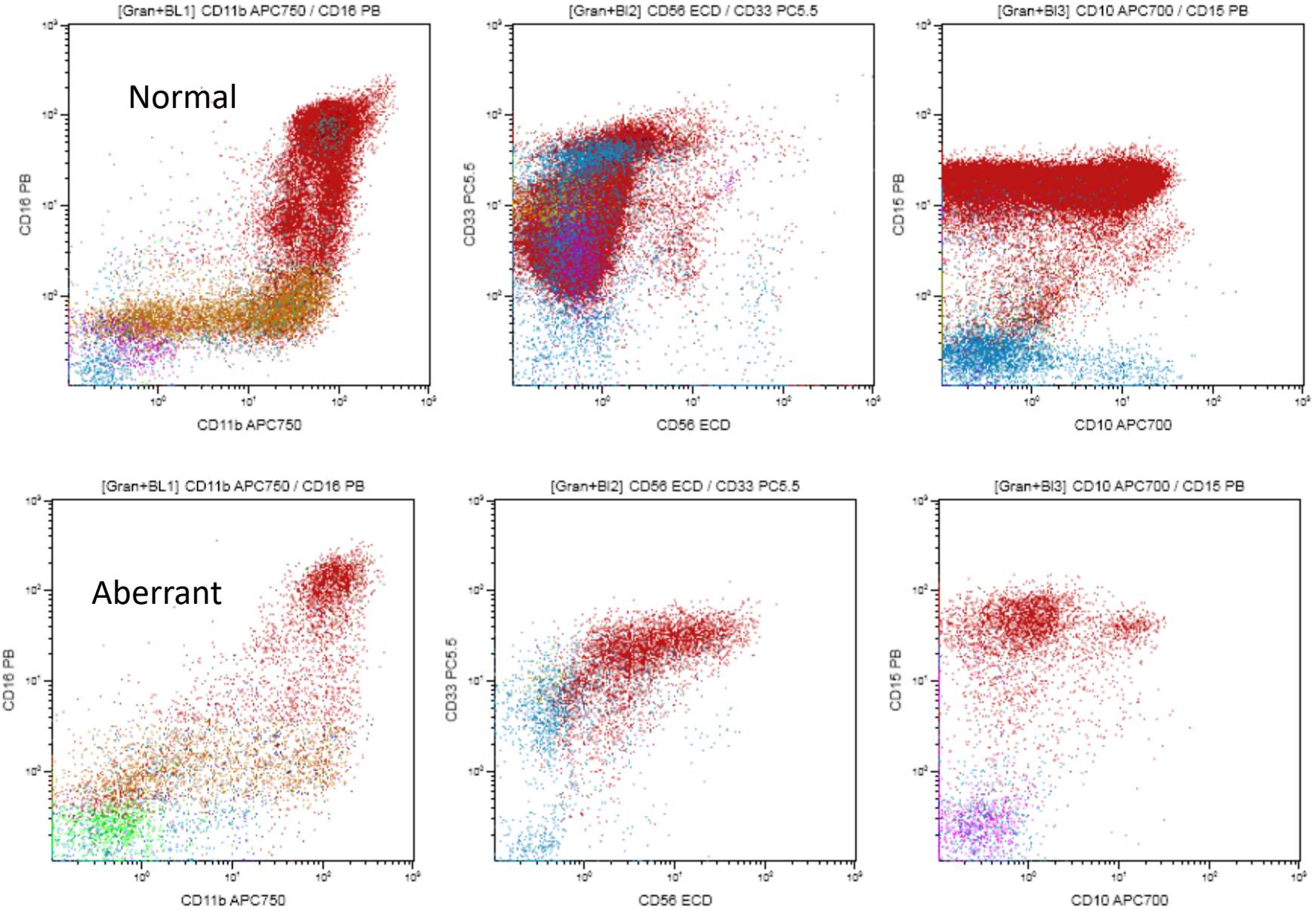
**Jafari K,et al. Cytometry B Clin Cytom. 2018 Mar;94(2):219-229**

# Aberrancies to be assessed by FCM recommended by ELN Workshops

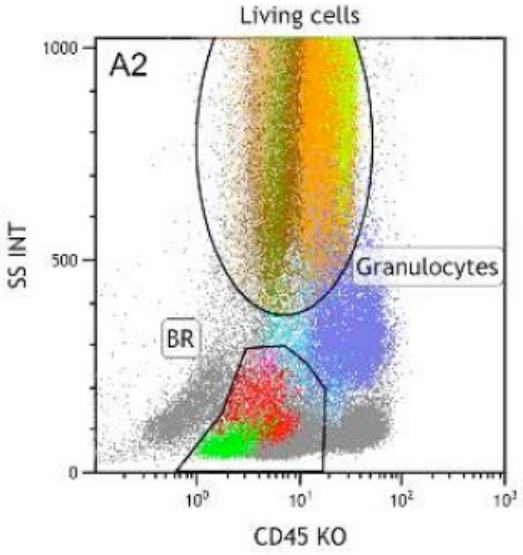


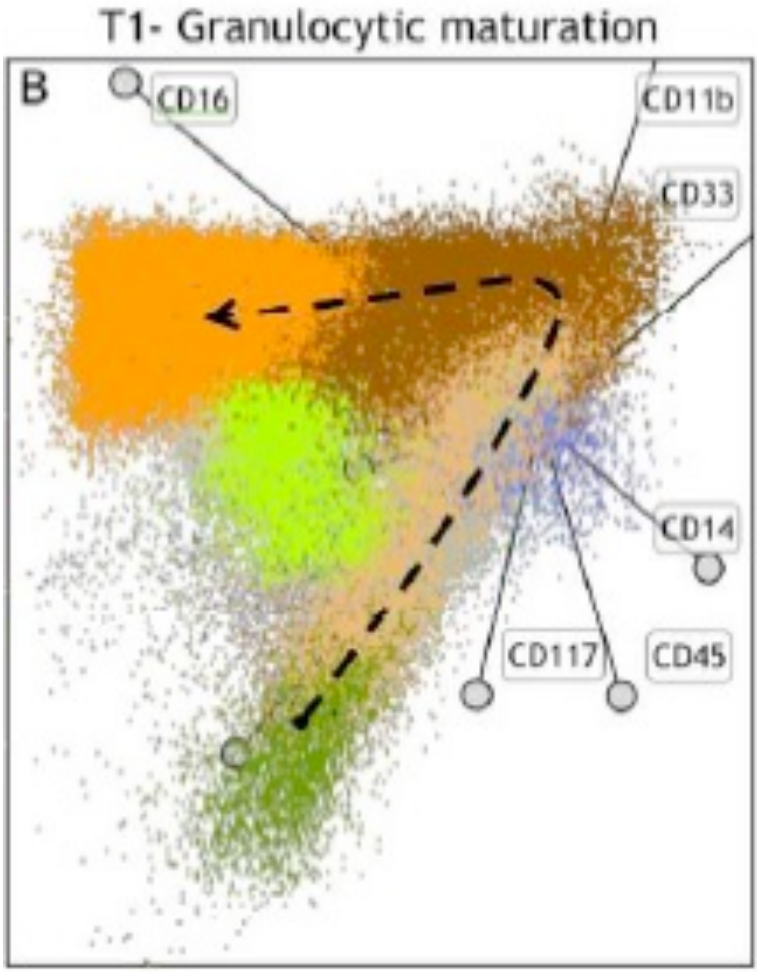
- **Granulopoietic dysplasia by flow:**
- **Abnormal scatter and CD45 expression**
- **Abnormal CD11b/CD13 pattern**
- **Abnormal CD13/CD16 pattern**
- Persisting expression of CD34 or CD117
- Abnormal expression of **CD13, CD33,** CD15, CD10, CD36, CD64
- Overexpression of HLA-DR
- Lineage infidelity markers CD7, CD2, CD5, CD19, CD5, CD56

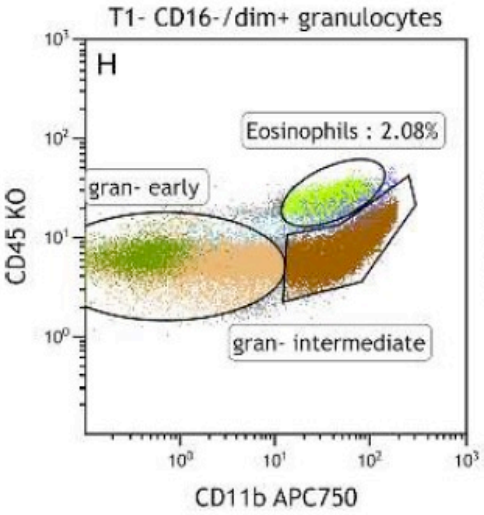
# Abnormal findings in neutrophils

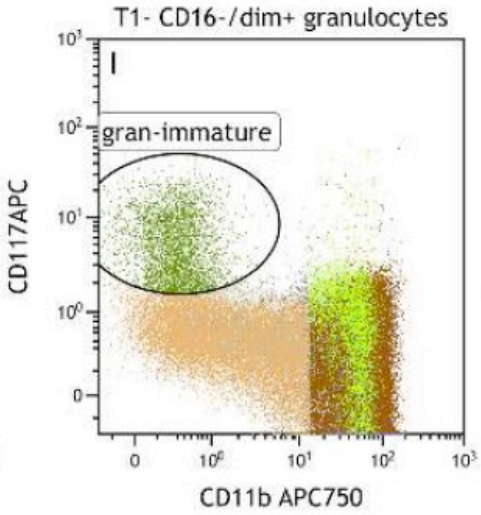


# Granulocytic maturation

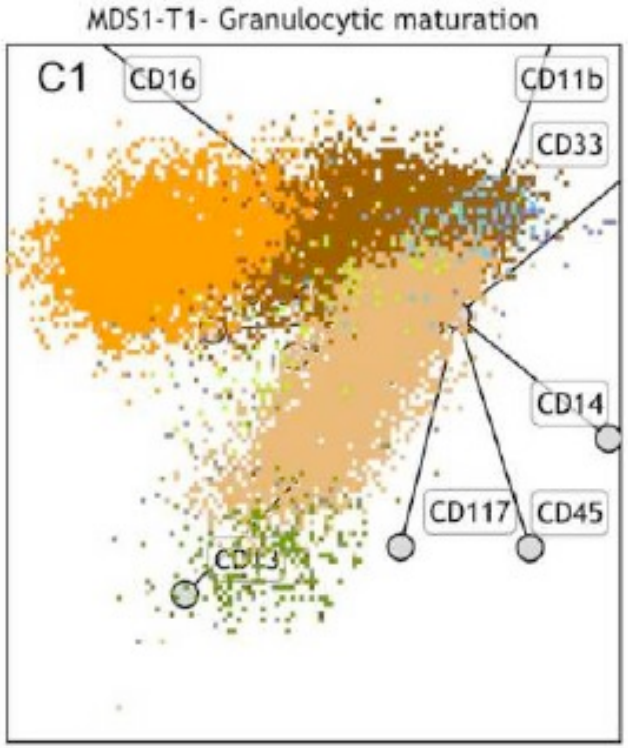


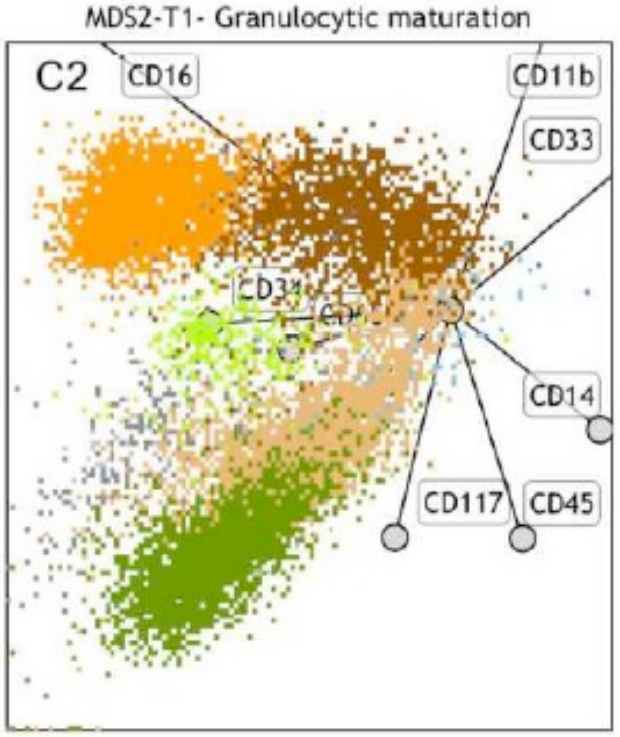



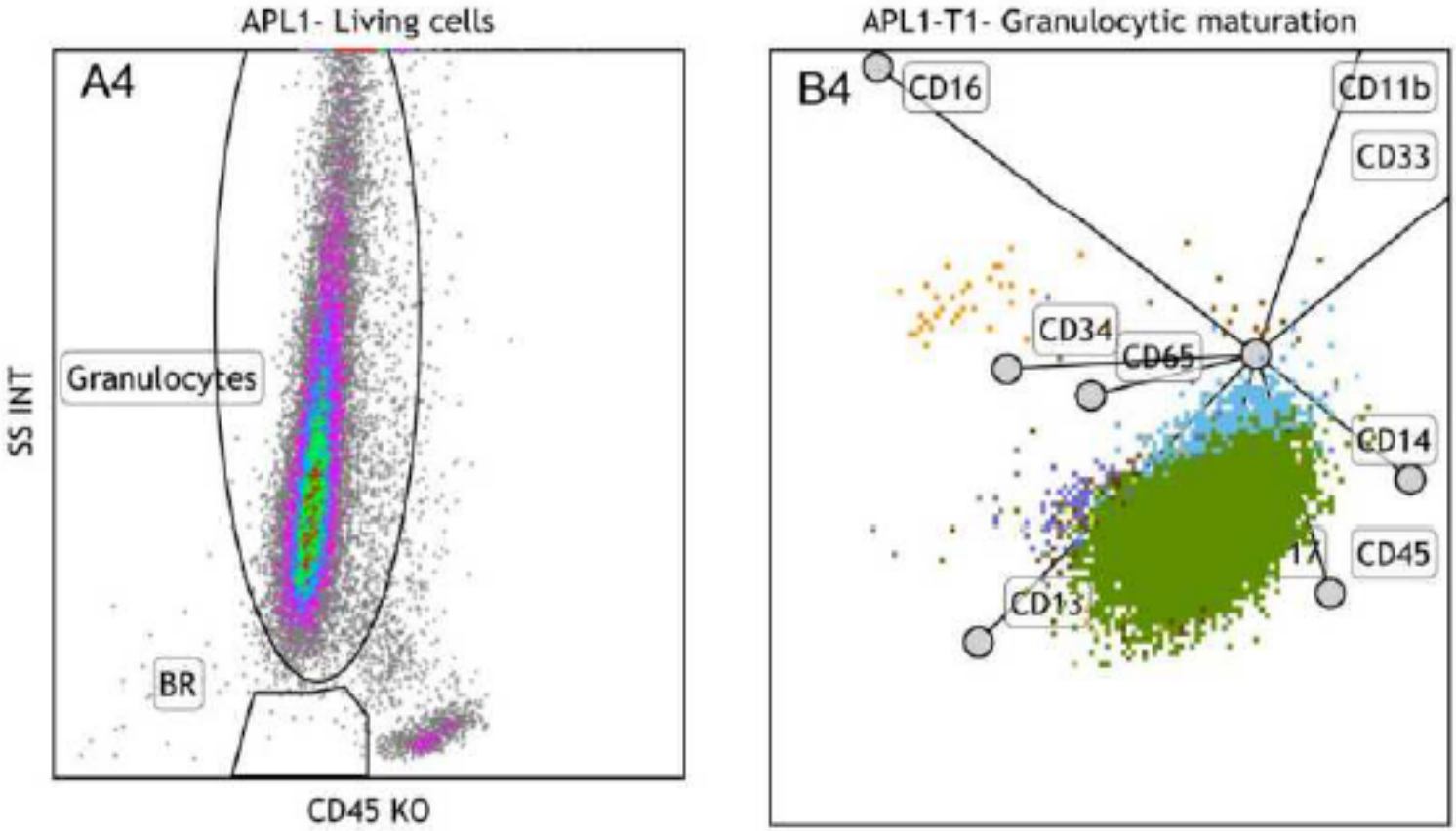



# RADAR patterns in MDS

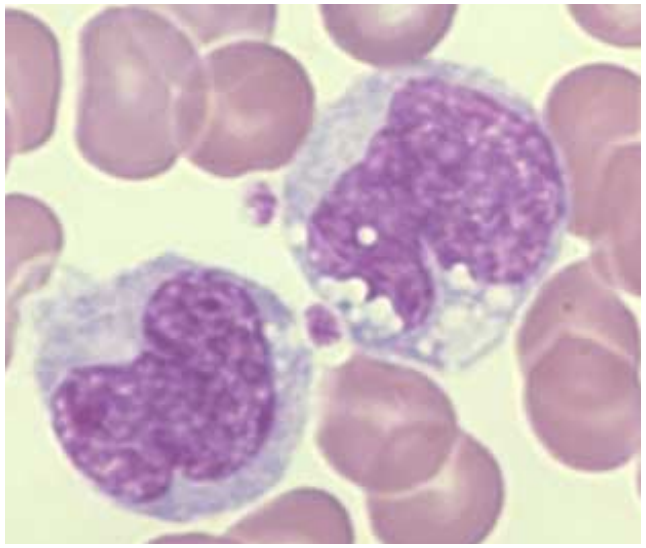


# RADAR pattern in acute promyelocytic leukemia

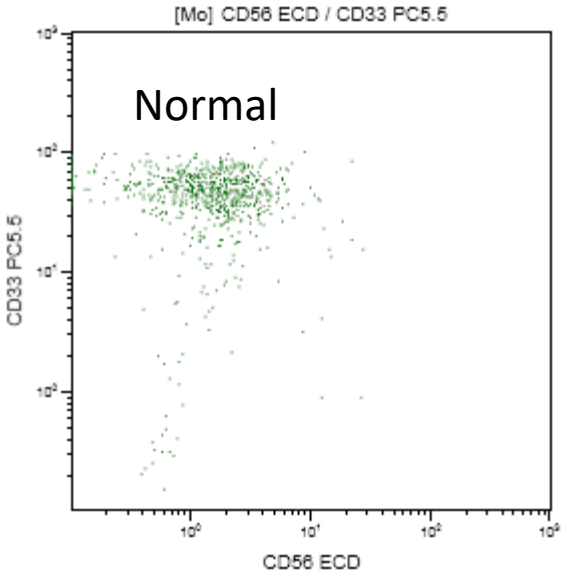


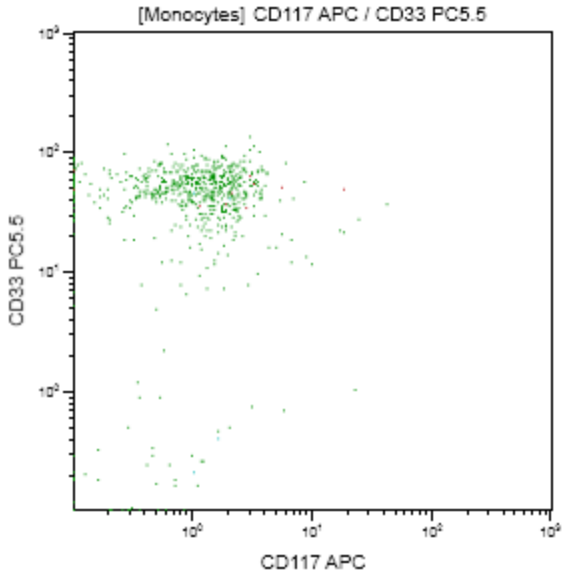
# Aberrancies to be assessed by FCM recommended by ELN Workshops

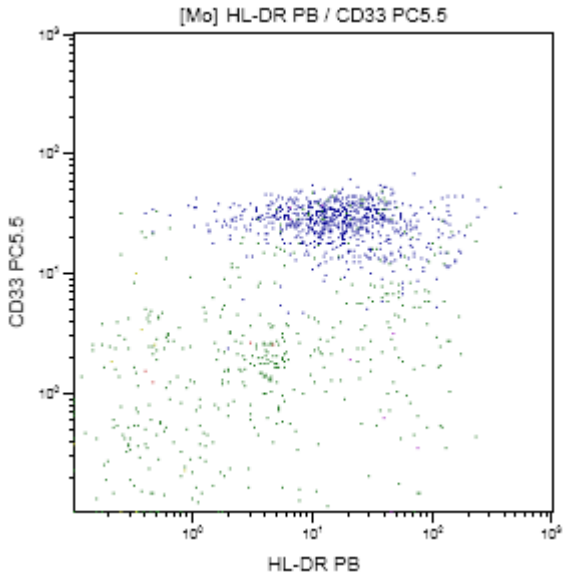


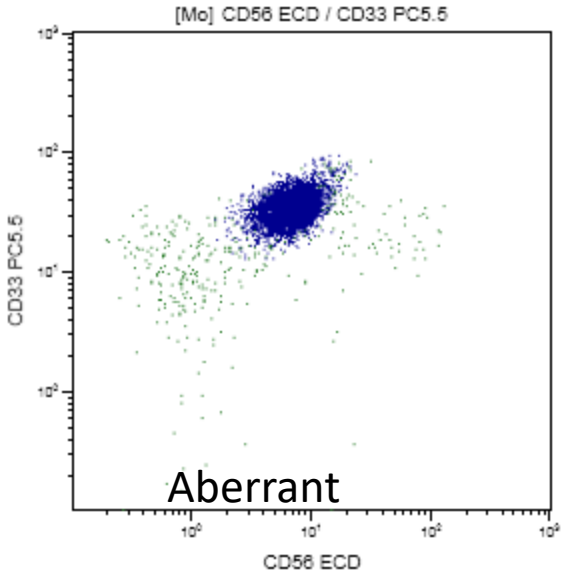
- **Abnormal Features of Monocytes:**
- Decreased/increased percentages
- Abnormal scatter and CD45 expression
- Abnormal expression of CD33, CD13, CD36
- Abnormal CD11b/HLA-DR pattern
- Expression of CD34
- Abnormal expression of CD14, CD16, CD64, CD11c, CD15
- Overexpression of **CD56 (2 log)**
- Lineage infidelity markers CD7, CD2, CD19

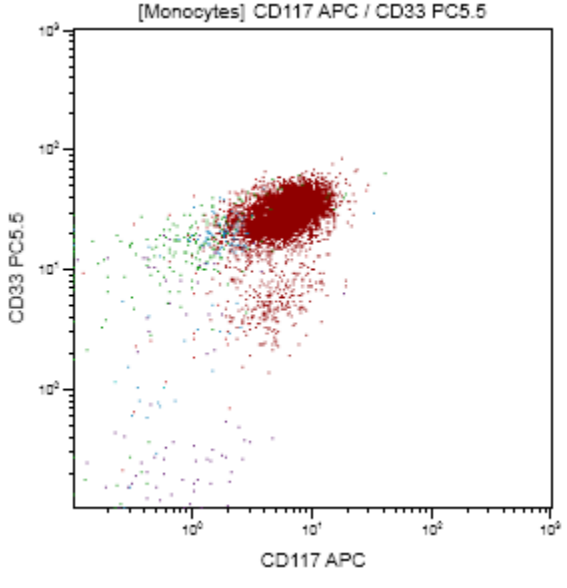
# Aberrant findings in monocytes

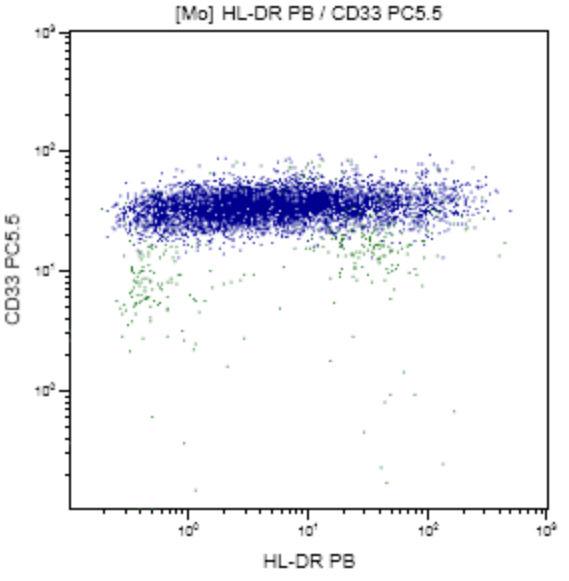


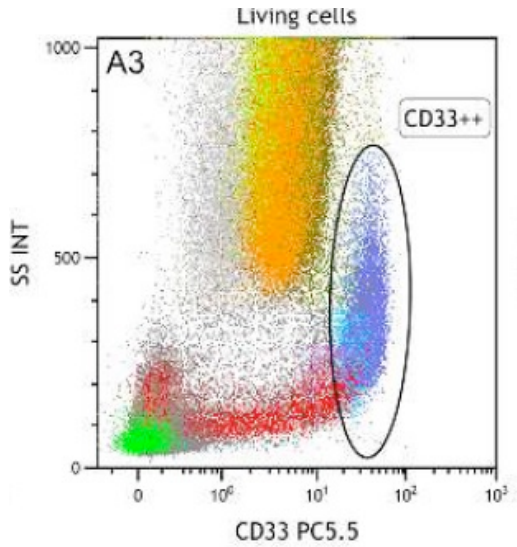



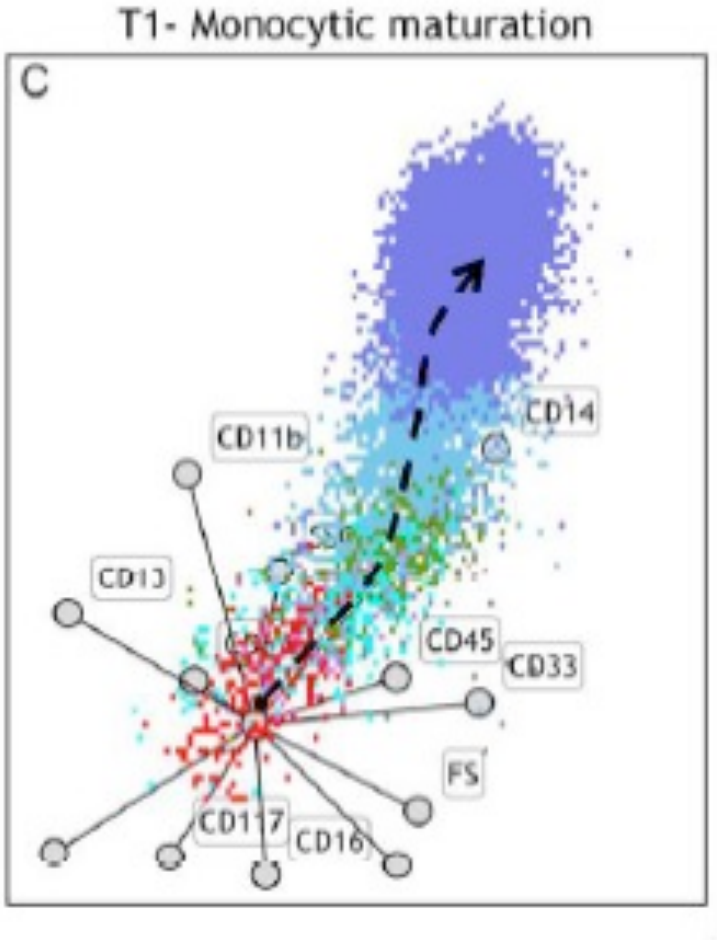



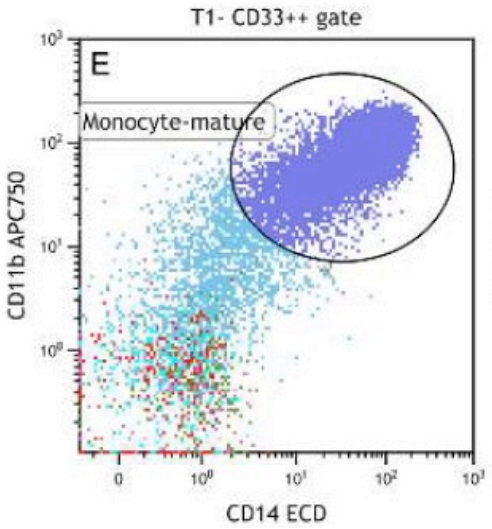



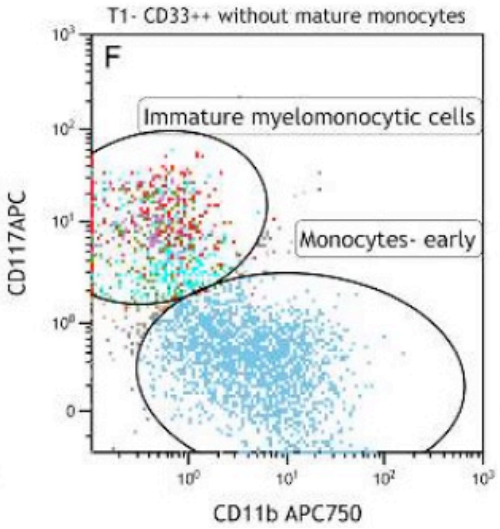



# Radar plots of monocytic maturation

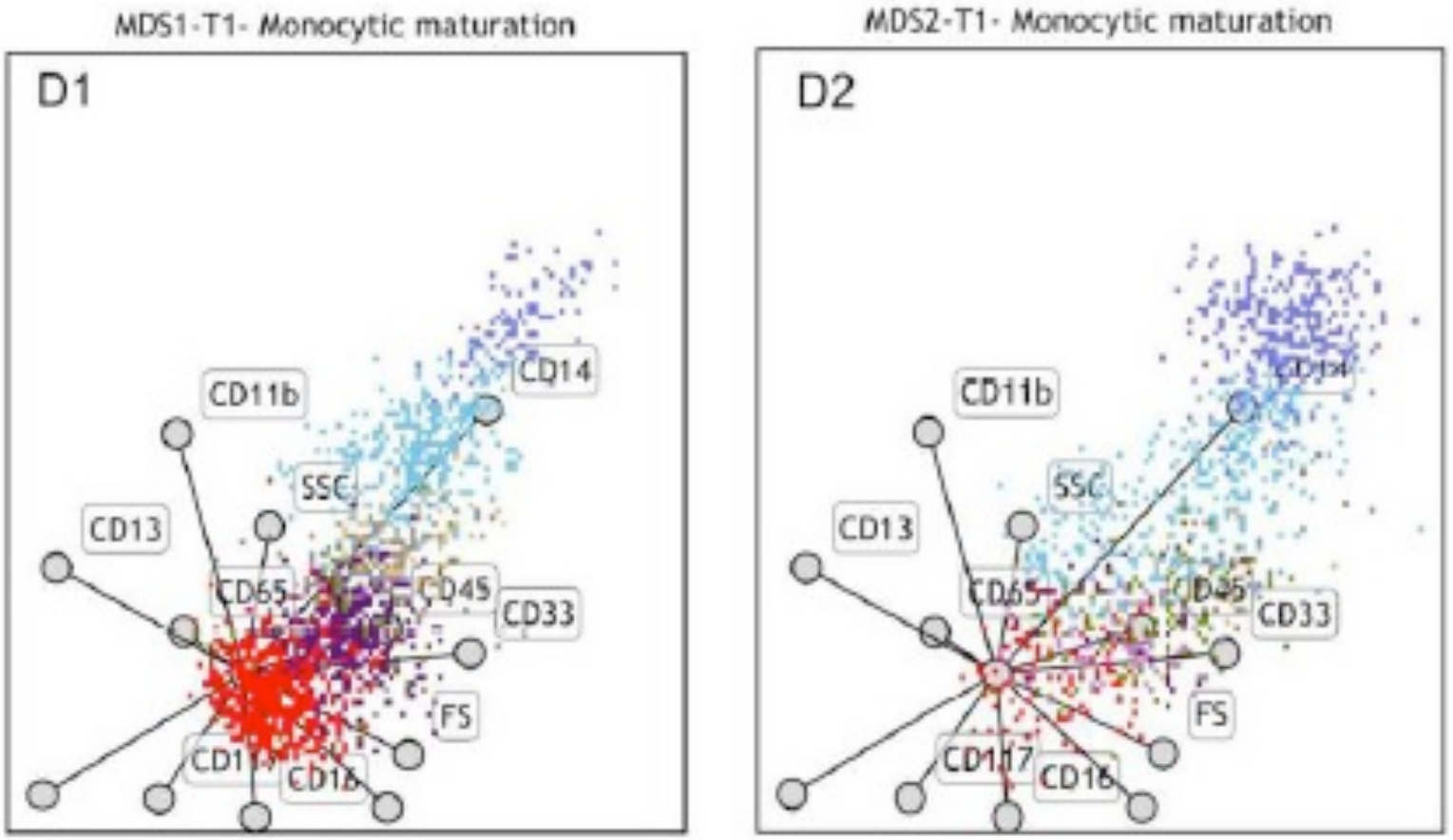


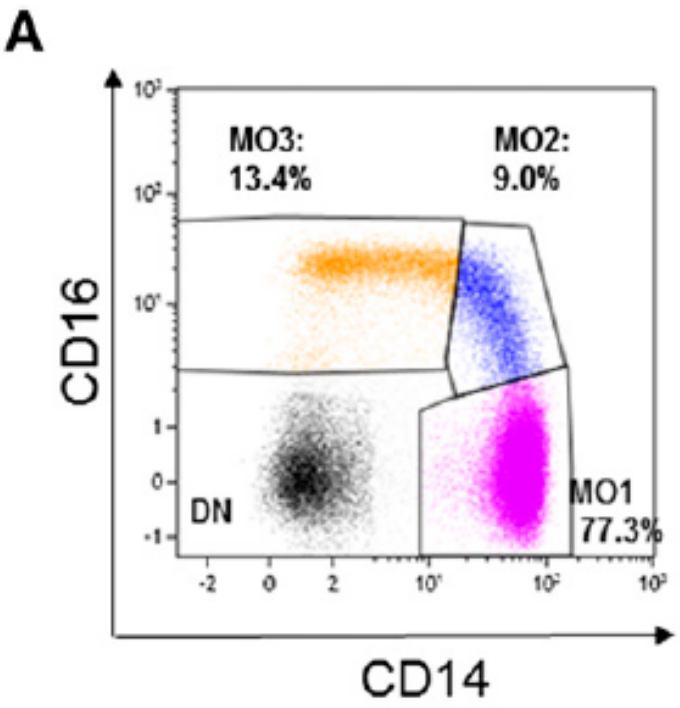
# Aberrant patterns of monocytic maturation in MDS

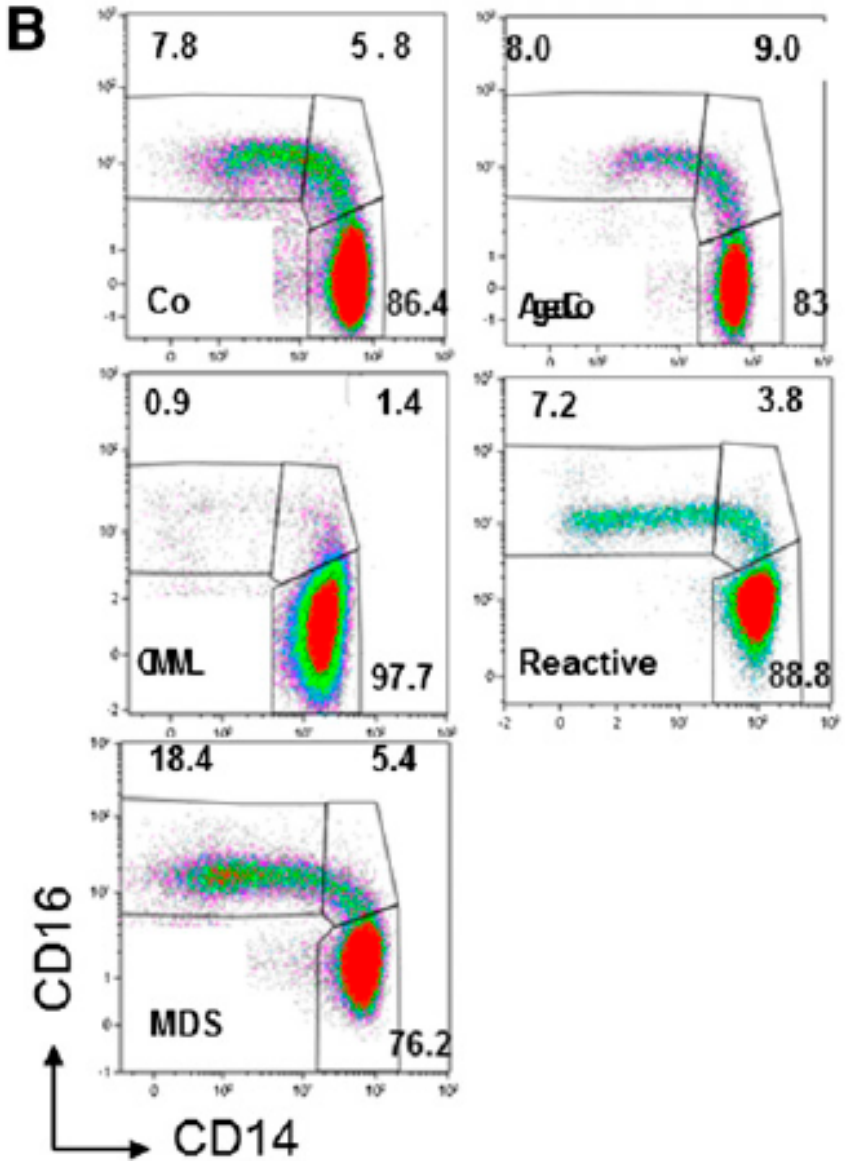


# Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia

Dorothée Selimoglu-Buet,[1,2] Orianne Wagner-Ballon,[3,4] Véronique Saada,[5] V

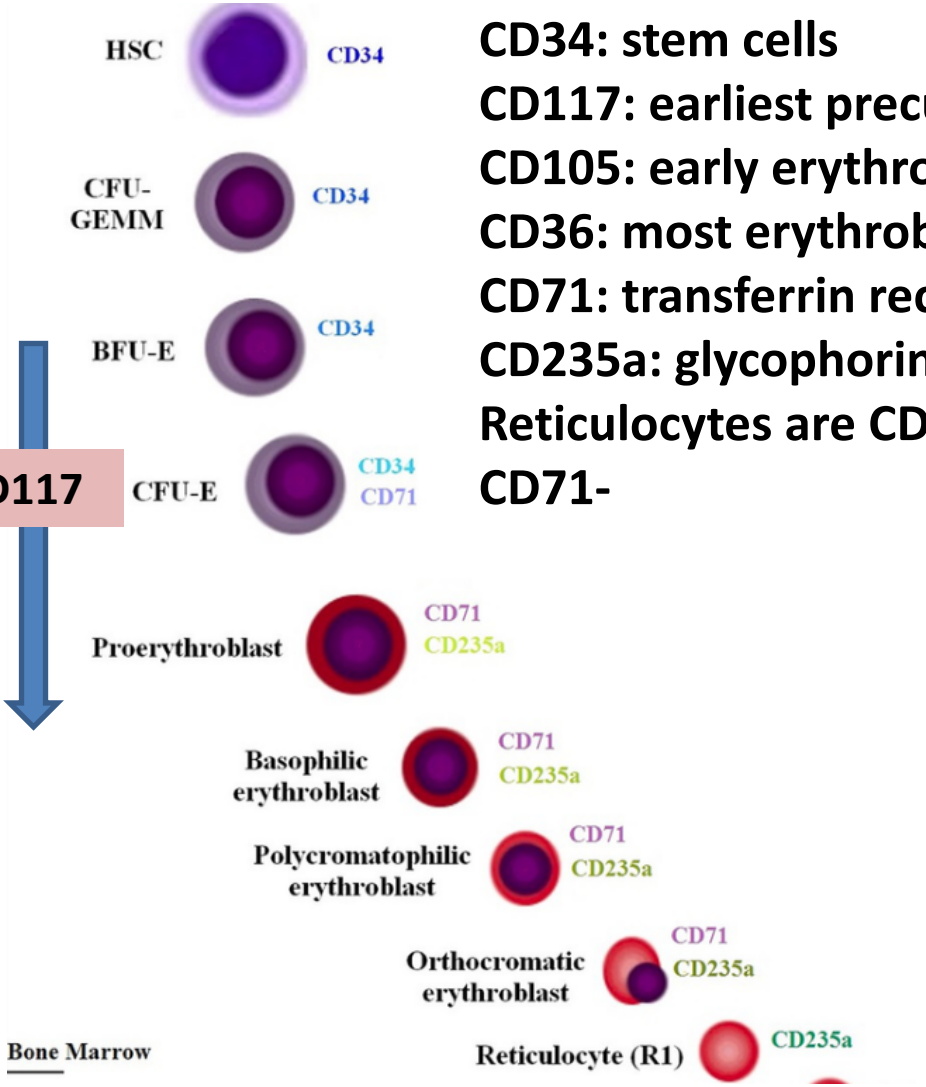
BLOOD, 4 JUNE 2015 • VOLUME 125, NUMBER 23



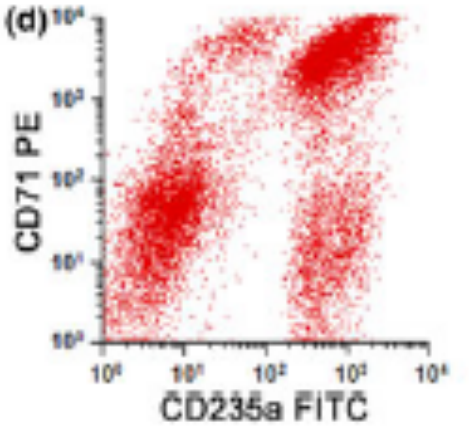




**Normal distribution of monocyte subsets in blood**

# Erythropoiesis by flow cytometry



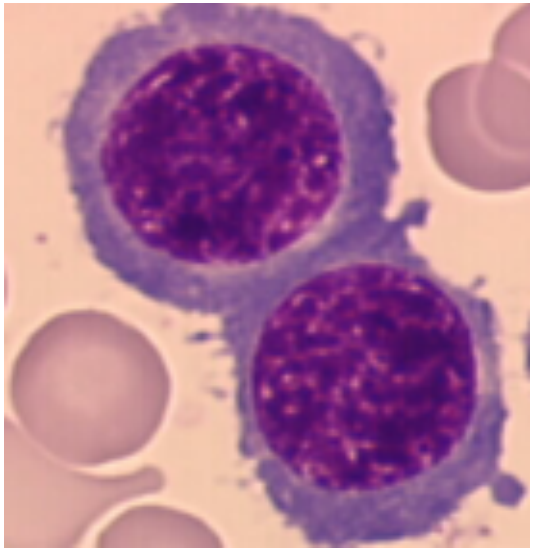

**CD34: stem cells**
**CD117: earliest precursors+ (both ery and myelo)**
**CD105: early erythropoietic precursors+**
**CD36: most erythroblasts+**
**CD71: transferrin receptor**
**CD235a: glycophorin**
**Reticulocytes are CD71 dim and mature erythrocytes are CD71-**




CD45neg/ low SSC gate

LUND UNIVERSITY

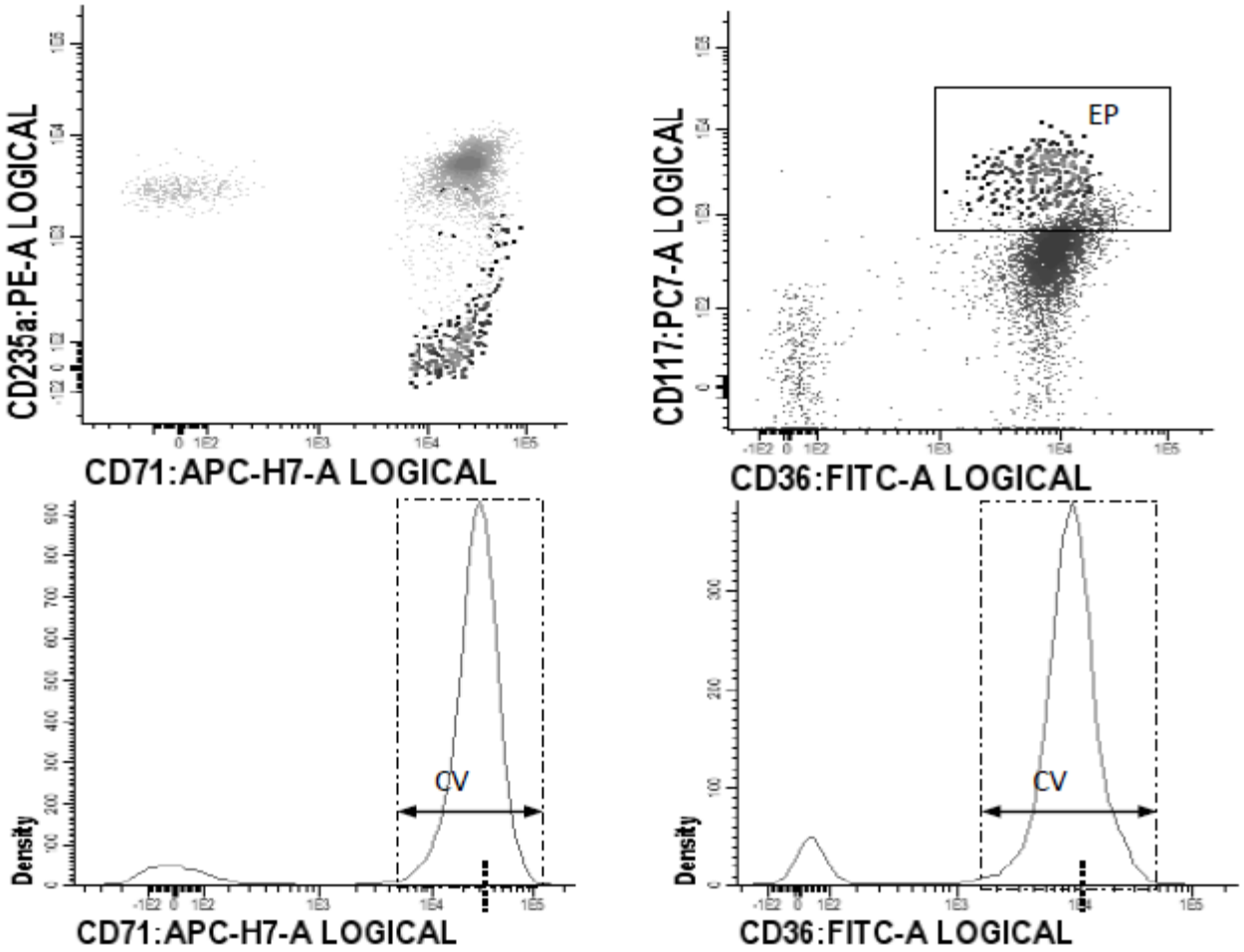
# Aberrancies to be assessed by FCM recommended by ELN Workshops



- **Erythroid dysplasia by flow:**
- Increased percentage after lysis
- Abnormal scatter and CD45 expression
- Abnormal pattern of **CD71**/CD235a (glycophorin A)
- Abnormal expression of CD105, CD34, **CD36**, CD117

# Normal

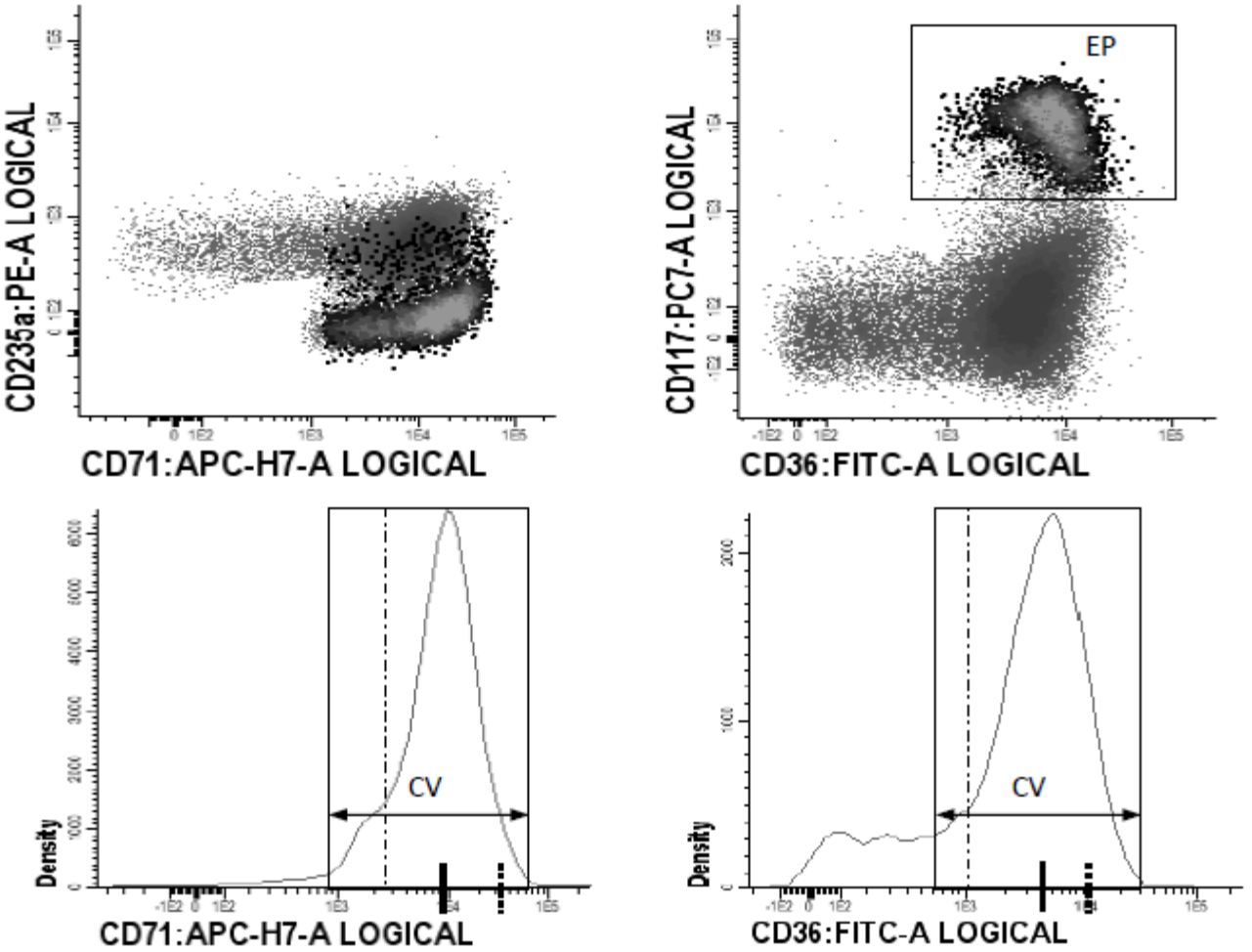
A




Cremers EM,et al; A study on behalf of the HOVON89 study group. Implementation of erythroid lineage analysis by flow cytometry in diagnostic models for myelodysplastic syndromes. Haematologica. 2017 Feb;102(2):320-326

# MDS

B




**Cremers et al, Hematologica 2017**

## Westers TM, et al. Haematologica. 2017 Feb;102(2):308-319

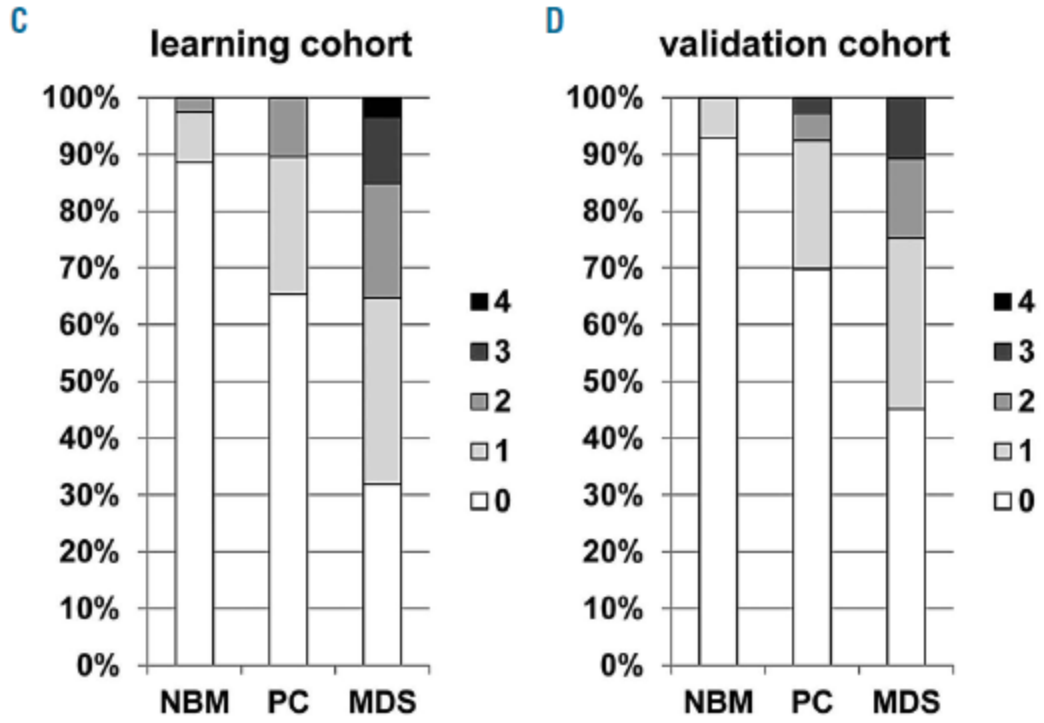
Table 4. Results of multivariate logistic regression analysis in learning cohort.

| Parameter | odds ratio | 95% CI | P |
|---|---|---|---|
| rel. CD36 CV | 3.7 | 1.6 – 8.5 | 0.003 |
| rel. CD71 CV | 3.2 | 1.6 – 6.4 | 0.001 |
| rel. CD71 MFI | 2.2 | 1.1 – 4.5 | 0.033 |
| rel. %CD117 | 1.7 | 0.92 – 3.2 | 0.084 |

Markers entered in the analysis were relative CD36 MFI, CD36 CV, CD71 MFI and CD71 CV, and the relative percentage of CD117+ erythroid cells (%CD117). 272/535 cases were available for analysis of which 153 pathological controls and 119 MDS cases in the learning cohort; $P<0.001$). CI: confidence interval; CV: coefficient of variation; MFI: mean fluorescence intensity; P: P-value; rel.: relative.

**Score 1 points each**

**Cut-off 2**



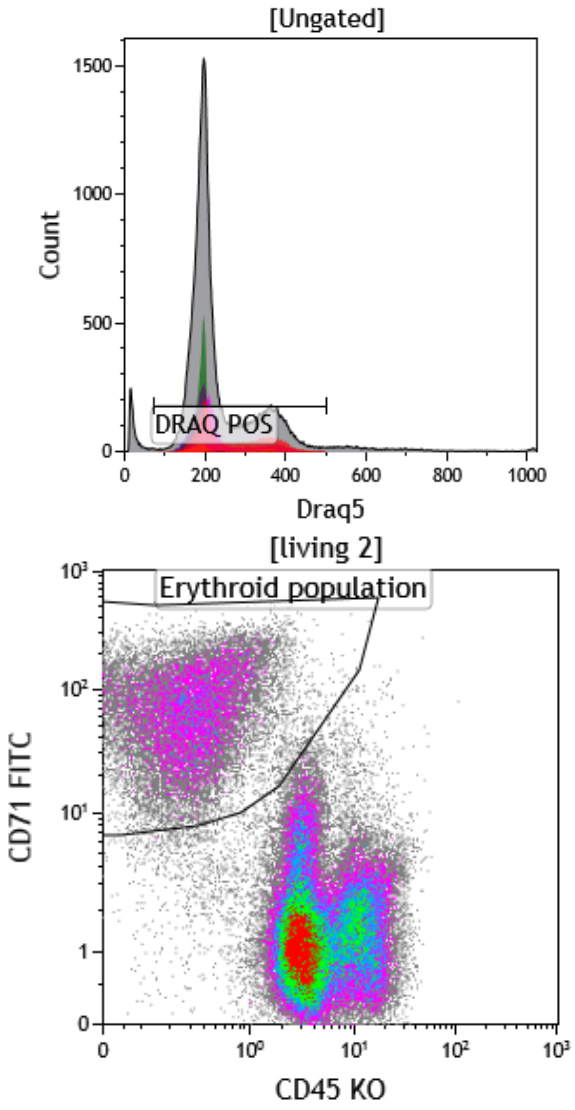

Sensitivity 90%
Specificity 35%

Validated

Sensitivity 92%
Specificity 25%

**Lysed BM samples**
**Variable lysis methods**
**17 labs**

# Erythropoietic tube on non-lysed sample

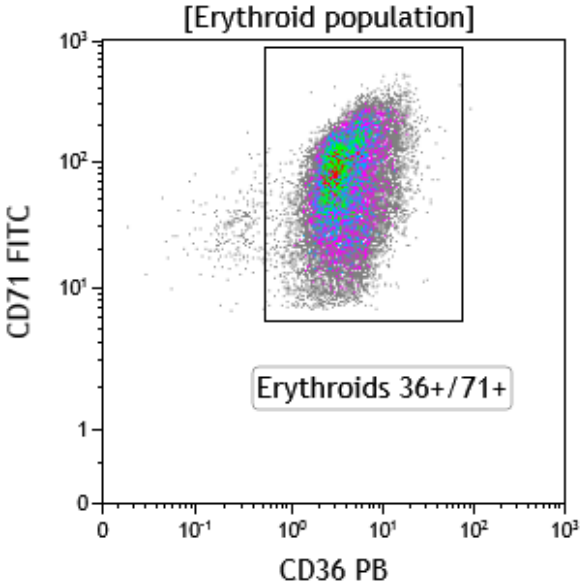


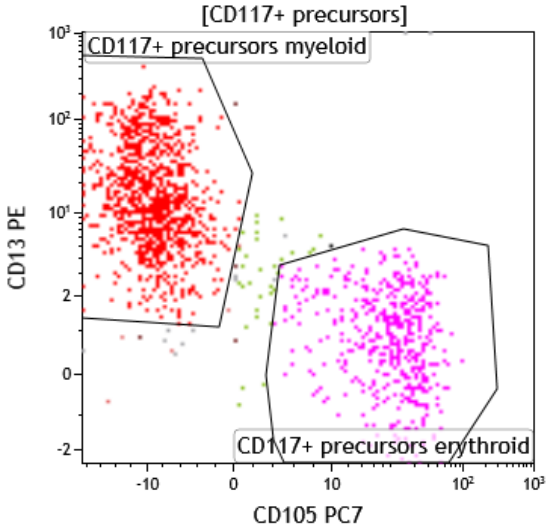

- 2.5 μl CD71-FITC,
- 2.5 μl CD13-PE,
- 5 μl CD117-ECD,
- 5 μl CD105-PE-Cy7,
- 5 μl CD36-PB,
- 2.5 μl CD45-KO

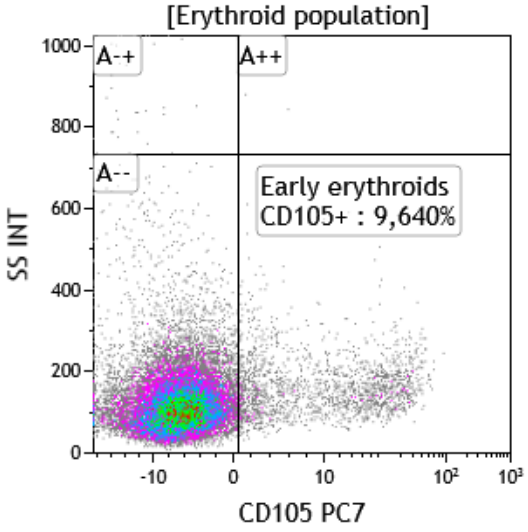
- DRAQ5

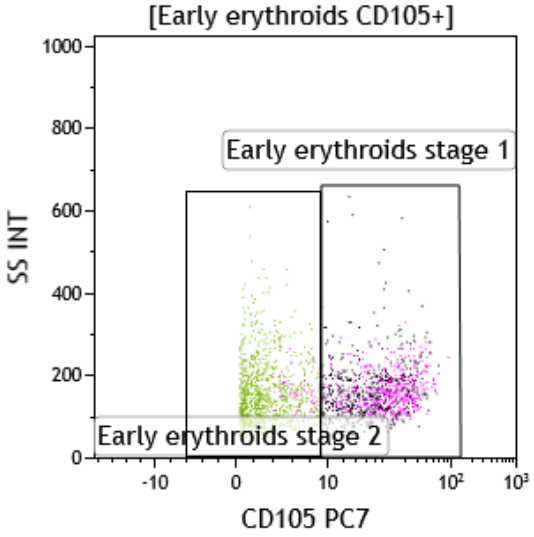
Violidaki et. al, Abstract ESCCA 2017

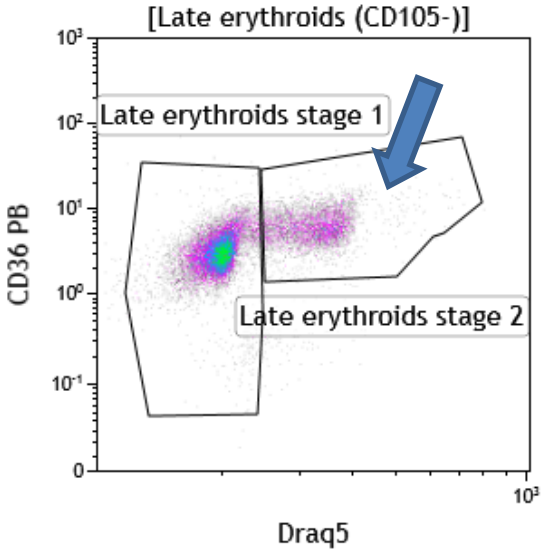
# Analysis Normal bone marrow



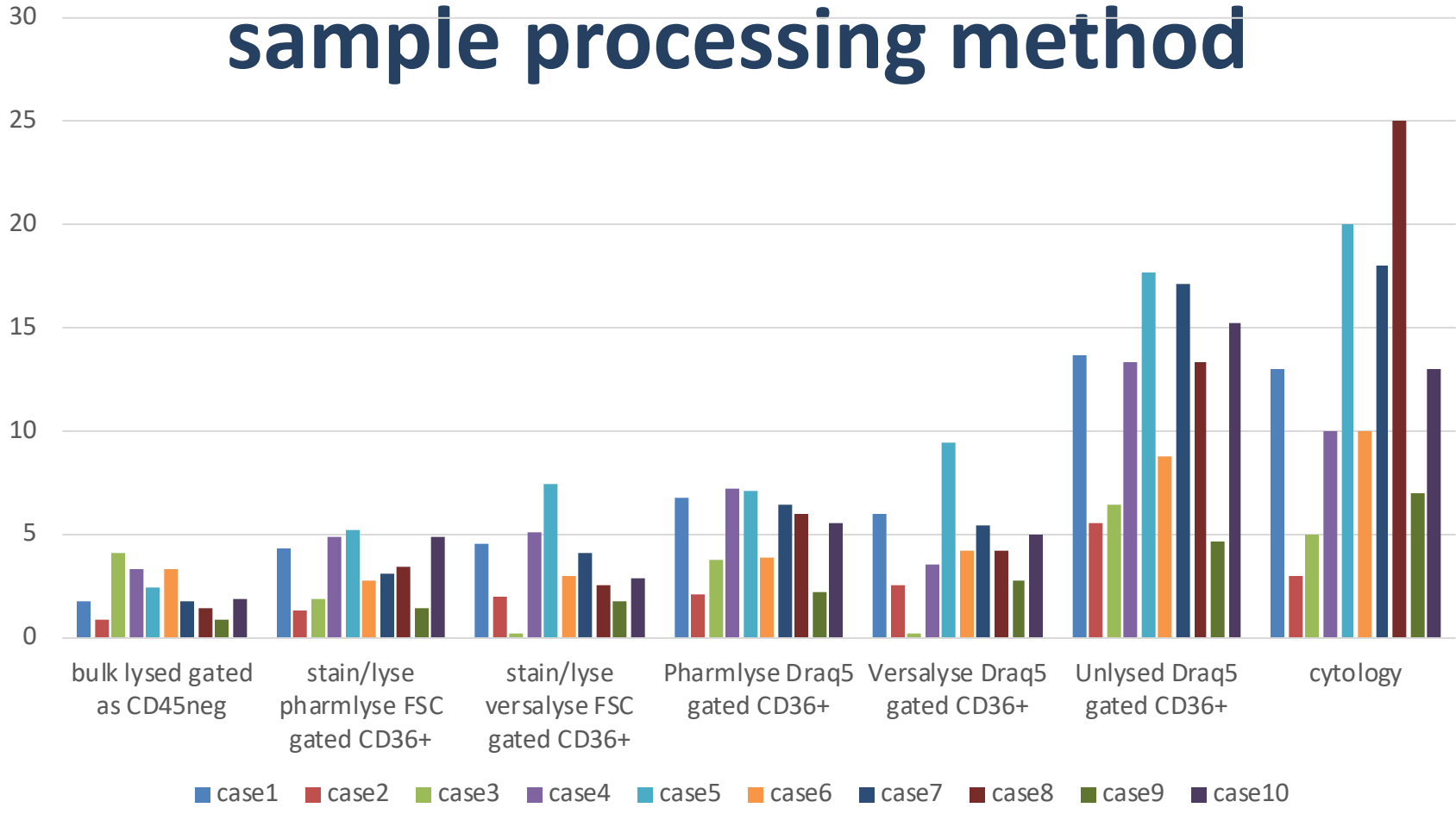



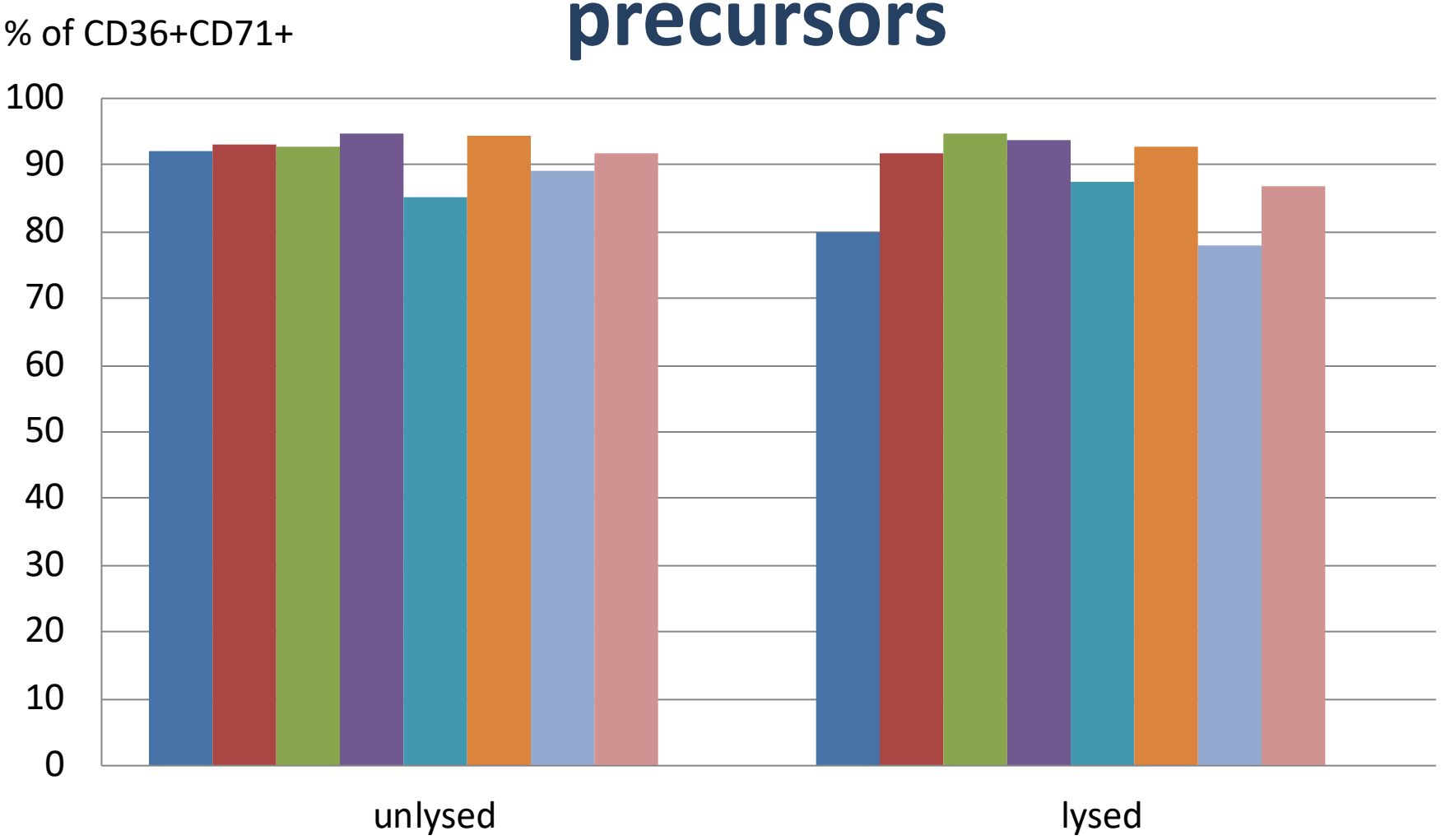




Violidaki et. al, Abstract ESCCA 2017

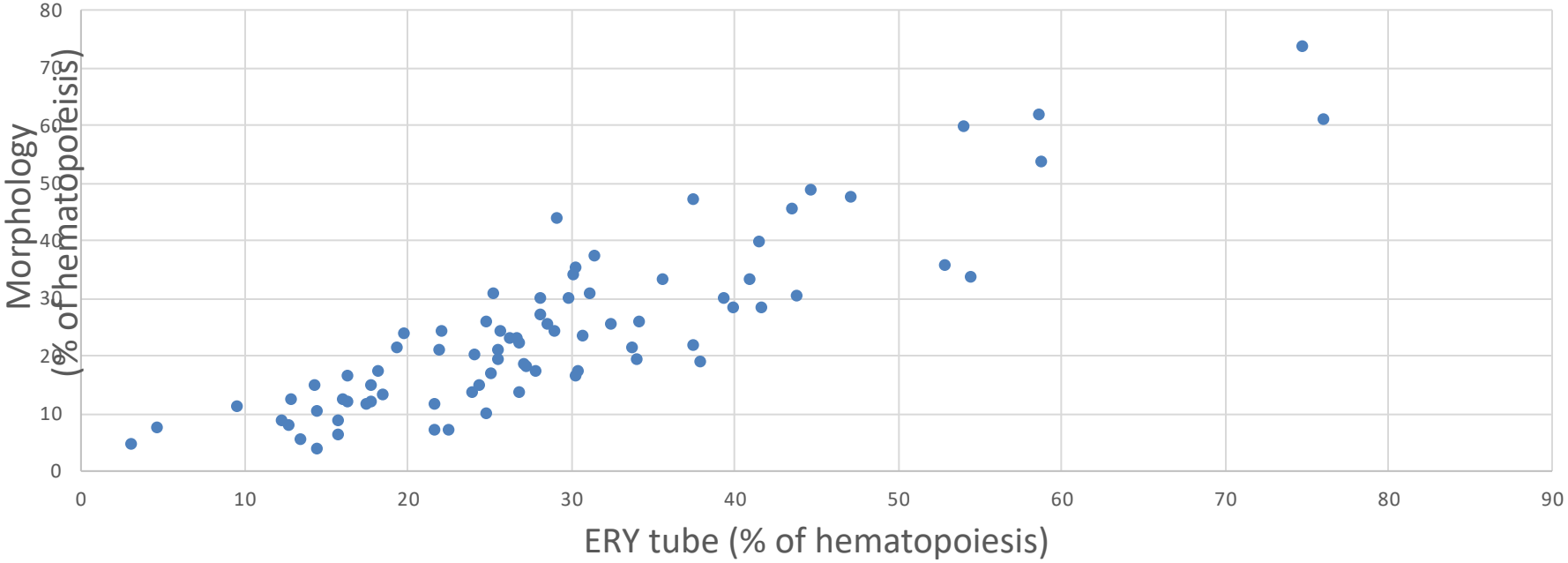
# Frequency of erythropoiesis in BM samples depends on sample processing method



# Fraction of most mature CD105-CD117-CD36+CD71+ cells of erythropoietic precursors

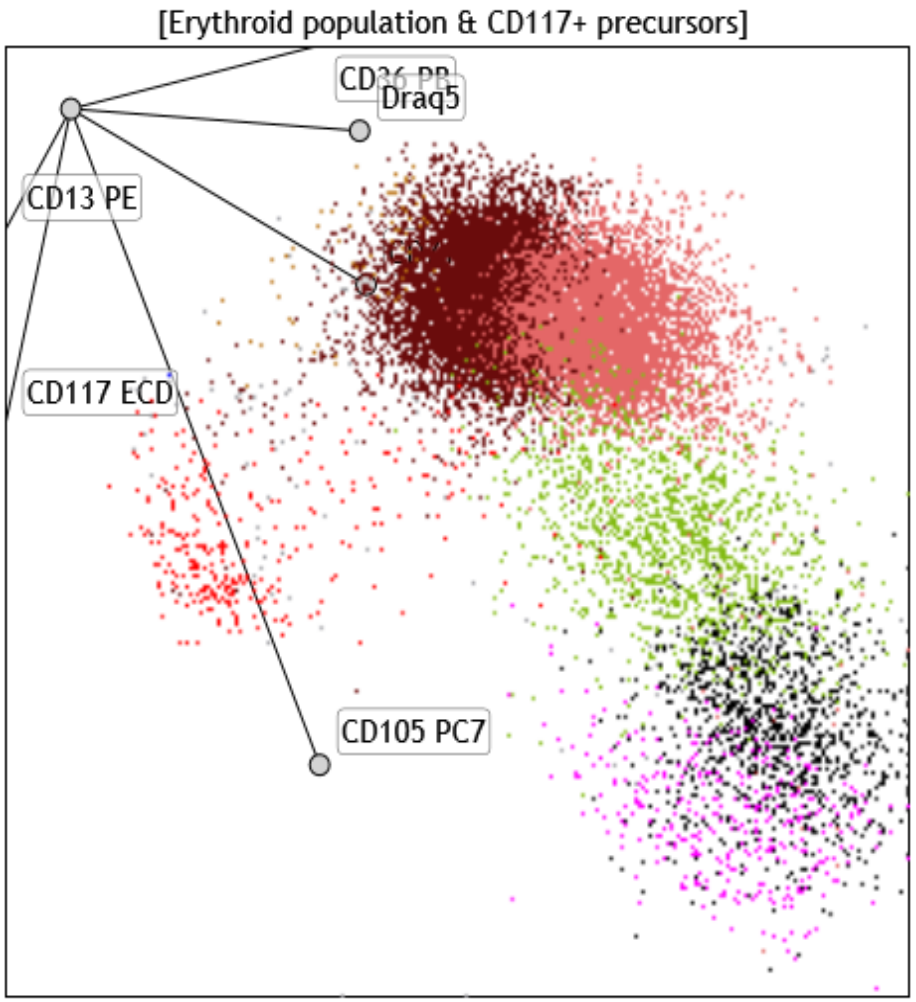


# Correlation of %erythropoiesis by morhological differential count and by ERY tube



# Radar analysis:

## erythroid population and CD117 myeloid blasts



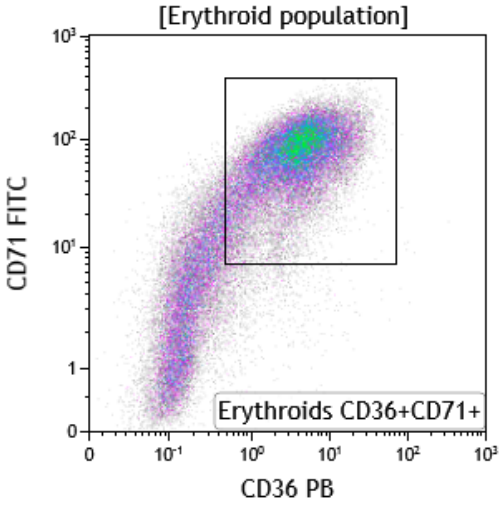

Well preserved in normal bone marrow

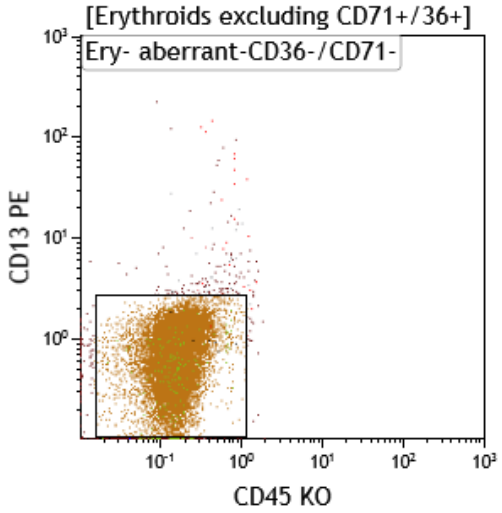
**Erythroid maturation pattern:**
**Two subsets of CD117+ precursors: myeloid (red), and erythroid (magenta, 1-2% of erythropoiesis)**

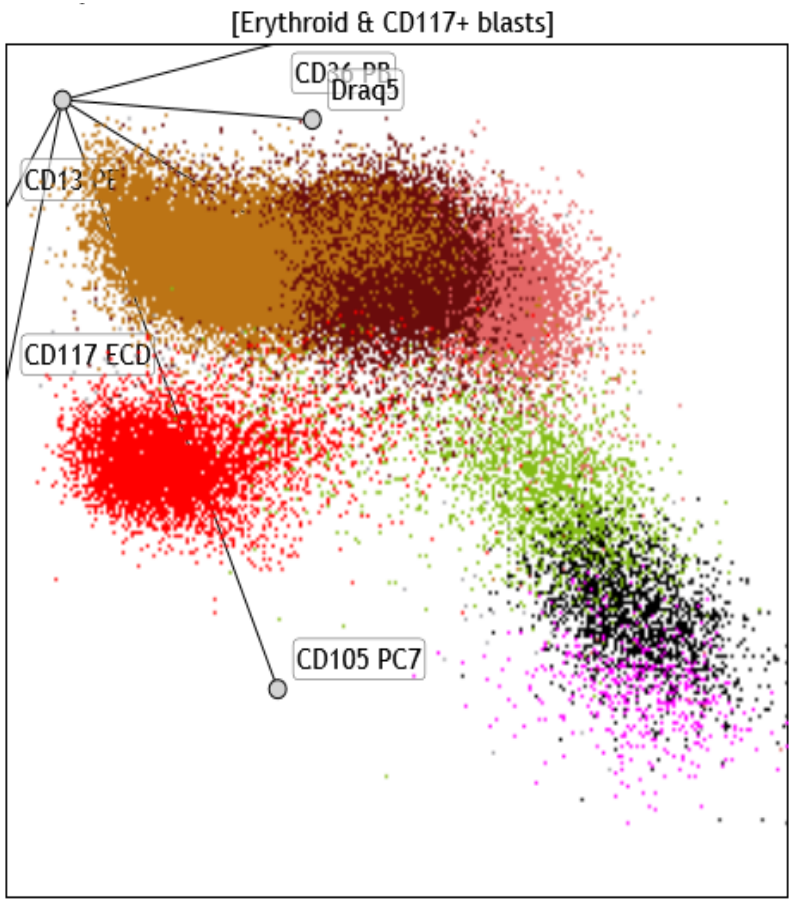
**Two subsets of early erythroid populations: with bright CD105 expression (black 4-5%) and weak CD105 expression (green 6-7%)**

**Two subsets of late CD36+CD105- erythroid populations: proliferating (salmon 25%) and final stage of non-proliferating nucleated erythroids (maroon 60% or erythropoiesis)**

# Aberrant maturation patterns in MDS

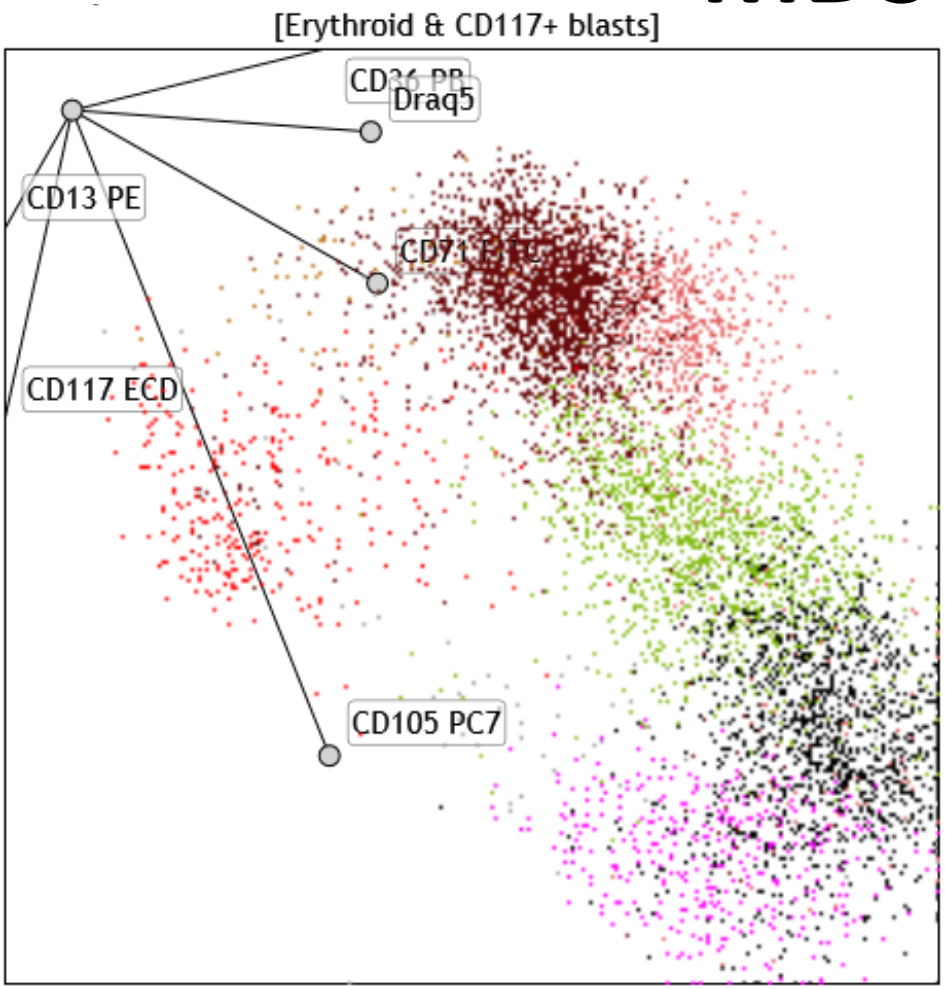



Pattern seen in 75% of tested MDS cases

# Aberrant maturation patterns in MDS




**MDS with ring sideroblasts show different pattern:**
**Left shifted erythropoiesis with decreased maturation**

# How to report FCM findings?

## Guidelines of the IMDSflow WG on FCM in MDS 2015

- A:
  **FCM analysis: *NO* MDS-related features**
- B:
  **FCM analysis: some changes often seen in MDS**
- C:
  **FCM analysis: consistent with MDS**

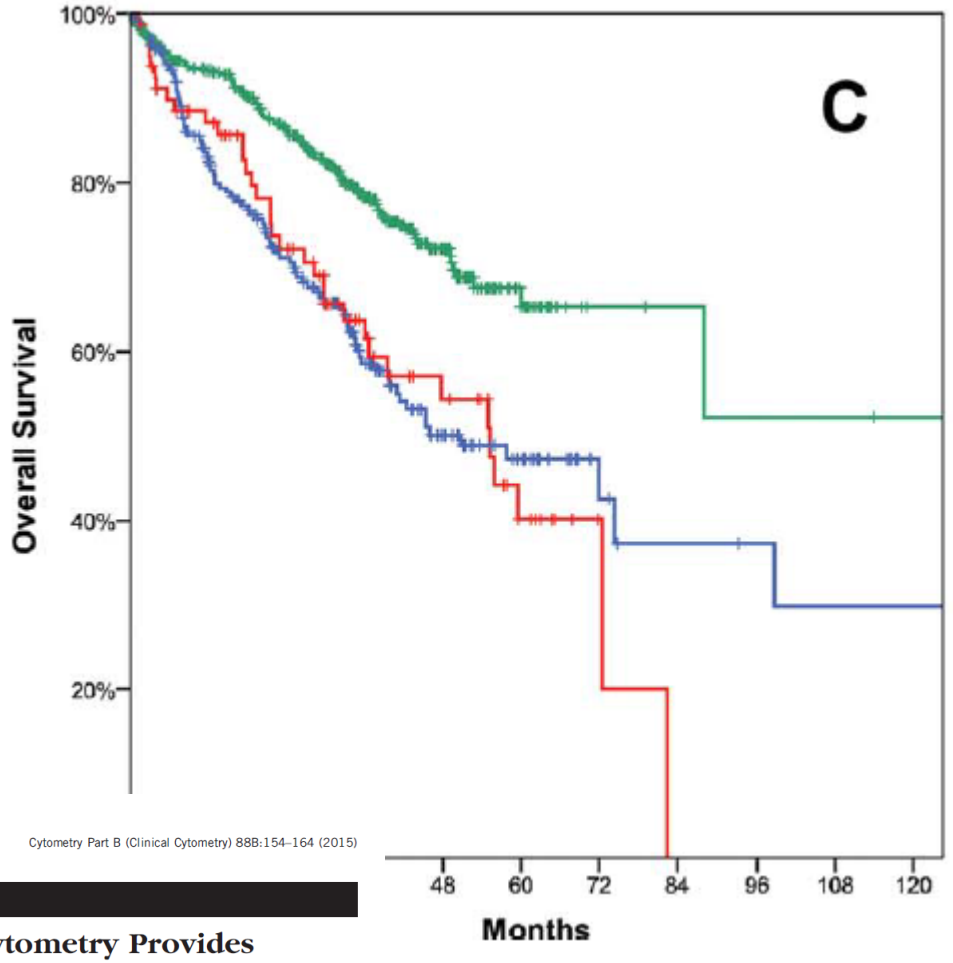
Loosdrecht AA van de, Westers TM. J Nat Compr Cancer Netw 2013;11:892-902
Westers TM, et al., Leukemia 2012;26:1730-41; Porwit A, et al., Leukemia 2014;28:1793-98

# Integrated Flow Cytometric diagnostic approach
# Scoring system

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Diagnostic flow score (Ogata et al.)** | **<2** | **<2** | **<2** | **<2** | | **≥2** | **≥2** | **≥2** | **≥2** |
| **Dysplasia by FC myeloid progenitors** | **-** | **-** | + | + | | **-** | **-** | + | + |
| **Dysplasia by FC**<br>**- Neutrophils (SSC or two or more other aberrancies)**<br>**- Monocytes (CD56 or two or more other aberrancies)**<br>**- Erythroid precursors (CD36 and/or CD71)** | **-** | + | **-** | + | | **-** | + | **-** | + |
| **Conclusion** | **A** | **A/B** | **A/B** | **C** | | **A/B** | **B/C** | **B/C** | **C** |

**Loosdrecht AA van de, Westers TM. J Natl Comp Canc Netw 2013;11:892-902;**
**Porwit A, Loosdrecht AA van de, et al., Leukemia 2014;28:1793-98**

# Flow cytometry and survival in patients with MDS




MDS score
0-2
3-4
5-11

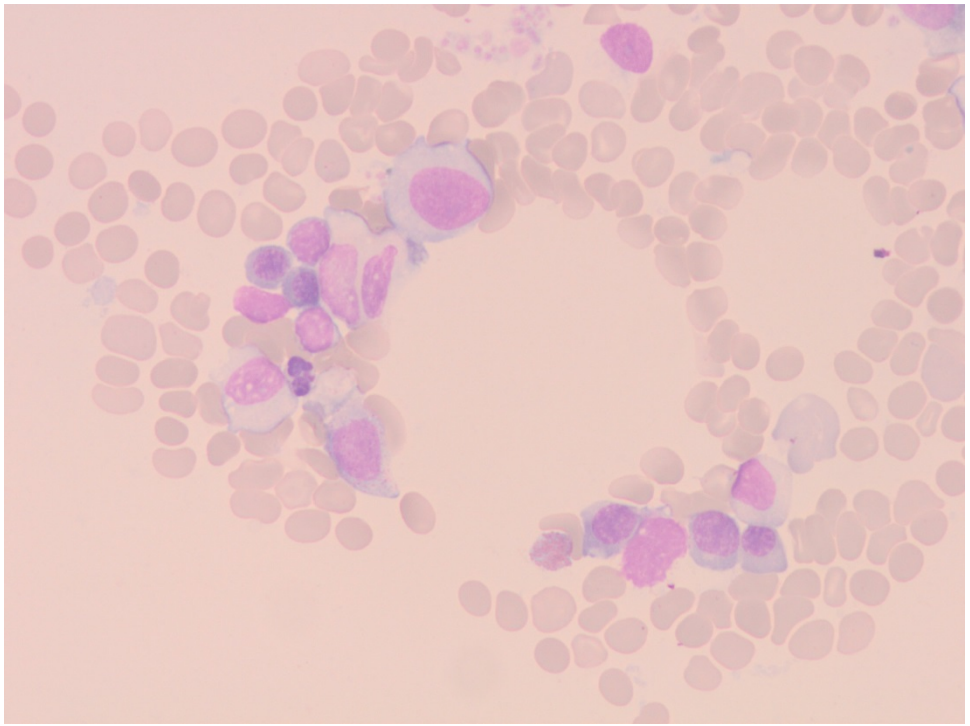
Cytometry Part B (Clinical Cytometry) 88B:154–164 (2015)

*Original Article*

**Multiparameter Flow Cytometry Provides Independent Prognostic Information in Patients with Suspected Myelodysplastic Syndromes: A Study on 804 Patients**

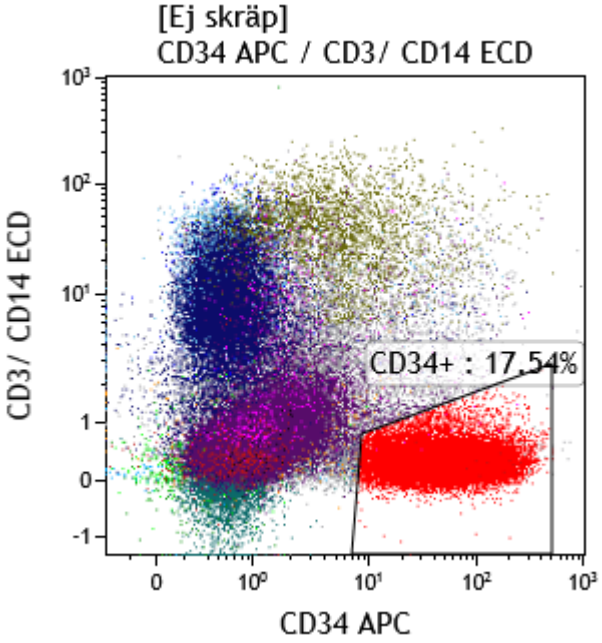
Wolfgang Kern,* Ulrike Bacher, Claudia Haferlach, Tamara Alpermann, Susanne Schnittger, and Torsten Haferlach
MLL Munich Leukemia Laboratory, Munich, Germany

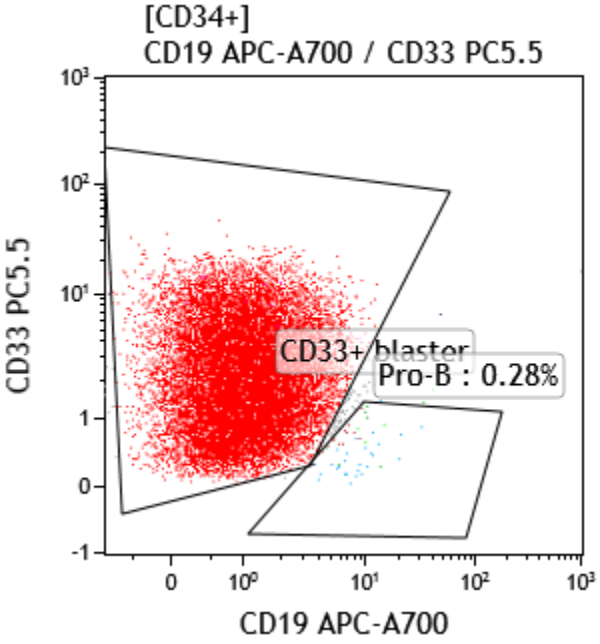
# **Case 1:** 52 year old male

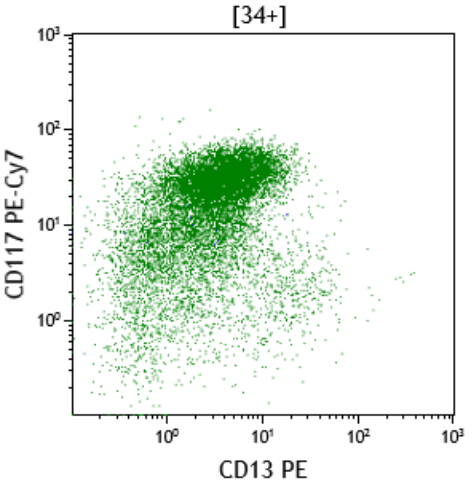


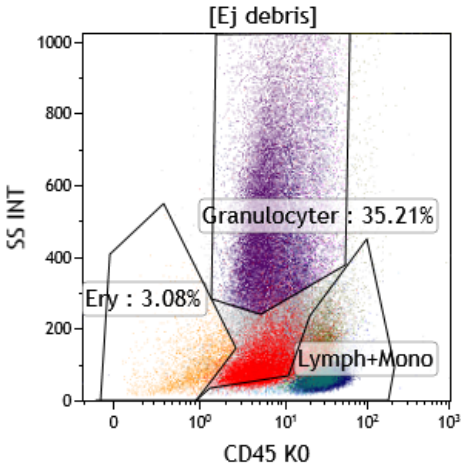
- presented with pancytopenia, fatigue, bleeding gums
- Hb 107 g/L, MCV 99,
- WBC 1,5x$10^9$/L, ANC 0.3x$10^9$/L, Plt 72x$10^9$/L
- BM Smears 6-9% blasts in various areas
- Erythropoiesis 48%
- dysplasia

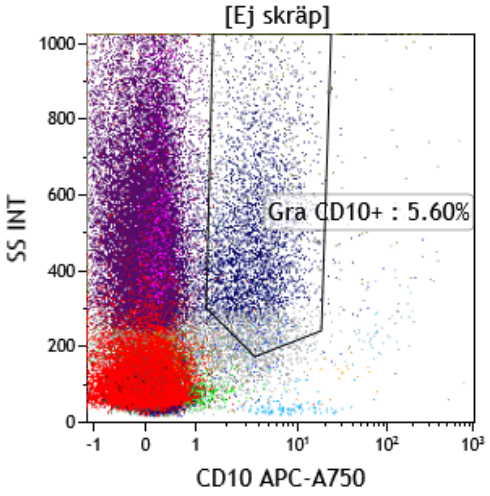
# Flow cytometry on lysed BM sample

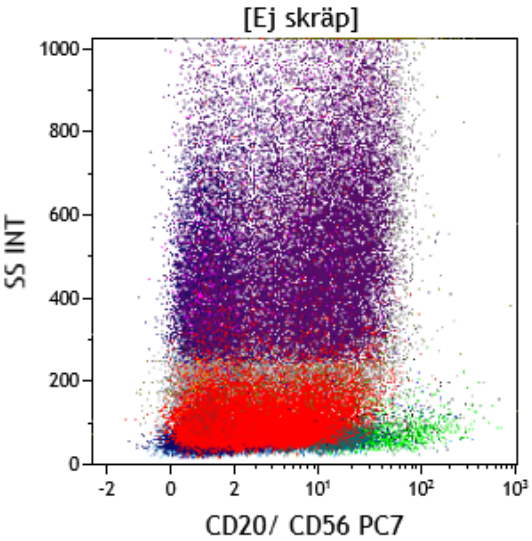


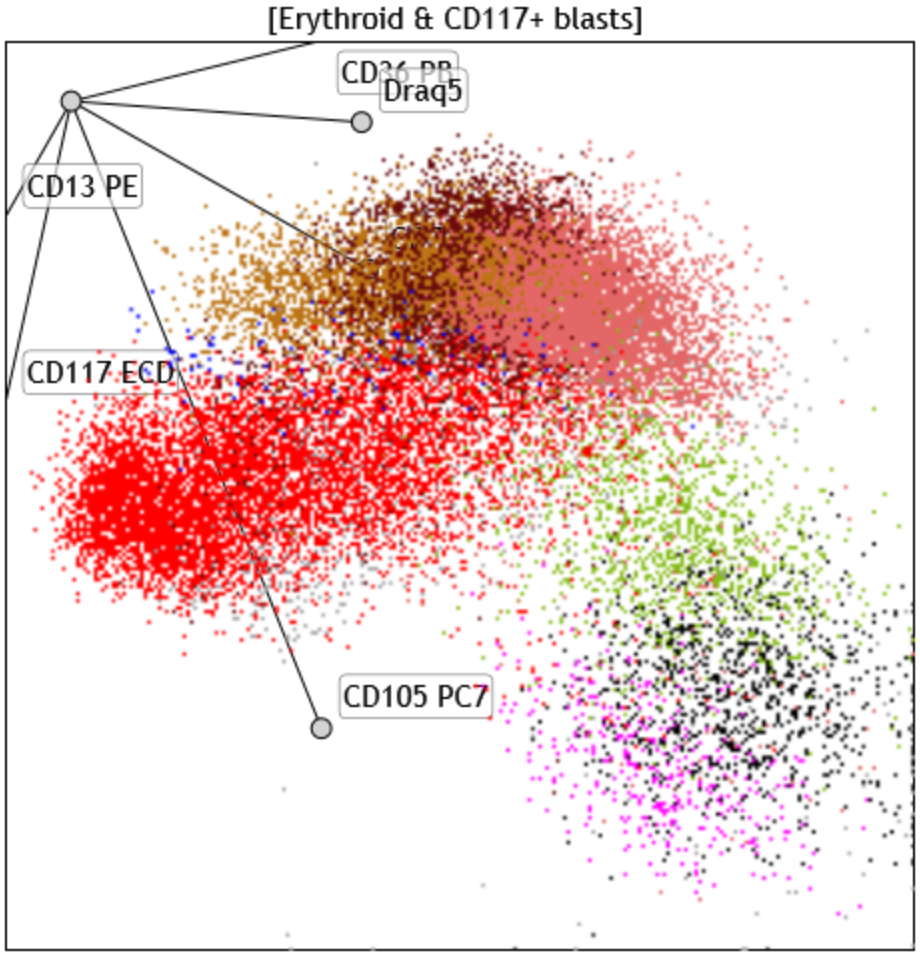



# Aberrant pattern of erythroid maturation



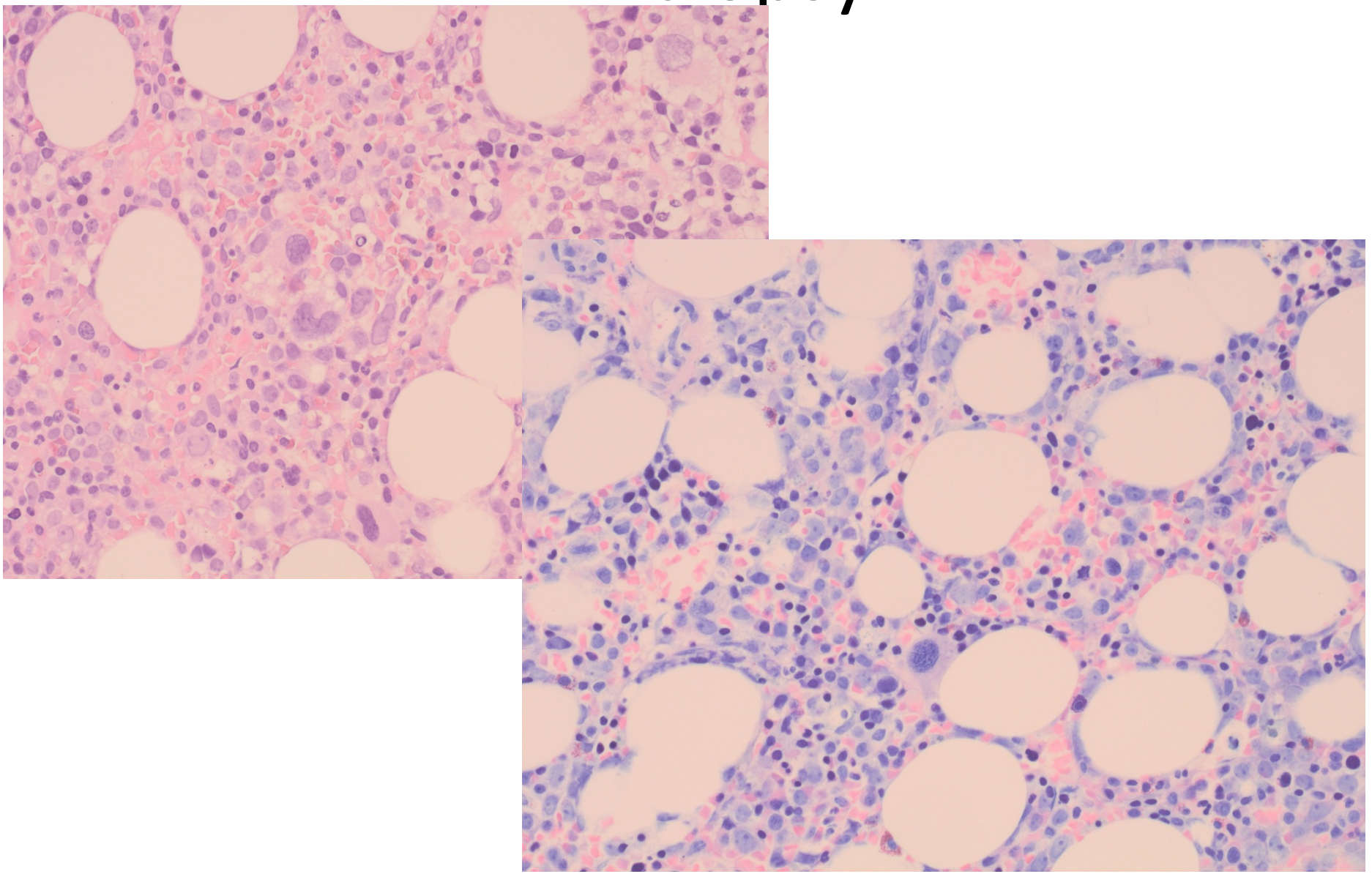

| Gate | %Total | %Gated |
|---|---|---|
| All | 100.00 | 100.00 |
| blasts CD117+ | 8.29 | 89.88 |
| blasts- CD117+/13+ | 7.07 | 85.32 |
| blasts-CD117+/13-/105- | 0.13 | 1.54 |
| Ery- CD117+/105+ | 0.36 | 4.36 |
| Ery- aberrant-CD36-/CD71- | 2.00 | 59.35 |
| Erythroids- CD45-/71+v | 21.68 | 25.93 |
| Ery- early/CD105++/+d | 2.58 | 11.90 |
| Ery- CD105++ | 1.12 | 43.22 |
| Ery- CD105+d | 1.40 | 54.11 |
| proliferating ery | 9.12 | 42.05 |
| erythroids excluding CD105+ | 19.10 | 19.10 |
| Ery- 105-/36+d | 10.06 | 52.67 |
| Ery-105-/36+ | 7.30 | 38.20 |

Erythropoiesis: 26%

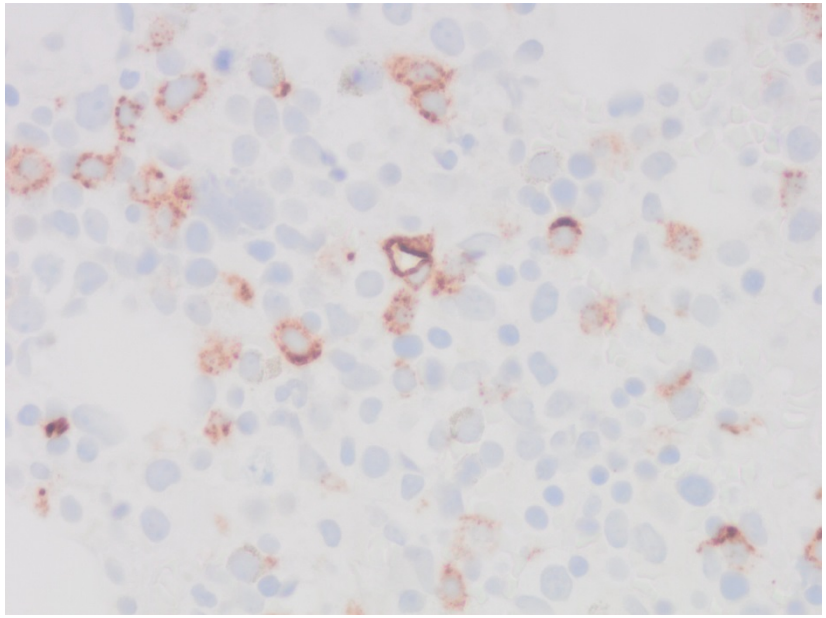
CD117+ 7%

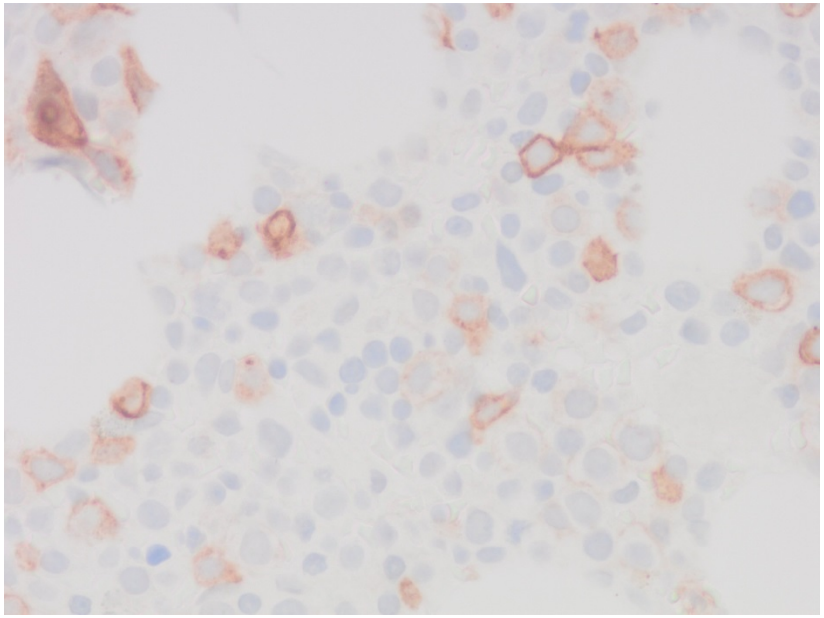
CD117+ erythroid 0.4%

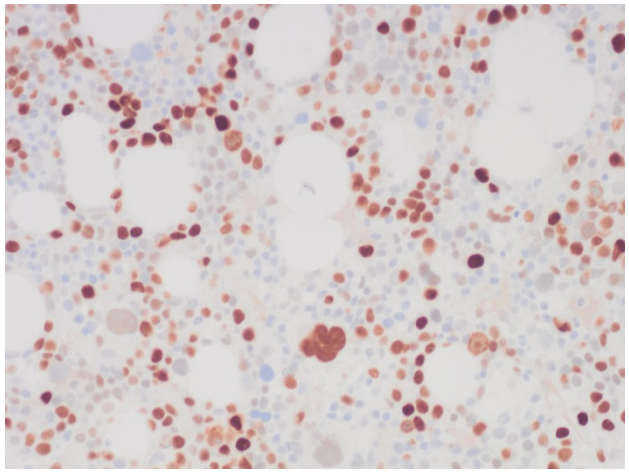
# BM biopsy



# IHC







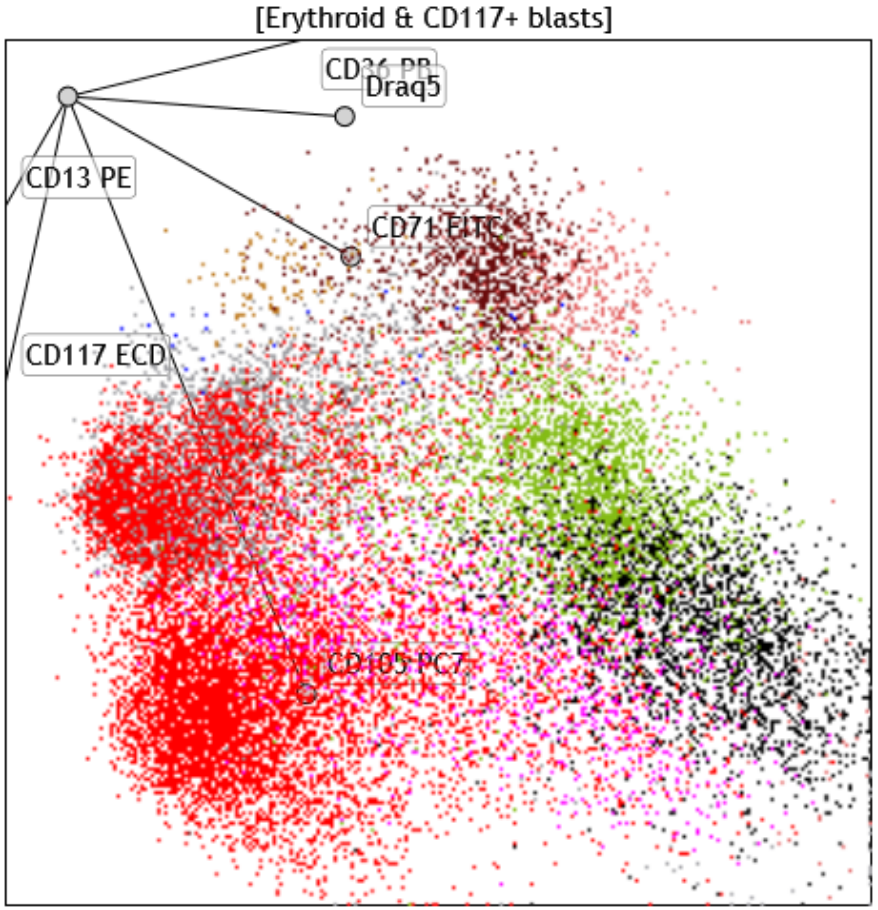
**CD34 and CD117 counted 11-13%**

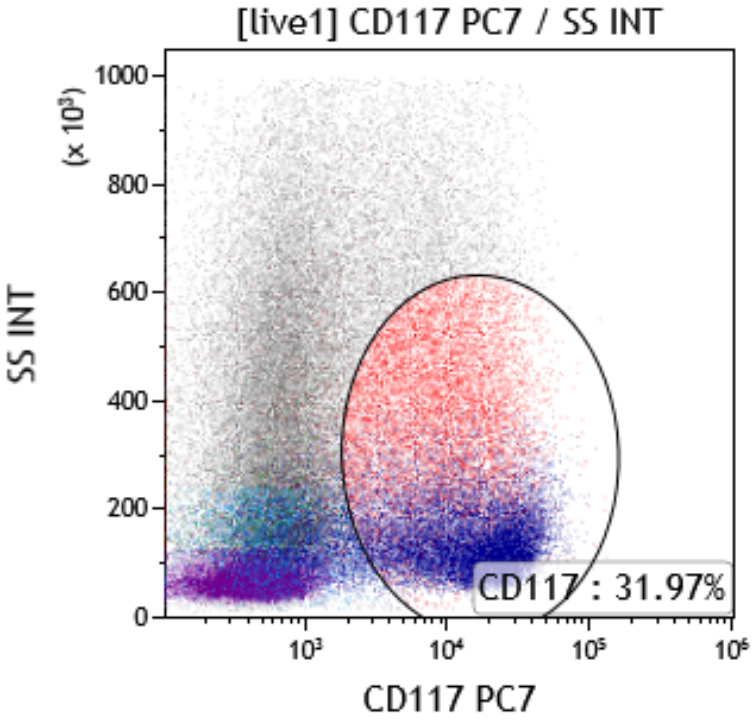
**P53 overexpressed**

# Cytogenetics/Molecular

- 41-44, X,-Y,-4,-5, der(7)t(?5;q31)p(1?5;q31),der(17)(t(7;17)(q31;p11),-18,-19,?der(20)t(5;20)(q31;p11),-22, +1-4mar [cp21]/46XY[4]
- Illumina Tru Sight 54 genes
- TP53 c747G>T Tier 1 57%

# Vidaza treatment
# Progressed to AML within 3 months



# Case 2: Male 59 years old

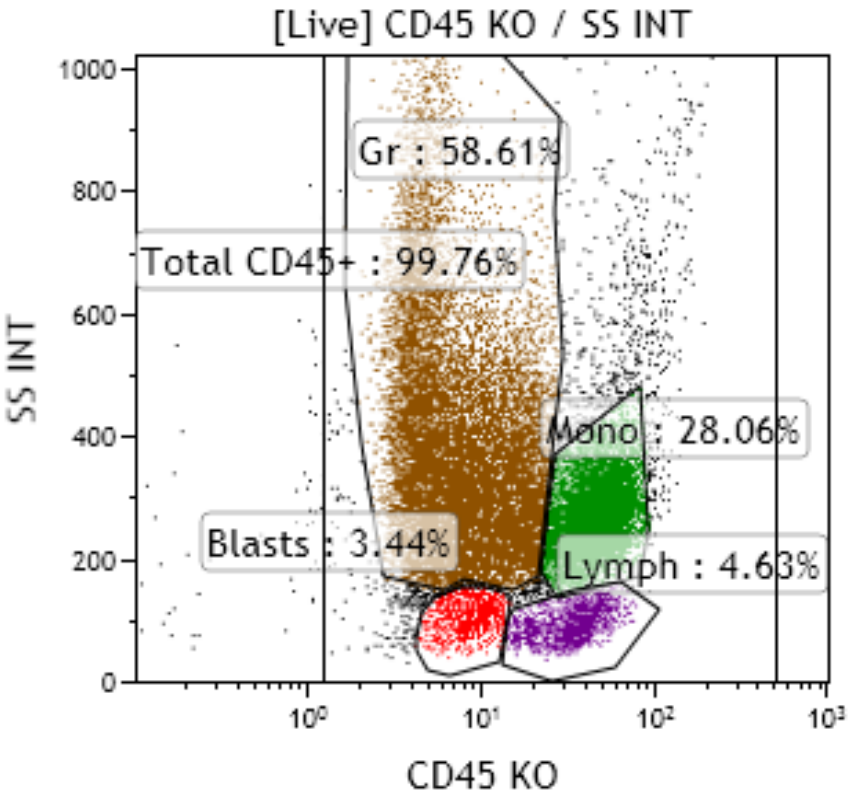
- Previously healthy
- Developed increasing fatigue about 2 months before presentation
- 6 weeks before presentation GP found anemia
- Patient went for vacation to Barbados
- Felt even more fatigue after coming back
- No fever, night sweats or weight loss

# Status and Lab

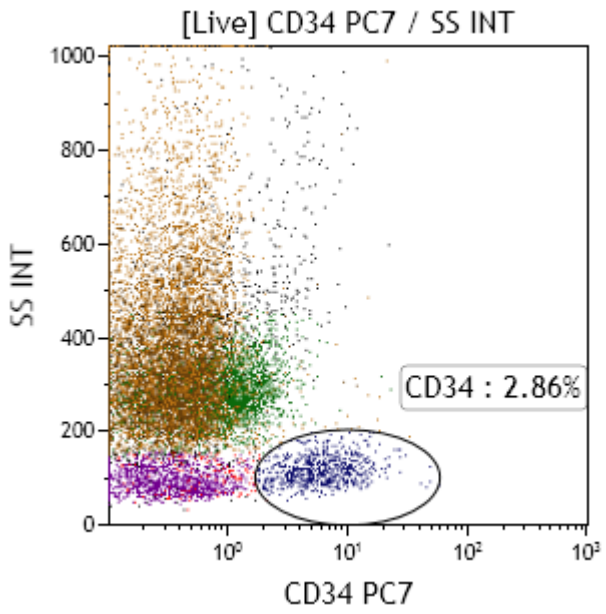
- No lymphadenopathy or organomegaly
- No bruising or rash, no neurological deficit
- Hb 70g/L, MCV 115, reticulocytes $33x10^9/L$
- WBC $24.4x10^9/L$, no eosinophilia or basophilia
- Neutrophils $17.5x10^9/L$, Monocytes $6.1x10^9/L$
- Plt $148x10^9/L$
- LDH and creatinine borderline
- Bone marrow biopsy with flow cytometry was performed

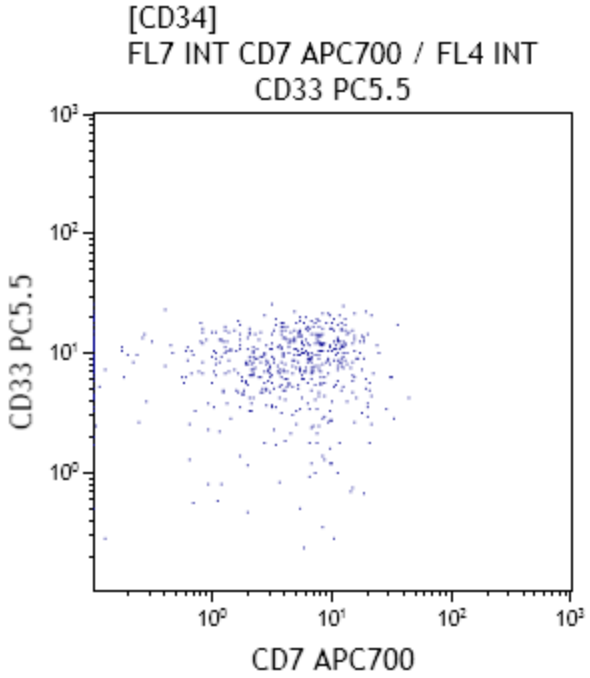
# CD45 vs SSC

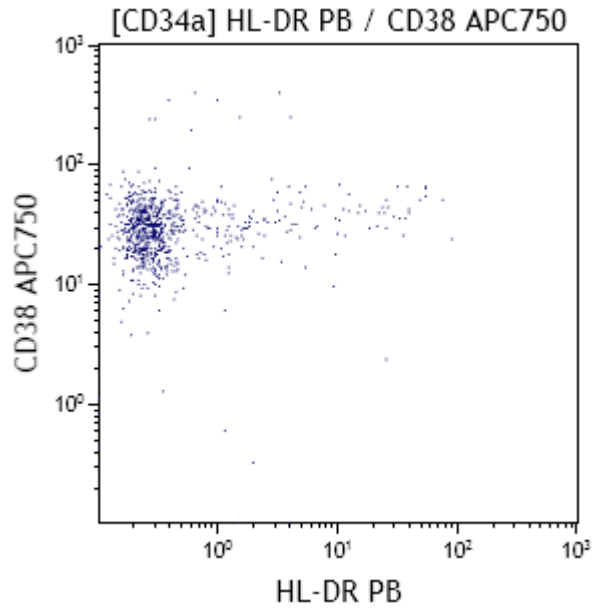
- Blasts are not increased
- Monocytes are increased
- Granulopoiesis shows abnormal scatter
- Lymphocytes are within normal limits

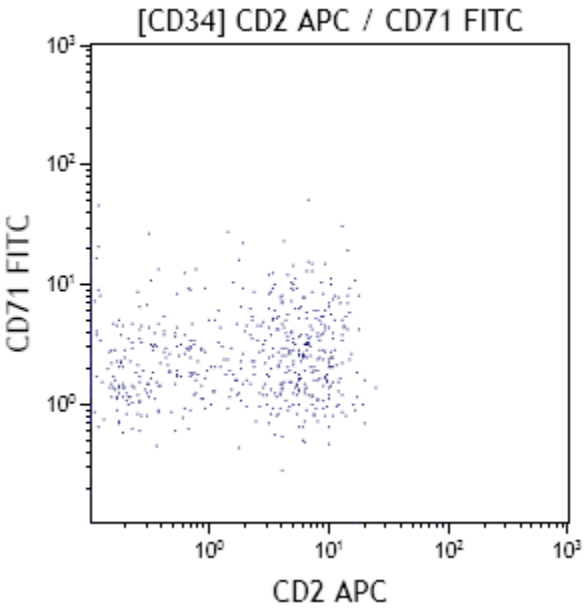


# CD34+ cells >2%



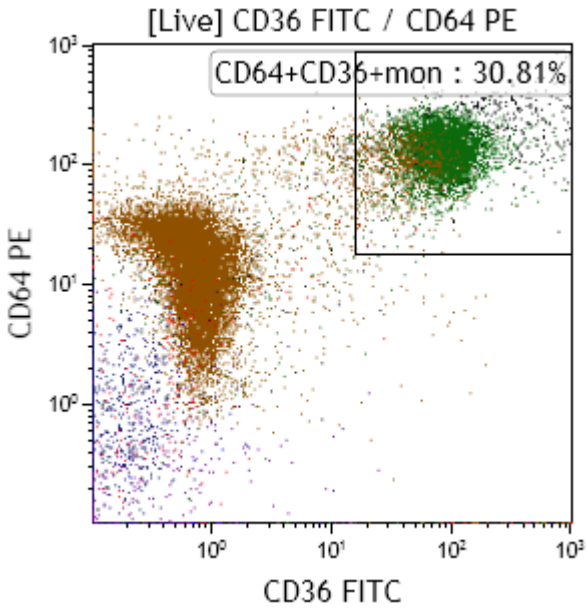



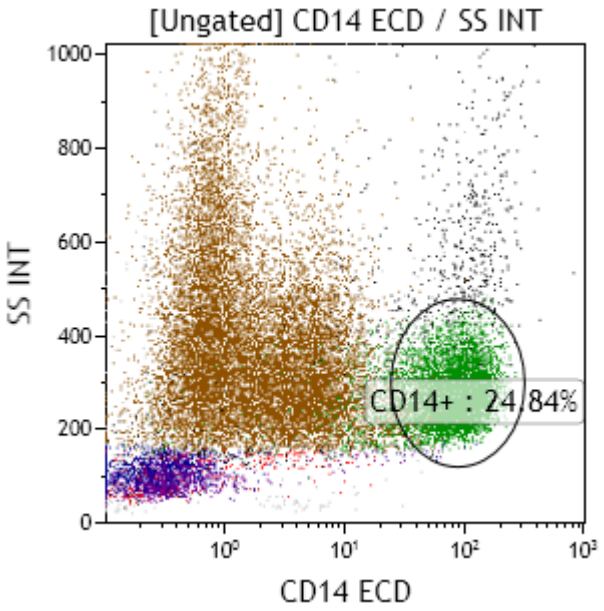



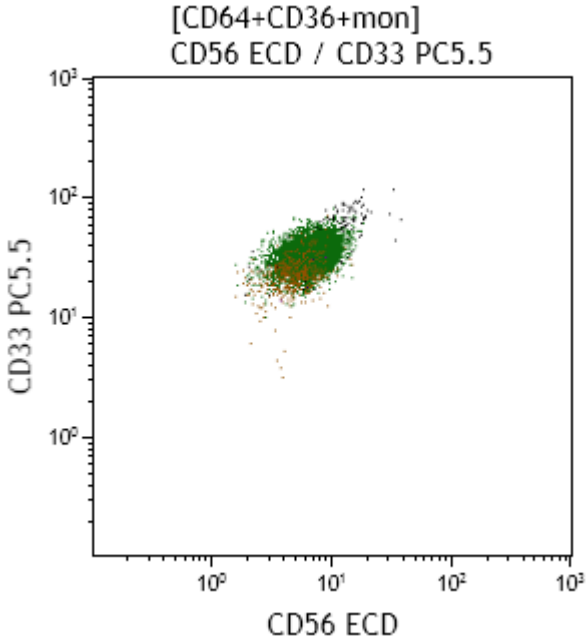



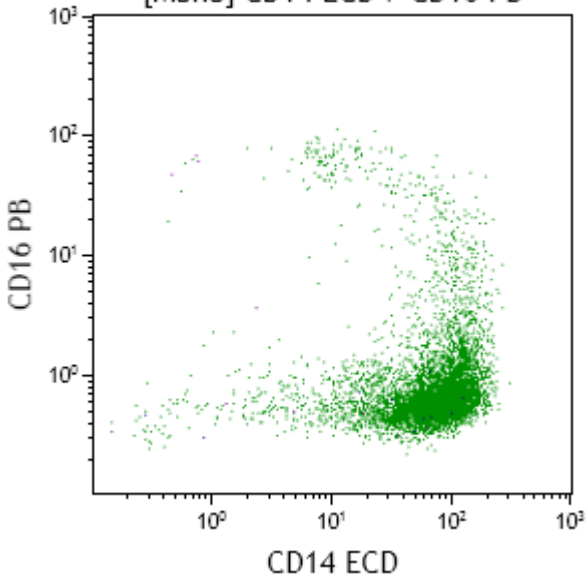

**CD7 expression**
**CD2 expression**
**Negative HLA-DR**
**No B-precursors**

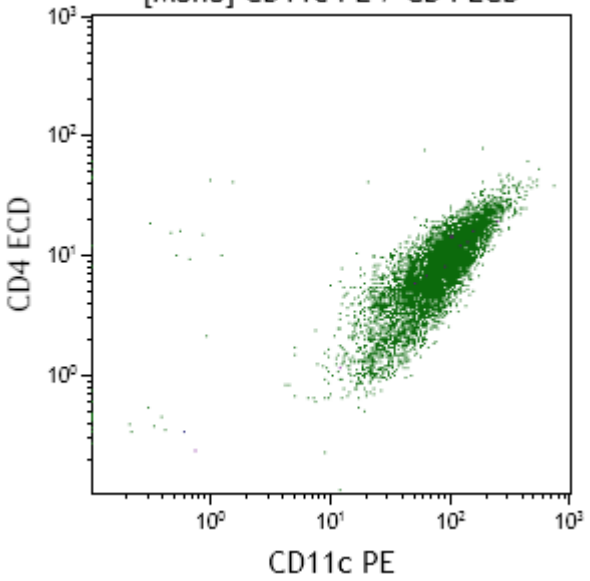
# Monocytes are increased and have abnormal expression of CD56 and HLA-DR

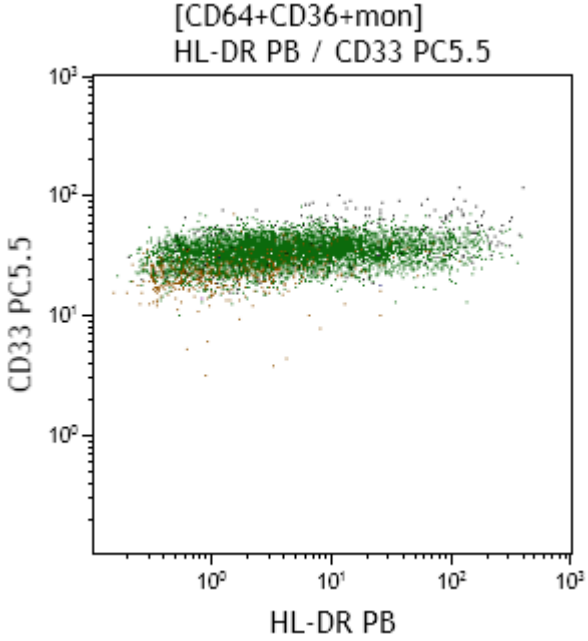


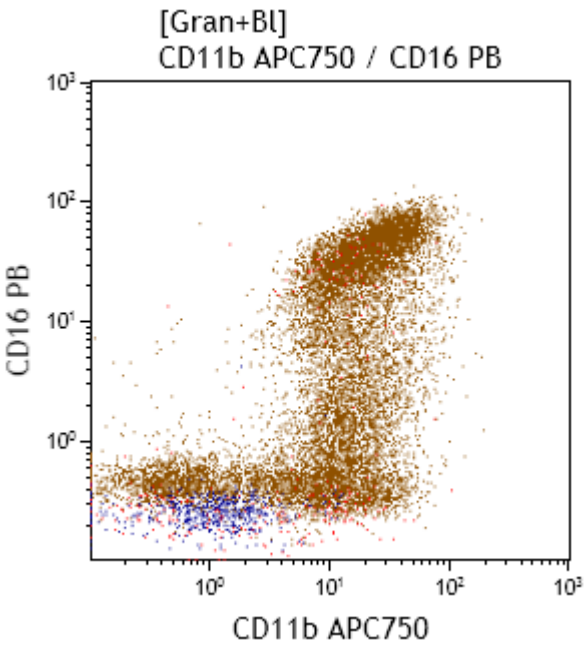



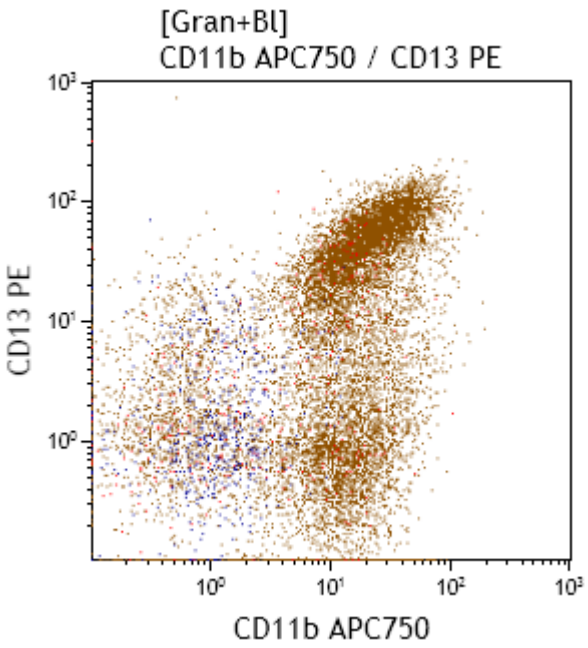



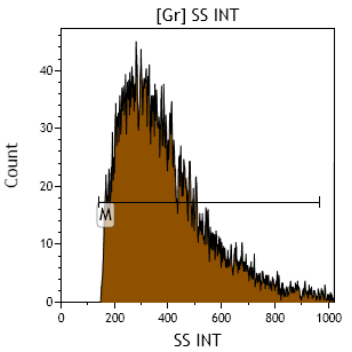



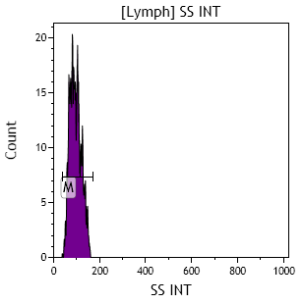
# A subset of granulocytes has abnormal scatter characteristics and upregulated CD14



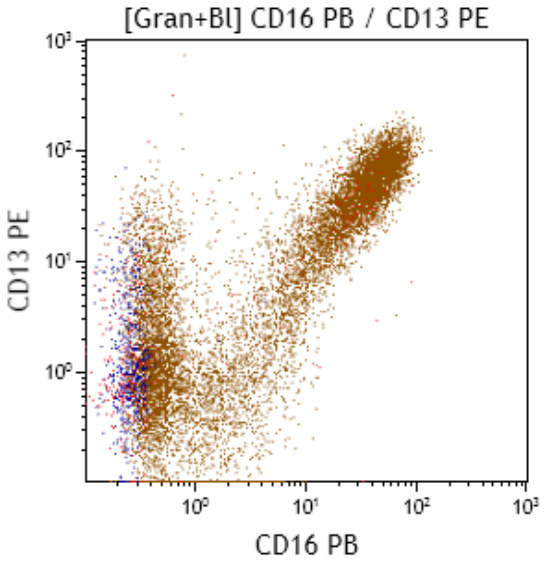



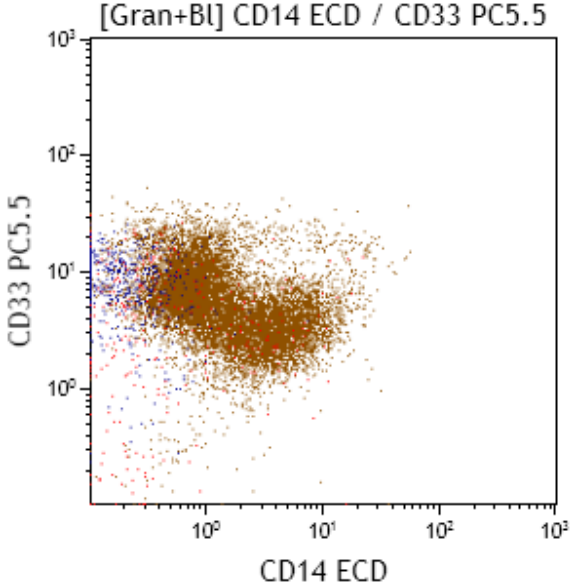




| Gate | X-Mode |
|---|---|
| All | 1,023.00 |
| M | 254.00 |



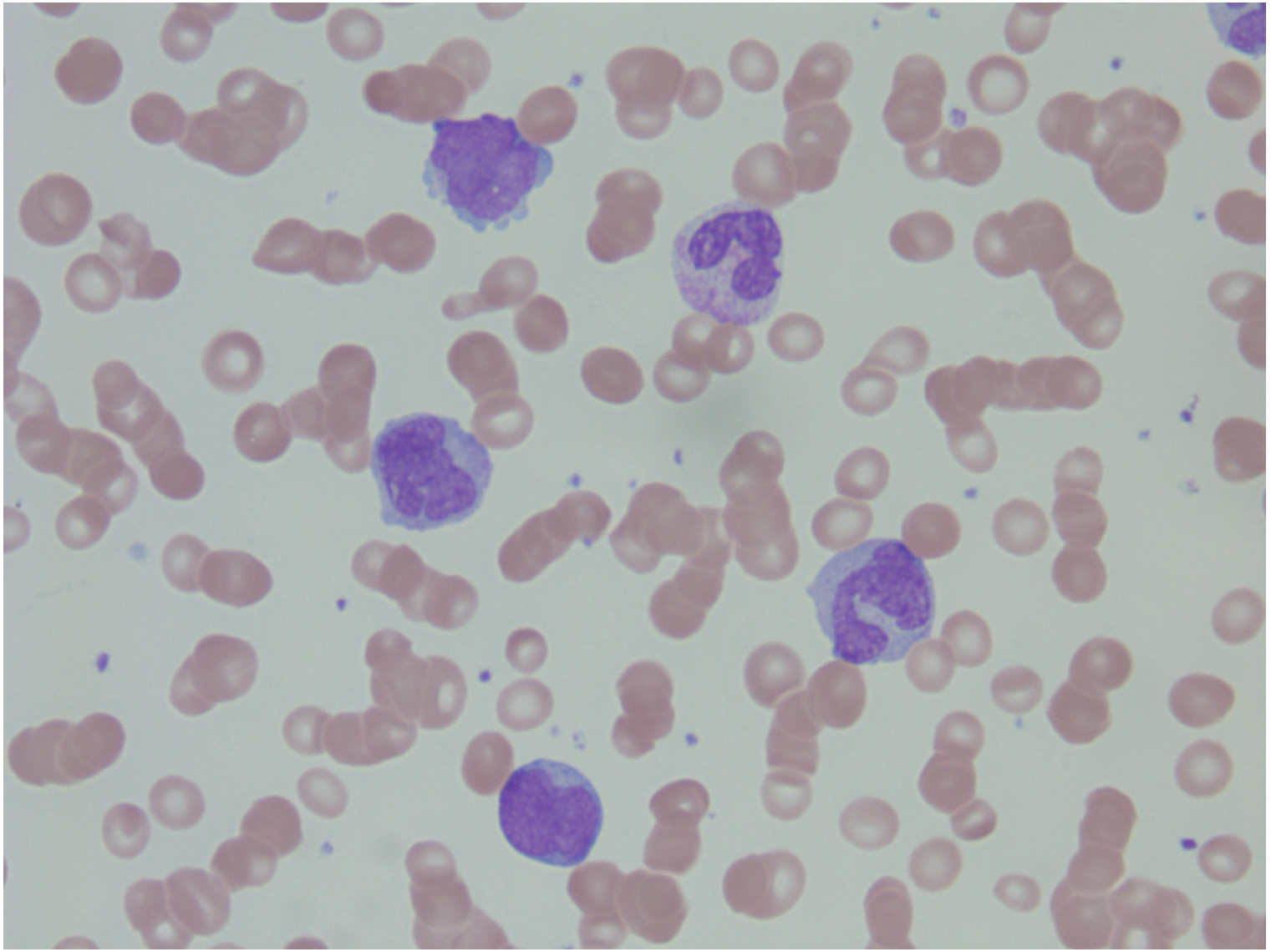

| Gate | X-Mode |
|---|---|
| All | 82.00 |
| M | 82.00 |



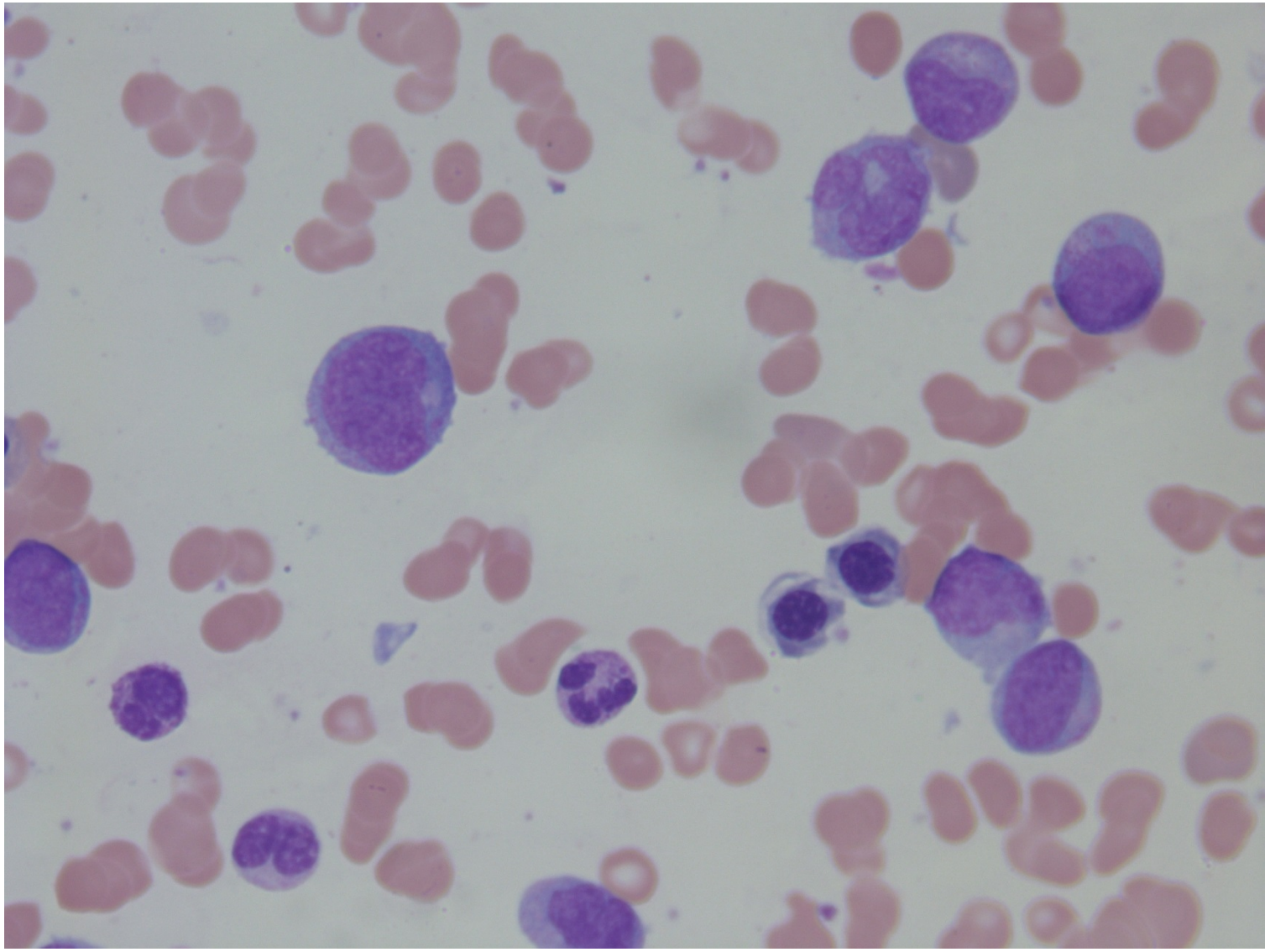




**SSC Gr/ly= 3**

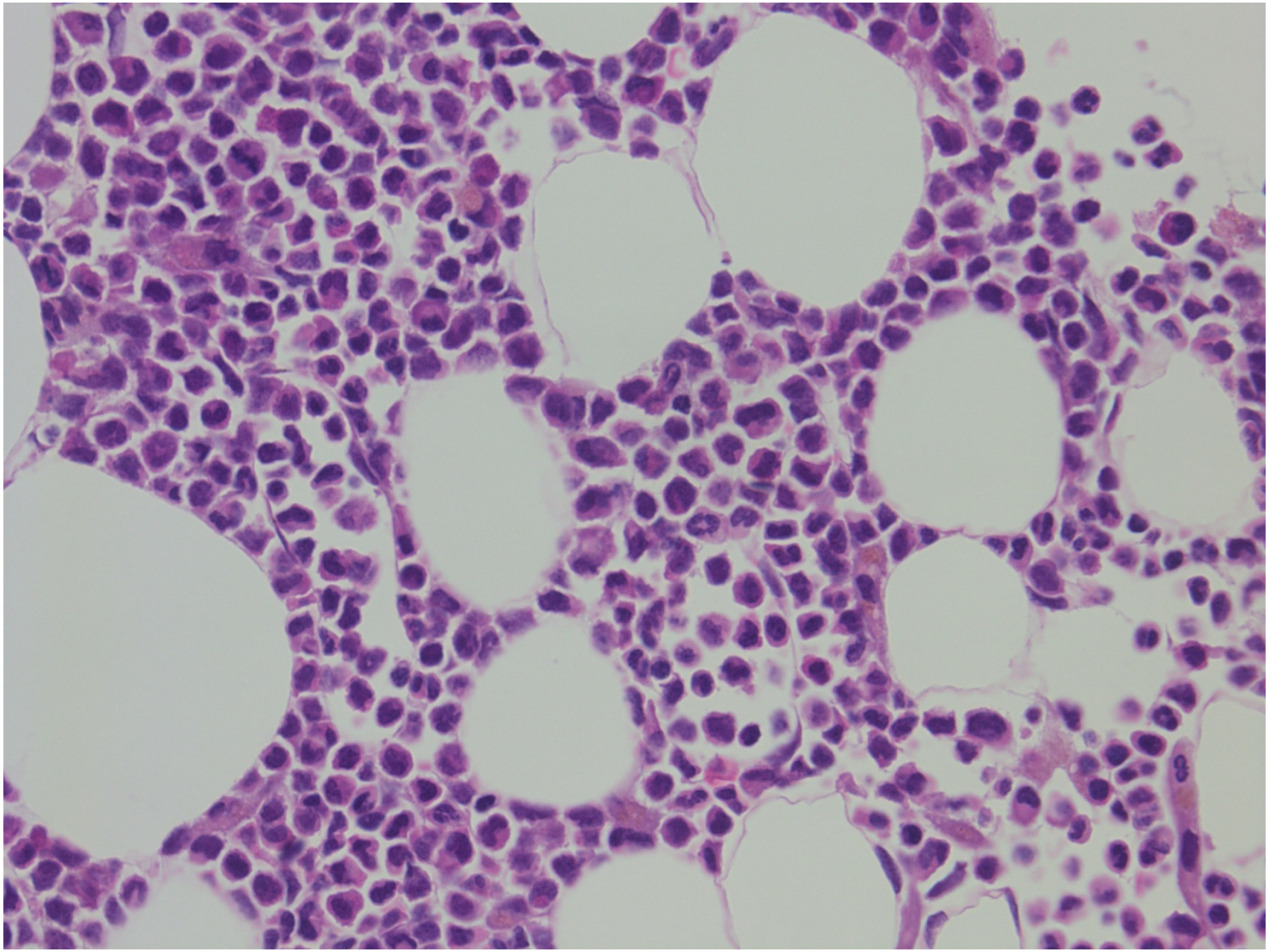
# Blood cytology, blasts 1%

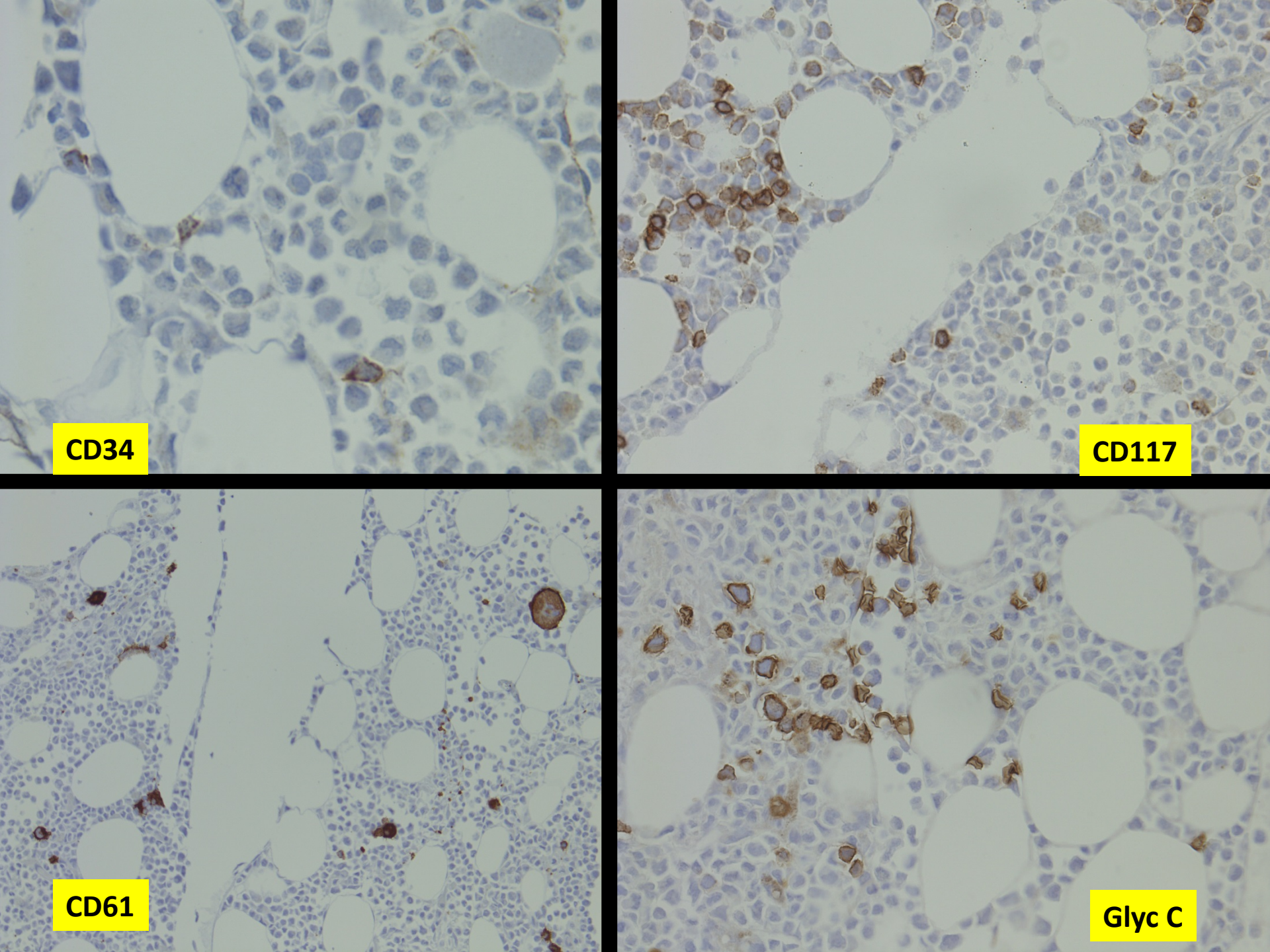


# Bone marrow cytology, blasts 5%



# Bone marrow biopsy

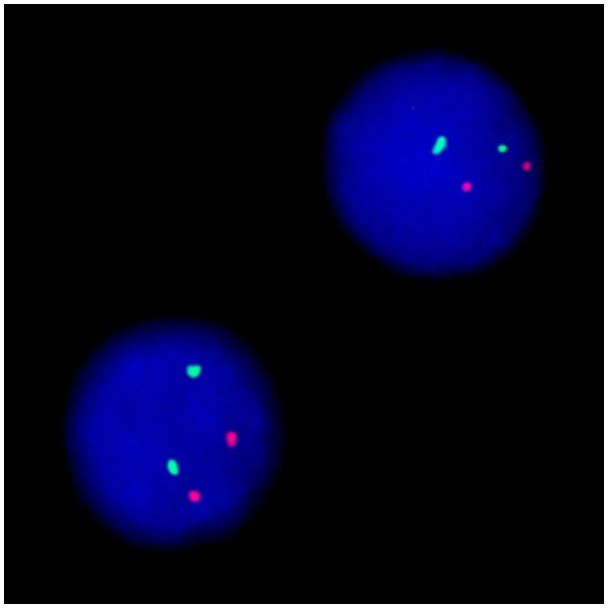



CD34
CD117
CD61
Glyc C

# Cytogenetics, FISH, molecular studies

- **t(11;19)(q23;p13.1), MLL-ELL**
- **FISH confirmed MLL rearrangement**
- ***JAK-2* mutation negative**
- ***BCR-ABL1* negative**

- **11q23 abnormalities**
- **leading to the *MLL* gene**
- **rearrangement are more**
- **common in AML than in CML**



# Diagnosis and Follow-up

- **Chronic myelomonocytic leukemia (CMML-1)**

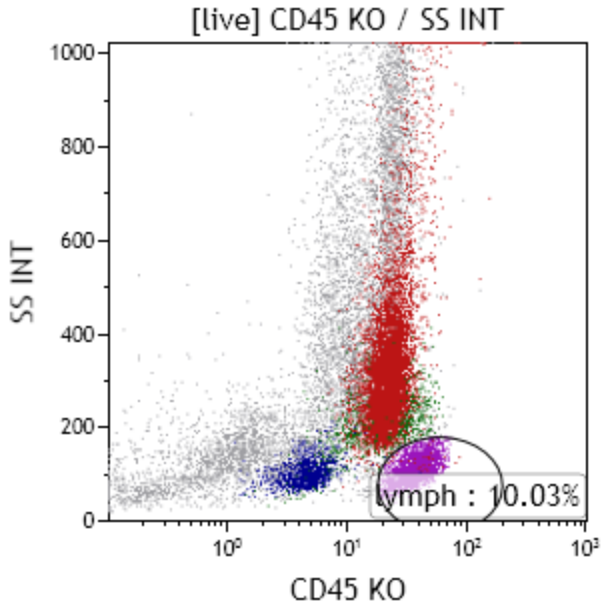
- **One month later blasts were 9%**
- **Due to cytogenetics this patient was at risk of rapid progression to AML**
- **Induction with FLAG-IDA**
- **Consolidation with 2 cycles of intensification protocol Dana-Farber**
- **Doing well after BMT with 10/10 matched unrelated donor**

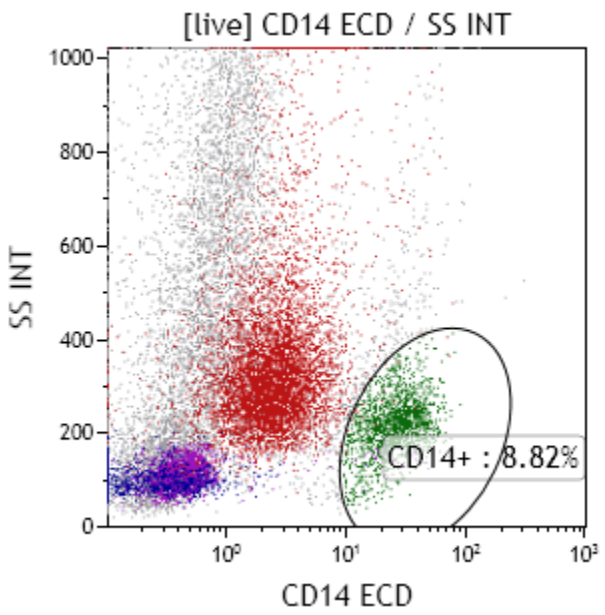
# Case 3

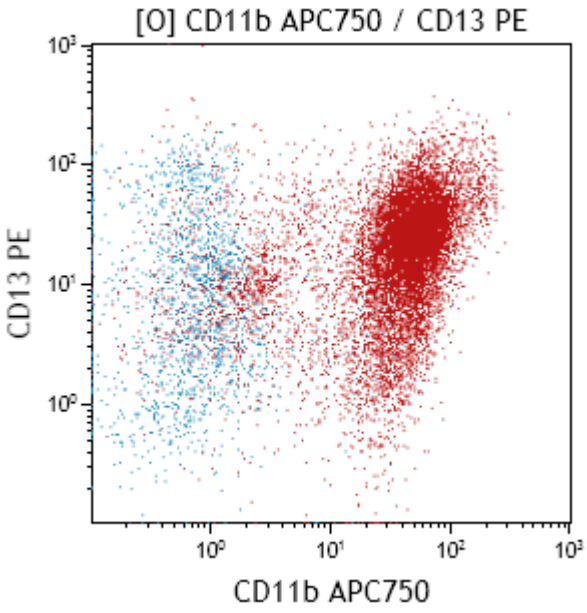
- 70 year old female
- Treated with R-CHOP 3 years ago for diffuse large B-cell lymphoma stage 2A
- In continuous CR
- Developed fatigue
- No fever, night sweats or weight loss

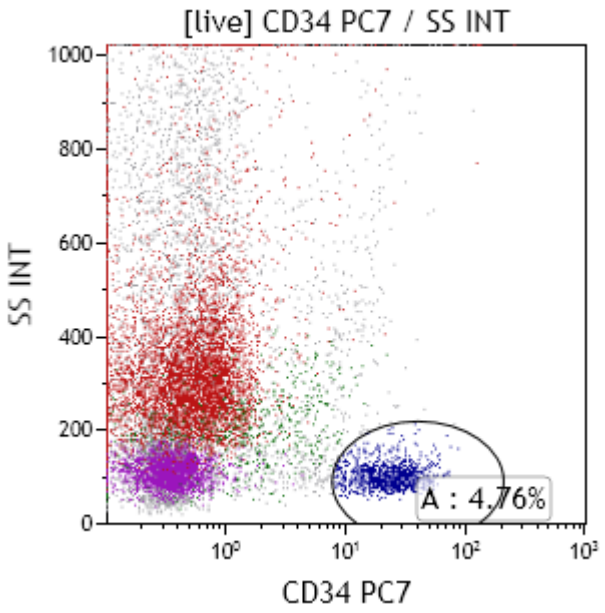
# Status and Lab

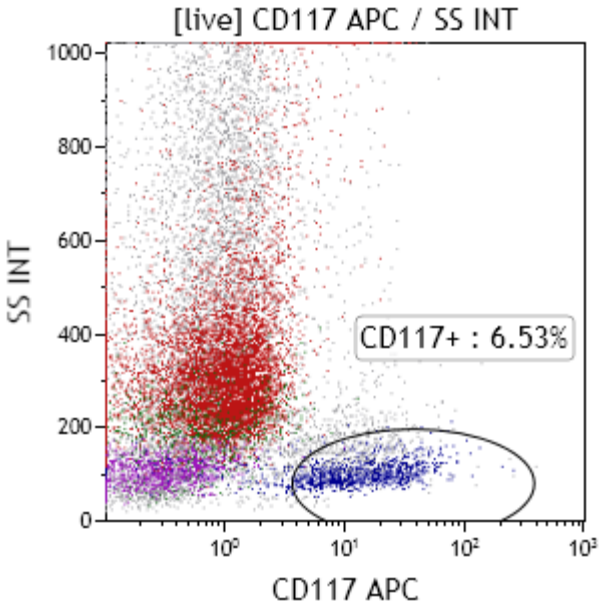
- No lymphadenopathy or organomegaly
- No neurological abnormalities, no skin rash
- Hb 101 g/L, MCV 88, reticulocytes $30x10^9/L$
- WBC $3.5x10^9/L$, Plt $106x10^9/L$
- Neutrophils $1.64x10^9/L$, monocytes $0.21x10^9/L$
- Eosinophils $0.31x10^9/L$, basophils $0.1x10^9/L$
- Lymphocytes $1.26x10^9/L$
- 1% blasts seen at review of the blood smears
- 1 nRBC/100 WBC
- RBC: anisocytosis with some microcytes and some macroovalocytes, occasional fragments
- Liver and kidney tests normal
- Bone marrow aspirate and biopsy with flow cytometry and cytogenetics was acquired

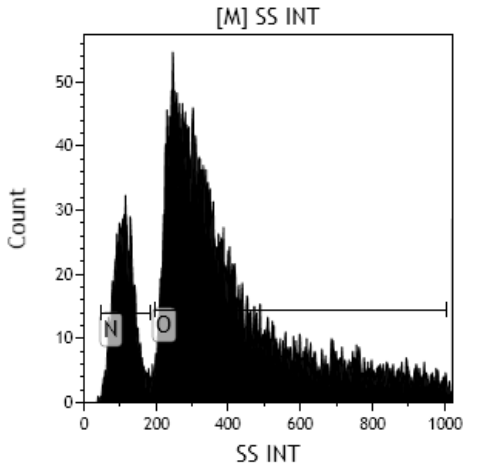



| Gate | X-Mode |
|---|---|
| All | 1,023.00 |
| N | 108.00 |
| O | 247.00 |

SSC Gr/lymph = 2.3

# Granulopoiesis

# Progenitors and granulocytes

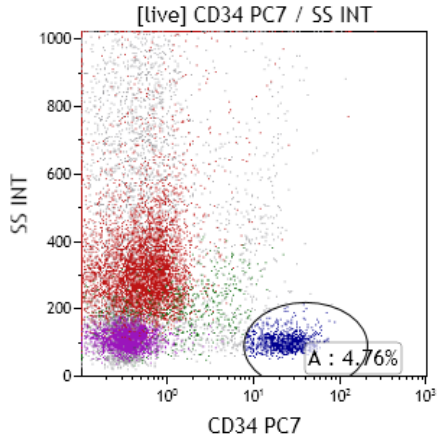
**CD34+ cells were increased**

**CD117+ cells were increased**

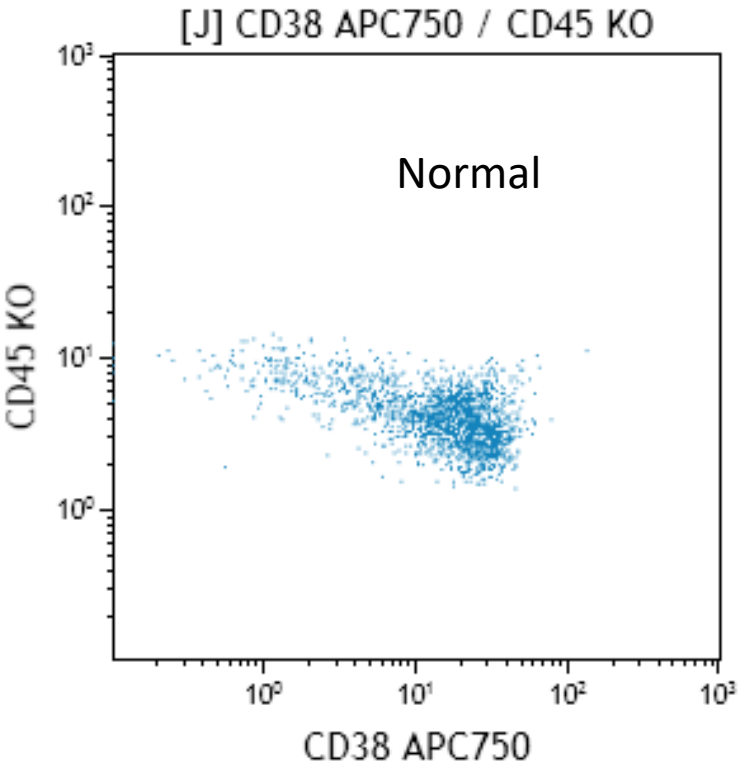
**Granulocytes had increased CD14 expression**

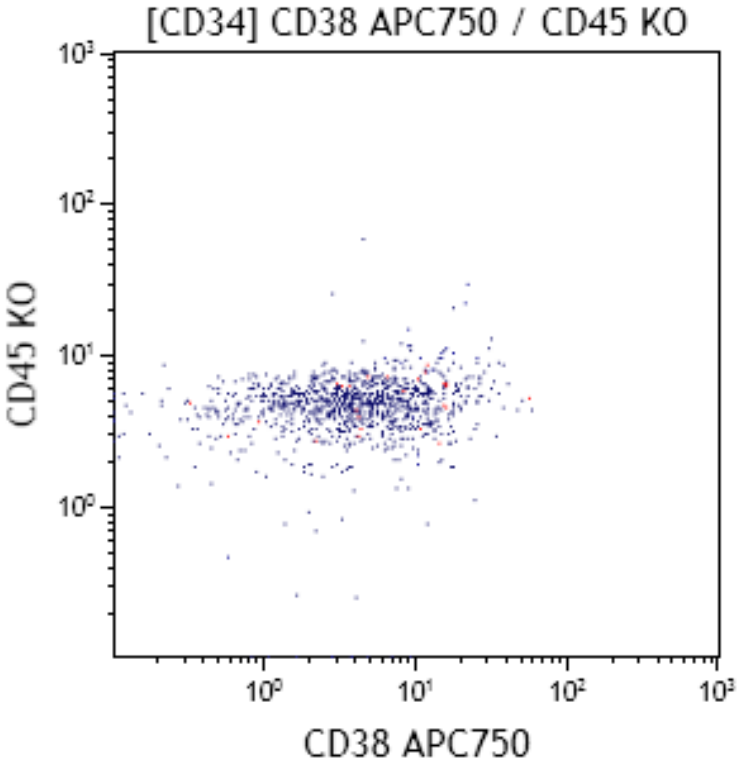
**Granulocytes had abnormal scatter characteristics**

# CD34+ cells: Decreased CD38 Normal CD45

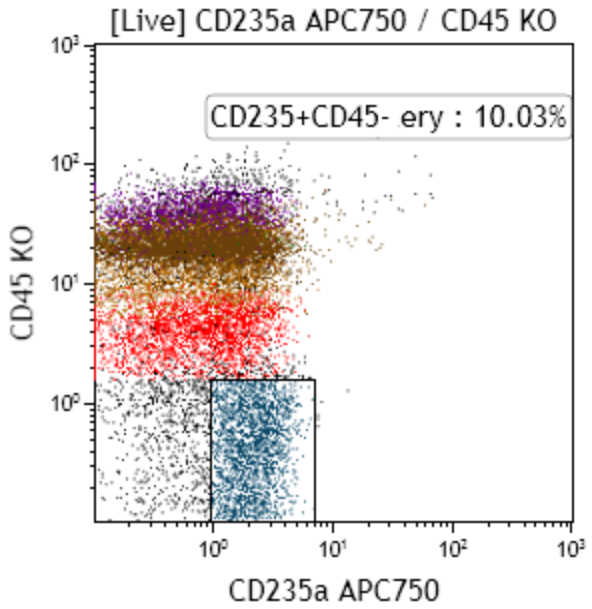


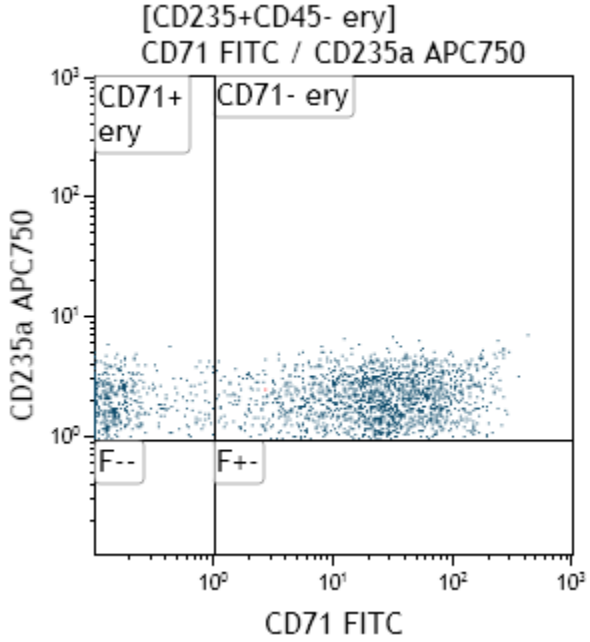

Patient

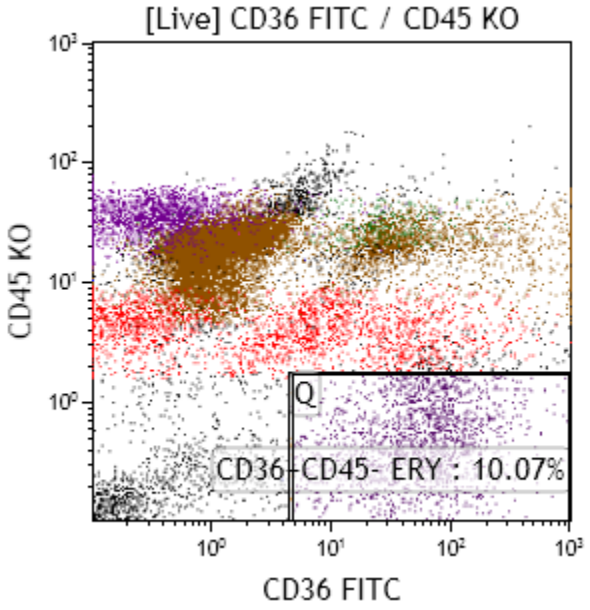


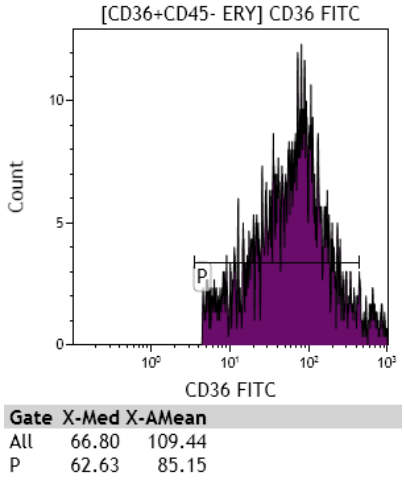



# Erythropoiesis



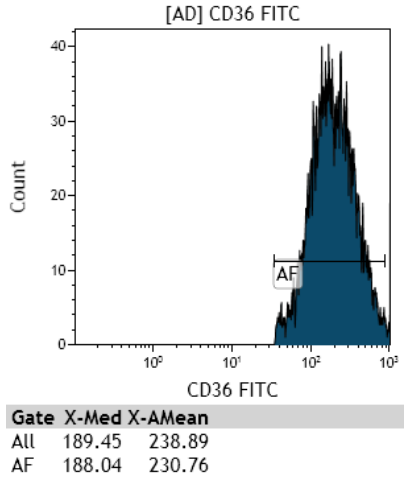




| Gate | X-Med | X-AMean |
|---|---|---|
| All | 66.80 | 109.44 |
| P | 62.63 | 85.15 |

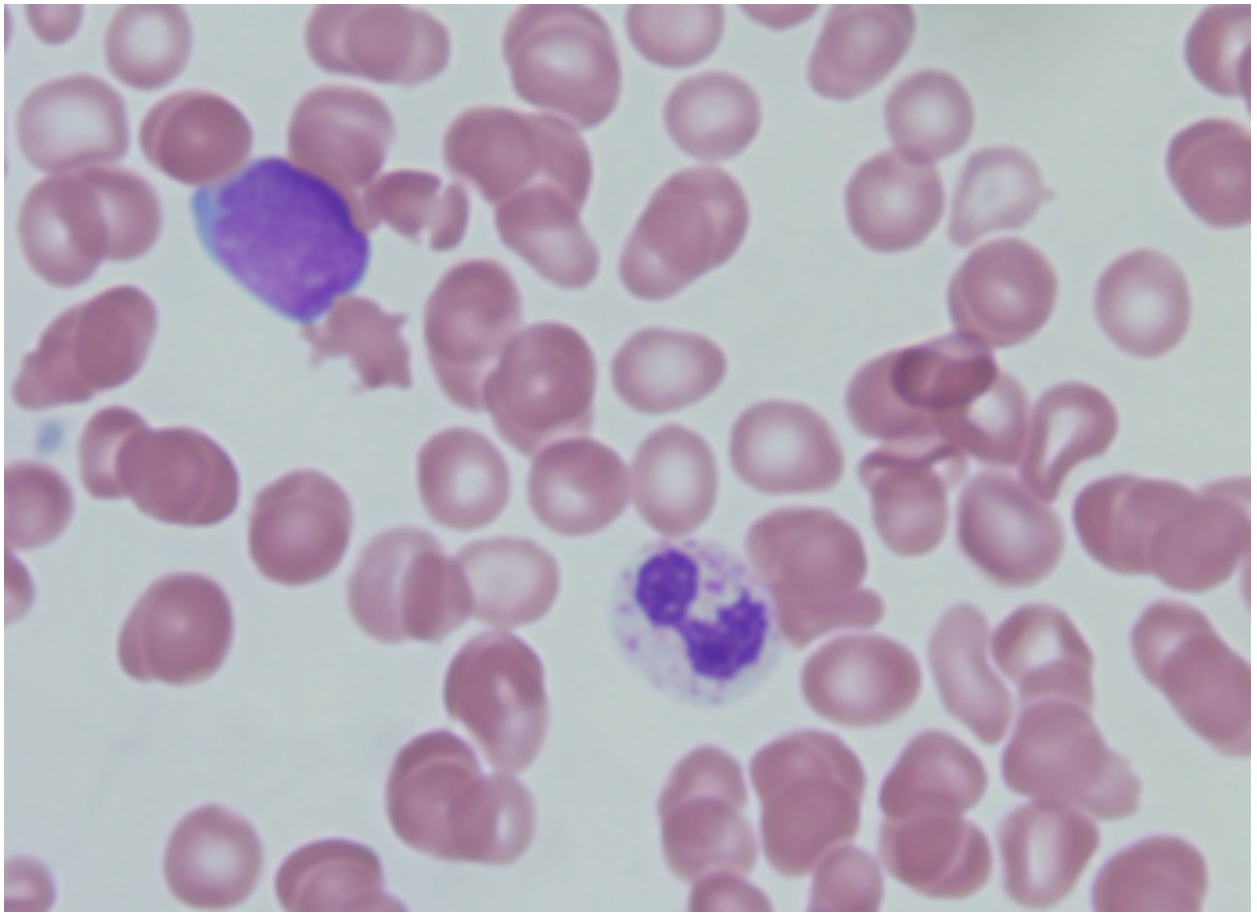
Patient




| Gate | X-Med | X-AMean |
|---|---|---|
| All | 189.45 | 238.89 |
| AF | 188.04 | 230.76 |

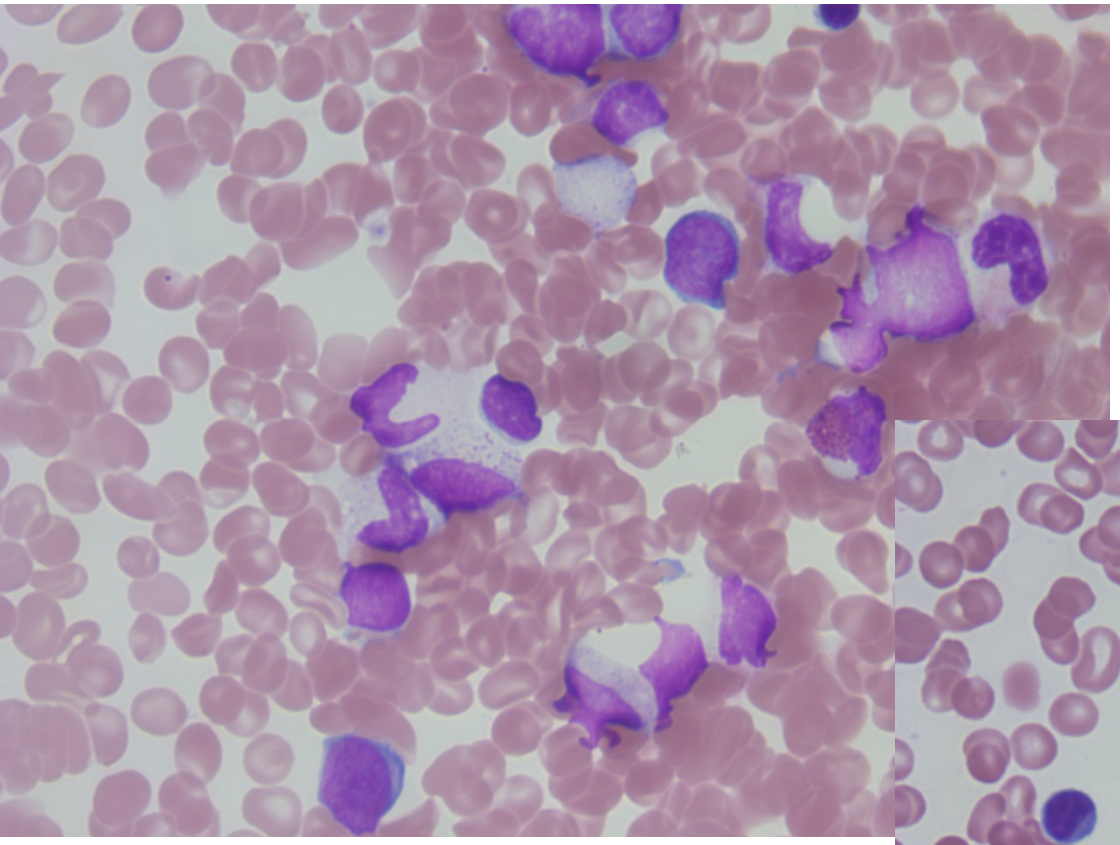
Normal

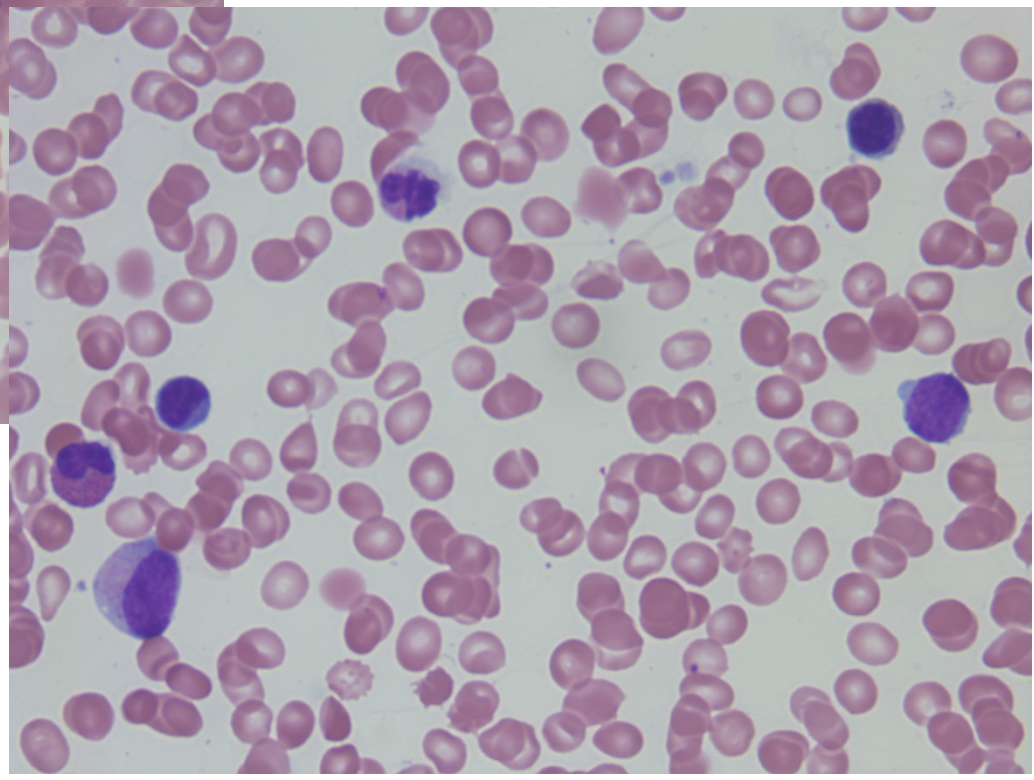
# Blood morphology, blasts 1%



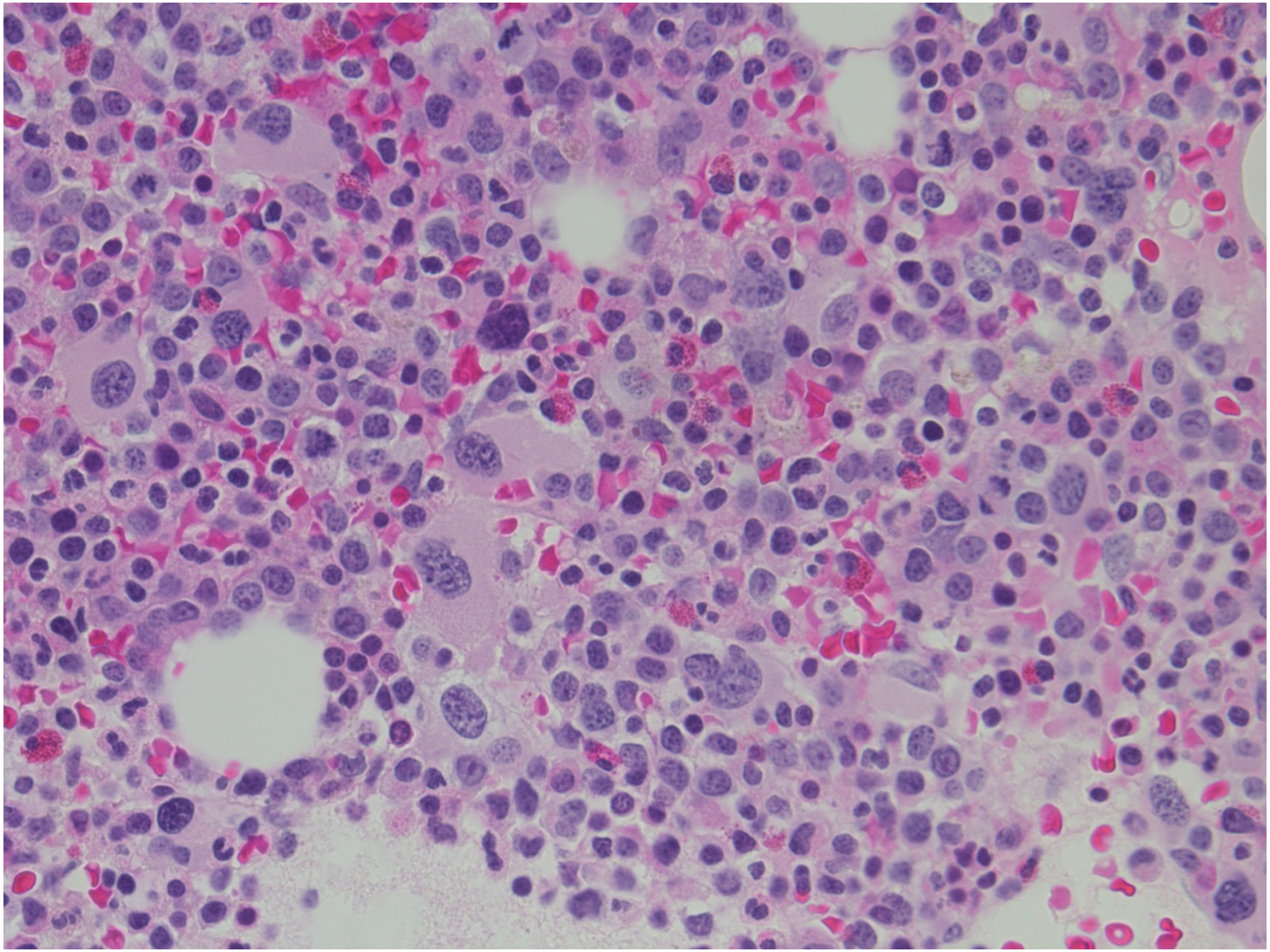
# Bone marrow smears

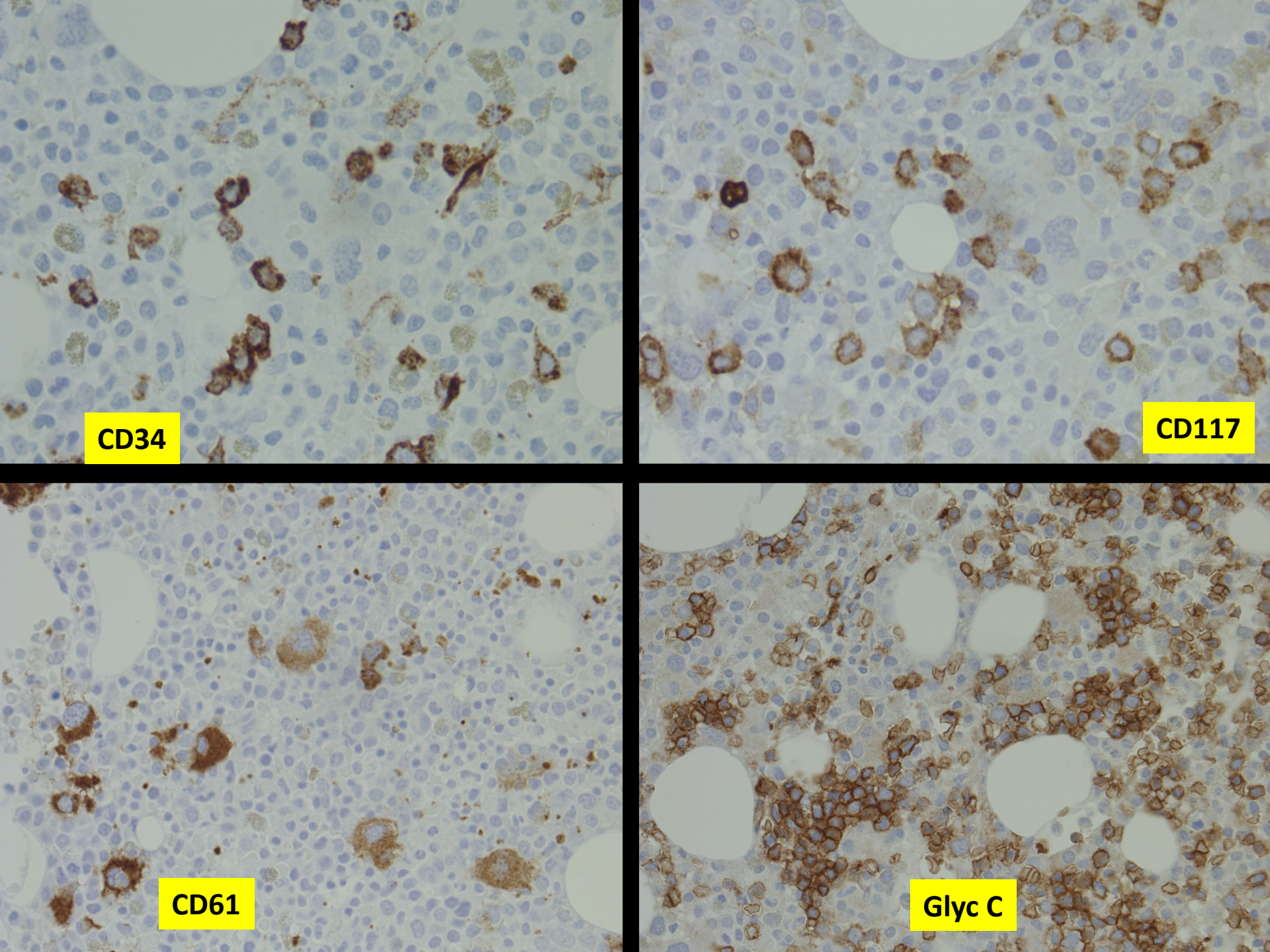
blasts 7%





# Bone marrow biopsy




CD34
CD117
CD61
Glyc C

# Cytogenetics

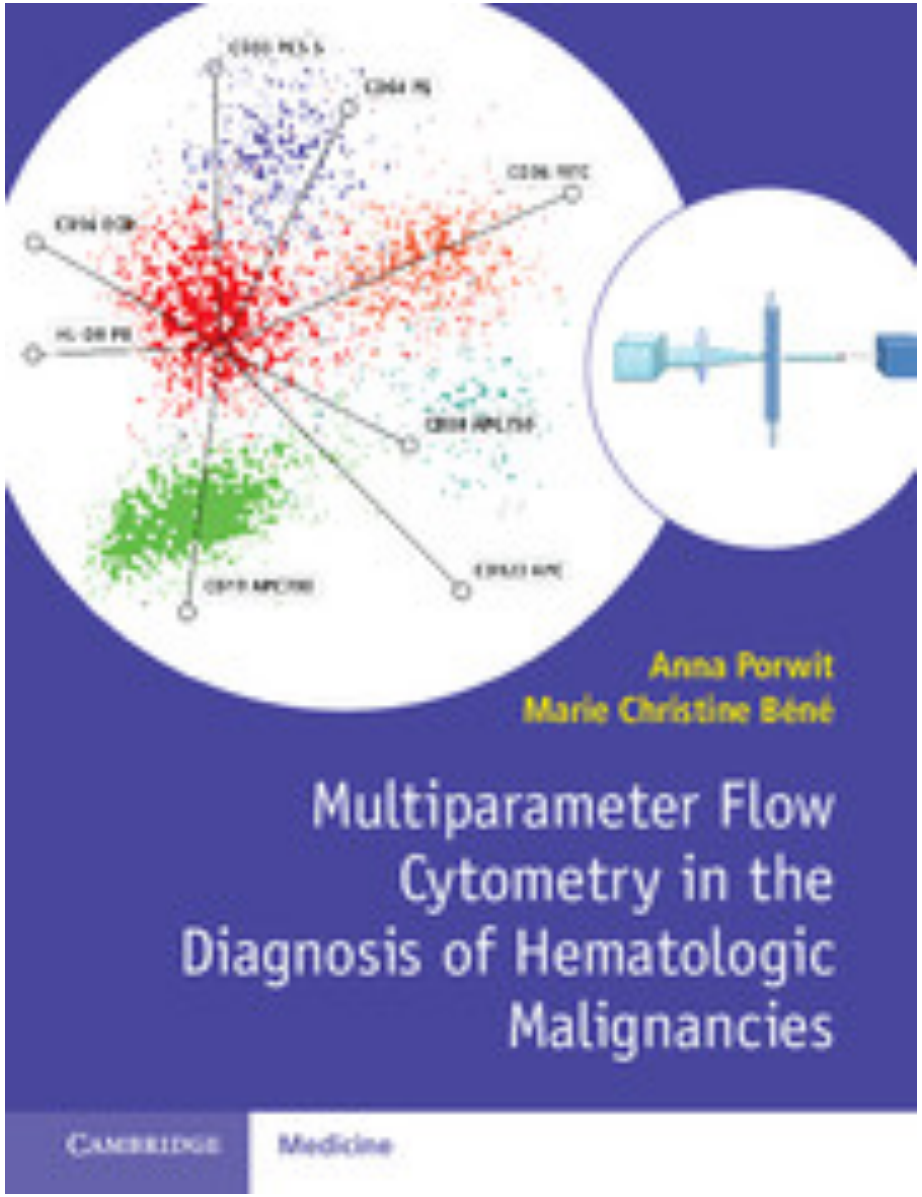
- **44-45, XX, add(1)(p36.1) [10], -5[10], del(7)(q22)[10], add(12)(p13)[10], add(13)(p11.2)[10], -15[10], -20[10], -22[7],+mar1~3[10][cp10]**
- **Abnormal karyotype, characterized by detection of multiple clonal anomalies in all analyzed metaphases. Some of the structural aberrations were too complex to elucidate**
- **Therapy related myeloid neoplasm**

# Summary of recommendations for FCM in Myelodysplastic syndromes

- For FCM application for MDS diagnostics
  - Follow International MDS Flow methodological recommendations
- For screening purposes
  - Follow a mini-panel based on the so-called ***Ogata score***
- For extended analysis: perform FCM in all cell compartments following ELN recommended antigen combinations
  - Myeloid and lymphoid progenitor cells
  - Maturing myelomonocytic cells
  - Immature and mature erythroid cells

    → ***generate integrated Flow Score (A;B;C)***
- **Integrate Flow cytometry findings in the bone marrow report (morphology, cytogenetics, flow cytometry, molecular methods)**

Malcovati L, et al., ELN guidelines 2013: 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; Van de Loosdrecht AA, Westers TM. J Natl Comp Canc Netw 2013;11:892-902; Porwit A, et al., Leukemia 2014;28:1793-98




**Cambridge University Press, 2018**


ELN
European LeukemiaNet
6th international ELN Workshop on Standardization of
Flow Cytometry in MDS, October 31-November 2, 2013
Munich, Germany, Chairs W. Kern, A.A. van de Loosdrecht

# Questions?

LUND UNIVERSITY