Data analysis in MDS: from pattern recognition to a glimpse of emerging computational tools

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Outline workshop

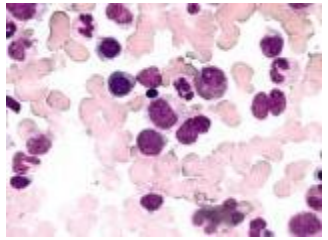
1. Introduction MDS
2. How to perform MDS-FC

3. Expert-based analysis
4. MDS flow cytometry reports

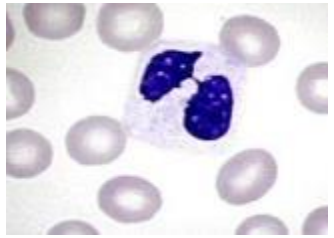
5. Multidimensional analysis and computational analysis

Myelodysplastic Syndromes (MDS)

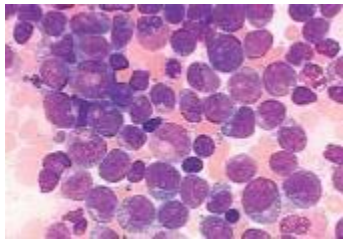
normal BM

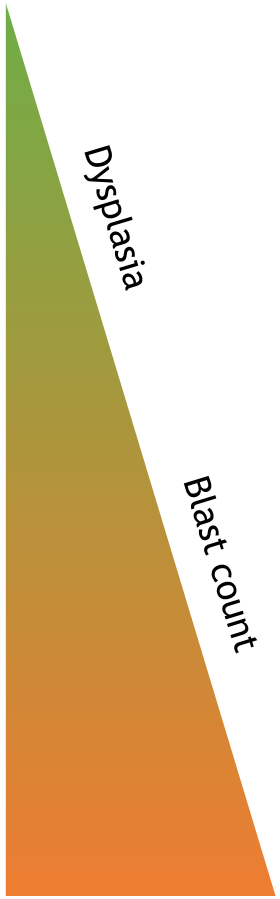


MDS

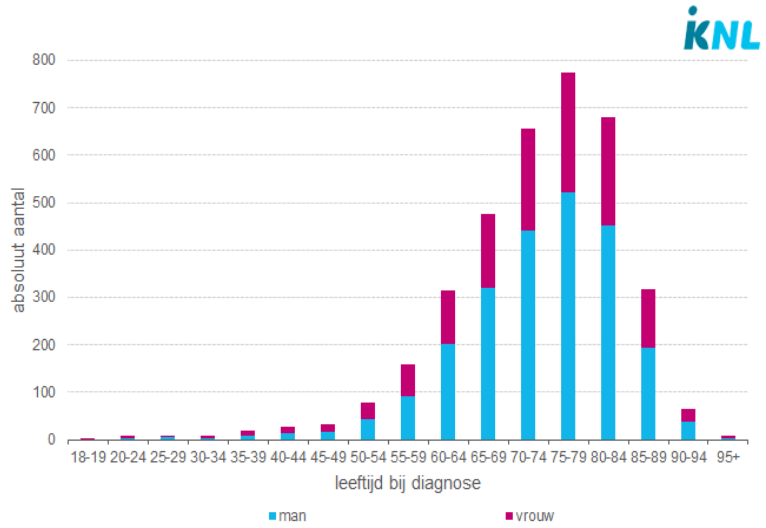


AML

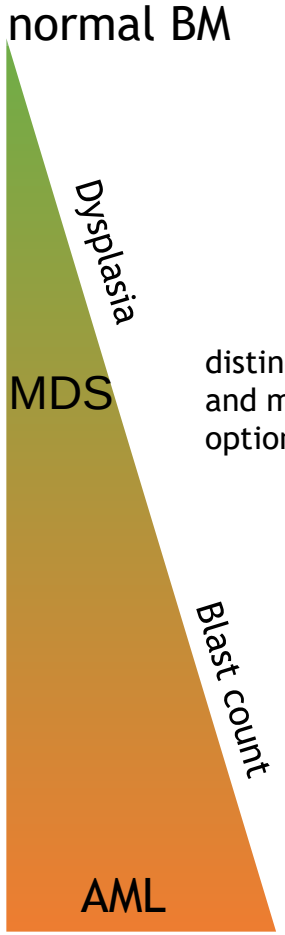




- characterized by cytopenia, dysplastic morphology and a varying percentage of blasts
- progression to leukaemia in approximately 30% of the cases
  - incidence of MDS 4-5/100,000 per year
  - clear increase of the incidence with higher age
    (>70 yrs: 30/100,000 per year)



WHO-2022 classification of MDS



distinction between single lineage and multi lineage dysplasia is optional

International Consensus Classification differs from WHO

MDS, morphologically defined

MDS-LB MDS with low blasts <5% BM and < 2% BL

MDS-h MDS-hypoplastic (<25% BM cellularity)

MDS with increased blasts

MDS-IB-1	5-9%	BM or 2-4% BL
MDS-IB-2	10-19%	BM or 5-19% PB or Auer rods
MDS-f (with fibrosis)	5-19%	BM; 2-19% PB

MDS with defining genetic abnormalities

MDS-5q MDS with low blasts and isolated del(5q) <5% BM and <2% BL

5q deletion alone or with 1 other abnormality other than monosomy 7 or 7q deletion

MDS-*SF3B1* MDS with *SF3B1* mutation

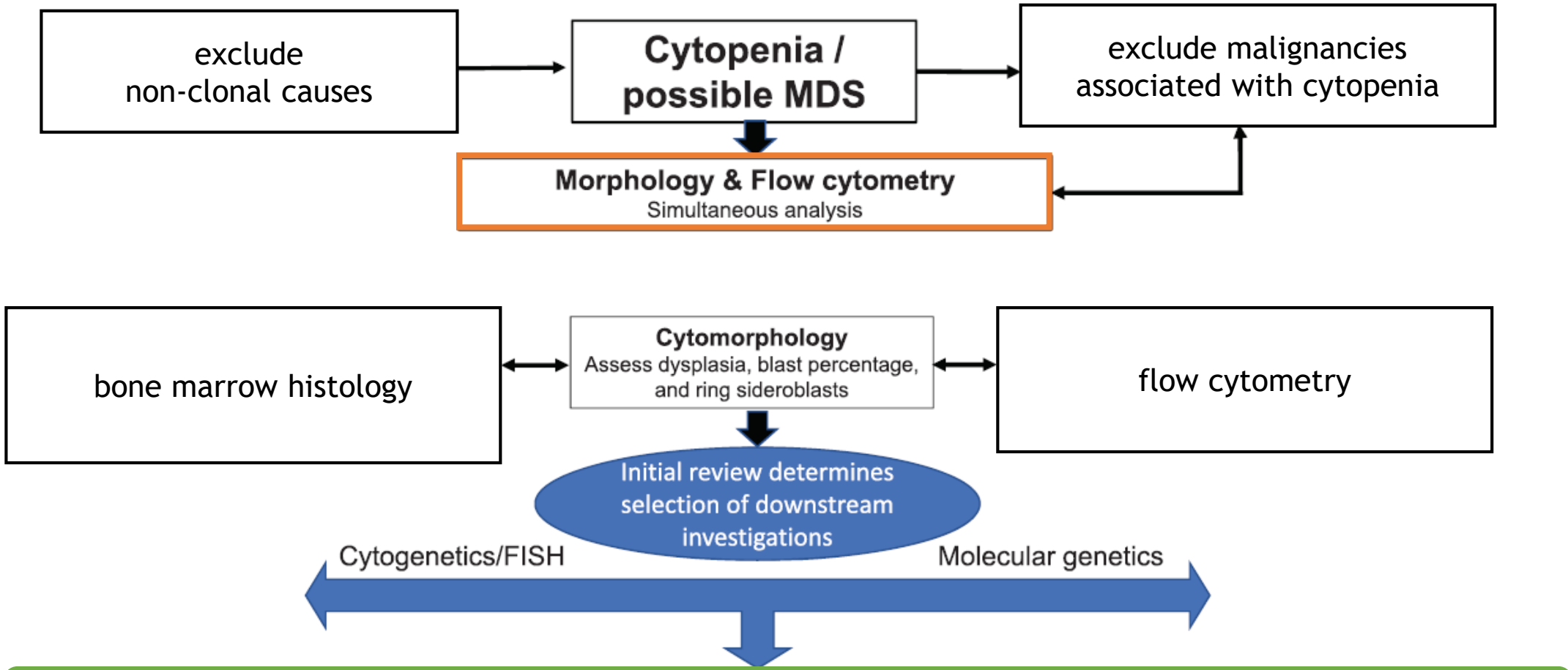
excluding del(5q), monosomy 7 or complex karyotype

(or MDS-LB with *SF3B1*-wild type with ≥15% ring sideroblasts)

MDS-bi*TP53* MDS with allelic *TP53* inactivation

Khoury et al., Leukemia 2022, 36(7):1703-1719; Arber et al., Blood 2022 140(11):1200-1228

Flow cytometry in the diagnostic algorithm for MDS



Adapted from Van de Loosdrecht et al., Cytometry B Clin Cytom. 2023;104(1):77-86

Recommendations on FC in (suspected)MDS

- apply international guidelines (ELNet/NCCN):
  - pre-analytical, analytical, post-analytical
  - screening
    - mini-panel based on *Diagnostic score (a.k.a. Ogata score)*
  - extended analyses of:
    - Myeloid and lymphoid progenitor cells
    - Maturing myelomonocytic cells
    - Maturing nucleated erythroid cells

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;
Van der Velden et al. Cytometry B Clin Cytom. 2023;104(1):15-26; Porwit et al. Cytometry B Clin Cytom. 2023;104(1):27-50

Recommendations on pre-analytical and technical issues

- adequate quality bone marrow aspirate (preferably in heparin)
- appropriate processing time (<24h)

    - appropriate lysing procedures
    - lyse-wash-stain procedure
    - bulk lysis with (preferably) NH_4Cl

- appropriate antibody combinations
- backbone of at least CD45 in every tube

    - appropriate instrument settings
        - stability of the cytometer's performance
    - adequate analysis strategy

ELN European LeukemiaNet

Van de Loosdrecht, Haematologica, 2009; 94:1124-34;
Westers et al., Leukemia 2012;26:1730-41;
Porwit A, et al., Leukemia 2014;28:1793-98

Van der Velden et al. Cytometry B Clin Cytom, 2023:104(1):15-26;
Porwit et al. Cytometry B Clin Cytom, 2023;104(1):27-50

Preanalytical: recommended antibodies (2023)

TABLE 1 Recommended antibodies for FCM analysis of various cell types

Cell subset	Backbone markers	Recommended markers	Optional
Myeloid progenitor	CD45, CD34, CD117, HLA-DR	CD13, CD33, CD10, CD11b, CD15, CD38, CD7, CD56	TdT, CD5, CD19, CD25, CD133
Lymphoid progenitor	CD45, CD34	HLA-DR, CD10, CD19	CD22
Granulocyte	CD45, CD117	HLA-DR, CD13, CD33, CD11b, CD16, CD10, CD15, CD14, CD64, CD56	CD34, CD5, CD7
Monocyte	CD45	HLA-DR, CD13, CD33, CD11b, CD14, CD34, CD36, CD64, CD16, CD56, CD117	CD2, MDC8 (Slan), CD300e
Erythroid	CD45, CD34, CD117	HLA-DR, CD36, CD71, CD105, CD13, CD33	CD235a
Optional cell subsets for analysis:			
Basophil	CD45	CD123, HLA-DR	CD203c
Mast cell	CD117	CD45, HLA-DR	CD2, CD25
Dendritic cell	CD45, CD34, CD117	HLA-DR, CD123	CD11c CD1c, CD141, CD303

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Van der Velden et al. Cytometry B Clin Cytom, 2023:104(1):15-26

Back bone markers: example of pipetting Ab combinations

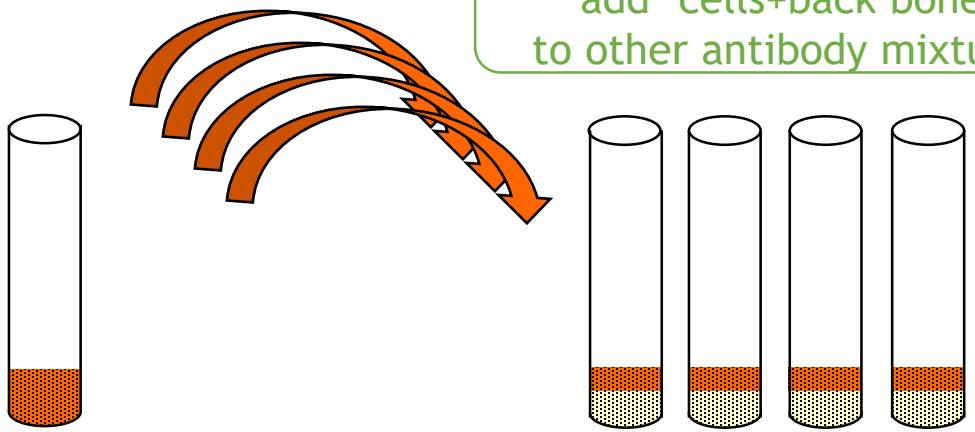
EuroFlow AML/MDS panel; Leukemia 2012

1	CD45	CD34	CD117	HLA-DR	CD16	CD13	CD11b	CD10	myelomonocytic
2	CD45	CD34	CD117	HLA-DR	CD35	CD64	CD300e	CD14	myelomonocytic
3	CD45	CD34	CD117	HLA-DR	CD36	CD105	CD33	CD71	myelomonocytic-erythroid
4	CD45	CD34	CD117	HLA-DR	TdT	CD56	CD7	CD19	lineage infidelity markers

Step 1
cell suspension + back bone

Step 2
add 'cells+back bone'
to other antibody mixtures

identical staining of
back bone markers in every tube
for **more optimal gating**



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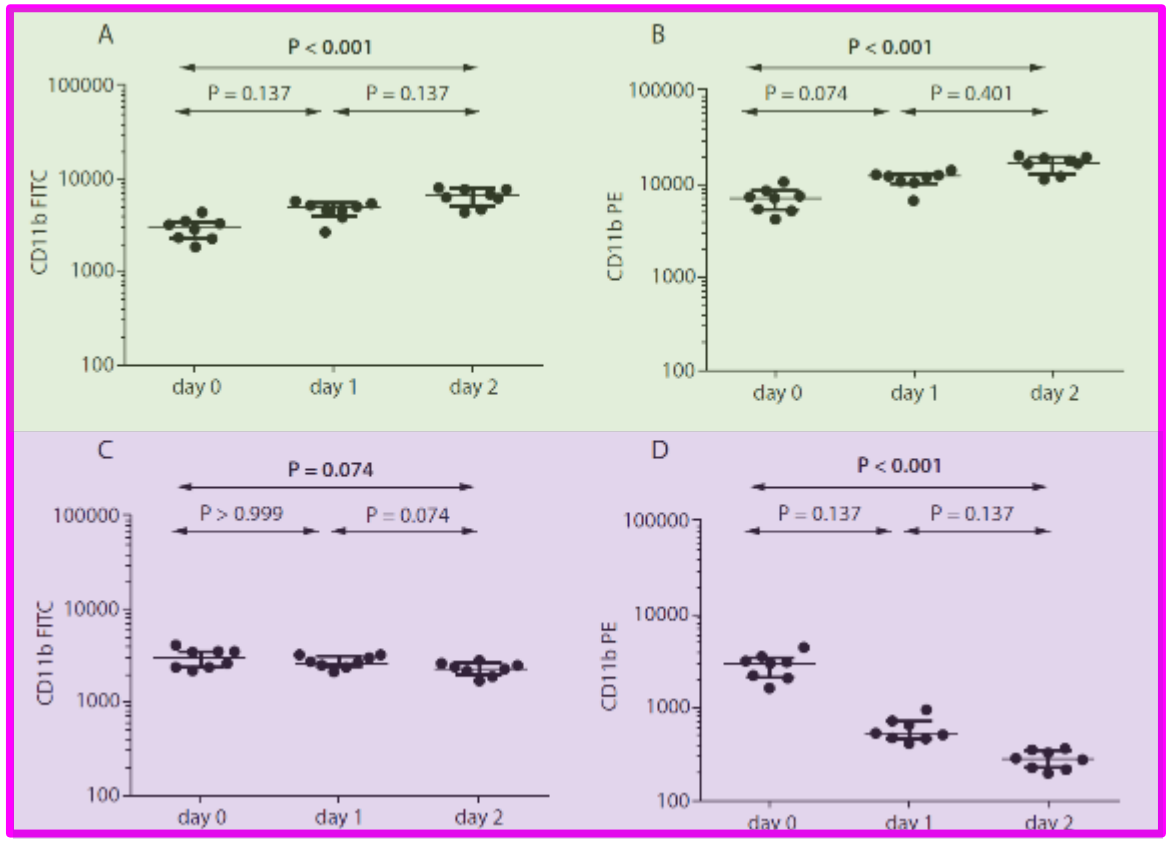
Preanalytical: anticoagulants and delay of processsing

granulocytes

Heparin

EDTA

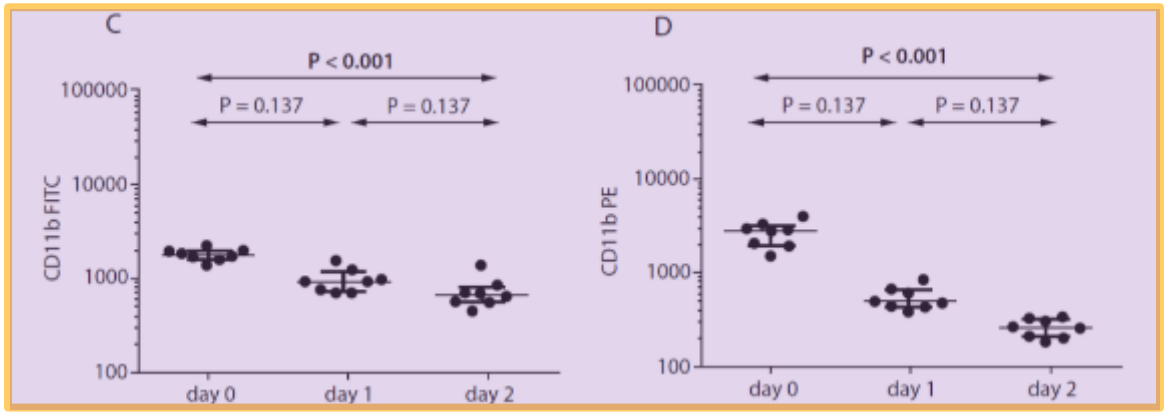


CD11b FITC clone ICRF44

CD11b PE clone D12

EDTA may affect expression of several antigens when samples are not processed immediately

monocytes



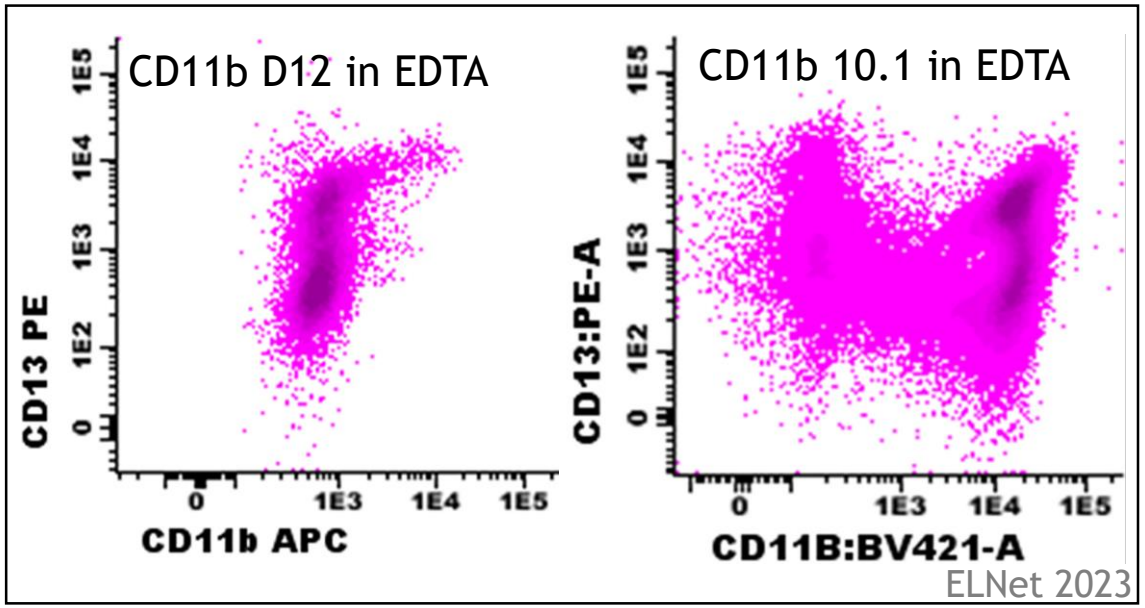
CD11b FITC clone ICRF44

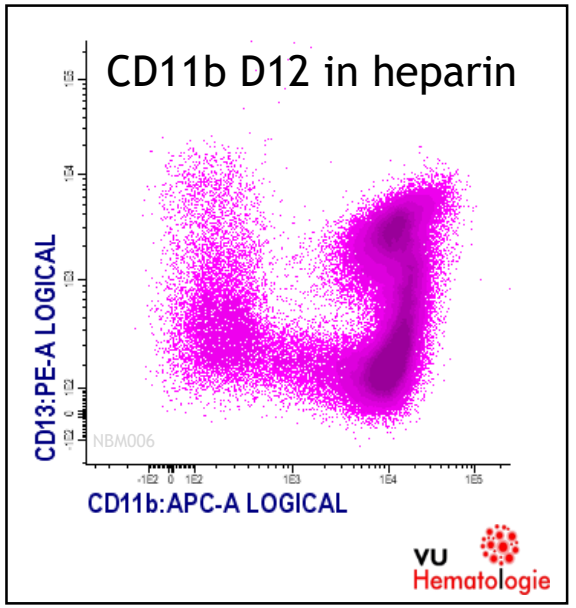
CD11b PE clone D12

Elghetany & Davis, Cytometry B Clin Cytom 2005;65(1):1-5;
Kárai B et al., Biochem Med 2018;28(2):020704; Van der Velden et al. Cytometry B Clin Cytom, 2023:104(1):15-26

Neutrophil CD11b expression in EDTA and heparin

- studying of CD11b expression in EDTA-anticoagulated specimens may be problematic
  - a calcium-dependent epitope in human neutrophils
  - binding of some antibodies to their ligand is Ca^{2+} dependent
  - need for other clone or higher concentration of CD11b (clone D12) in EDTA samples





Repo et al. J Immunol Methods 1995; 185: 65–79 and Leino et al. Biochem Biophys Res Commun 1992; 187: 195–200; Elghetany & Davis, Cytometry B Clin Cytom 2005;65(1):1-5;
Van der Velden et al. Cytometry B Clin Cytom, 2023:104(1):15-26

Recommendations on pre-analytical and technical issues

- adequate quality bone marrow aspirate (preferably in heparin)
- appropriate processing time (<24h)

    - appropriate lysing procedures
    - lyse-wash-stain procedure
    - bulk lysis with (preferably) NH_4Cl

- appropriate antibody combinations
- backbone of at least CD45 in every tube

    - appropriate instrument settings
        - stability of the cytometer's performance
    - adequate analysis strategy

standardize !!

ELN European LeukemiaNet

Van de Loosdrecht, Haematologica, 2009; 94:1124-34;
Westers et al., Leukemia 2012;26:1730-41;
Porwit A, et al., Leukemia 2014;28:1793-98

Van der Velden et al. Cytometry B Clin Cytom, 2023:104(1):15-26;
Porwit et al. Cytometry B Clin Cytom, 2023;104(1):27-50

How to process stained samples

- Acquisition preferably within 1 h. of completing staining procedure
- Acquisition of a **minimum of 100,000 CD45+** events per tube
  - at least **250 CD34+** events should be present
  - preferably at least **500 CD34+** events for the '**diagnostic score**'

- Adequate analysis strategy
  - gating of subpopulations by means of
    CD45, FSC and SSC characteristics in combination with other specific markers

    Increase in amount of backbone markers can facilitate more accurate gating procedures
    - CD45 vs. CD45/CD34 or
    - CD45/CD34/HLA-DR/CD117 or
    - CD45/CD34/HLA-DR/CD117/CD33 or
    - ….

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Van der Velden et al. Cytometry B Clin Cytom, 2023:104(1):15-26

Data analysis

Data analysis

Low number of dimensions

- Expert-based manual gating:
  - step-by-step evaluation of multiple parameters and patterns in one or two-dimensional plots (histogram and scatter plots)

- Dimensionality reduction and clustering algorithms
  - mapping of cell clusters of normal BM on multidimensional plots (e.g. Kaluza radar plots, Infinicyt's APS plots, FlowSOM)
    - provide insight into BM cell composition and maturation
    - may serve as reference map to assess abnormal hematopoiesis in MDS

Multi-dimensional (->numerous two-dimensional plots)

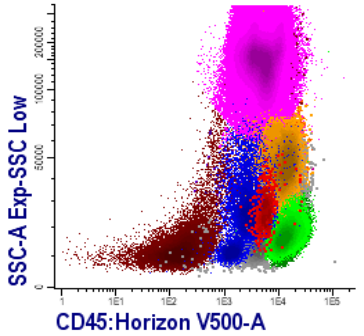
- Dimensionality reduction and clustering algorithms
- Computational analysis

Manual analysis - Gating strategy

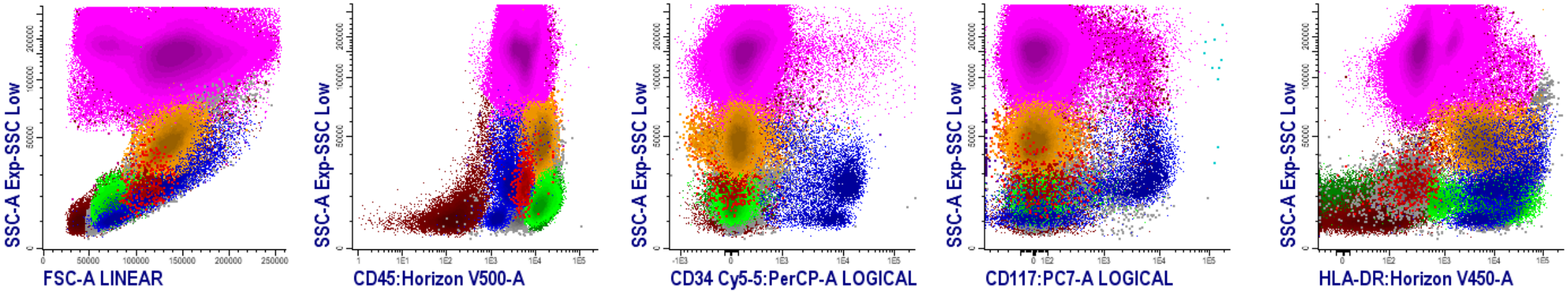
- exclusion of doublets (if applicable)
- exclusion of debris and non-nucleated erythroid cells
- definition of subsets by CD45 and scatter characteristics

- advice to use:
  - Fixed X- and Y-axes for interpretation of patterns

  - Fixed colors for defined subsets, e.g.:
    - progenitors
    - granulocytes
    - monocytes
    - lymphocytes
    - erythroid cells



Gating strategy -result based on back bone markers



lymphocytes	based on CD45 and FSC/SSC	
progenitors	based on CD45, CD34, SSC	(CD117/CD19/CD71)
“monocytes”	based on CD45, HLA-DR and SSC	(CD64, CD33)
“granulocytes”	based on CD45, SSC (, HLA-DR)	(CD33)
basophils	based on CD45, SSC, HLA-DR	(CD123)
erythroid	based on CD45	(CD71, CD36, CD13/CD33^{-})

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See definitions in Porwit et al. Cytometry B Clin Cytom, 2023;104(1):27-50

Gating strategy - some examples

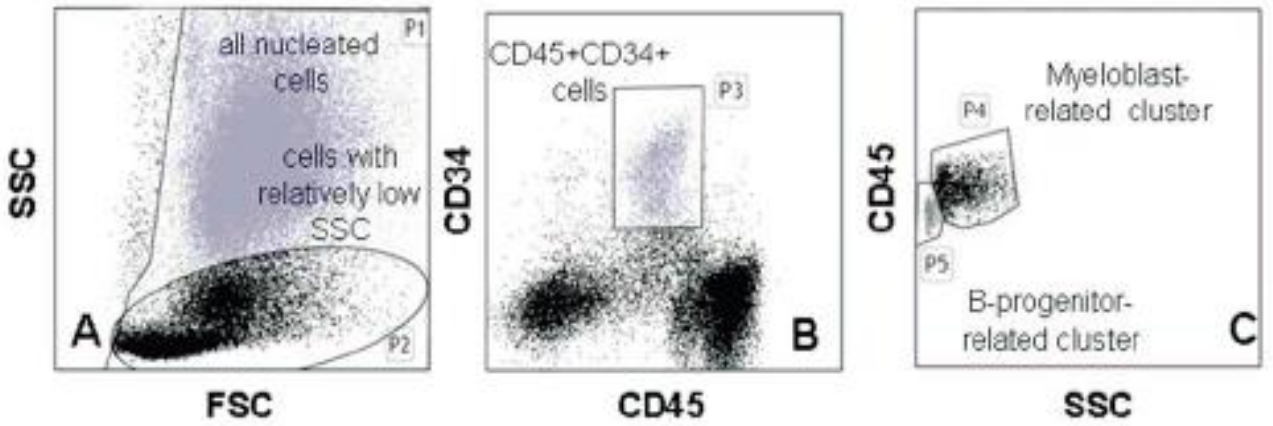
Manual analysis – Myeloid progenitor cell count by FC

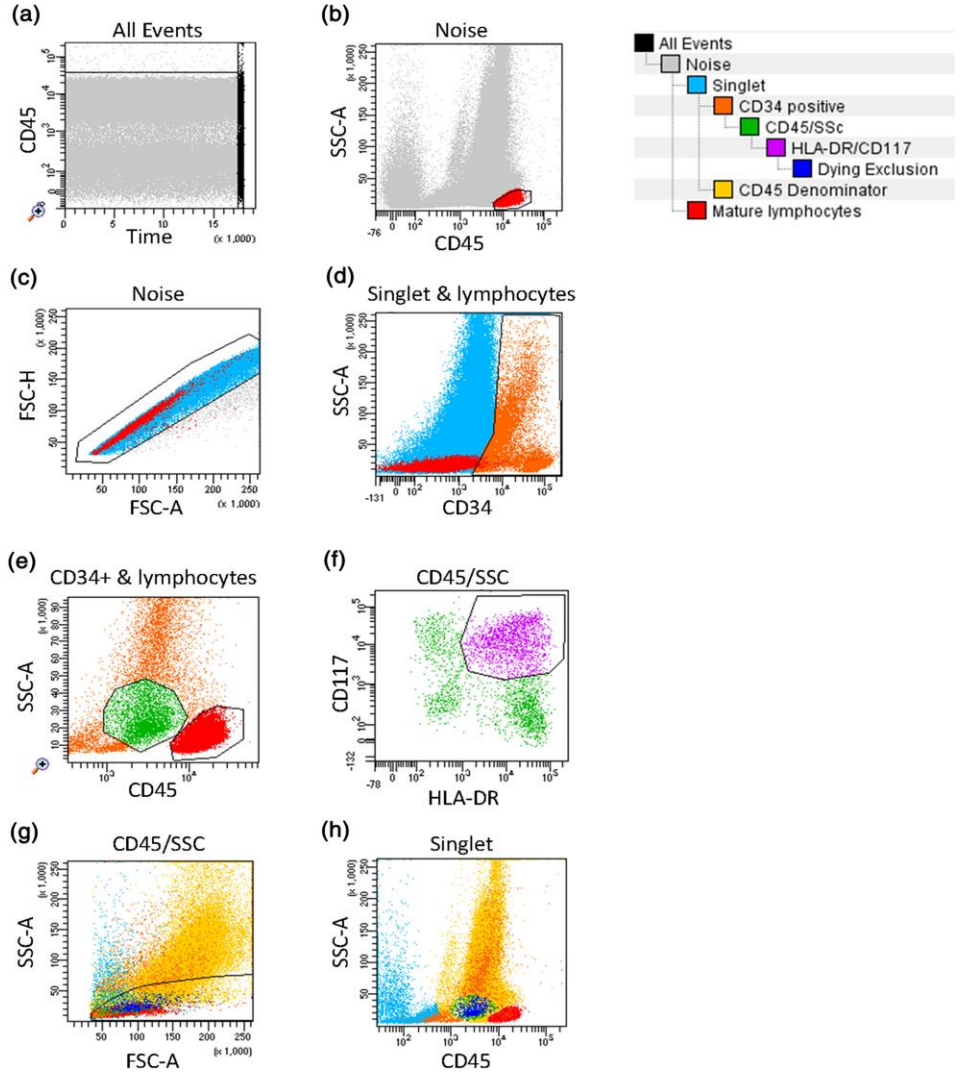
Cut-off blasts by cytomorphology ≥5%

Cut-off MyProg according to Ogata score ≥2%

Cut-off %MyProg indicative of MDS (Kern et al.) >3%

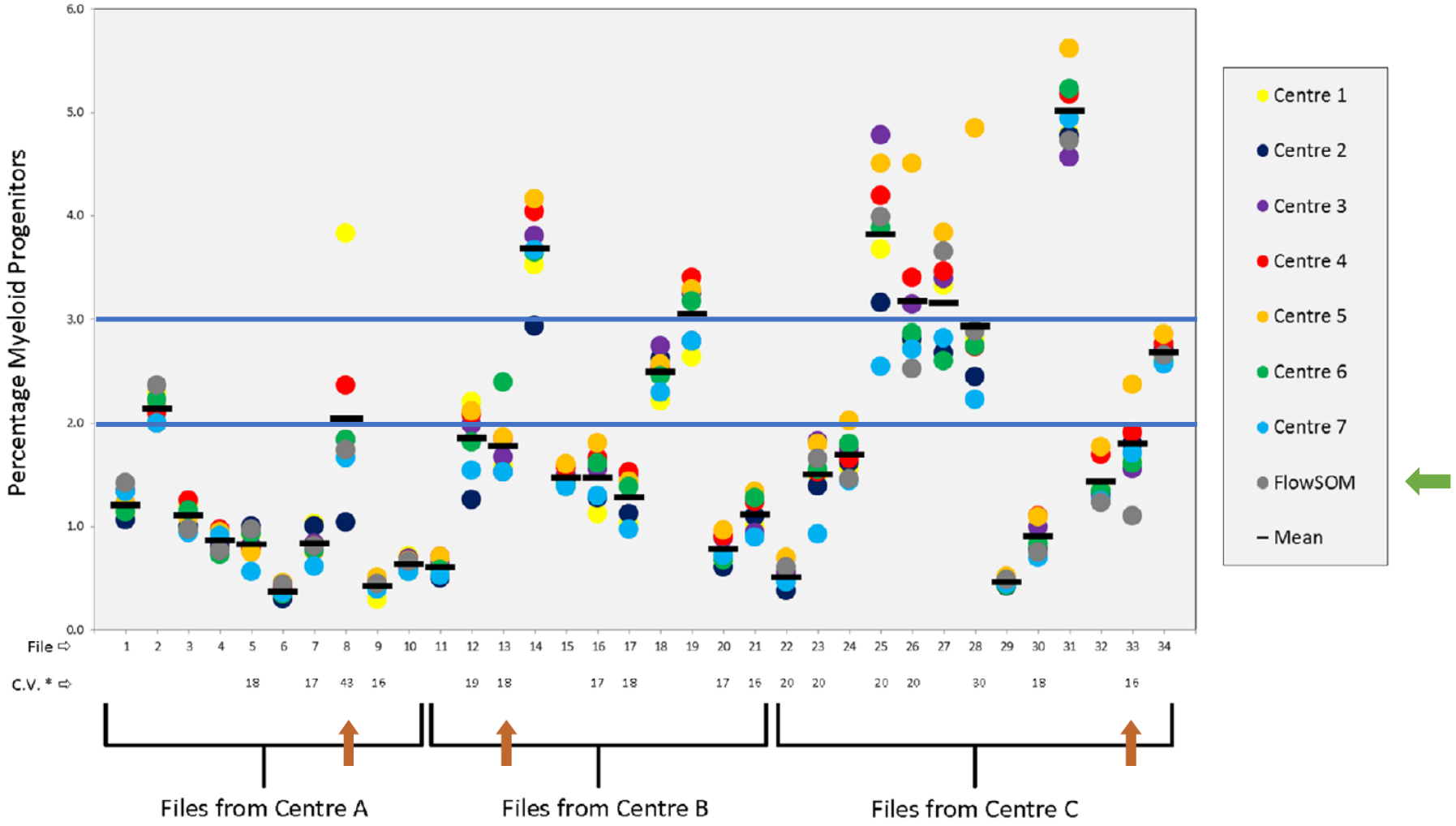
4-parameter Ogata score





Johansson et al. Cytometry B Clin Cytom 2023;104(2):115-127; Ogata et al., Haematologica 2009; 94(8):1066-74
Della Porta et al., [ELNet] Haematologica 2012;97:1209-17

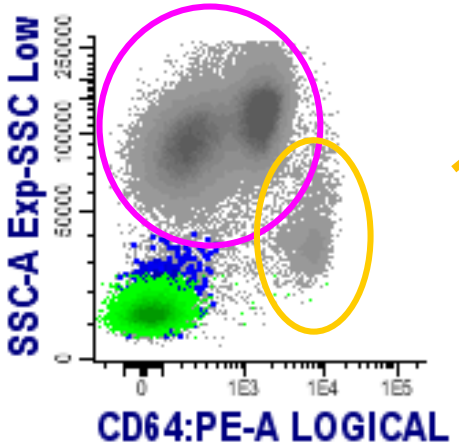
Reproducibility of CD34+ MyProg cell count in MDS by FC



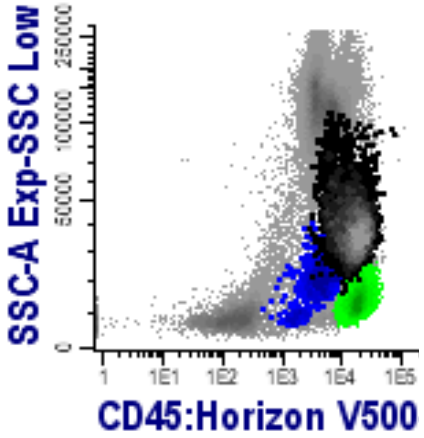
Johansson et al. Cytometry B Clin Cytom 2023;104(2):115-127

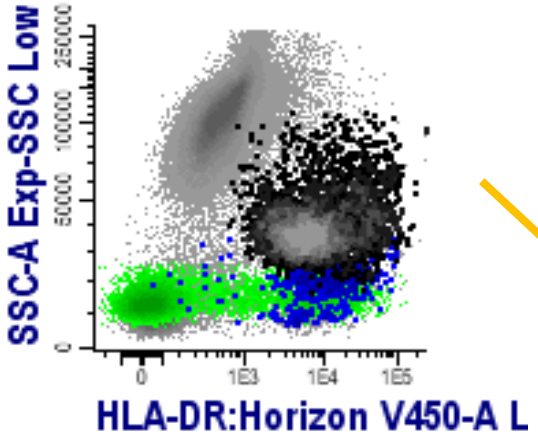
Optional gating strategy: ‘mono- vs. granulocytes’

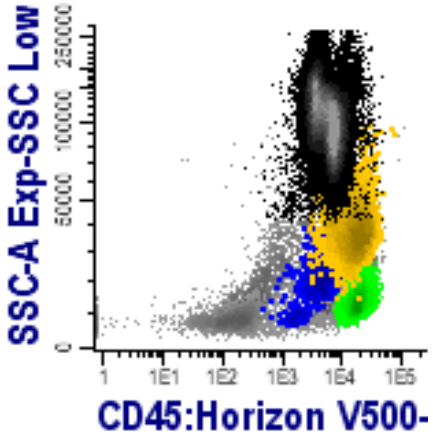
Applying PNH guidelines:
use CD64 to spot their location,
but gate them based on CD45/SSC

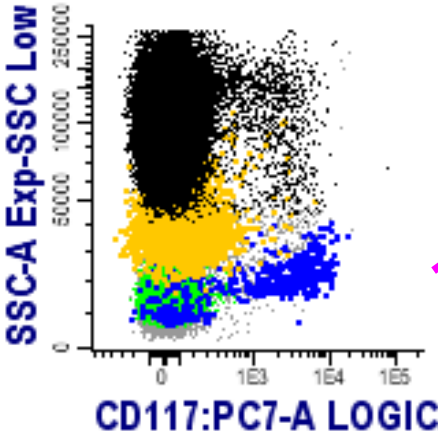


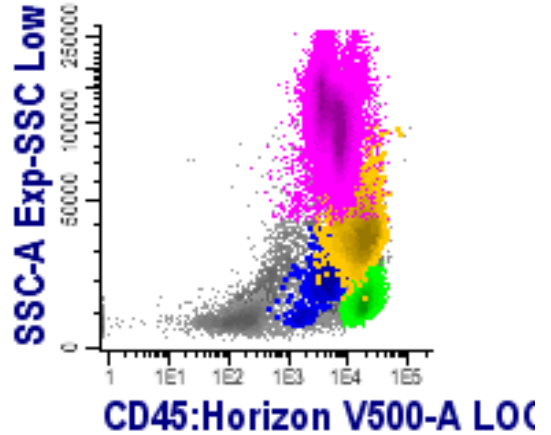
CD33 as alternative











Fine tuning by e.g.
HLA-DR/SSC and
CD117/SSC … mast
cells

- lymphocytes
- progenitors
- “monocytes”
- “granulocytes”

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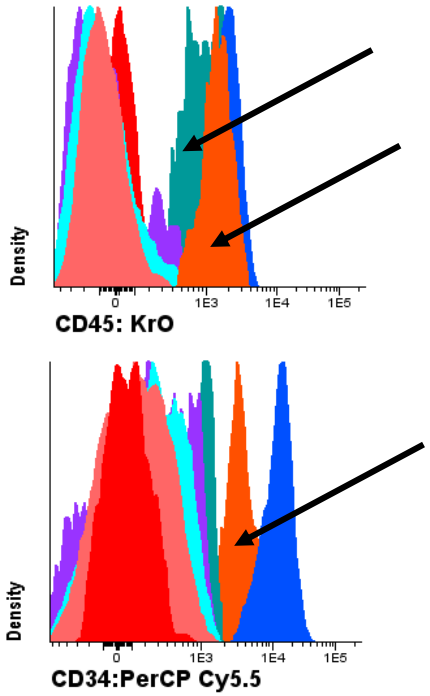
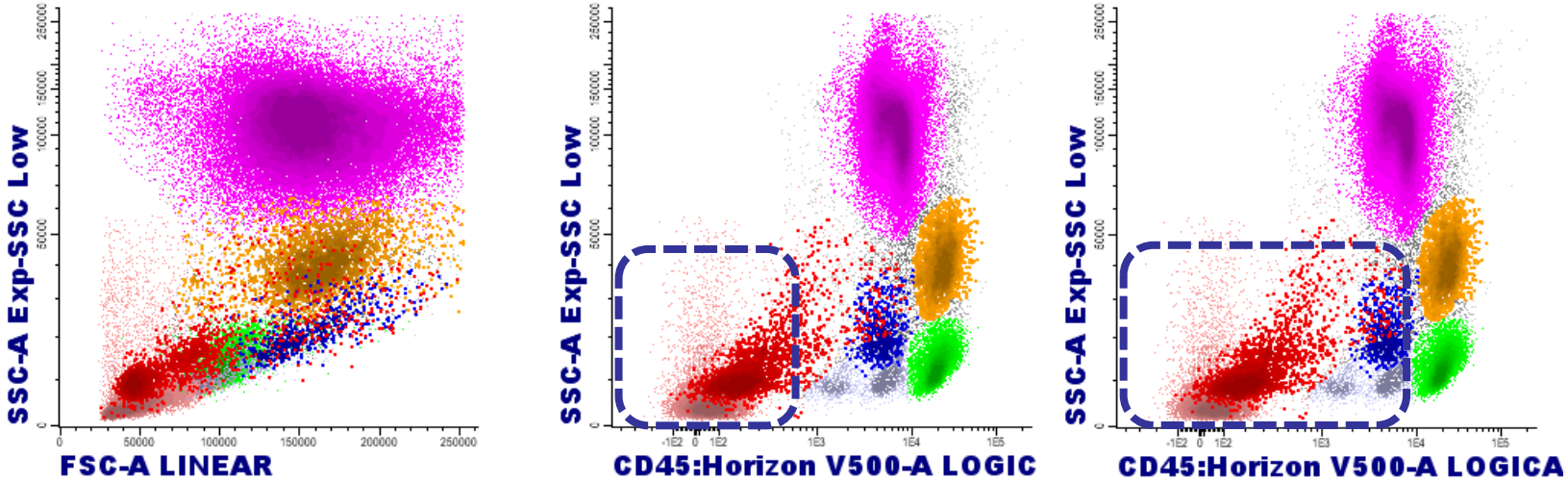
Proper gating strategy for erythroid lineage





Myeloid progenitor

Proerythroblast
Erythroblast
Basophilic erythroblast
Polychromatic erythroblast
Orthochromatic erythroblast
Mature erythrocyte

Early erythroid progenitors mingle with $CD34^{+}/CD45^{dim}$ MyProg
So, a $CD45^{negative}$ gate excludes early erythroid progenitors.

Note that, erythroid gate should be $CD45^{dim\text{-}to\text{-}neg}$!
Use a myeloid marker CD13, CD33 to remove MyProg

Evaluation of aberrancies

Evaluation of aberrancies

Only possible when adequate knowledge on age-matched normal and related pathological controls is acquired !!

- patterns … altered differentiation or distribution of subsets
  … altered expression levels (0.5log/1log or <> 2*SD)
- aberrant marker expression … lineage infidelity (cut-offs under evaluation)
  … asynchronous (cut-offs under evaluation)
- apply fixed X- and Y-axes to evaluate patterns

QC important!
instrument, antibodies, processing, analysis

ELN European LeukemiaNet

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Porwit et al. Cytometry B Clin Cytom, 2023;104(1):27-50

Aberrancies in maturing myeloid cells

ELNet recommendations

Recommended analyses	Aberrancy
Percentage of cells as ratio to lymphocytes	Decreased
SSC as ratio vs. SSC of lymphocytes	Decreased (note, haemodilution and aging samples)
Relationship of CD13 and CD11b	Altered pattern (note, aging samples)
Relationship of CD13 and CD16	Altered pattern (note, aging samples)
CD10	Decreased or loss (note, aging samples)
CD15	Decreased
CD33	Increased/decreased
Relationship HLA-DR and CD11b	Altered pattern
CD14	Presence
Lineage infidelity markers (CD56)	Presence

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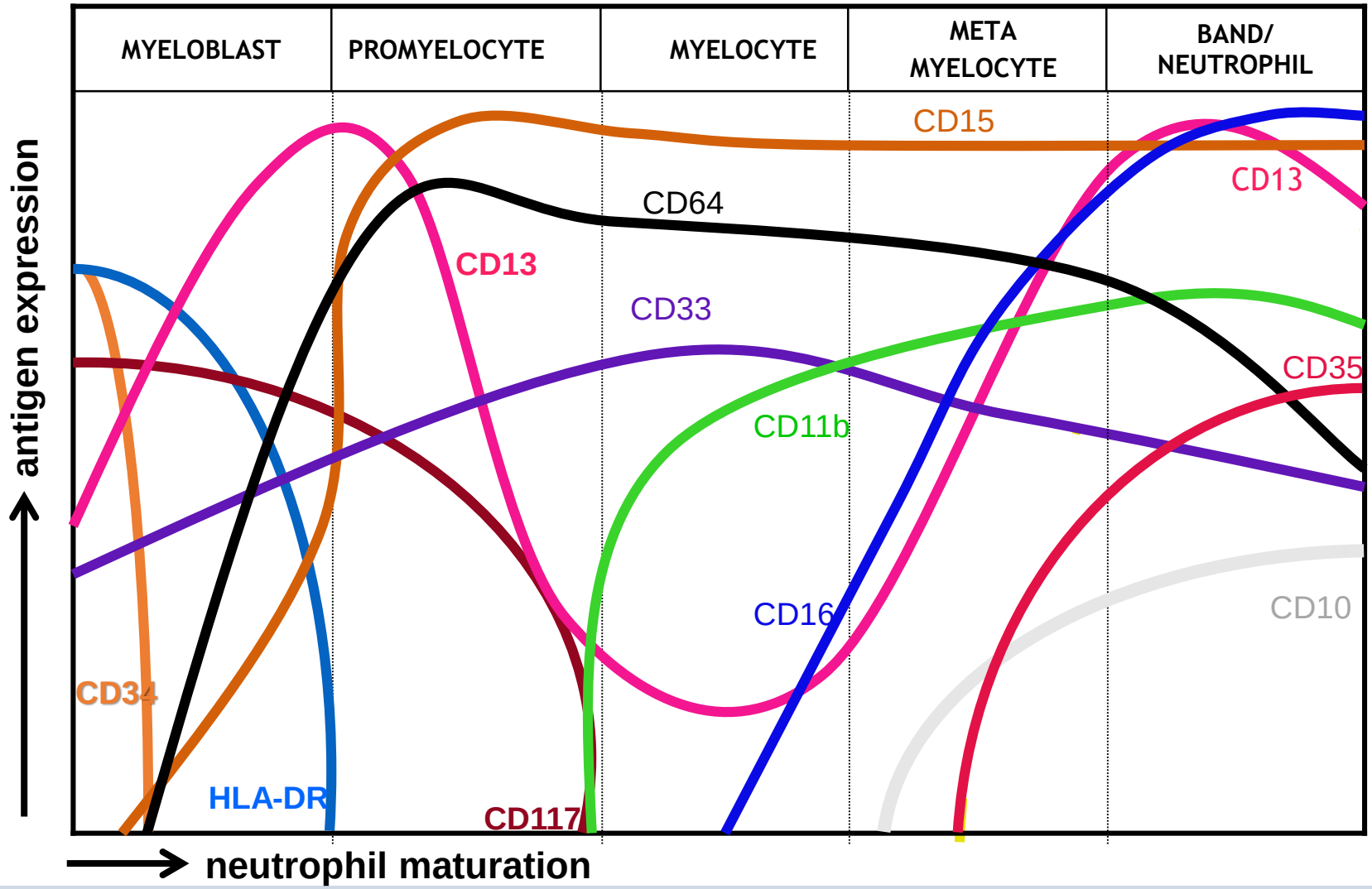
Kern et al., Cytometry B Clin Cytom. 2023; 104(1):51-65;
Porwit et al. Cytometry B Clin Cytom 2023; 104:27-50

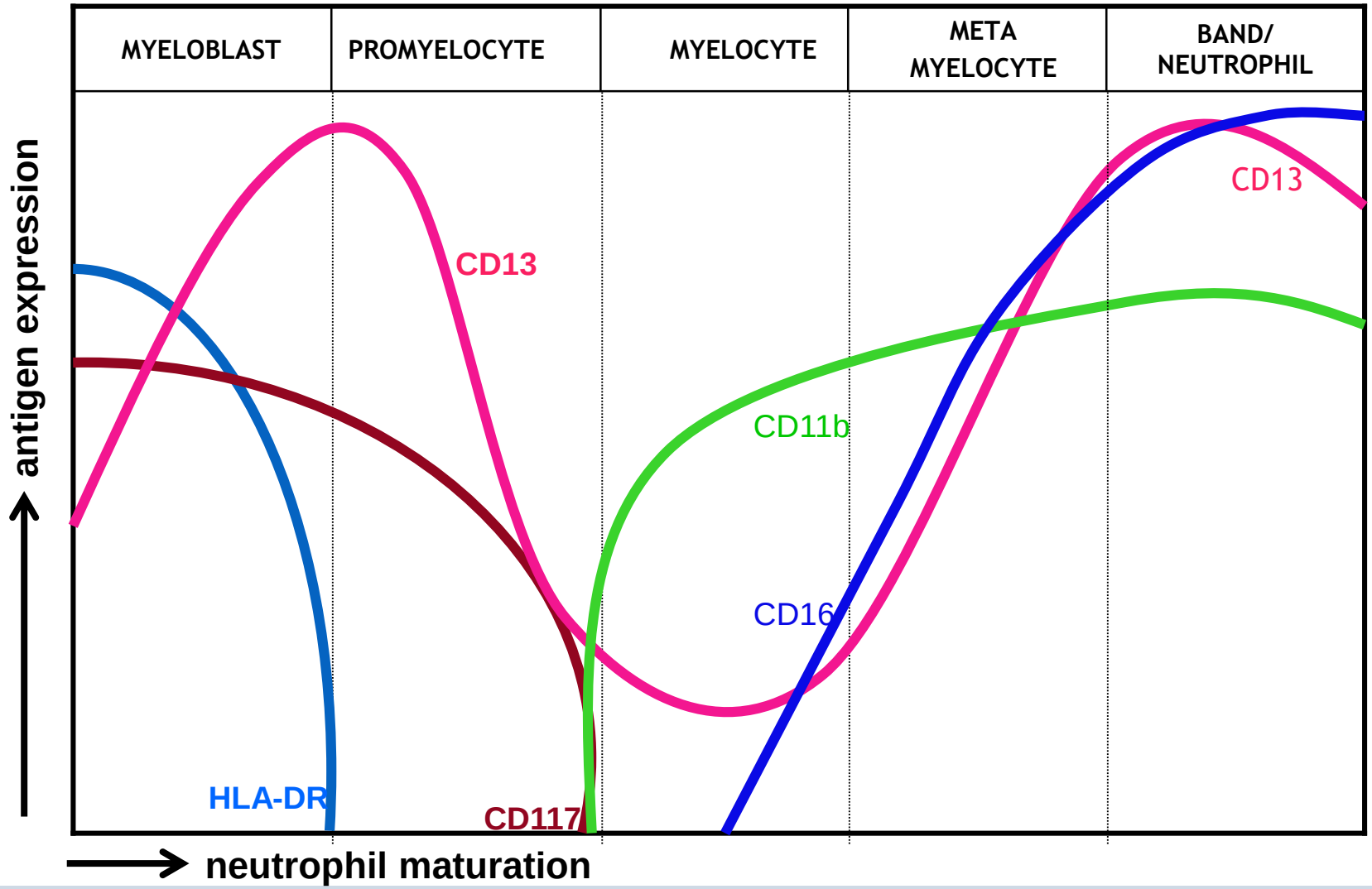
Antigen expression levels on maturing myeloid cells



Adapted from: Orfao, ELNet Flow MDS 2008, Amsterdam

Orfao et al. J Immunol Methods 2019;475:112684

Antigen expression levels on maturing myeloid cells



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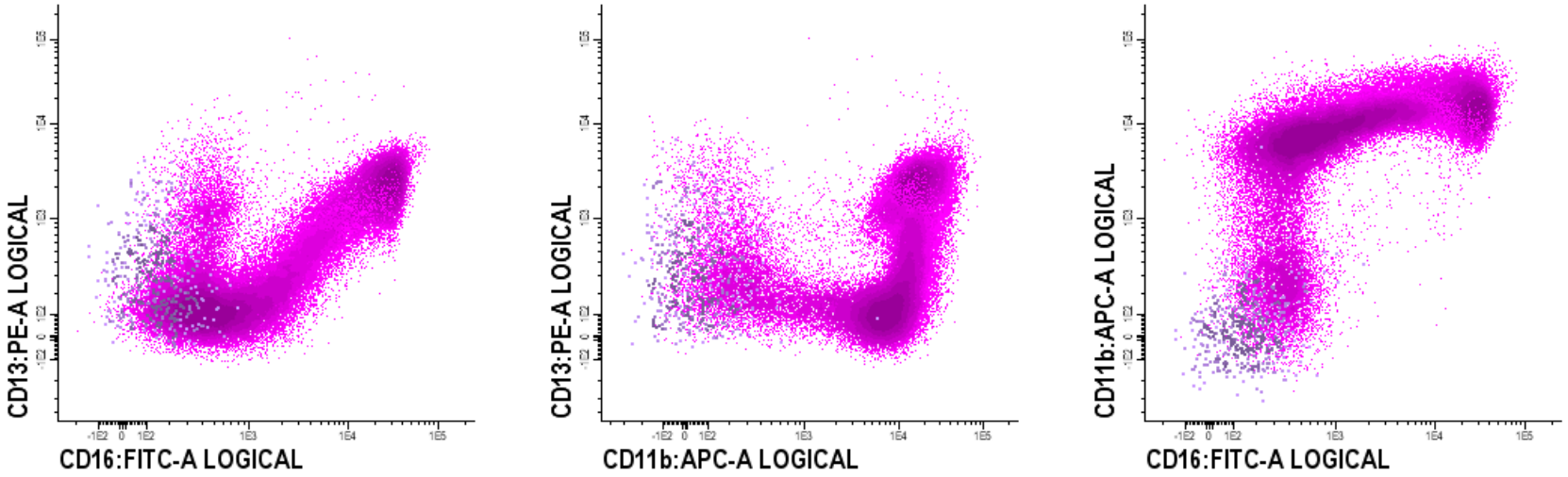
Orfao et al. J Immunol Methods 2019;475:112684

Differentiation patterns of myeloid cells

CD16/CD13/CD11b/CD45



CD34+ My

CD13+
CD117+
HLA-DR+

→

CD34-

CD13+ CD11b- CD16- CD117+ HLA-DR+ → CD13+/- CD11b- CD16- CD117+ HLA-DR- → CD13- CD11b- CD16- CD117- HLA-DR- → CD13- CD11b dim CD16- CD117- HLA-DR- → CD13- CD11b+ CD16-/dim → CD13 dim CD11b+ CD16 dim → CD13+ CD11b+ CD16+

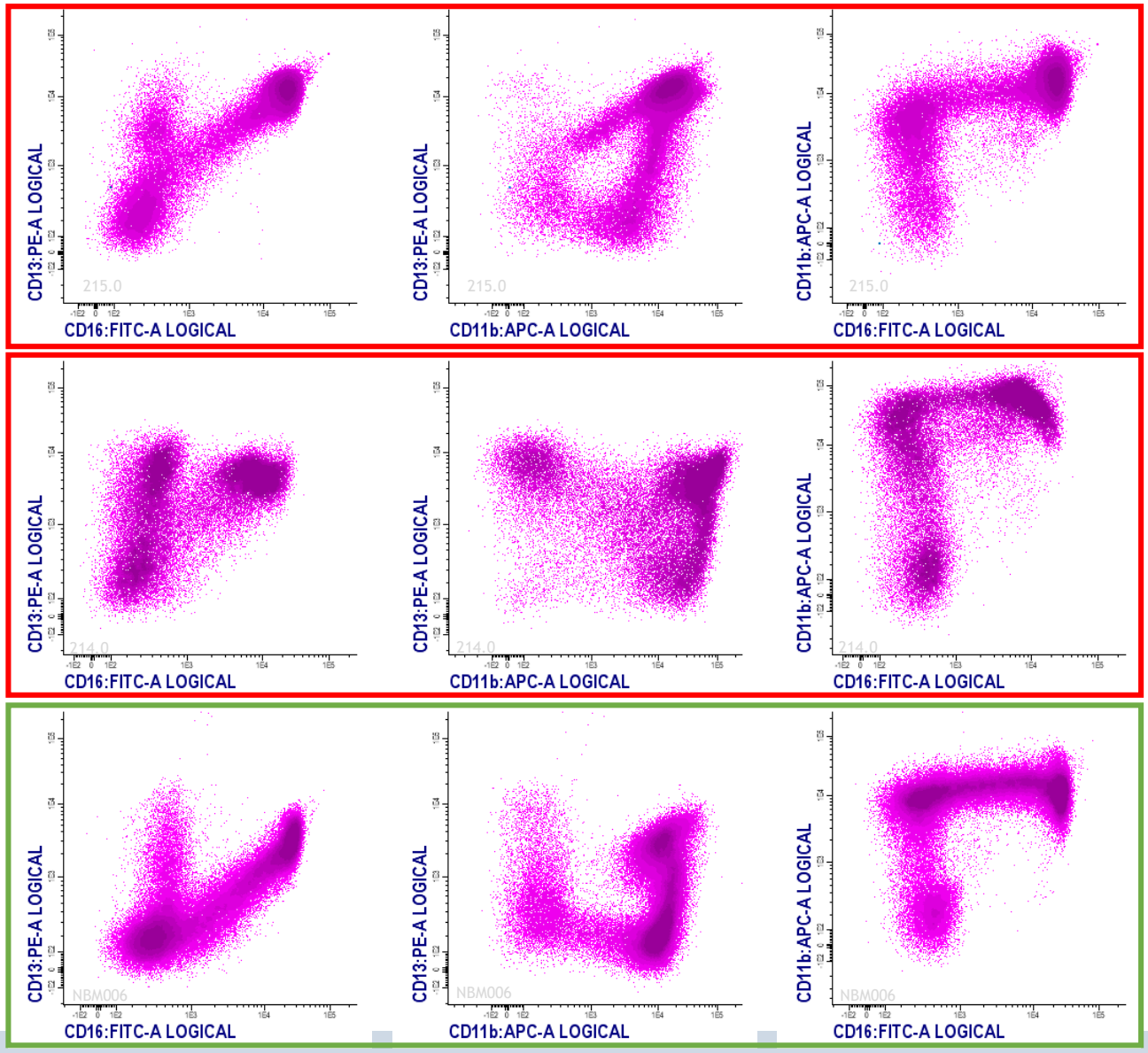
Upon maturation CD45 increases, FSC decreases, SSC first increases then decreases

Granulocytes: aberrant maturation patterns

MDS

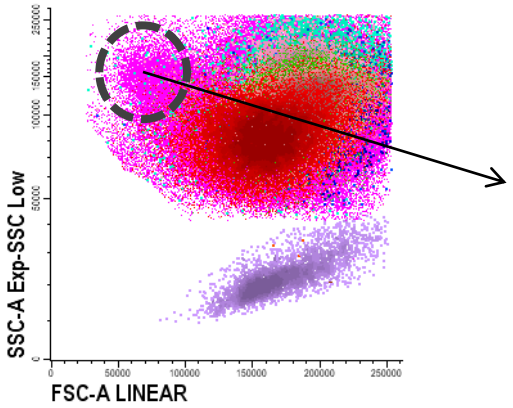
MDS

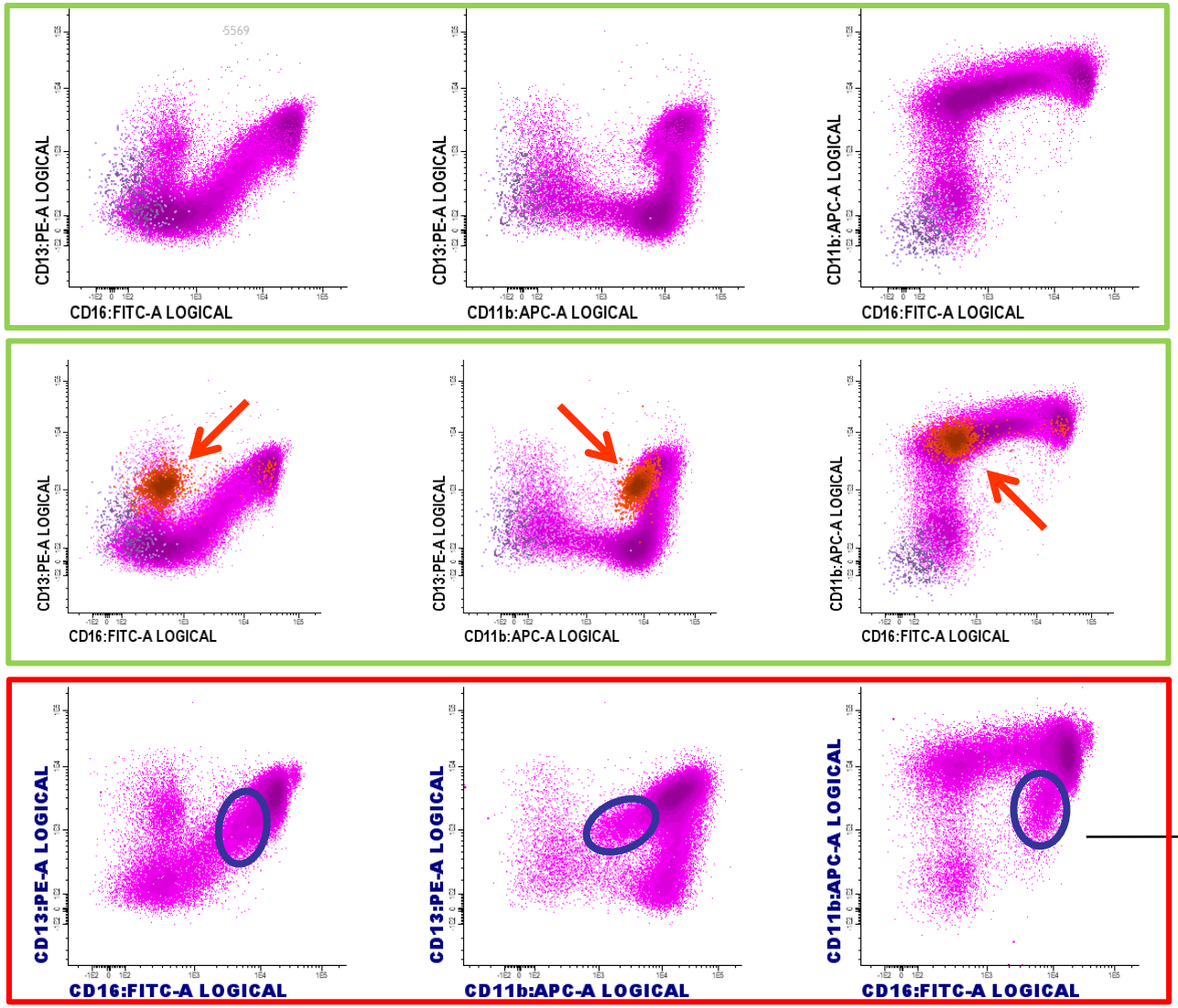
normal



Apoptosis and eosinophils can disturb maturation patterns

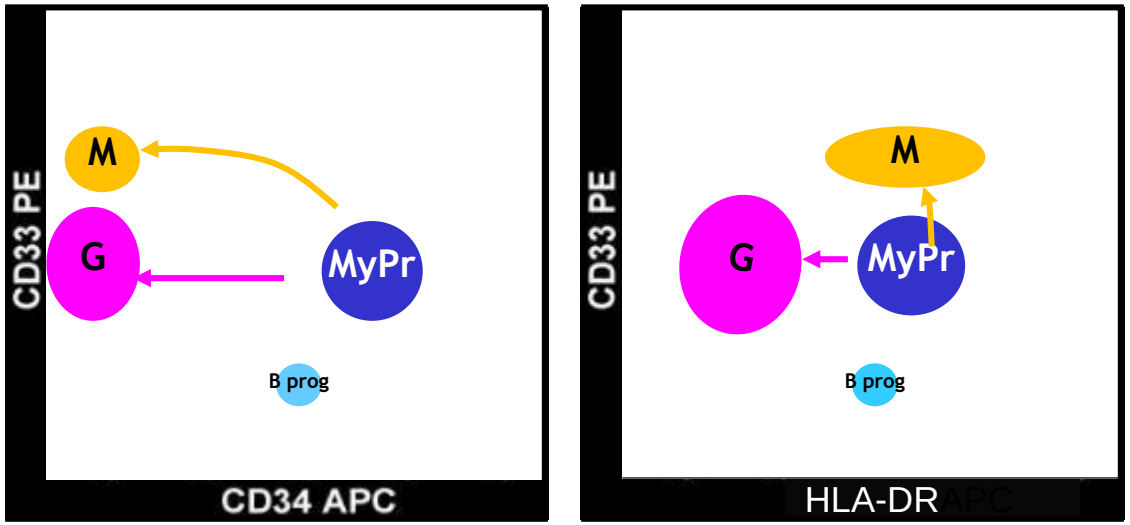
eosinophils





apoptosis !

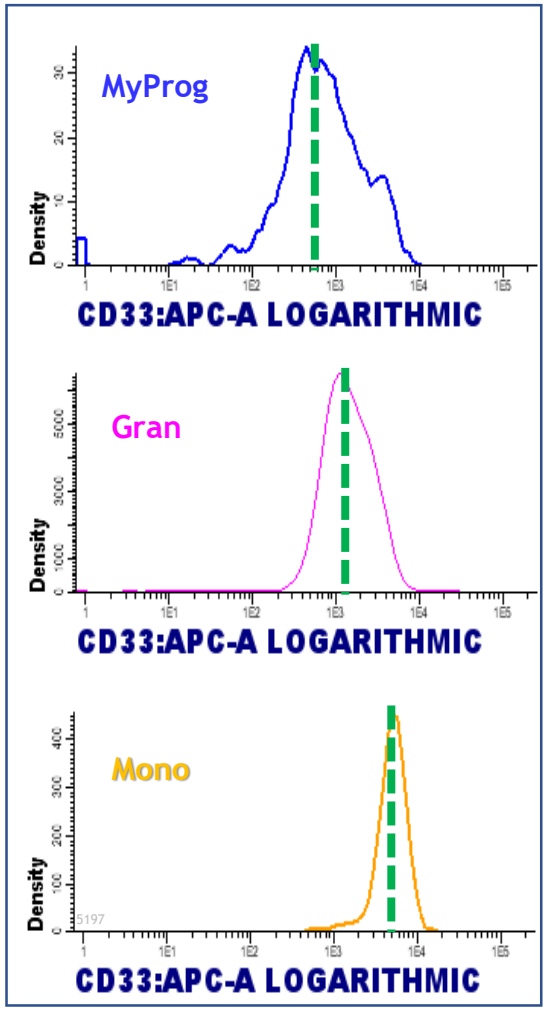
CD33 in granulocytic and monocytic differentiation

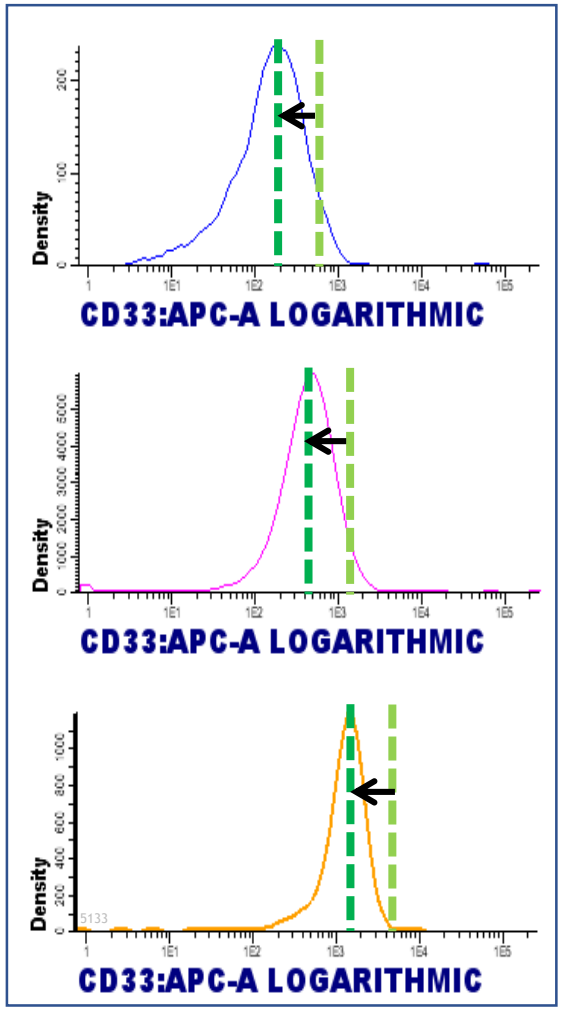


progenitors
“monocytes”
“granulocytes”

CD33 expression and CD33 polymorphism

approx. 10% of Caucasian population has a genetic polymorphism in CD33 antigen resulting in lower expression





Similar decrease in CD33 expression in all subsets:

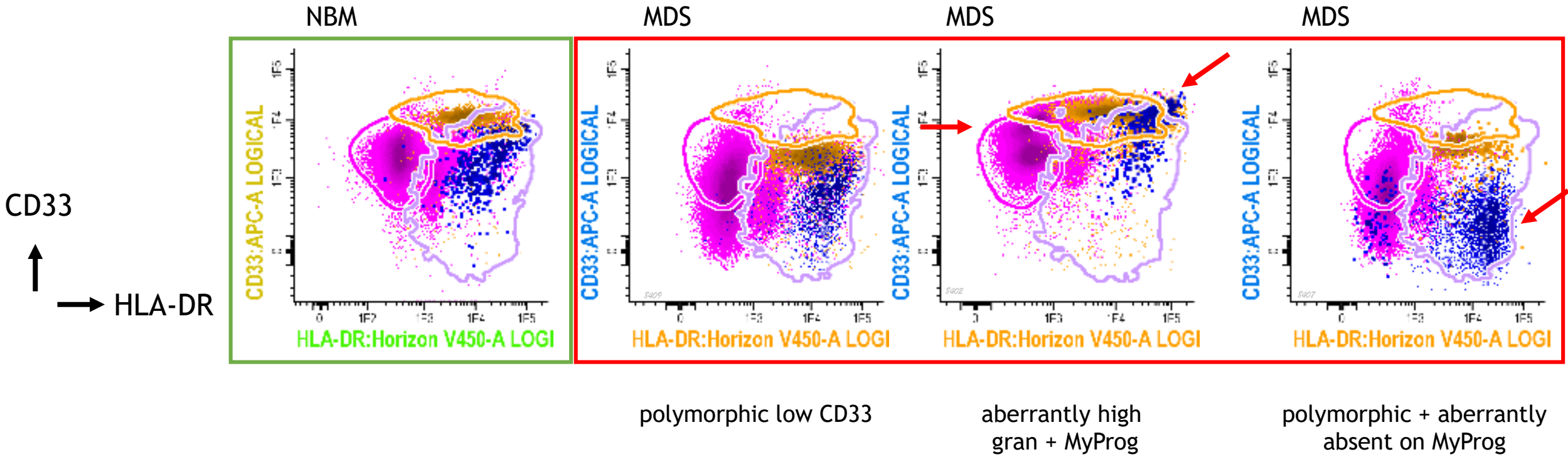
no aberrancy but CD33 polymorphism!

Calculate ratio between …

- CD33 Mo vs. Gr
- CD33 Mo vs. MyProg
- CD33 Gr vs. MyProg

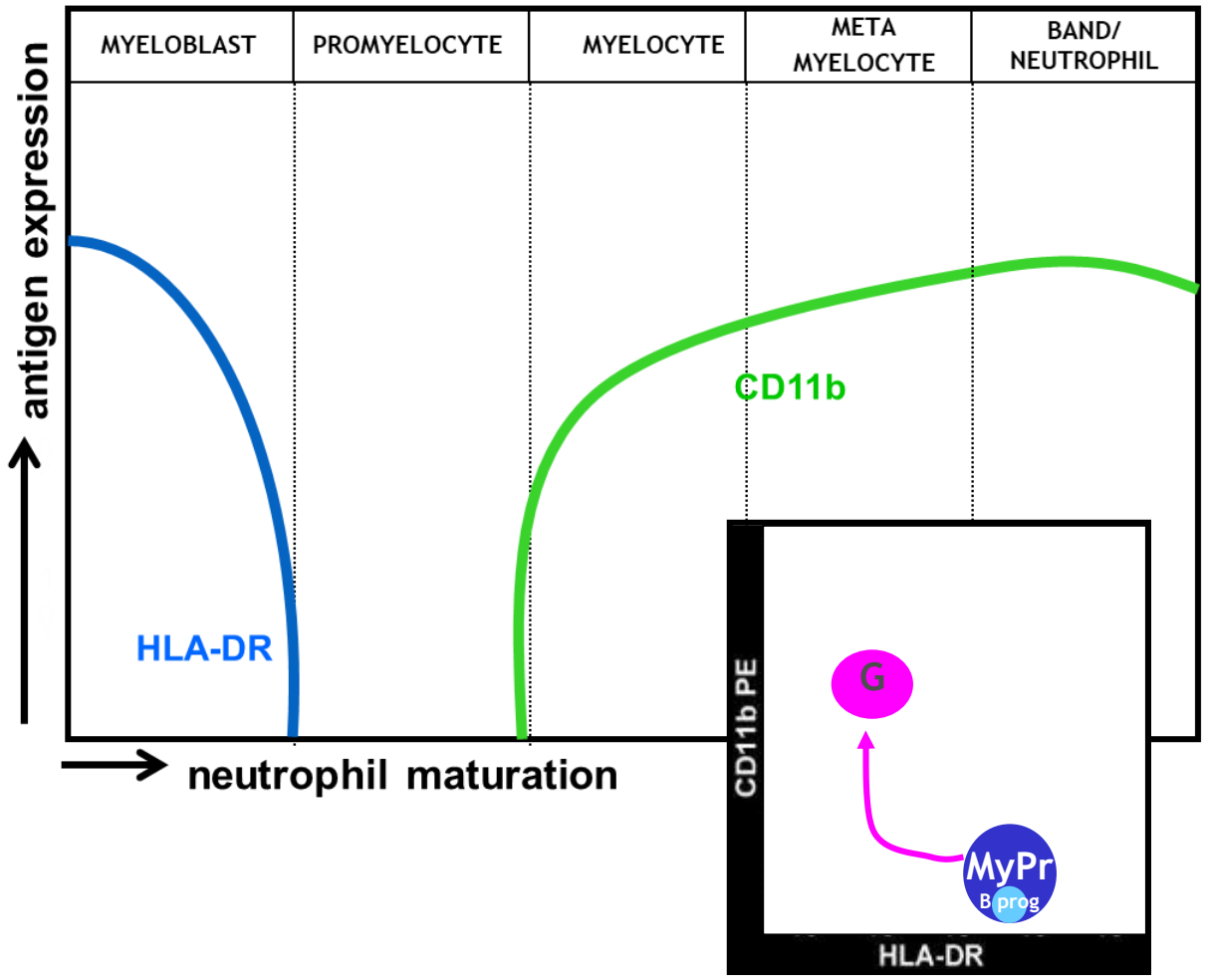
myloid progenitors
“monocytes”
“granulocytes”

Examples of FC aberrancies: CD33 and HLA-DR

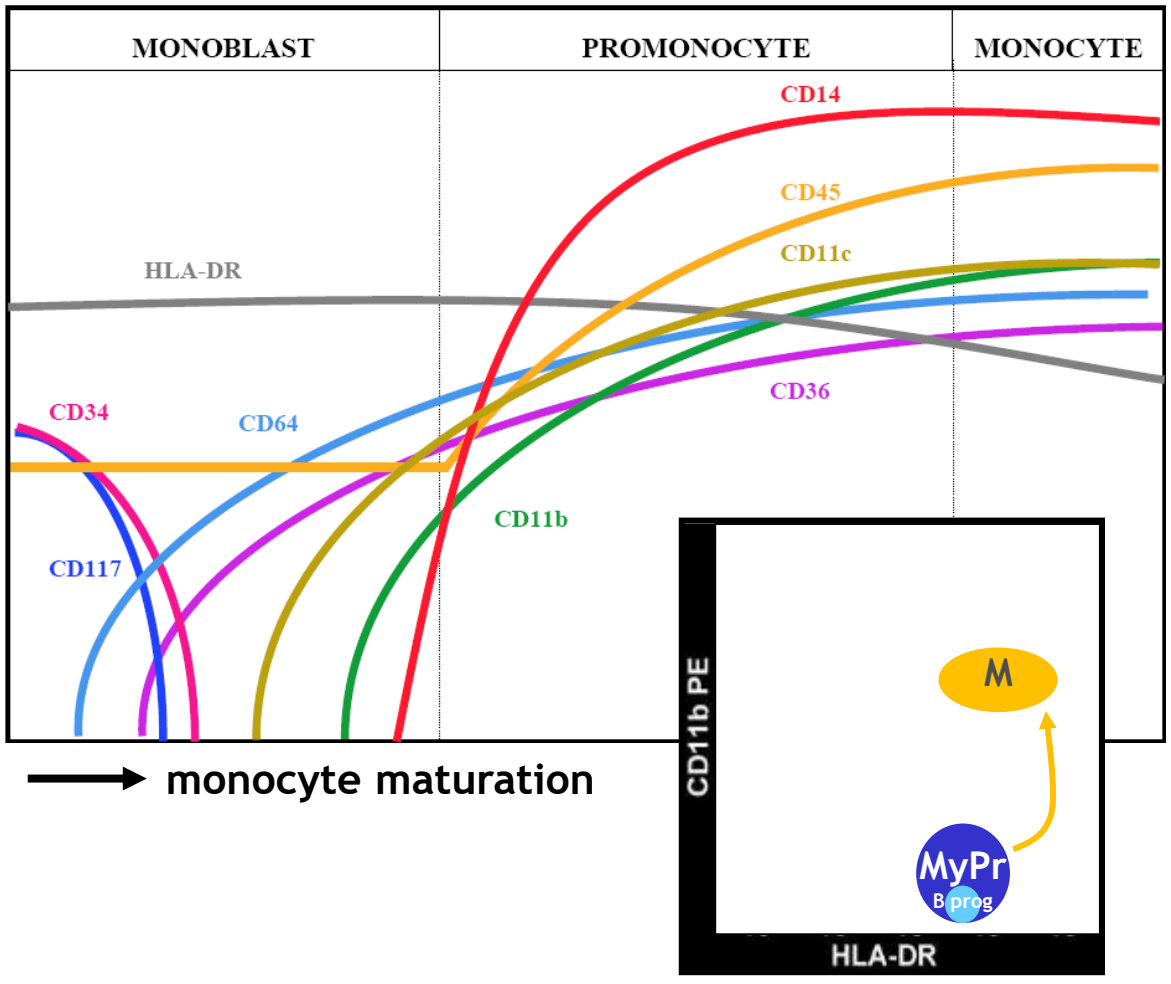


myeloid progenitors
"monocytes"
"granulocytes"

HLA-DR/CD11b expression on maturing myelo-monocytic cells



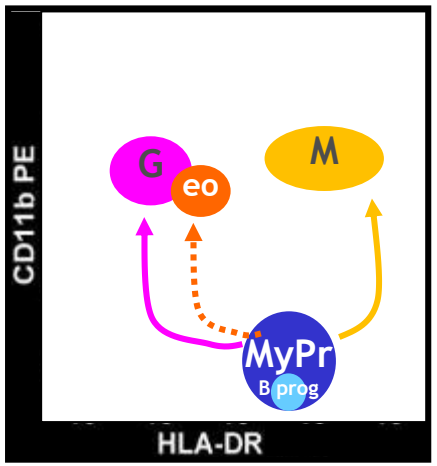
clock wise

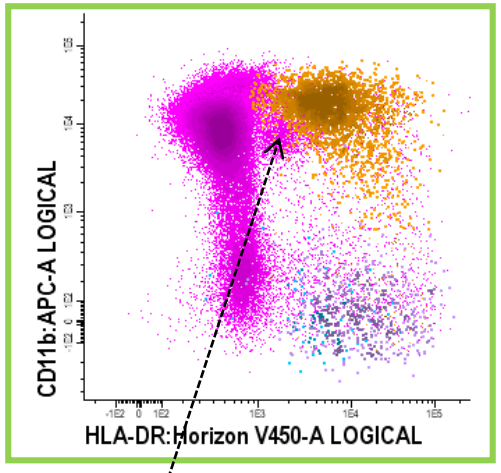


counter clockwise

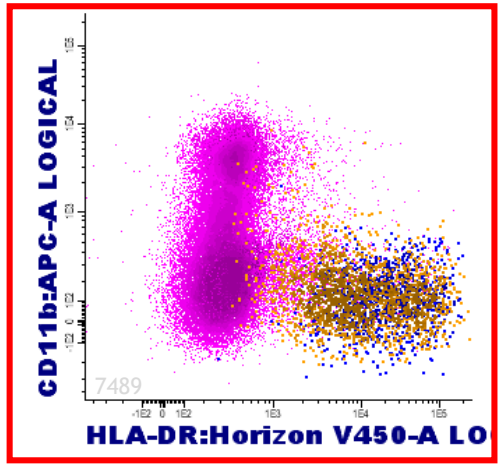
Adapted from: Orfao, ELNet Flow MDS 2008, Amsterdam

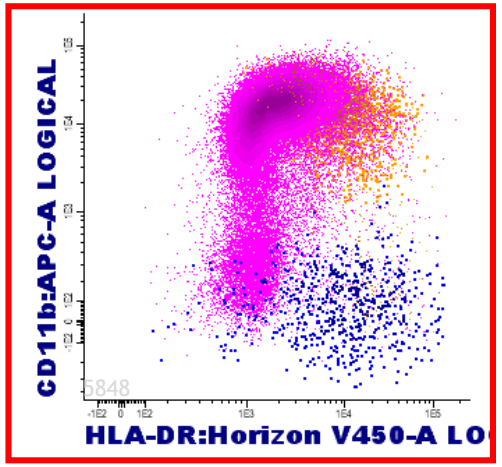
Monocytic vs. granulocytic differentiation: HLA-DR/CD11b

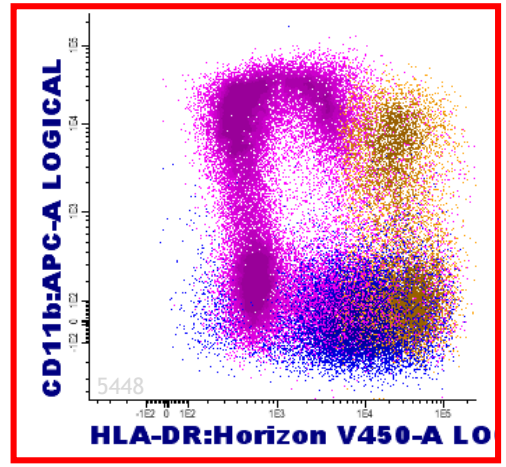




eosinophil







aberrant HLA-DR and/or CD11b expression

- progenitors
- “monocytes”
- “granulocytes”
- eosinophils

Aberrancies in maturing monocytic cells

ELNet recommendations

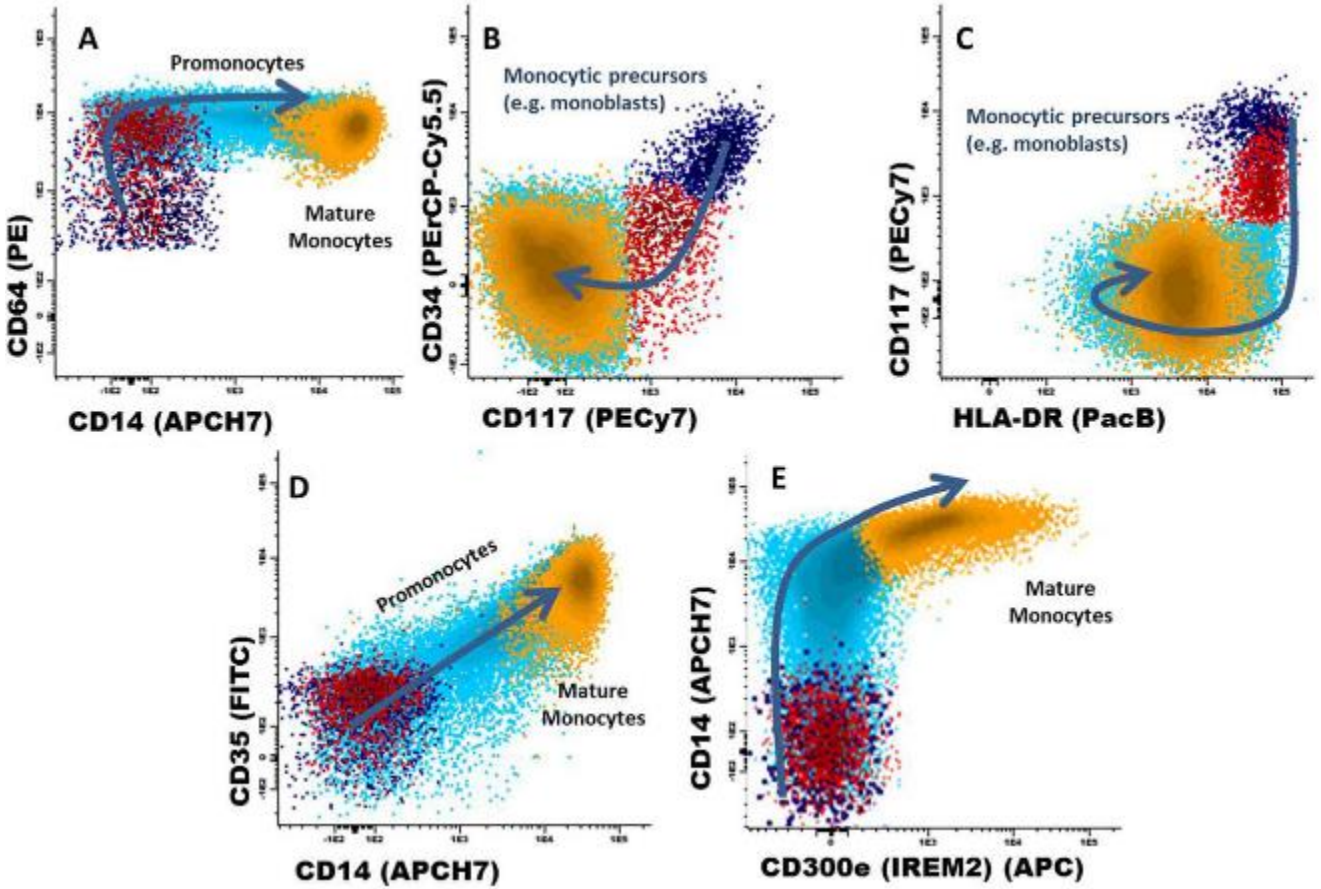
Recommended analyses	Aberrancy
Number of cells as ratio to lymphocytes	Decreased/Increased
Distribution of maturation stages	Shift towards immature
Relationship of HLA-DR and CD11b	Altered pattern
Relationship of CD14 and CD300e (IREM2)	Altered pattern
Relationship of CD14 and CD64	Altered pattern
Expression of CD13	(Homogenous) over/under expression
Expression of CD33	(Homogenous) over/under expression
Lineage infidelity markers: CD56	Presence

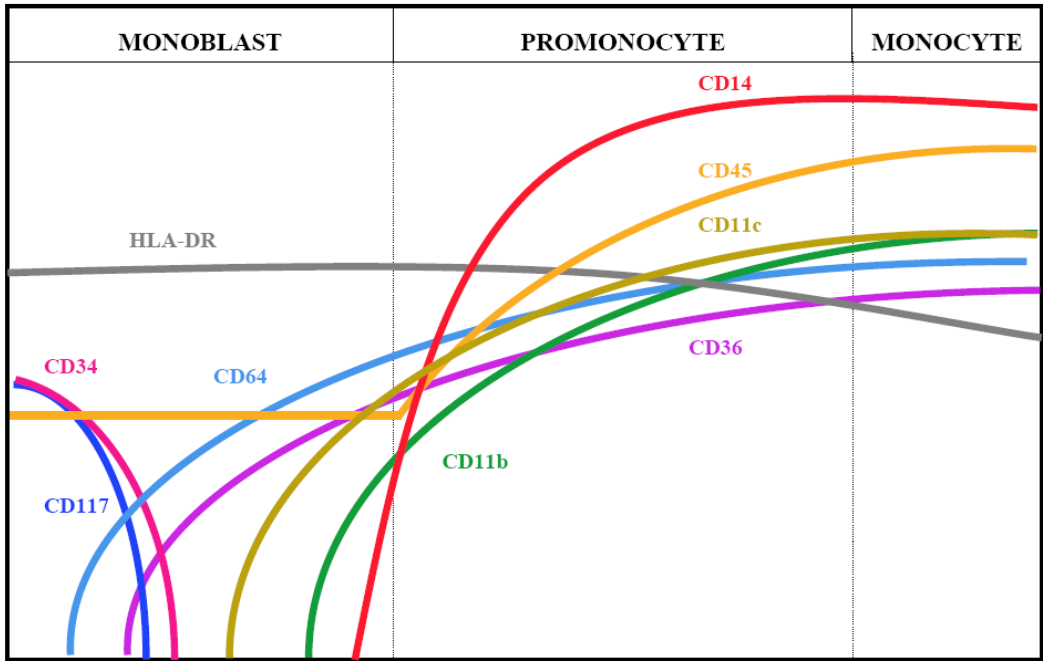
ELN European LeukemiaNet

Kern et al., Cytometry B Clin Cytom. 2023; 104(1):51-65;
Porwit et al. Cytometry B Clin Cytom 2023; 104:27-50

Normal monocytic differentiation

mix	CD45	CD34	CD117	HLA-DR	CD35	CD64	CD300e	CD14

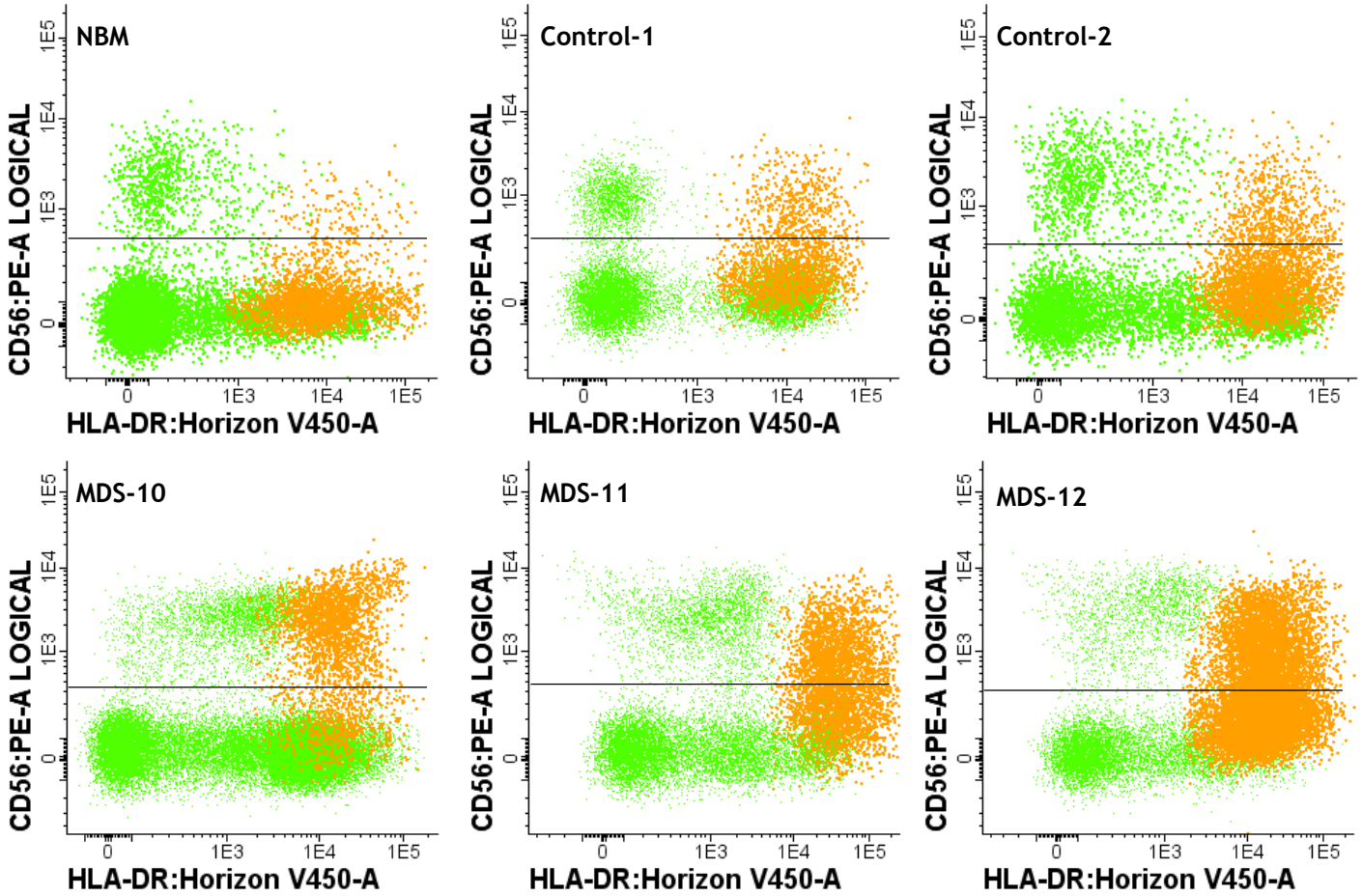




Matarraz et al. Cytometry B Clin Cytom. 2017 May;92(3):218-227

Adapted from: Orfao, ELNet Flow MDS 2008, Amsterdam

Lineage infidelity marker expression (CD56)



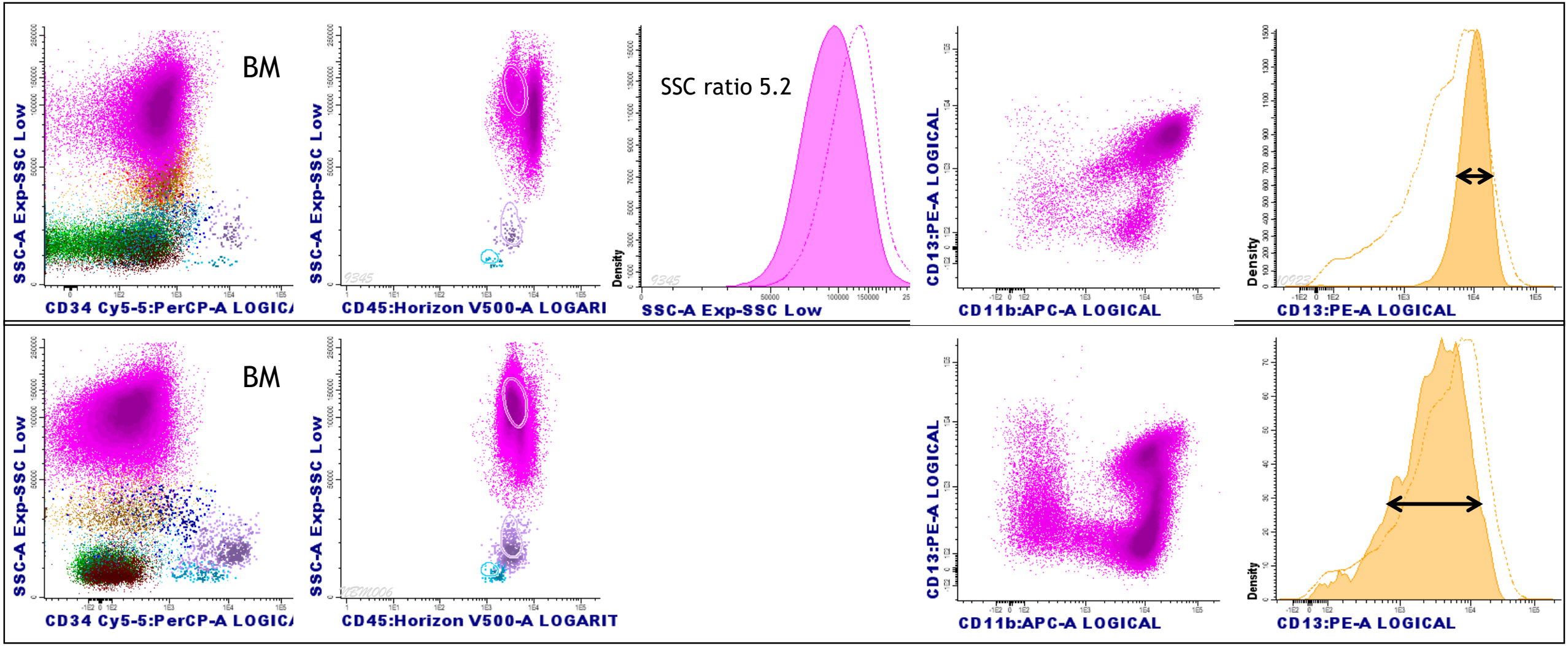
lymphocytes
"monocytes"

explore your own lab's cut-off based on activated monocytes …

1 log difference from normal is considered aberrant

Westers and Van de Loosdrecht, 2018 in book review
(Multiparameter Flow Cytometry in the Diagnosis of Hematologic Malignancies; ed. Porwit and Bené)

Haemodilution: examples of affected ‘markers’



#CD34⁺ MyProg - - - %CD34⁺ B-cell Prog gran SSC gran differentiation mono CD13

Aberrancies in immature (myeloid) cells

ELNet recommendations

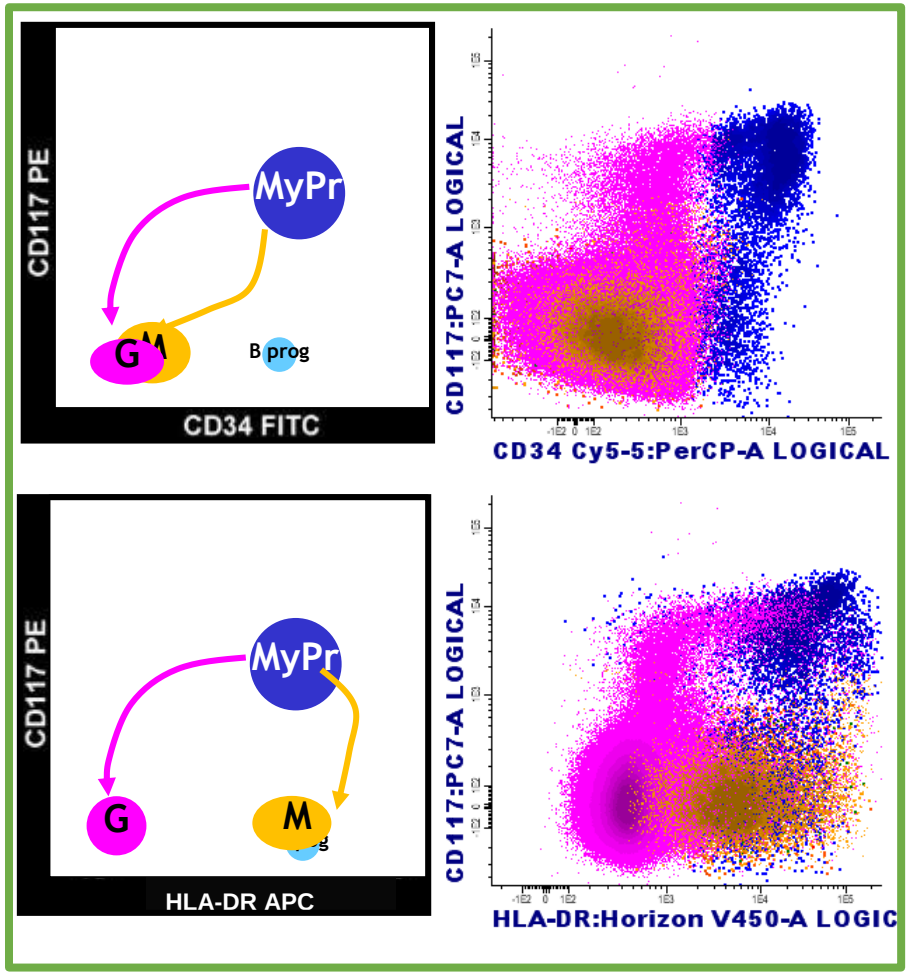
Recommended analyses	Aberrancy
Percentage of cells in nucleated cell fraction	Increased
Expression of CD45	Lack of/decreased/increased
Expression of CD34	Lack of/decreased/increased
Expression of CD117	(Homogeneous) over/under expression
→ CD34/CD117 ratio	Abnormal ratio (e.g. $CD34^{-}CD117^{+}$ cells)
Expression of HLA-DR	Lack of/decreased/increased
Expression of CD13 and CD33	Lack of/decreased/increased
→ Expression of CD38	Decreased expression
→ % $CD34^{+}CD123^{+}CD38^{-}$ cells	Increased percentage
Asynchronous expression of CD11b, CD15	Presence of mature markers
Expression of CD5, CD7, CD19, CD56	Presence of lineage infidelity marker
Progenitor B cells as fraction of total CD34+	Decreased/absent

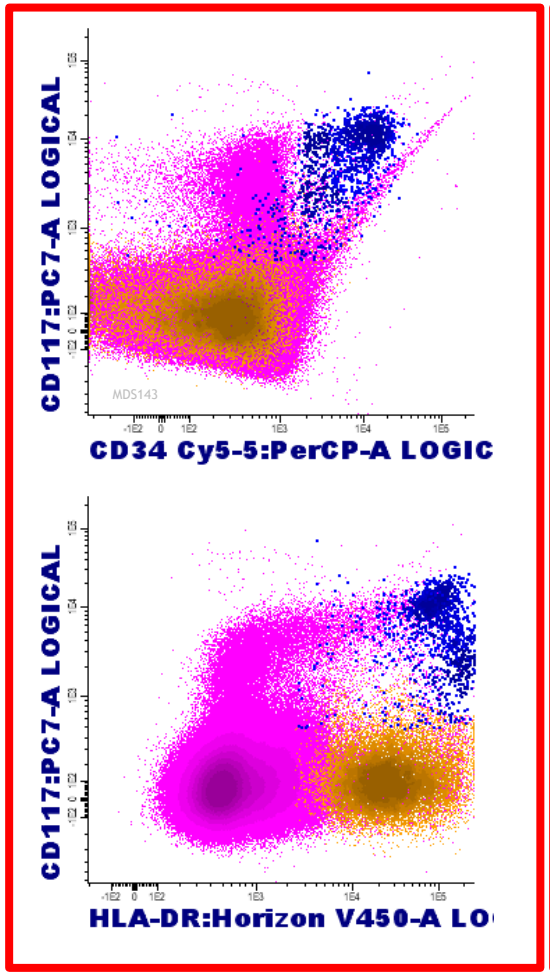
2023 update

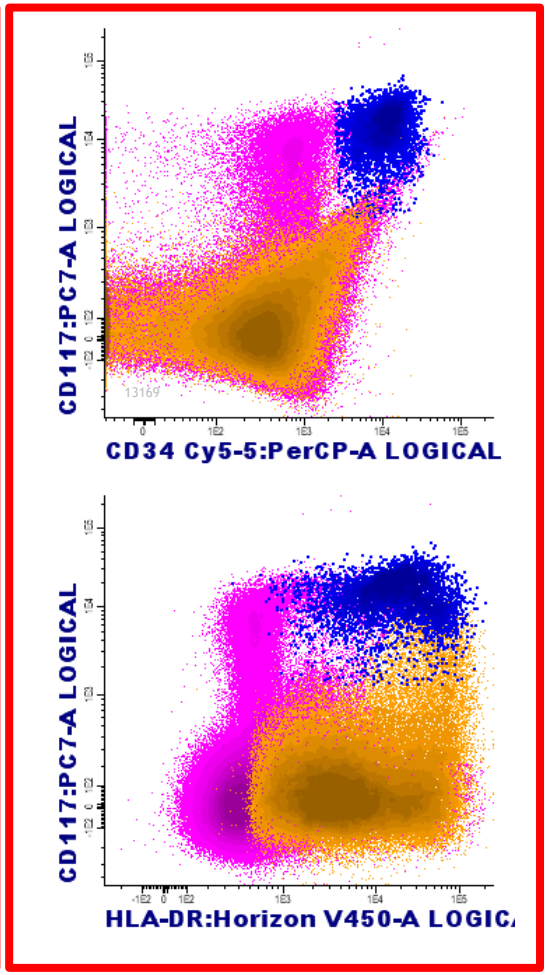
ELN European LeukemiaNet

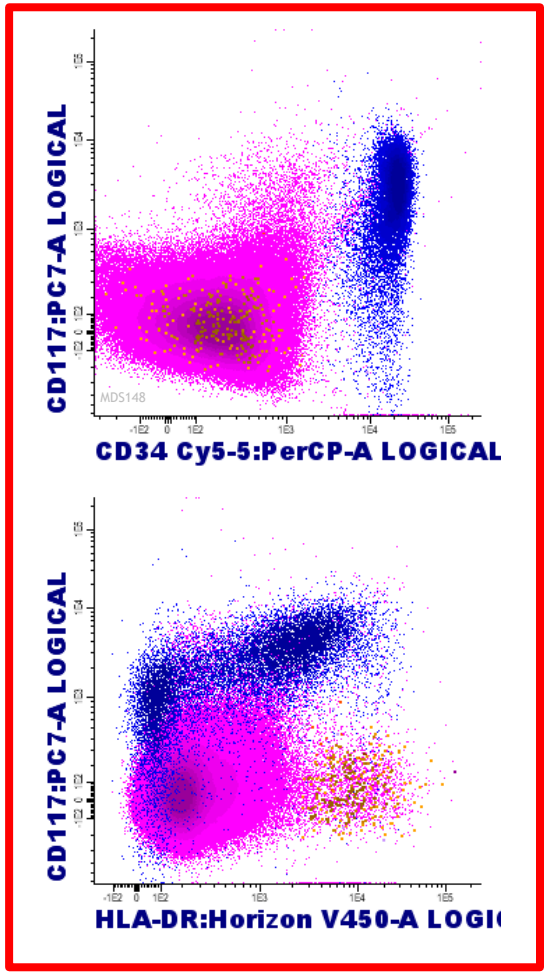
Kern et al., Cytometry B Clin Cytom. 2023; 104(1):51-65;
Porwit et al. Cytometry B Clin Cytom 2023; 104:27-50

Aberrant differentiation patterns ? CD34/CD117/HLA-DR

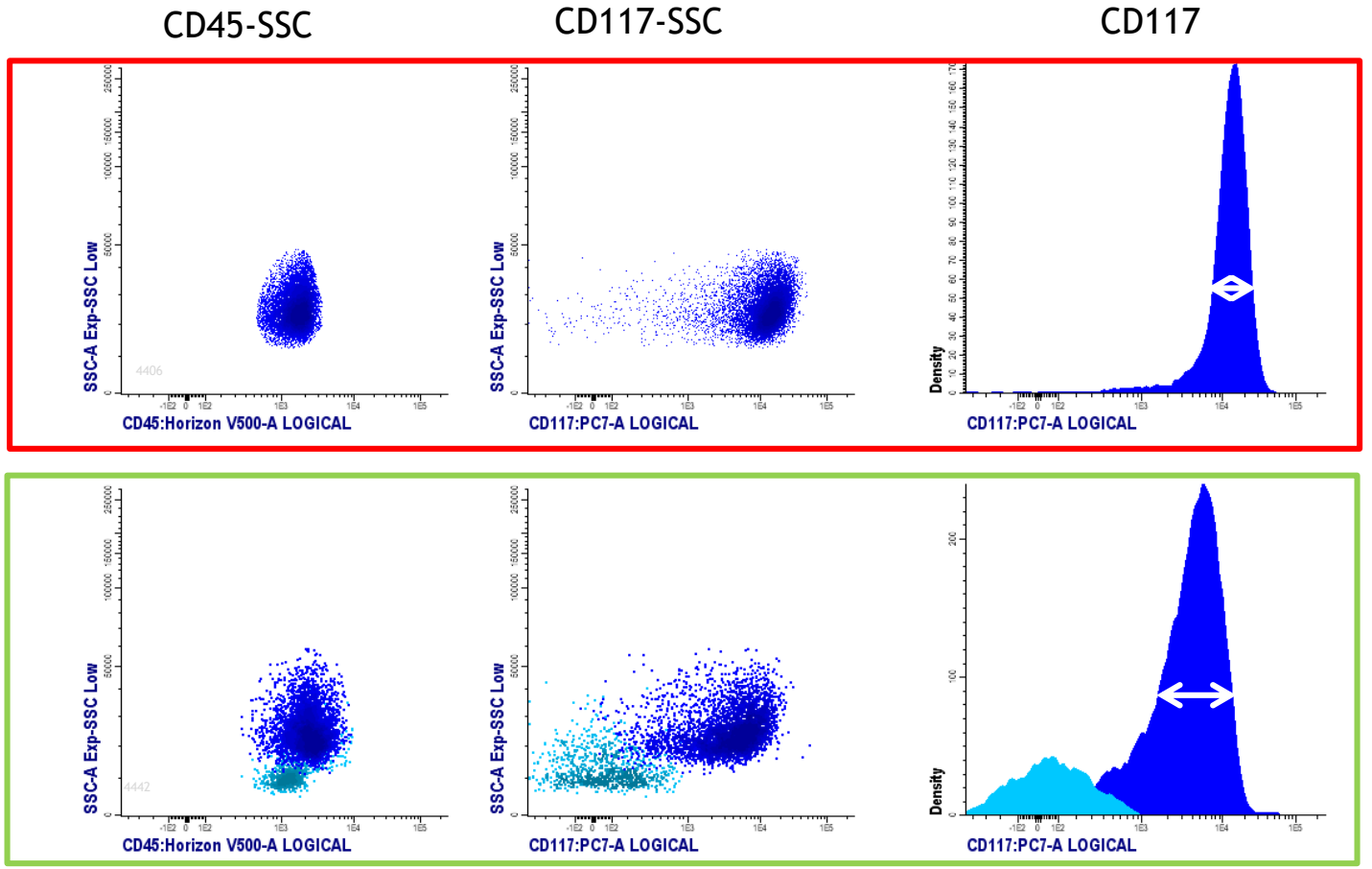








Homogeneous vs. heterogeneous CD117: standardization of interpretation



myeloid progenitors:

- CD117 over expression and
- decreased CV … homogeneous

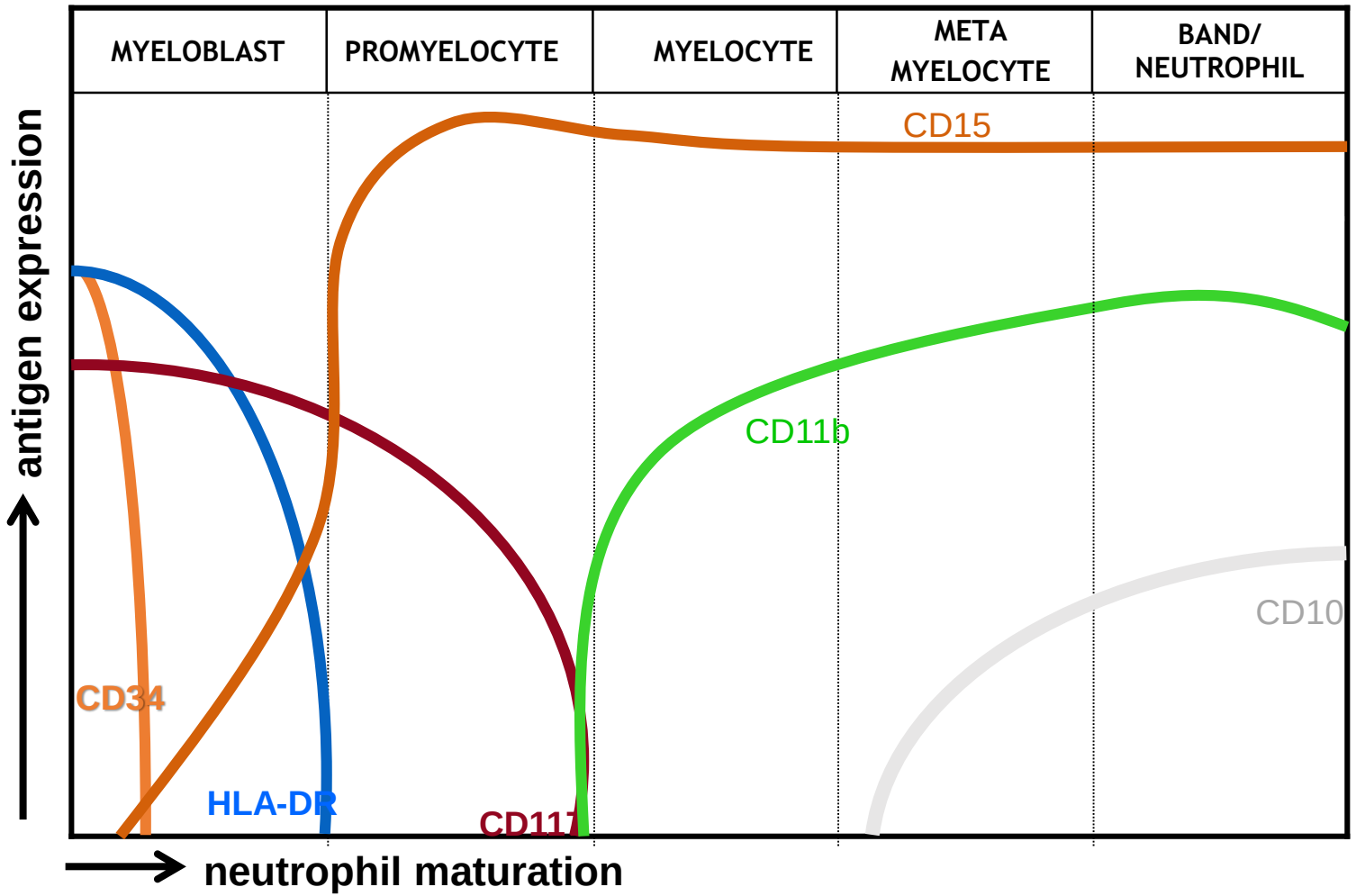
↓

homogenous over expression

Use CV values

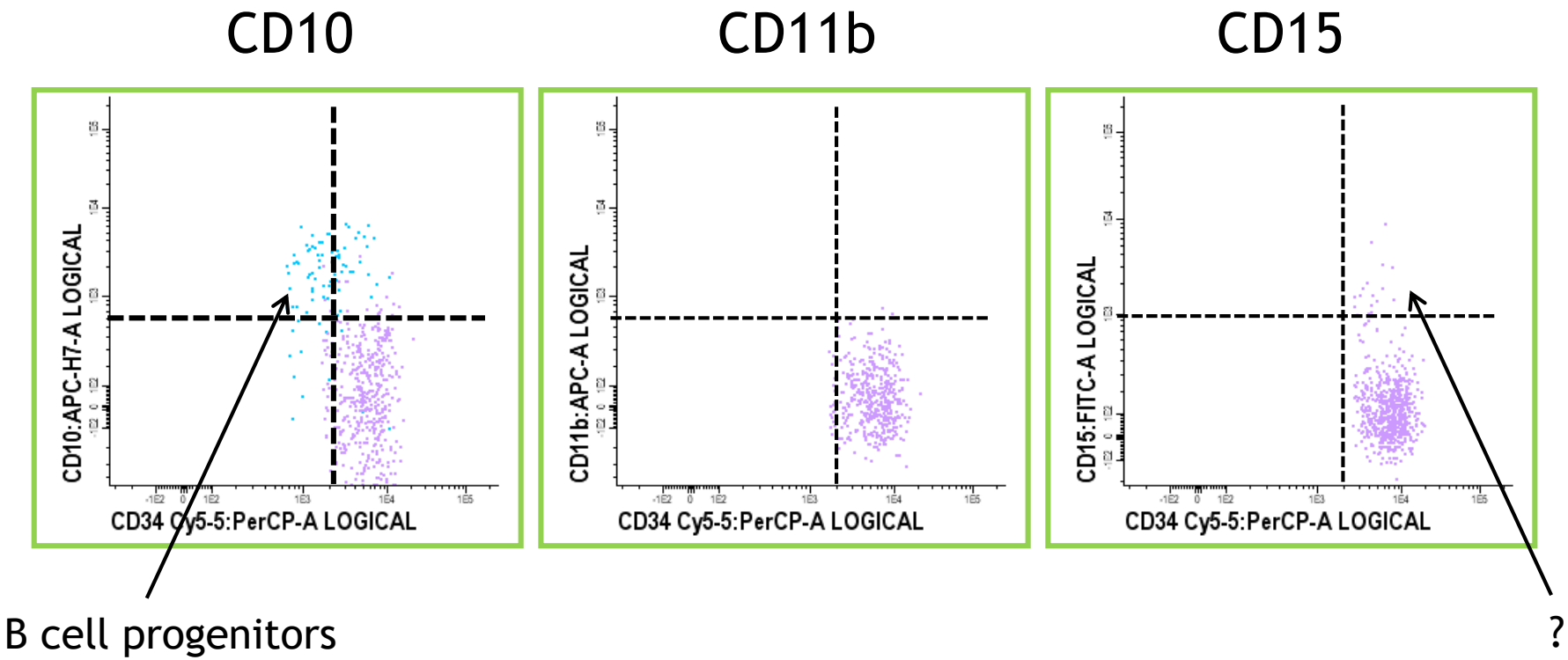
< normal: homogeneous
> normal: heterogeneous

Expression levels of maturing myeloid cells

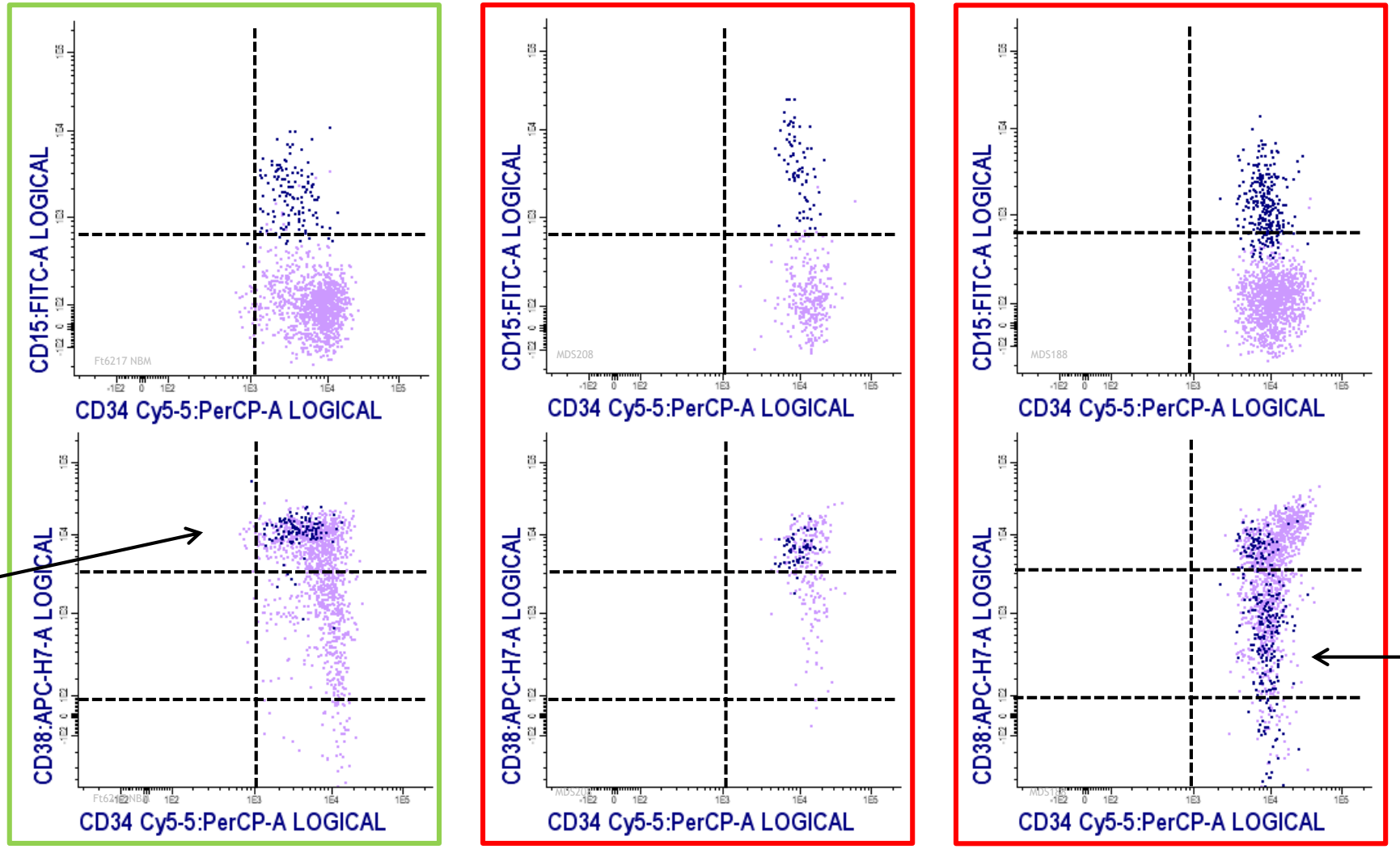


Adapted from: Orfao, ELNet Flow MDS 2008, Amsterdam

Asynchronous marker expression (normal BM)



Asynchronous CD15 expression ?



CD15 normally only on most mature CD34+

CD15 on more immature CD34+: aberrant

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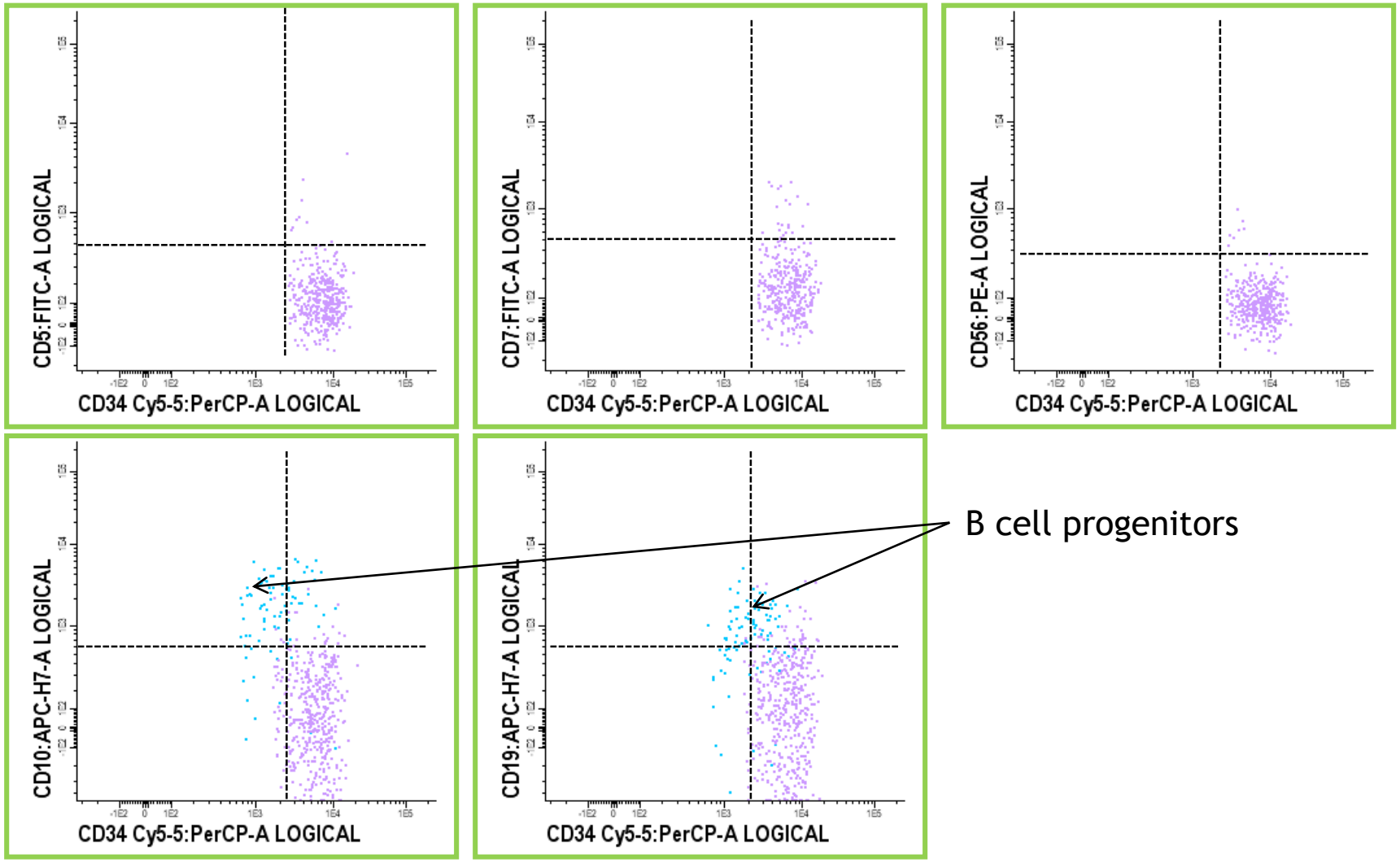
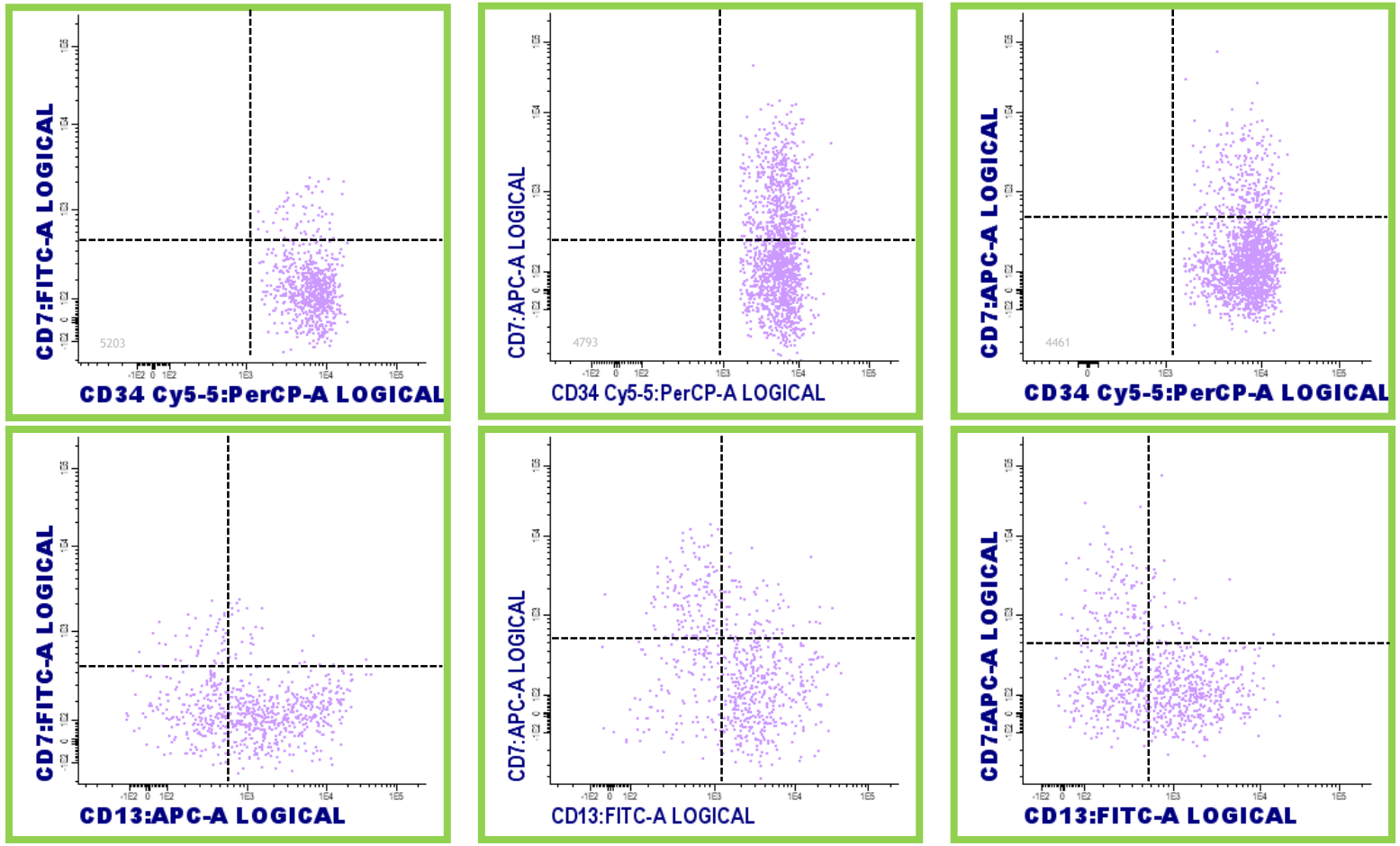
Lineage infidelity marker expression (normal BM)



Lineage infidelity marker expression (normal BM)

CD13/CD7/CD45/CD34



L-shaped pattern of
CD13 vs. CD7 on normal CD34^{+}

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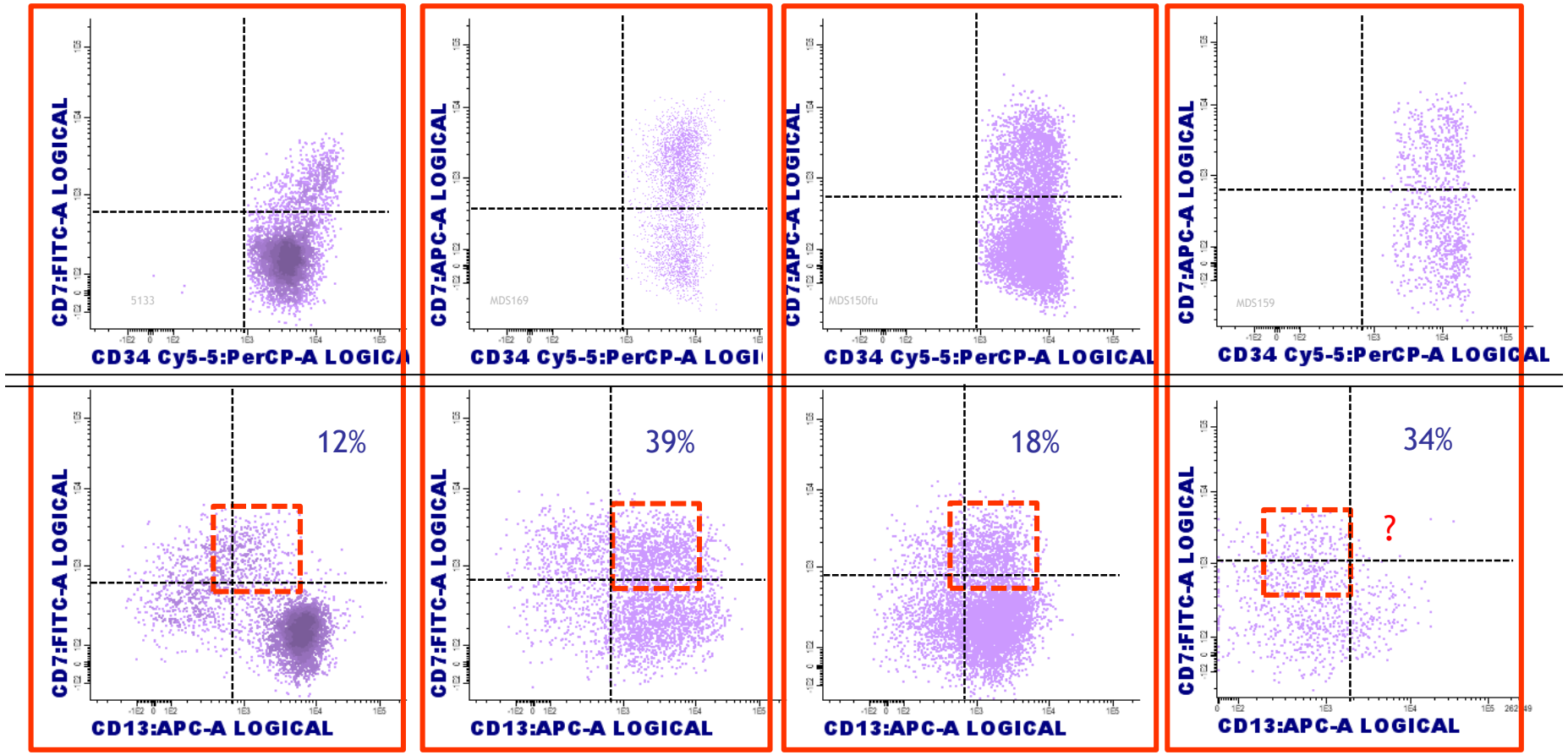
Aberrant marker expression (lineage infidelity)

CD13/CD7/CD45/CD34



L-shaped pattern CD13/CD7 lost

Aberrancies in maturing erythroid cells

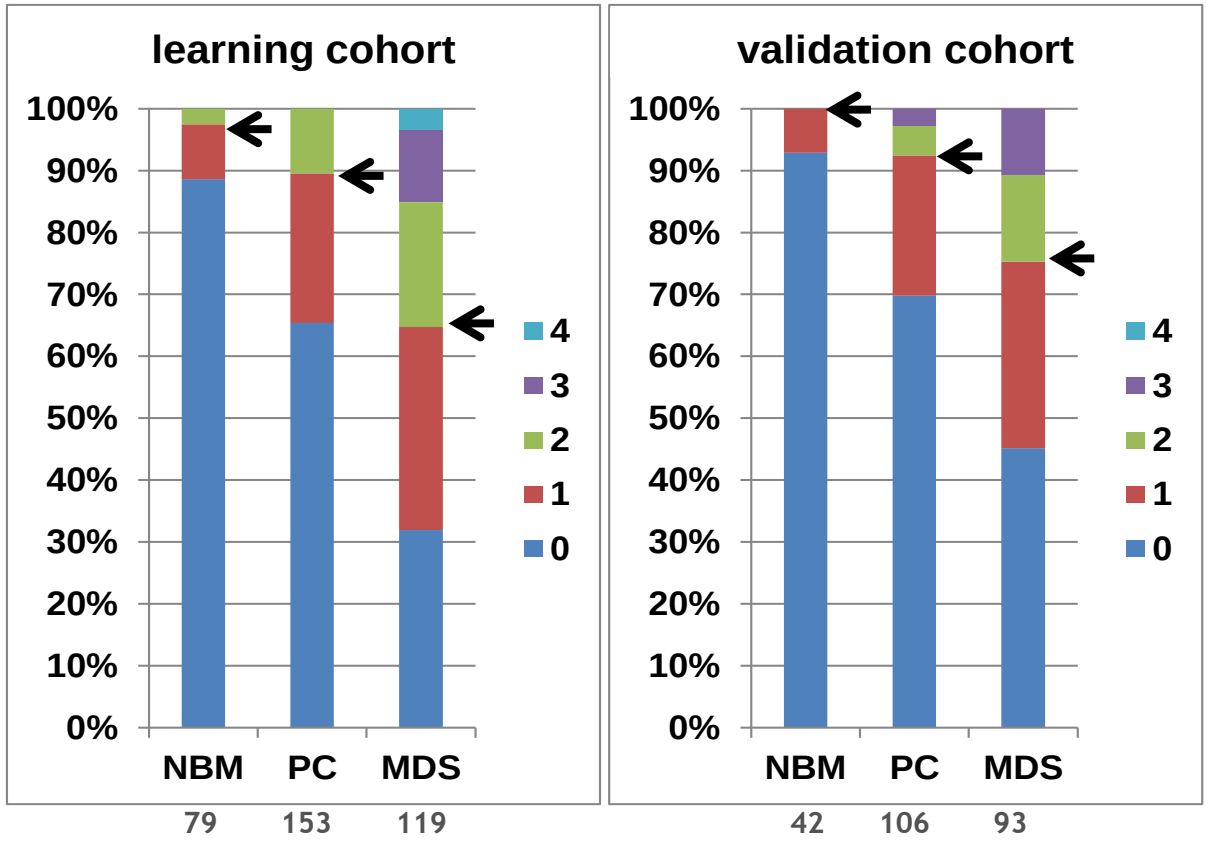
Recommended analyses	Aberrancy
Percentage of nucleated erythroid cells	Increased
Relationship of CD71 and CD235a	Altered Pattern
Expression of CD71	Decreased
Expression of CD36	Decreased
Percentage of CD117-positive precursors	Increased/decreased
CD71 CV	Increased
CD36 CV	Increased
Percentage CD105-positive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

selected parameters associated with erythroid dysplasia in MDS (ELNet 2017)

ELN European LeukemiaNet

Westers et al., Haematologica, 2017, 102(2):308-319
Van der Velden et al. Cytometry B Clin Cytom, 2023:104(1):15-26;
Porwit et al. Cytometry B Clin Cytom, 2023;104(1):27-50

Dysplastic erythroid immunophenotypes associated with MDS



Cut-off ≥ 2 aberrancies

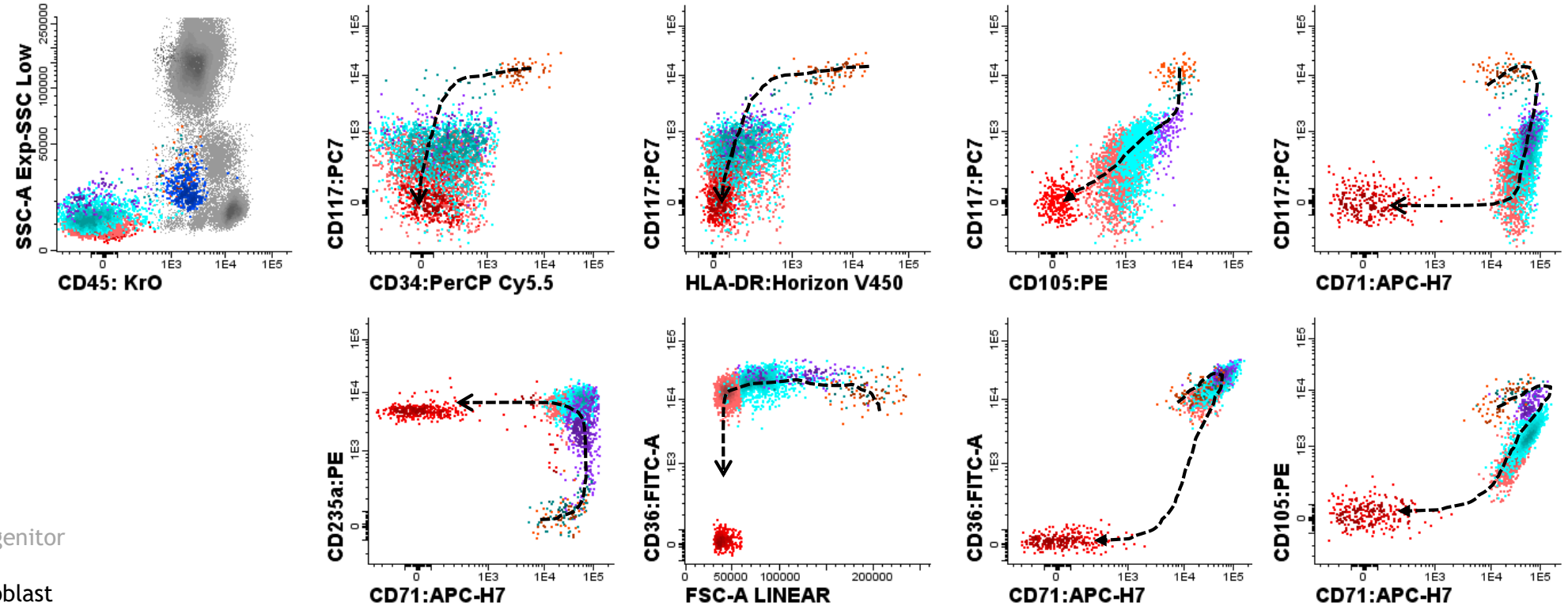
Note!
This is not a diagnostic score

Note! CD71-MFI and % CD117 not enough to indicate MDS-associated erythroid dysplasia

ELN European LeukemiaNet

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

Normal erythropoiesis by flow cytometry (after lysis)



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

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CD36	CD105	CD34	CD117	CD33	CD71	HLA-DR	CD45

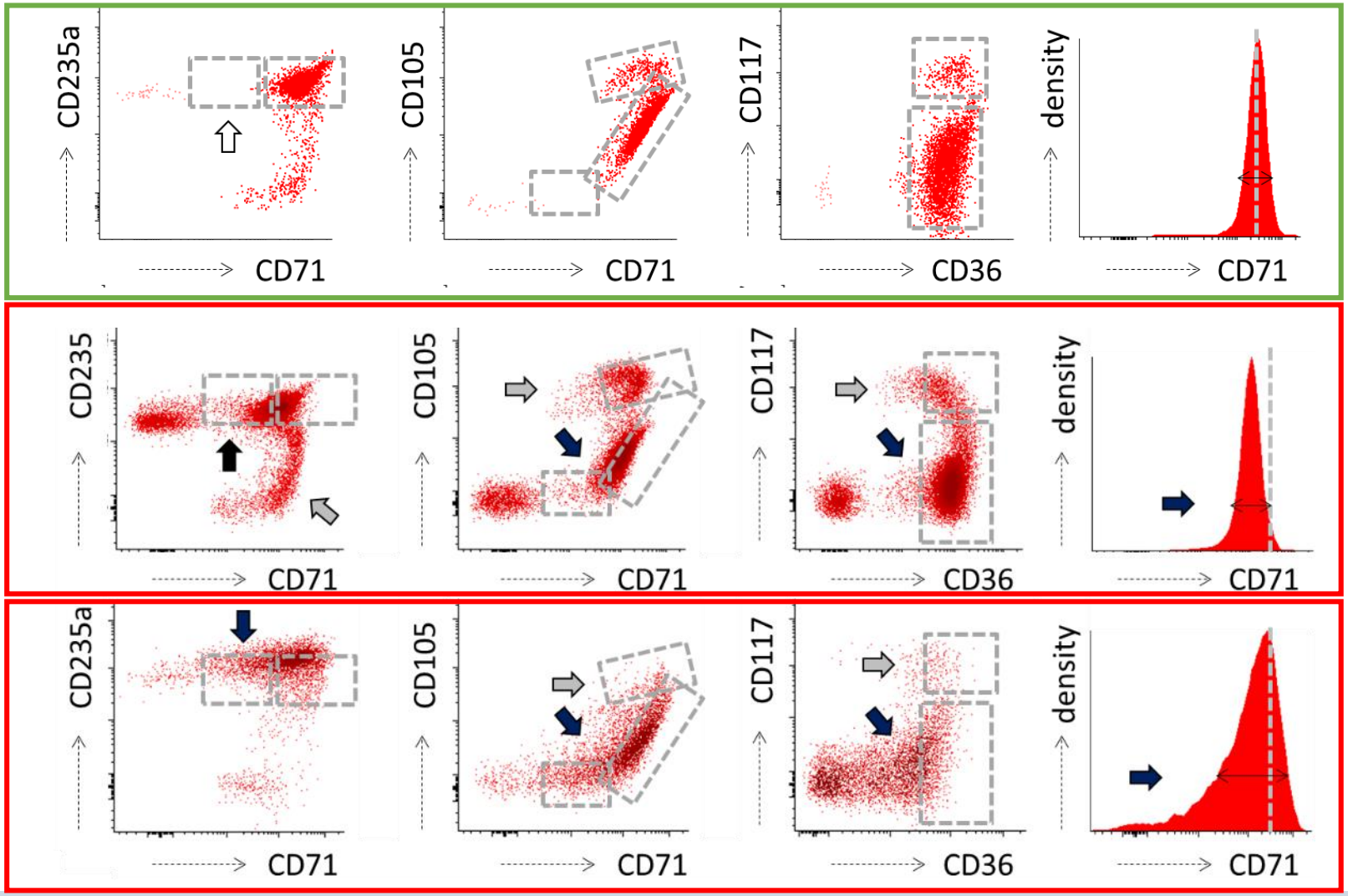
Westers, et al., Haematologica 2017;102(2):308-19

Dyserythropoiesis by flow cytometry in MDS

normal

MDS

MDS



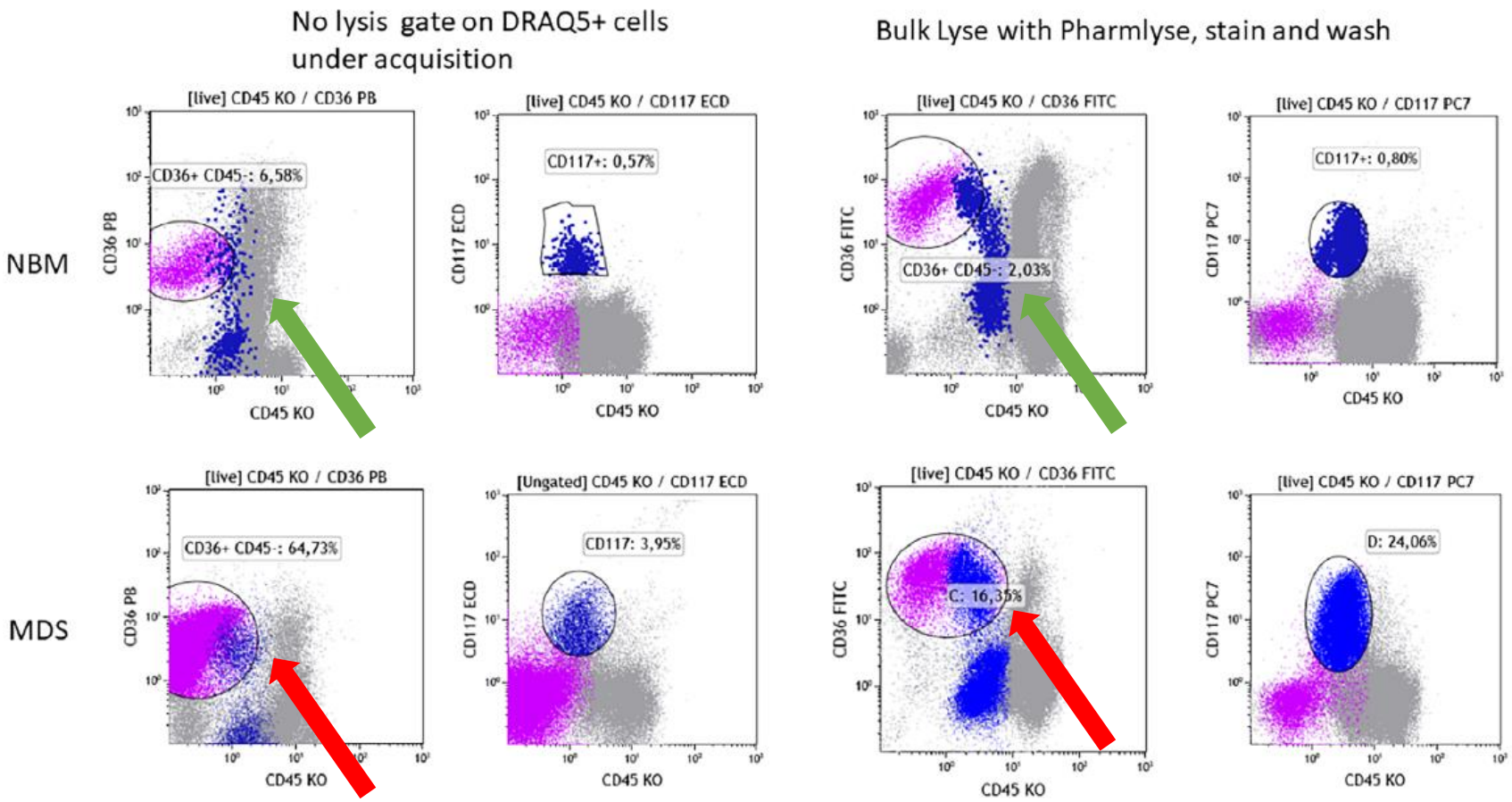
All data from ammoniumchloride-based lysed bone marrow aspirates

VU Hematologie

Porwit et al. Cytometry B Clin Cytom, 2023;104(1):27-50

Pre-analytical and technical issues - Impact of lysis

Lysing and type of lysing solution may affect results (scatter[1,3], erythroid compartment[2])



Both preparation methods have been shown to give diagnostic info, so:

standardize !!

and
determine your own reference values

ELN European LeukemiaNet

[1]Mathis et al. Leukemia, 2013, 27, 1981–1987; [2]Westers et al., Haematologica 2017, 102(2):308-319; [3]Wangen et al. Int J Lab Hem 2014;36(2):184-196

Van der Velden et al. Cytometry B Clin Cytom, 2023:104(1):15-26;
Porwit et al. Cytometry B Clin Cytom, 2023;104(1):27-50

Aberrancies in maturing erythroid cells

Note!

- determine reference values for each standardized work-up, on either non-lysed or lysed BM

- various conditions (e.g., reactive changes, medication-induced anomalies, vitamin B12, and folate deficiencies) may induce dysplastic features in the BM erythroid lineage

Thus,

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

Lu et al. Sci Rep 2023;1;13(1):8930

macrocytic anemia: CD36 RMFI increased in $CD105^+$

MDS: CD36 RMFI decreased in $CD105^+$

MDS flow cytometry reports

- %CD34+ myeloid progenitors
- descriptive

- concluding remark

- disclaimer: only one diagnostic test, FC should be part of integrated report

Schematic representation of FC results: Ogata + FCSS -> the integrated Flow Score (iFS)

Ogata score	**<2**	**<2**	**<2**	**<2**	**<2**	**<2**	**<2**	**<2**	**≥2**	**≥2**	**≥2**	**≥2**	**≥2**	**≥2**	**≥2**	**≥2**
Dysplasia by FC myeloid progenitors	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Dysplasia by FC - Neutrophils - Monocytes	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Dysplasia by FC - Nucl. erythroid	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Conclusion	**A**	**B**	**B**	**C**	**B**	**C**	**C**	**C**	**B**	**C**	**C**	**C**	**C**	**C**	**C**	**C**

- : no or only 1 aberrancy
+ : >1 aberrancy

A = 'results show no MDS-related features'
B = 'results show limited number of changes associated with MDS' } not enough to consider MDS

C = 'results are consistent with MDS'

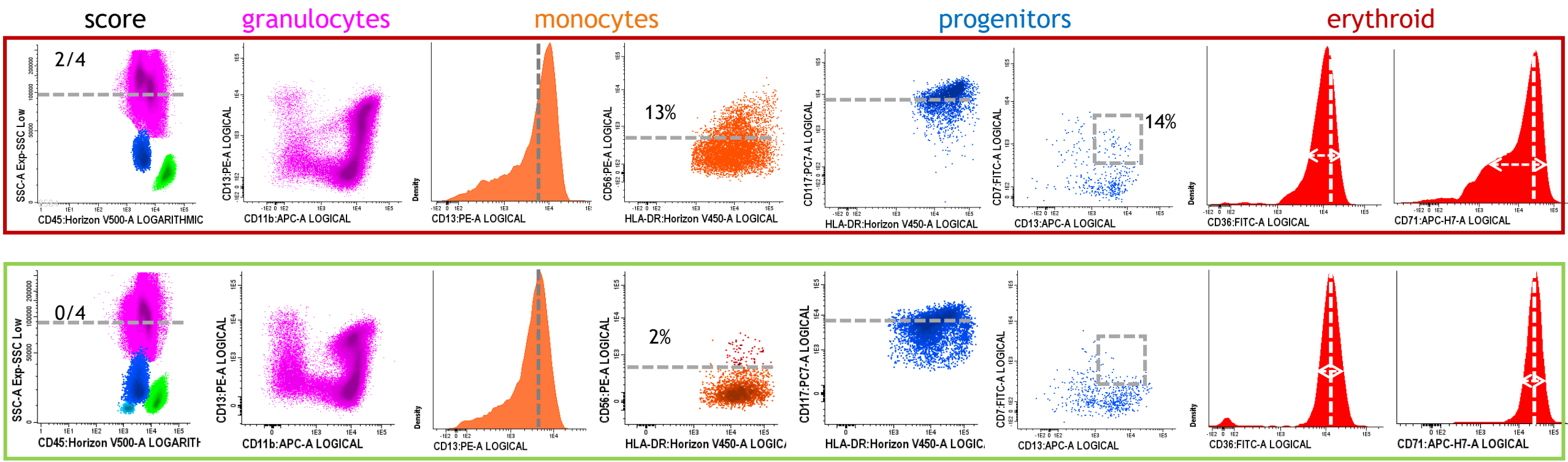
VU Hematologie

Wells et al. Blood 2003;102(1):394-403; Ogata et al., Haematologica 2009; 94(8):1066-74;
Della Porta et al., [ELNet] Haematologica 2012;97:1209-17

Cremers EMP, et al., Haematologica 2017, 102(2)
based on Van de Loosdrecht and Westers. J Nat Compr Cancer Netw 2013;11:892-902)

Case of suspected MDS vs. normal BM

Hb 9.3
T 241
ANC 3.6



Diagnostic score: 2 (SSC 5.4, B cell Prog)

Aberrancies in myeloid progenitor cells (homogeneous high CD117, CD7+, SSC high), neutrophil SSC decreased, aberrancies in monocytes (number increased, aberrant CD13?), and in erythroid lineage (CD36 CV and CD71 CV increased)

VU Hematologie

Schematic representation of FC results: Ogata + FCSS -> the integrated Flow Score (iFS)

Ogata score	<2	<2	<2	<2	<2	<2	<2	<2	≥2	≥2	≥2	≥2	≥2	≥2	≥2	≥2
Dysplasia by FC myeloid progenitors	-	-	-	-	+	+	+	+	-	-	-	-	+	+	+	+
Dysplasia by FC - Neutrophils - Monocytes	-	-	+	+	-	-	+	+	-	-	+	+	-	-	+	+
Dysplasia by FC - Nucl. erythroid	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Conclusion	**A**	**B**	**B**	**C**	**B**	**C**	**C**	**C**	**B**	**C**	**C**	**C**	**C**	**C**	**C**	**C**

- : no or only 1 aberrancy
+ : >1 aberrancy

Cytomorphology
RS-SLD (mega)

A = 'results show no MDS-related features'
B = 'results show limited number of changes associated with MDS'
} not enough to consider MDS

C = 'results are consistent with MDS'

VU Hematologie

Wells et al. Blood 2003;102(1):394-403; Ogata et al., Haematologica 2009; 94(8):1066-74;
Della Porta et al., [ELNet] Haematologica 2012;97:1209-17

Cremers EMP, et al., Haematologica 2017, 102(2)
based on Van de Loosdrecht and Westers. J Nat Compr Cancer Netw 2013;11:892-902)

Data analysis

Low number of dimensions

- Expert-based manual gating:
  - step-by-step evaluation of multiple parameters and patterns in one or two-dimensional plots (histogram and scatter plots)

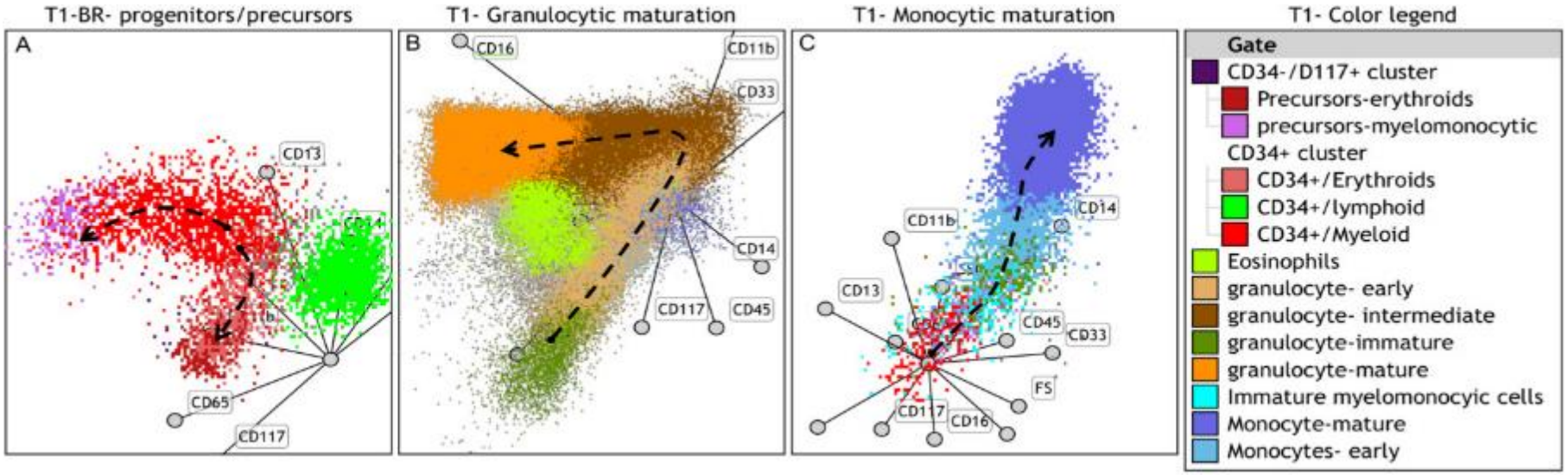
- Dimensionality reduction and clustering algorithms
  - mapping of cell clusters of normal BM on multidimensional plots (e.g. Kaluza radar plots, Infinicyt's APS plots, FlowSOM)
    - provide insight into BM cell composition and maturation
    - may serve as reference map to assess abnormal hematopoiesis in MDS

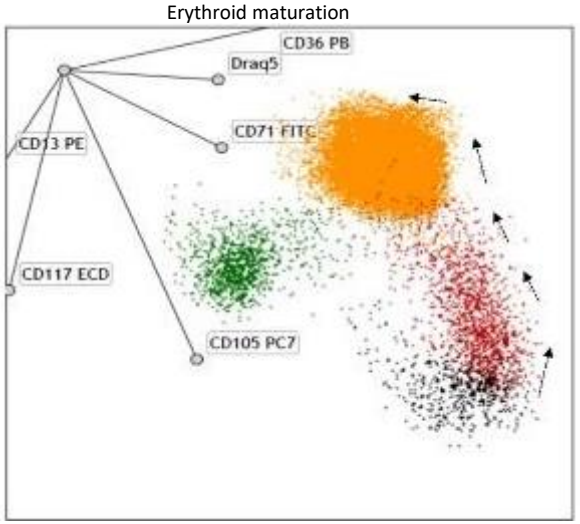
Multi-dimensional (->numerous two-dimensional plots)

- Dimensionality reduction and clustering algorithms
- Computational analysis

Radar plots

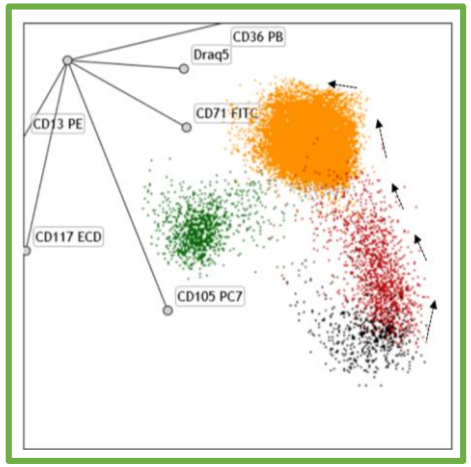
reference maps showing normal hematopoiesis

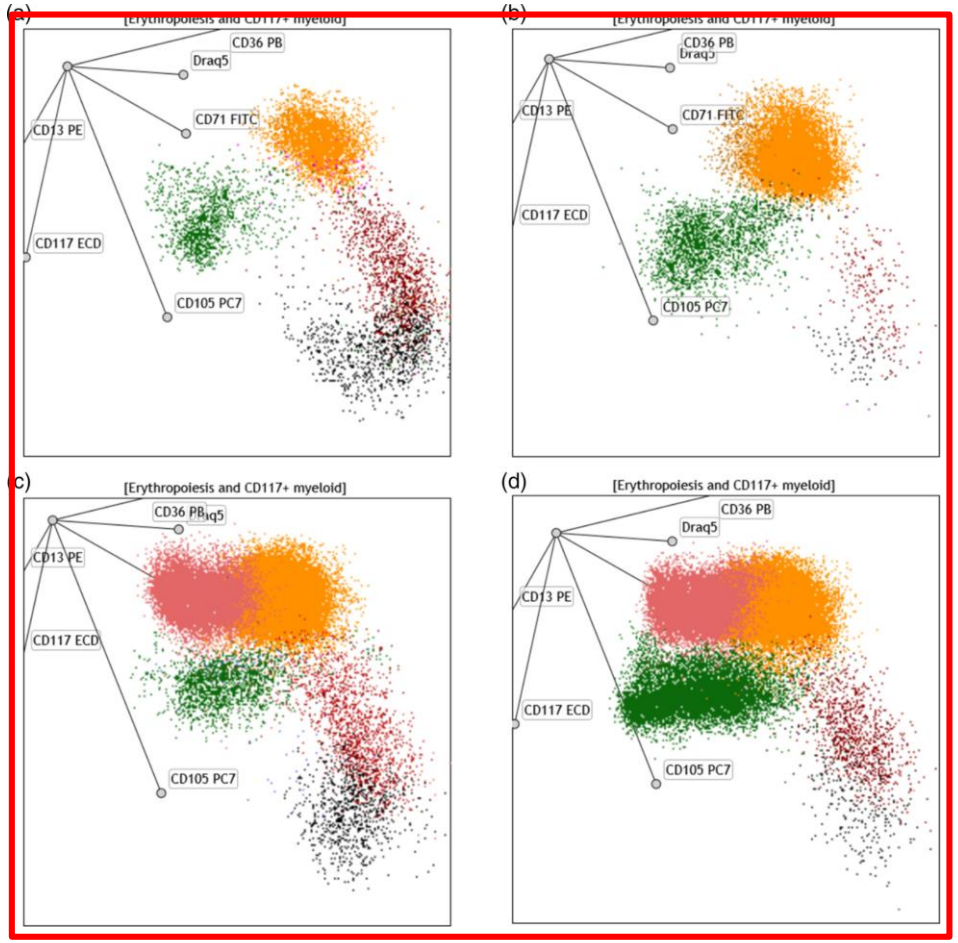




Jafari et al. Cytometry B Clin Cytom 2018, 94(2):219-229;
Violidaki et al. Cytometry B Clin Cytom, 2020, 98:399-41

Application of radar plot reference maps to assess abnormal hematopoiesis in MDS

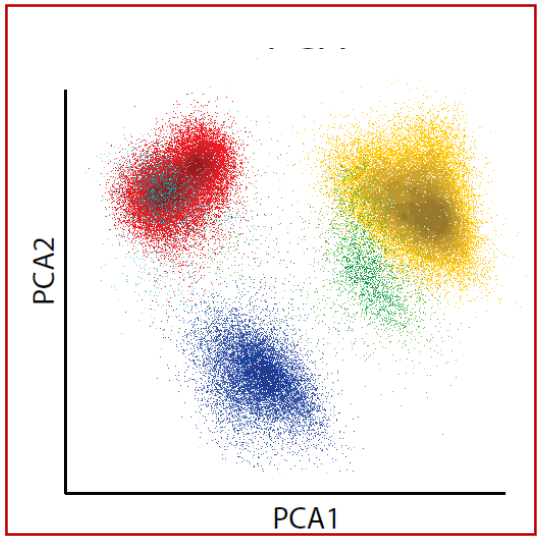




Examples of abnormal erythroid differentiation in MDS cases

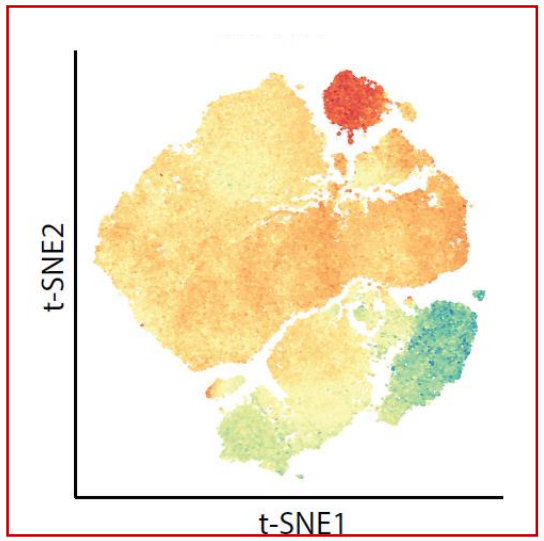
Dimensionality reduction and clustering algorithms

Principal component analysis (PCA)



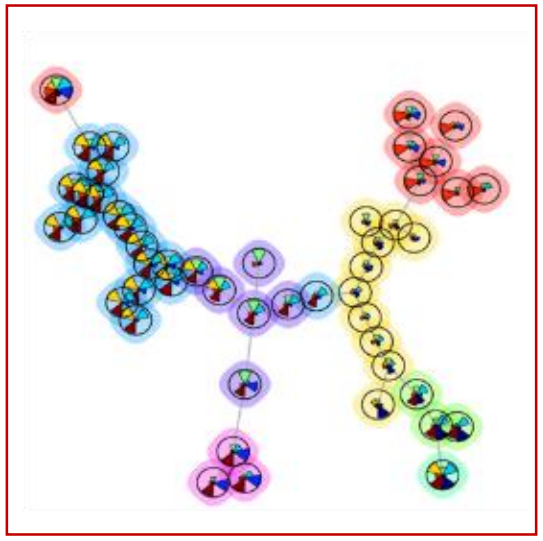
Jolliffe in Lovric M, editor. (2011) International Encyclopedia of Statistical Science

t-Distributed stochastic neighborhood embedding (tSNE)



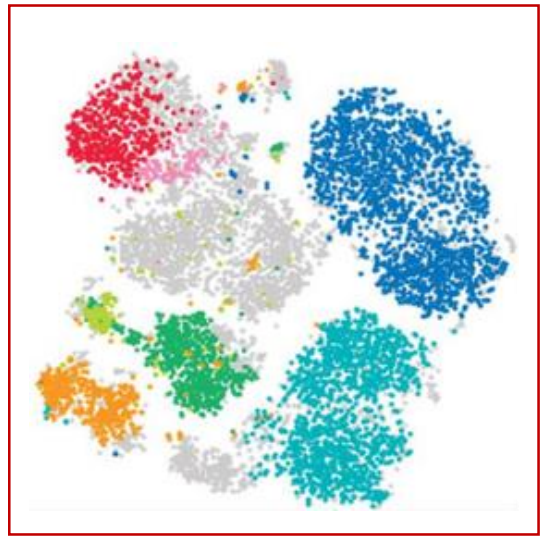
Amir, et al. (2013) Nature biotechnology

FlowSOM



Van Gassen et al. (2015); Cytometry Part A. 87(7):636-45

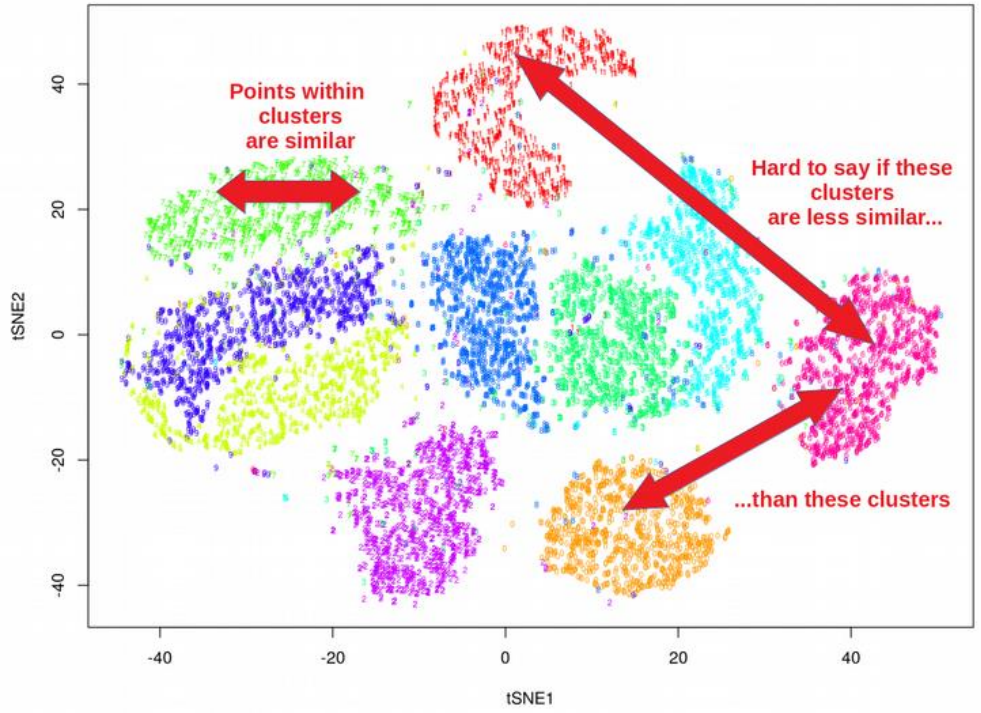
Phenograph



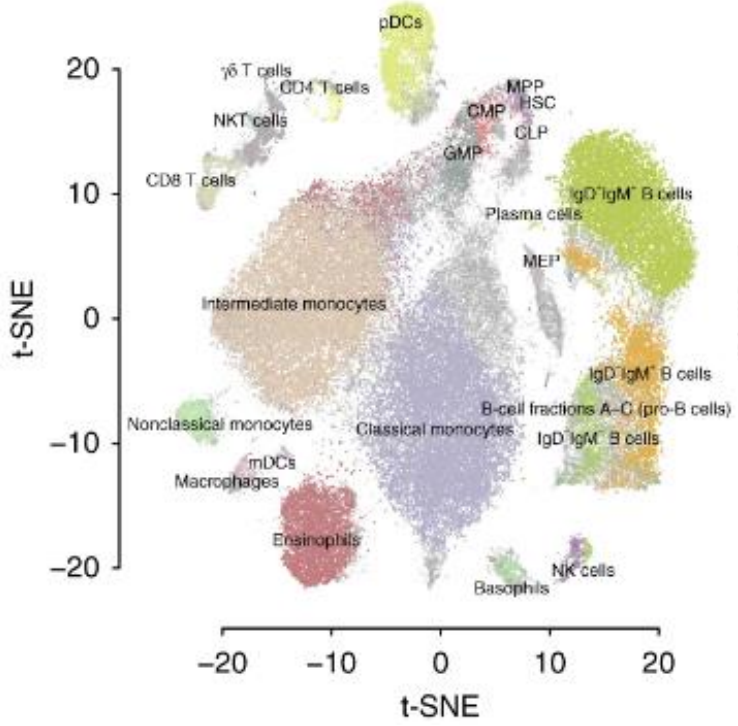
Levine et al. Cell. 2015;162(1):184-97

Dimensionality reduction and clustering algorithms

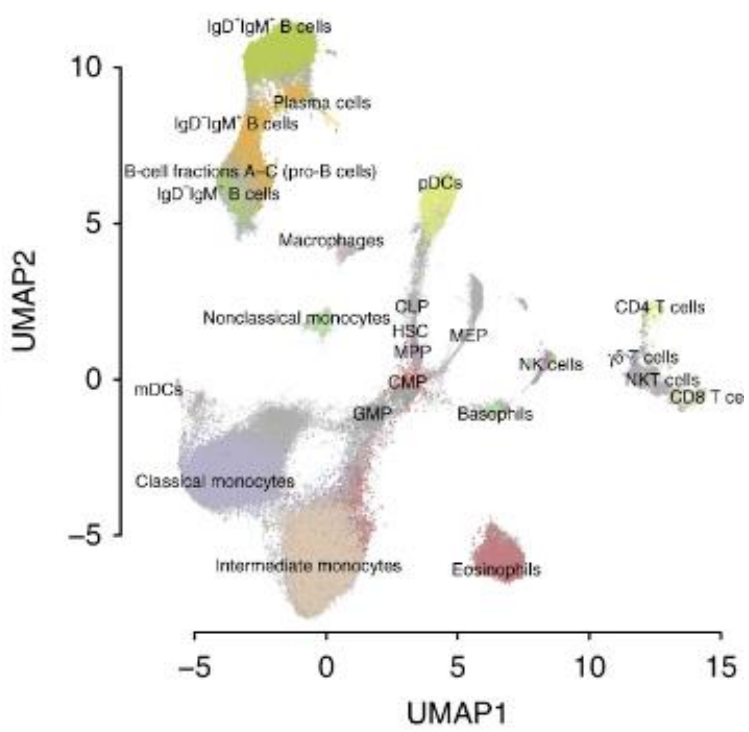
tSNE



tSNE



UMAP:
uniform manifold approximation and projection



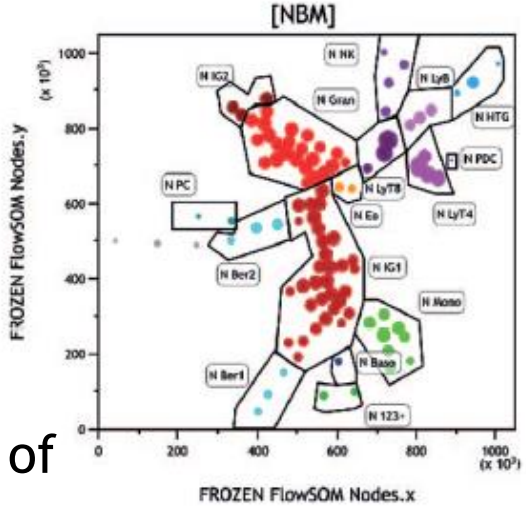
towardsdatascience.com/how-exactly-umap-works-13e3040e1668

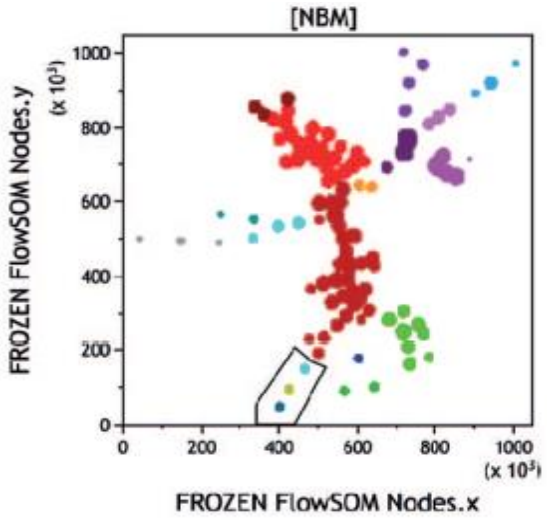
Becht et al. Nature Biotechnology 2018;37:38-44

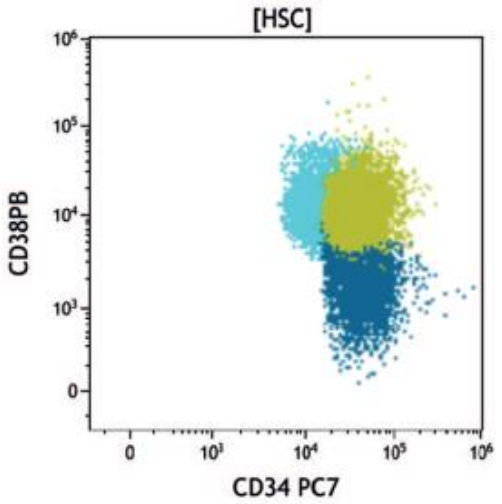
FlowSom

R-scripts were developed to obtain a representation of the global unsupervised multiparametric analysis

FlowSOM provides bidimensional graphic representations of clusters delineated in a highly multidimensional space



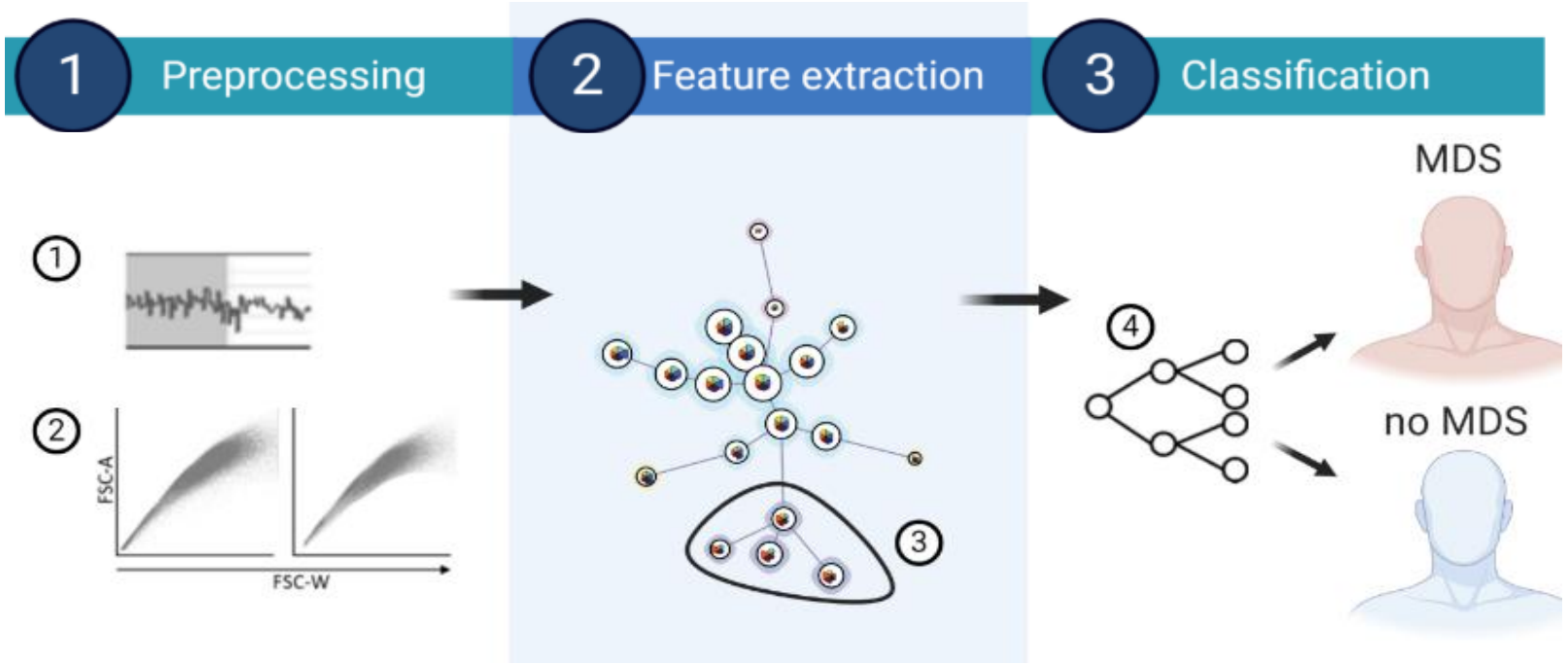




- normal BM subsets vs. AML and AML-MRD
- normal erythropoiesis
- feature selection for diagnosis of (suspected) MDS

Lacombe et al. HemaSphere 2019; 3(2): e173
Bene et al. Hemasphere 2021; 5(1): e512
Duetz, et al. 2021; Cytometry A. 2021;99(8):814-824

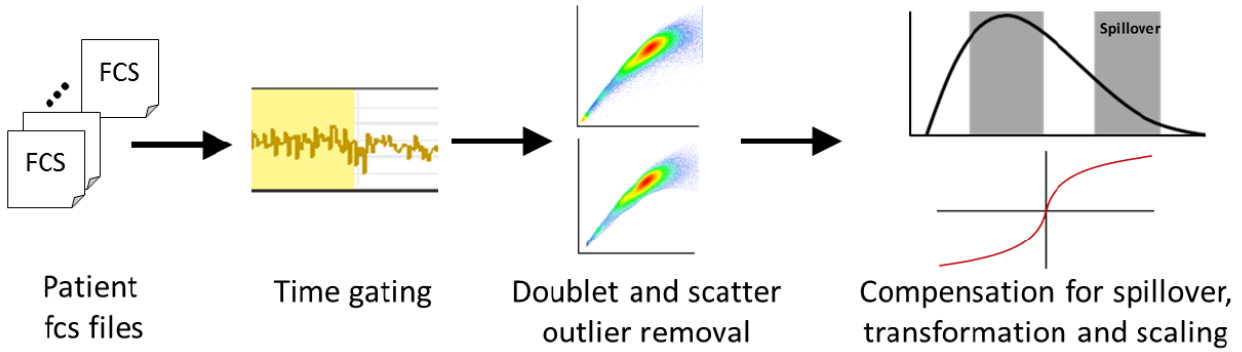
Computational analysis in (suspected) MDS



- Analysis of all markers simultaneously
- Less subjective and biased
- Reproducible
- Time-efficient

Duetz, et al. Curr Opin Oncol. 2020;32:162-169
Duetz, et al. 2021; Cytometry A. 2021;99(8):814-824

Preprocessing of FC data



- **assessing quality of data**
  - time-gating, removal of doublets
  - changes in marker expression in time across samples
  - compensating for spectral overlap

- **transformation of data** (e.g. arcsinh)
  - to turn negative signals and log-normal distribution onto scales for optimal visualization and analysis

- **normalization of data**
  - to harmonize data across samples and experiments, e.g. by scaling.

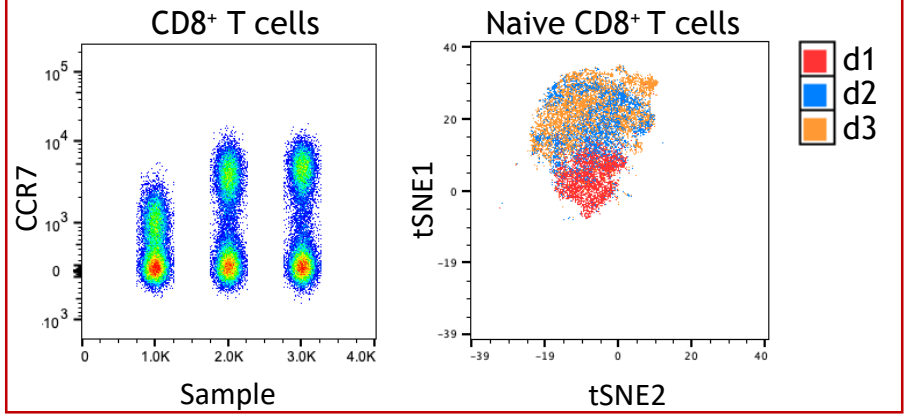
Duetz, et al. Curr Opin Oncol. 2020;32:162-169
Duetz, et al. 2021; Cytometry A. 2021;99(8):814-824

Computational analysis: pitfalls - data quality

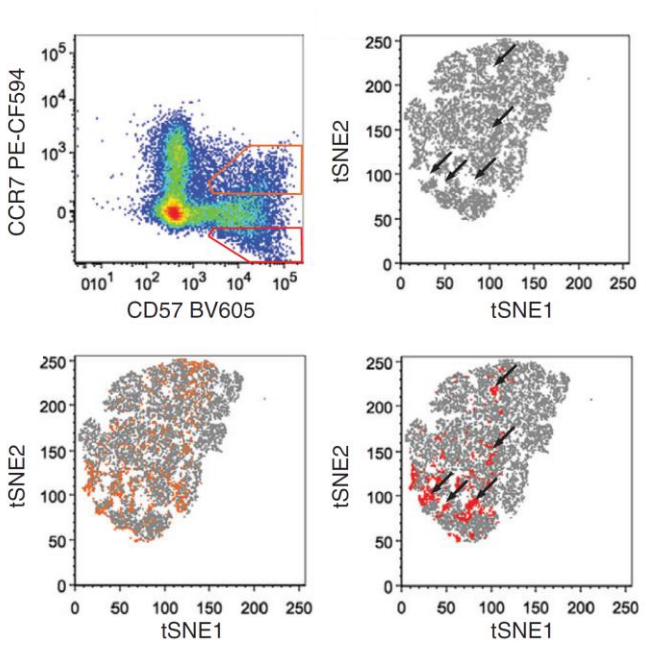
Sources of artifacts

- instrument performance
- batch effect (e.g. Ab lots)
- compensation and spreading errors
- background fluorescence

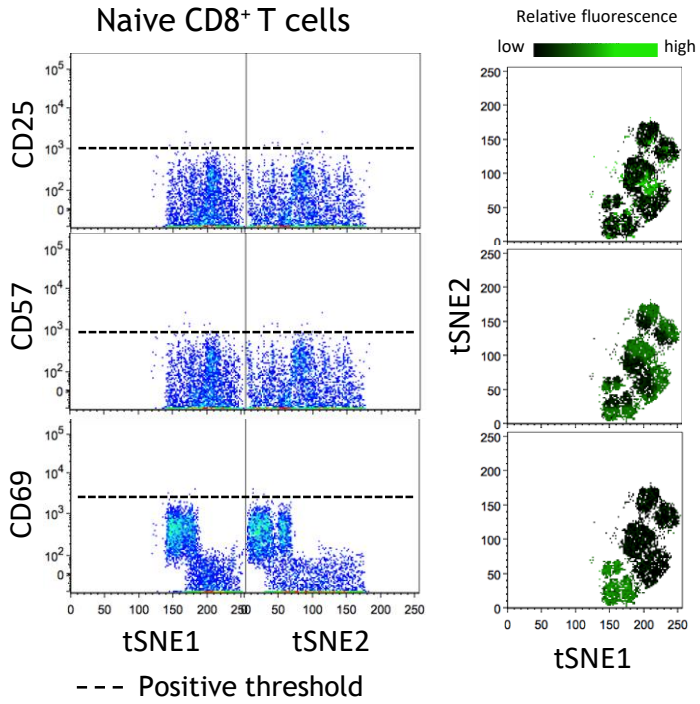
Batch effect (different lot d1)



Spreading error



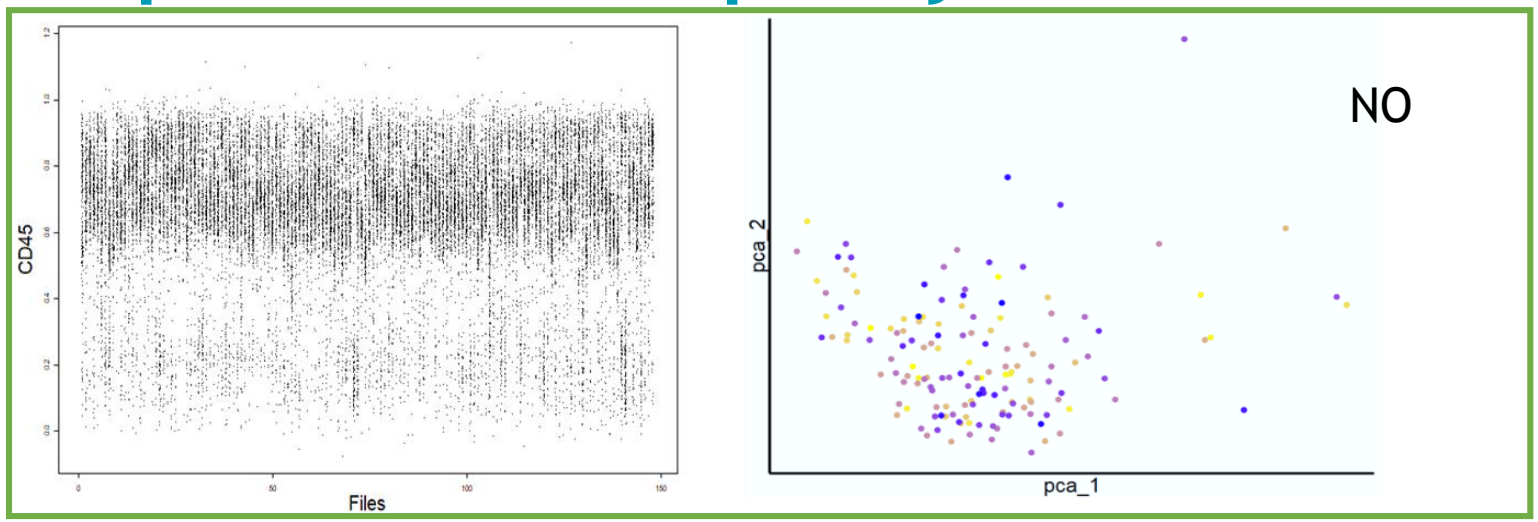
Background fluorescence



Mazza and Brummelman et al. (2018) Cytometry Part A

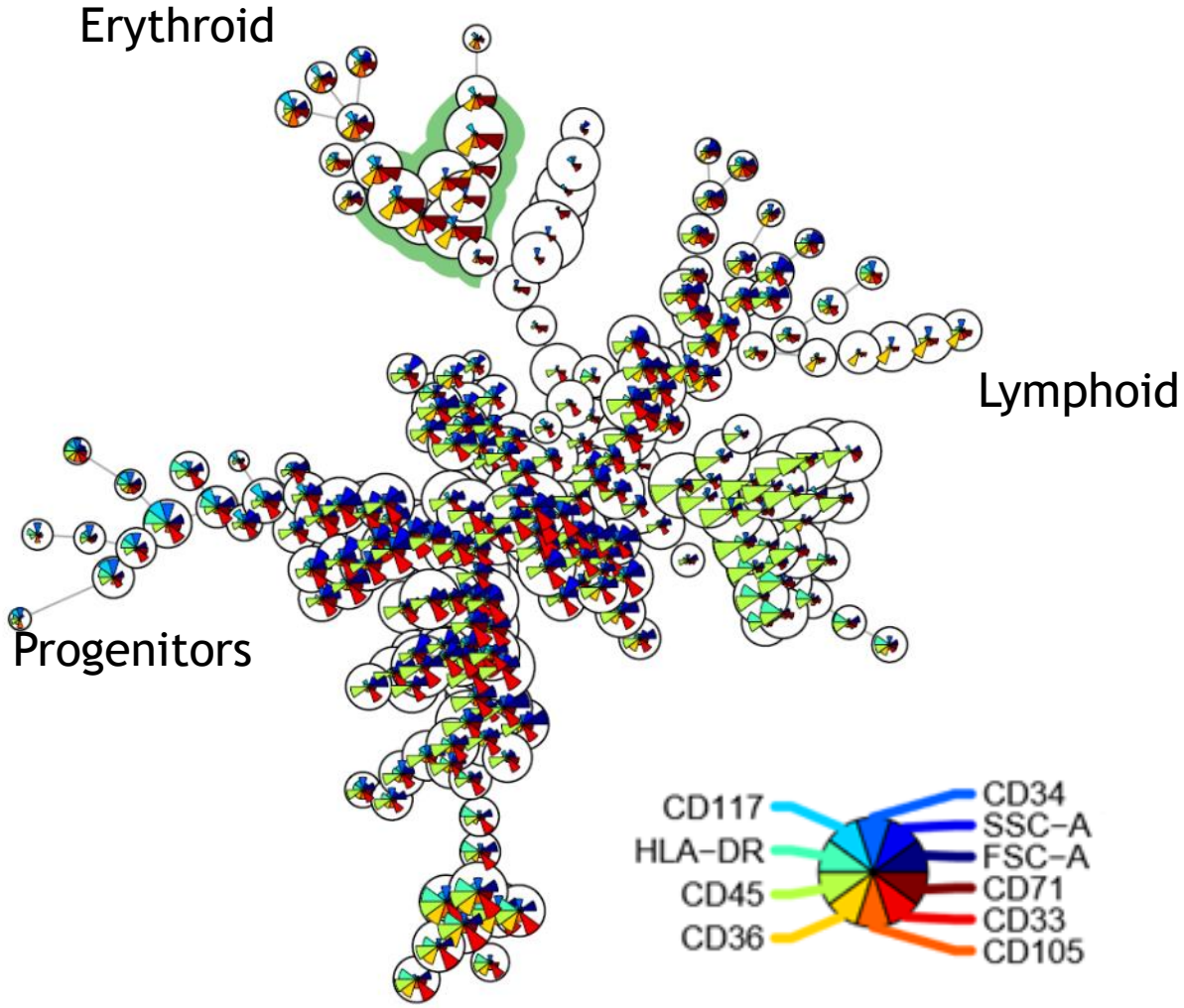
Computational analysis: pitfalls - data quality: variations in time

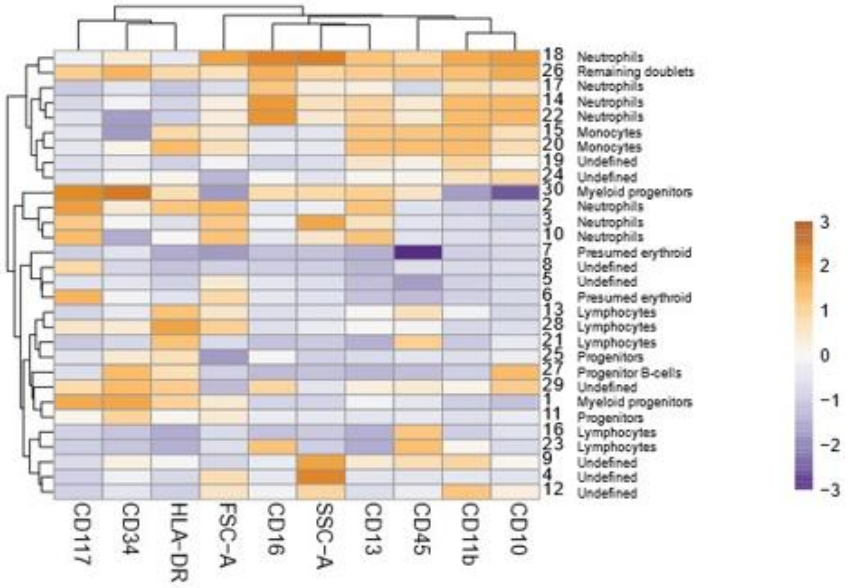
Does sample set show changes in marker expression in time?



Duetz, et al. Curr Opin Oncol. 2020;32:162-169
Duetz, et al. 2021; Cytometry A. 2021;99(8):814-824

Computational analysis in MDS: feature selection





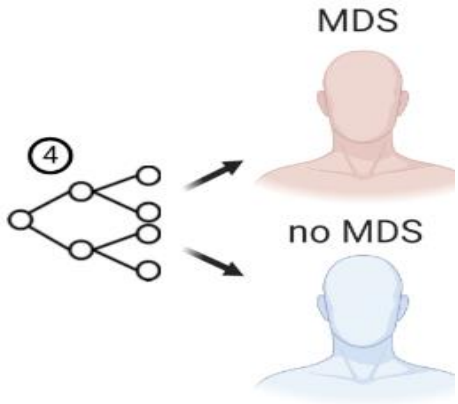
Features selection from metaclusters:

- Relative abundance
- MFI
- CV

Van Gassen et al. (2015) Cytometry part A
Weber et al. (2016) Cytometry part A

Duetz, et al. 2021; Cytometry A. 2021;99(8):814-824

Classification: AI-workflow outperforms expert manual analysis



machine learning – random forest

	manual analysis	single tube (ery)	complete 6-tube panel
Training cohort	sensitivity = 82% specificity = 87%	sensitivity = 85% specificity = 95% AUC = 0.96	sensitivity = 90% specificity = 96% AUC = 0.97
Validation cohort	sensitivity = 80% specificity = 86%	sensitivity = 97% specificity = 95%	sensitivity = 90% specificity = 93%

Duetz et al. Cytometry A. 2021;99(8):814-824

Where to find algorithms and scripts

Software programs such as:

Cytobank, FlowJo, Kaluza (radar plots), …

R-Packages:

Most R-scripts are available from GitHub

- FlowAI, FlowSOM, Phenograph, GMM, …
- MEM (marker enrichment modelling)
- Generic tools for data analysis such as feature selection and machine learning:

  Random Forest, Support Vector Machine, mRMR, GLMnet, …

FlowJo (https://www.flowjo.com/)

Cytobank (https://www.cytobank.org/)

R and R studio (https://www.r-project.org/)

R scripts are available from GitHub (https://github.com/)

Weber et al. Cytometry A. 2016 Dec;89(12):1084-1096
Brummelman et al. (2019) Nature protocols

Unsupervised computational FC in (suspected) MDS: Conclusions and future directions

- encompasses known and unknown cellular features
- high diagnostic accuracy when QC is optimal
- fast results

- (prospective) multi-center validation warranted
    - challenges with scatter harmonization
    - differences in sample preparation
    - exploration of more MDS-specific panels
    - optimization of the computational workflow

- computational analyses may reveal new features/biomarkers that help to predict response to therapy, leukaemic evolution, …

Questions?