From stem cell to blood cell: flow cytometry of the differentiation pathway

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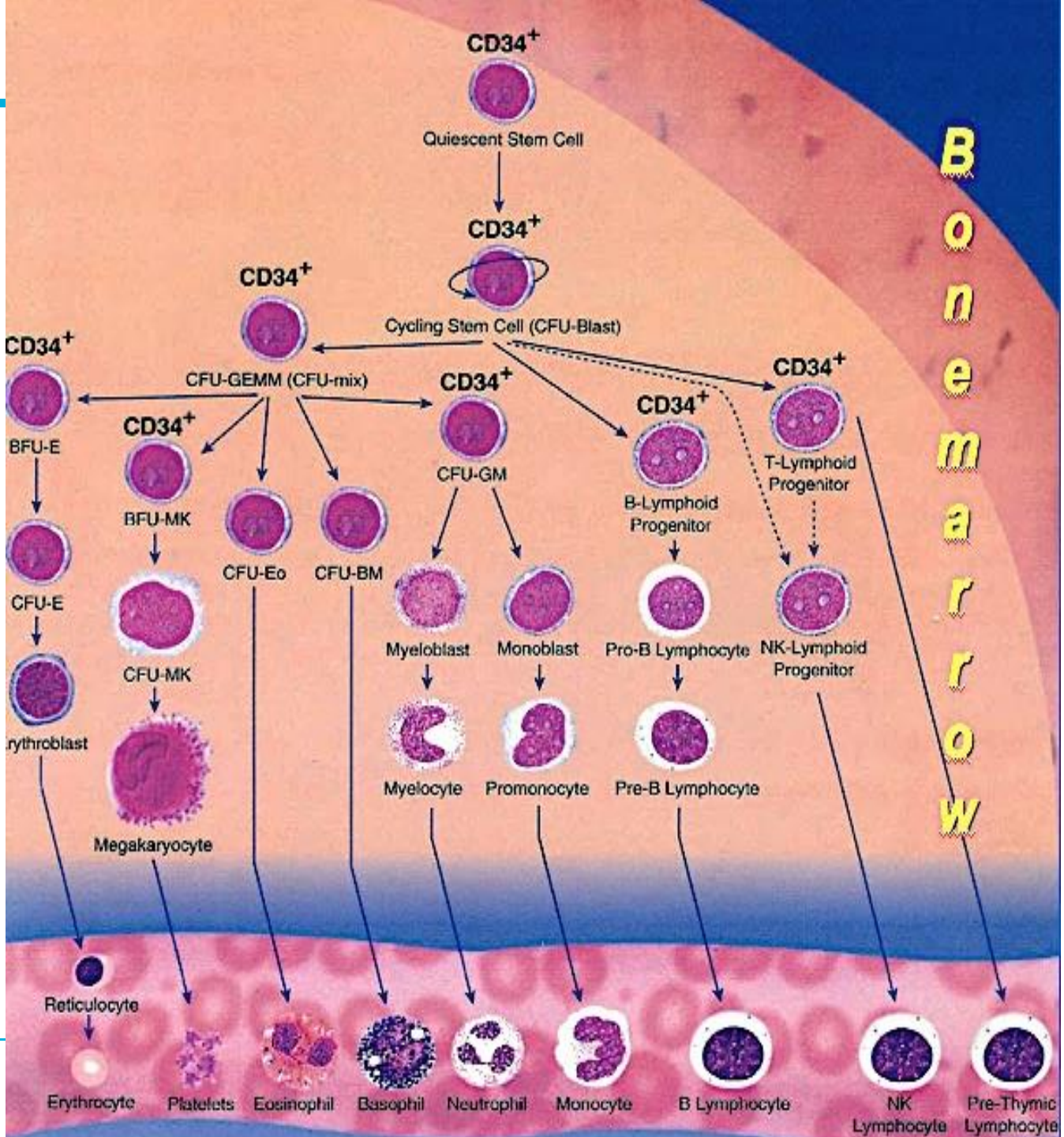
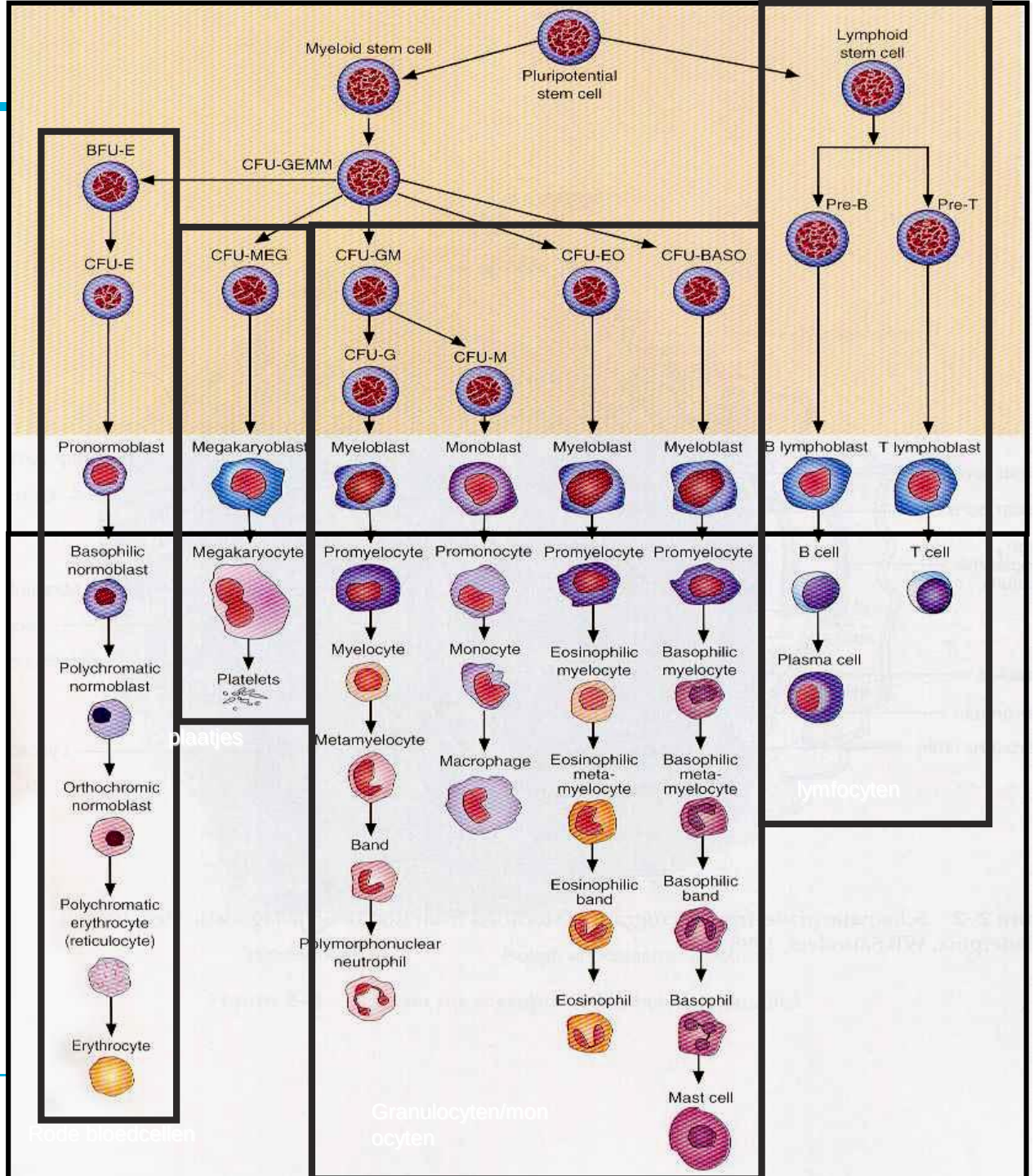
Maturation of Blood cells



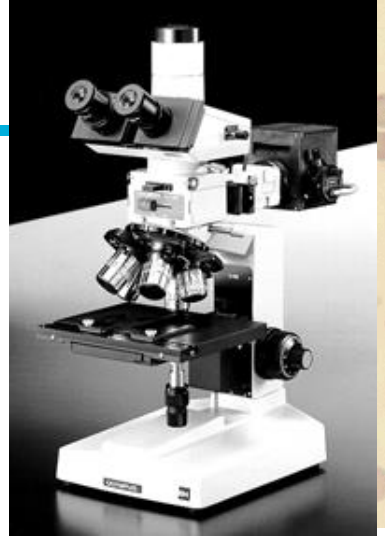
Intensity of hematopoiesis

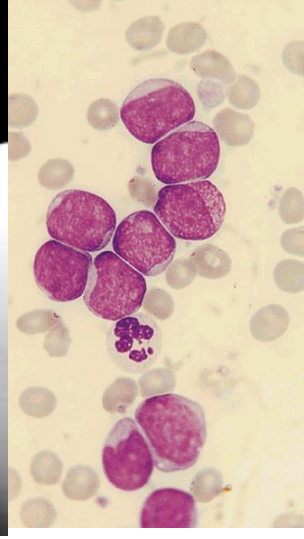
- RBCs: 3.5×10^{11} cells/day
- Neutrophils: 10^{11} cells/day
- Monocytes: 8.4×10^{9} cells/day
- Thrombocytes: 10^{11} cells/day

Maturation of Blood cells

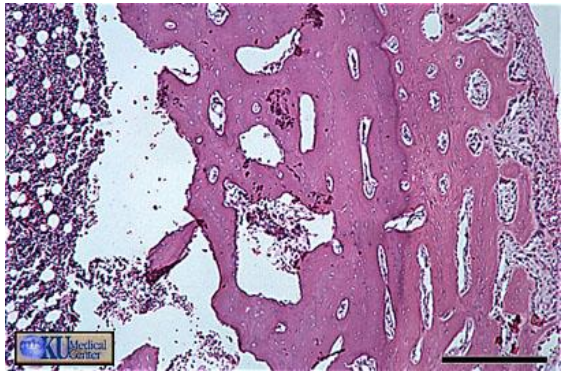


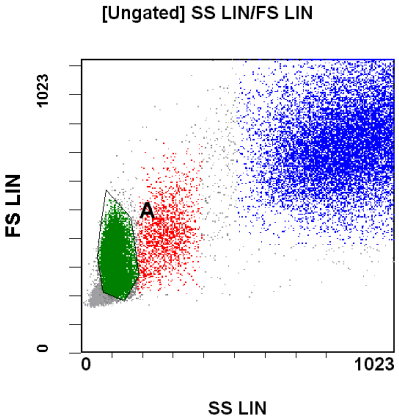
Diagnostic tools to identify cells

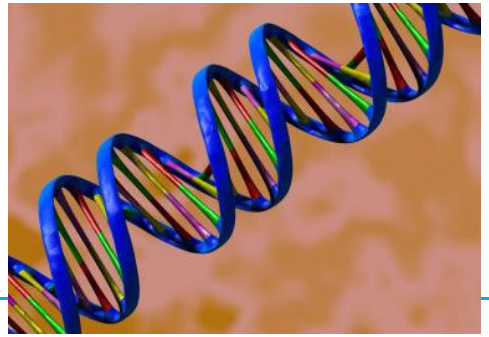




- Morphology
- Cytohisto-chemistry
- Cytogenetics
- Molecular analysis
- ***Immunophenotyping***







Flow Analysis Today

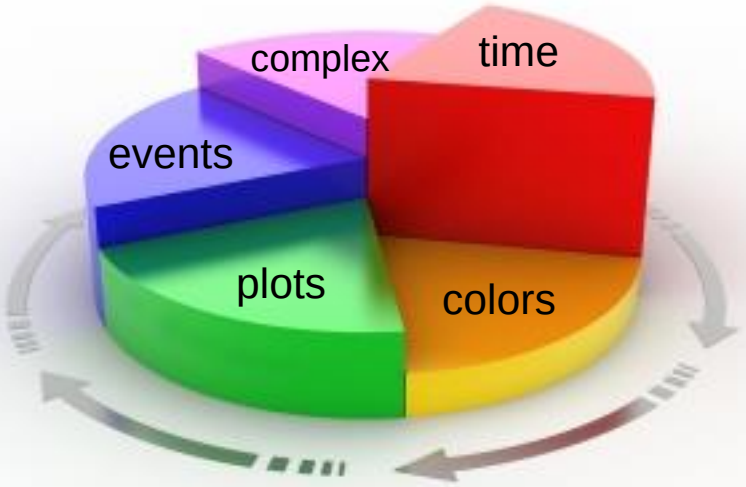
Flow cytometric immunophenotyping anno 2018

- Sophisticated multi-color FCMs

- Bright fluorochromes

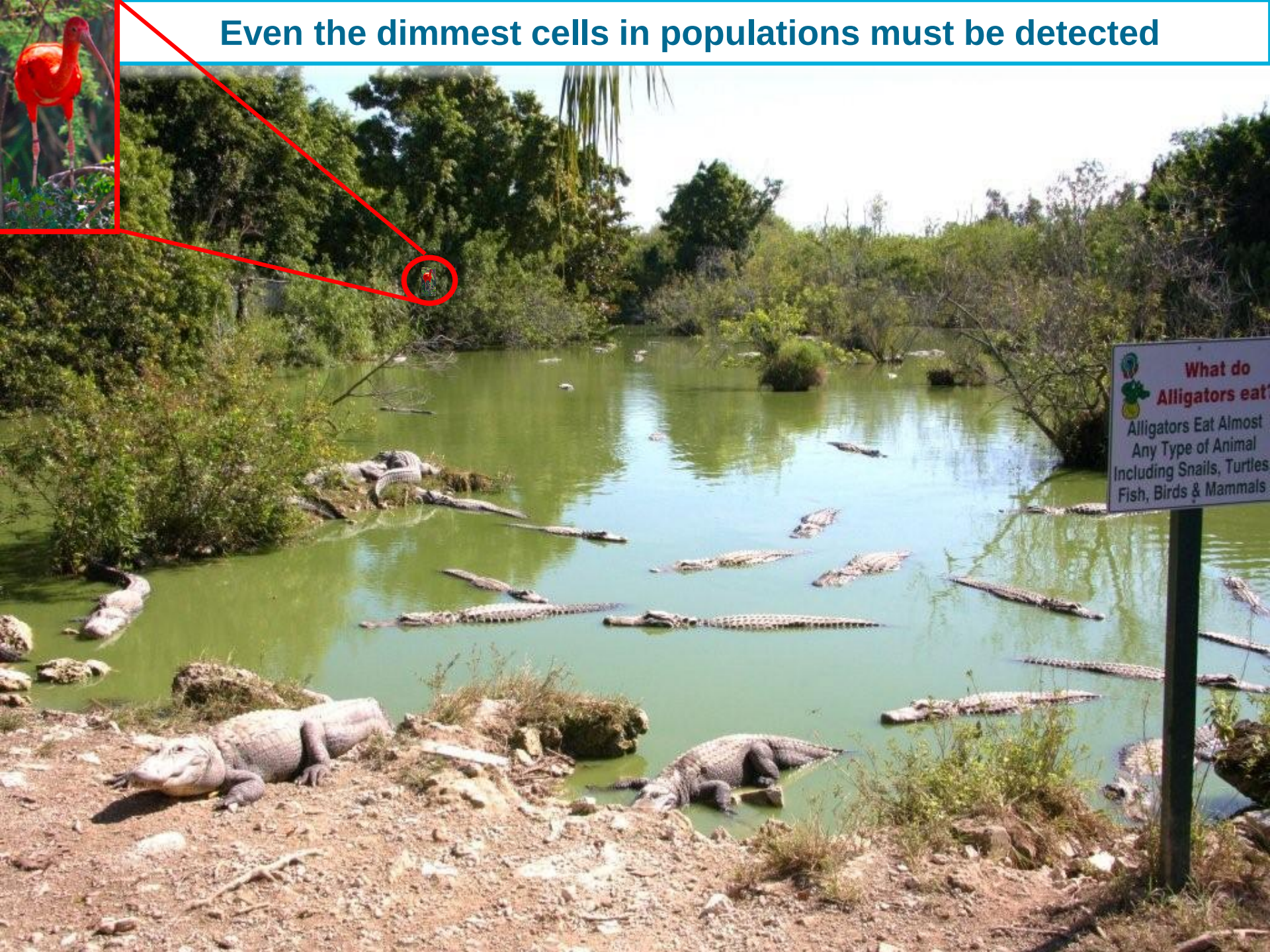
- Highly specific MoAbs

More colors requires more plots, events, protocol complexity and ultimately increased analysis time



How can we control and apply this multi-color FCM??

Even the dimmest cells in populations must be detected

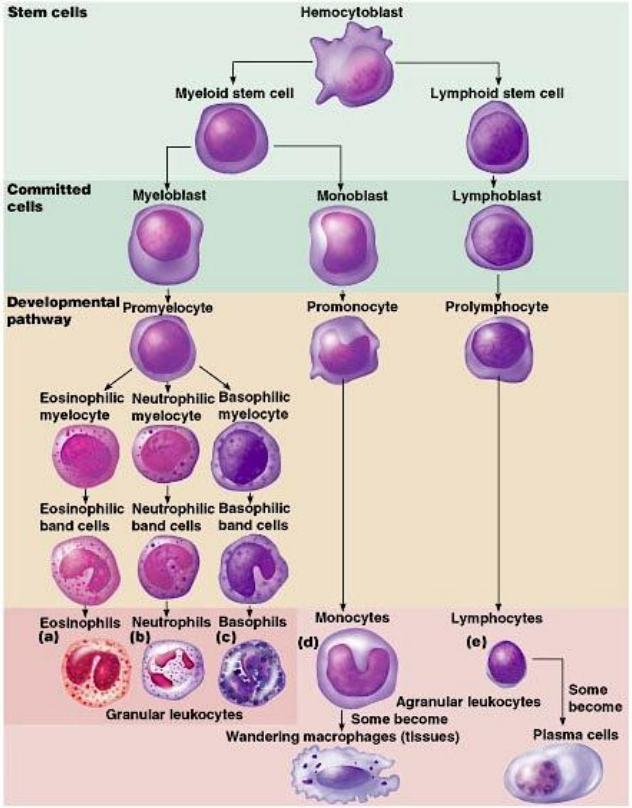


How do you differentiate between normal and malignant populations

- Pattern recognition:
  - what is the normal pattern of expression?
  - What is the aberrant pattern of expression?

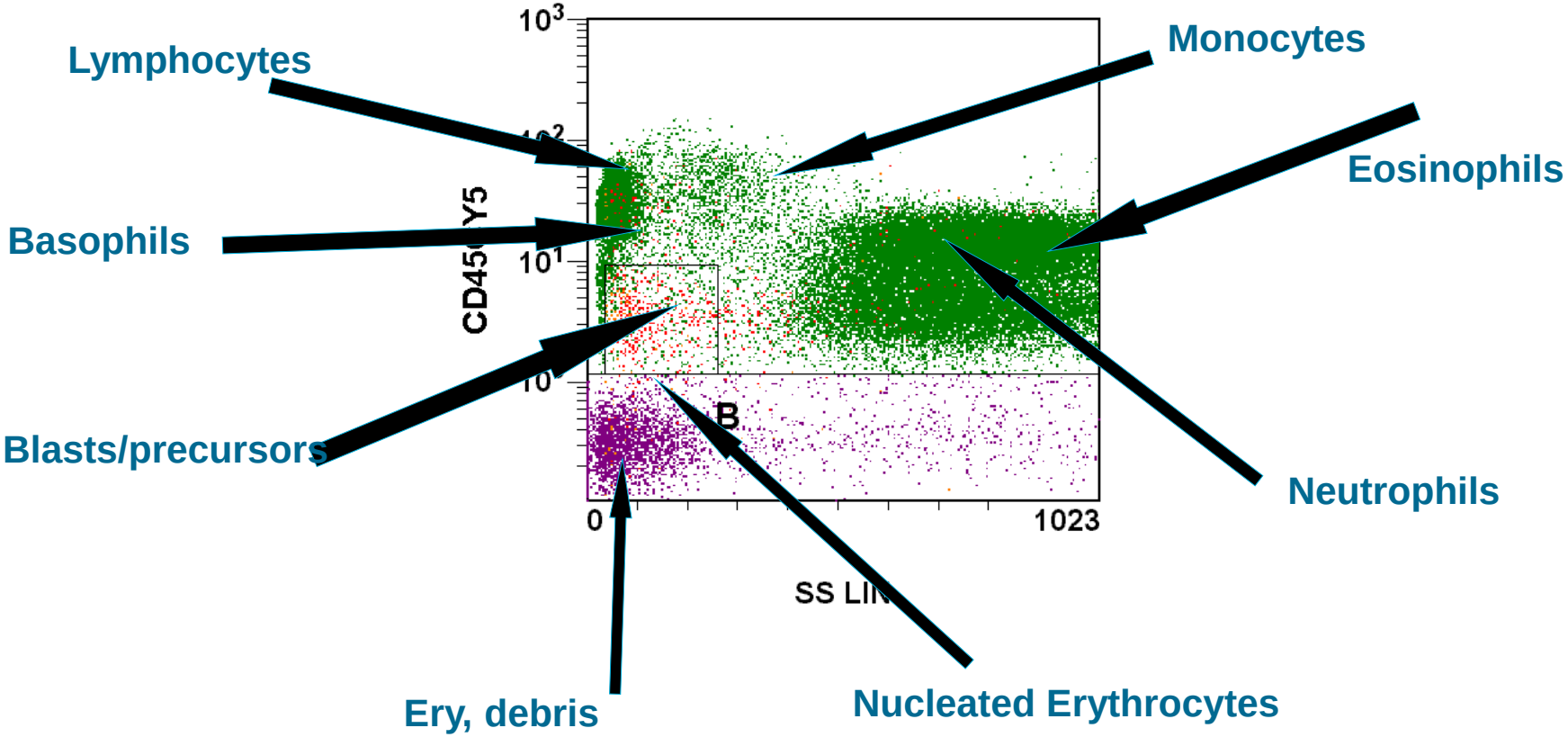
??How to Discriminate between Cells And cell differentiation stages??

How to start??



Cell population in CD45/SS plot of bone marrow

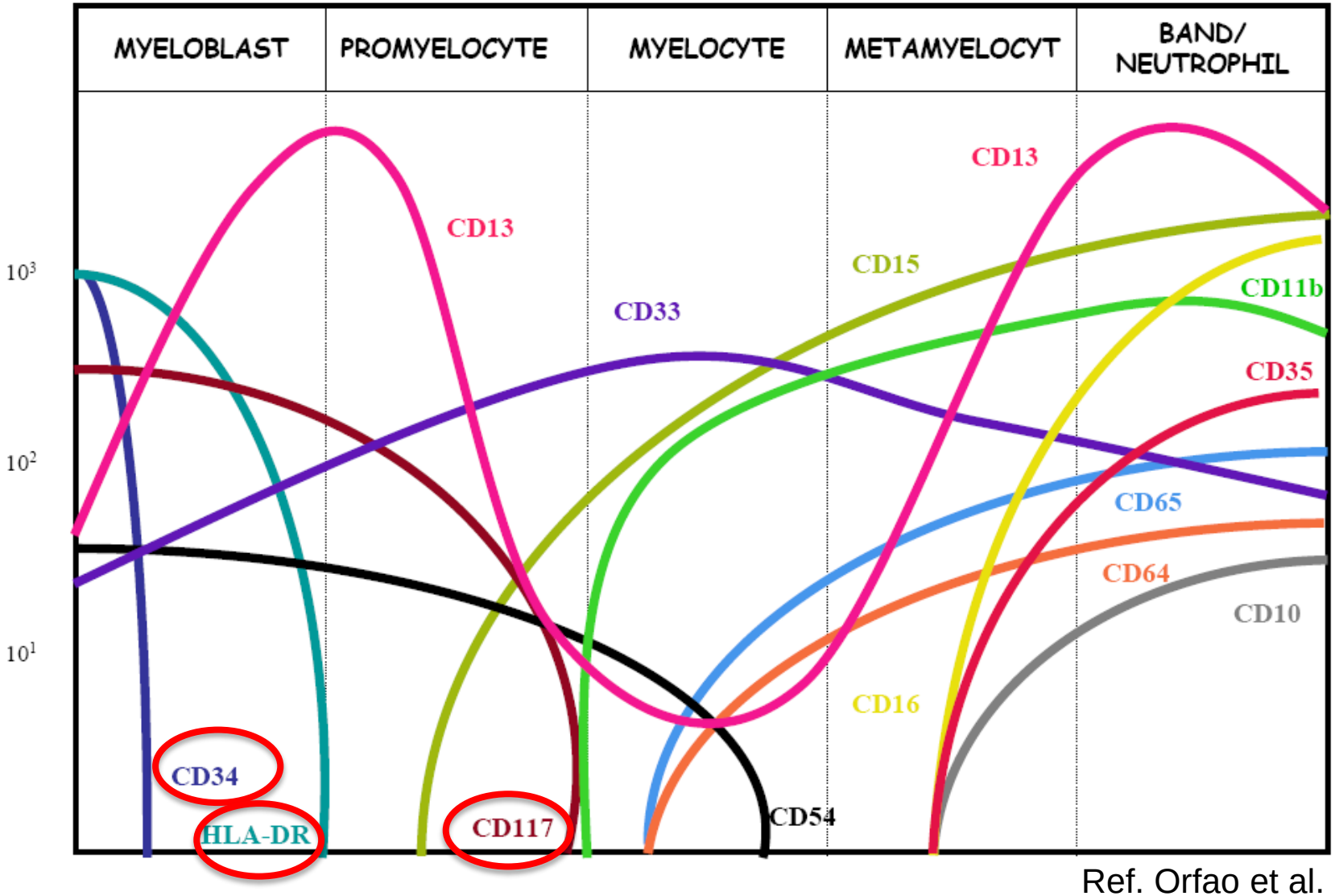
CD45: medium to high Ag density
CD45: available in most dyes



Phenotyping of the Myeloid Lineage to identify the different maturation stages

Search for the myelo-monocytic progenitor cells

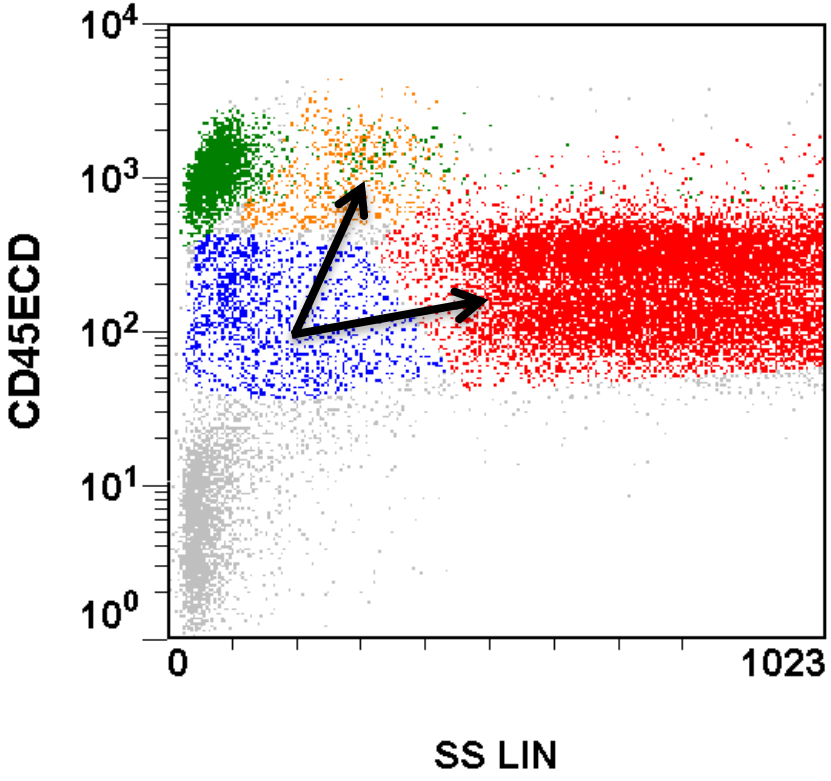
Phenotypic changes in the neutrophilic differentiation pathway

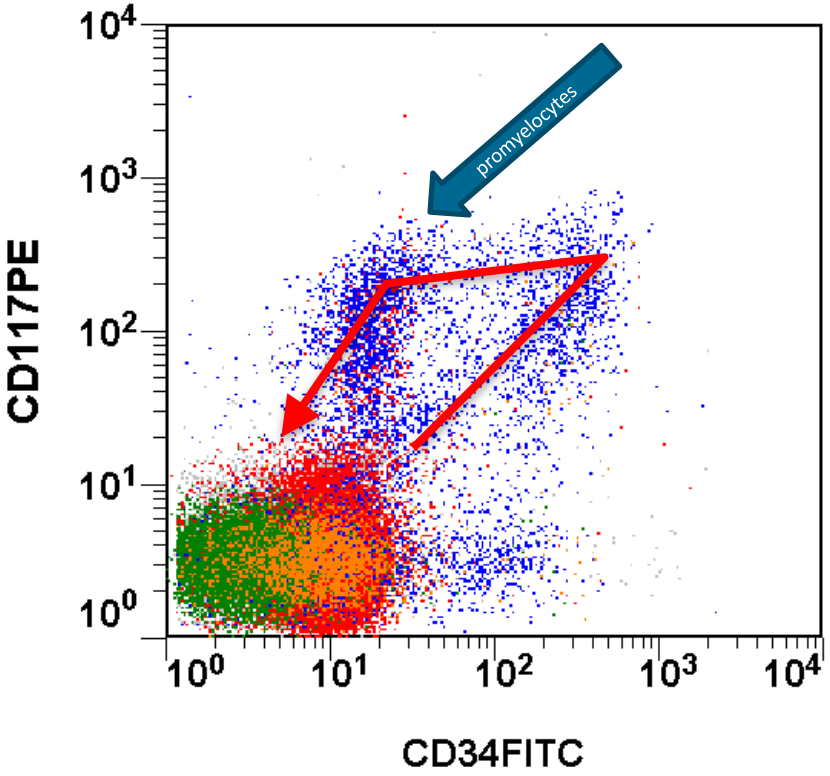


Ref. Orfao et al.

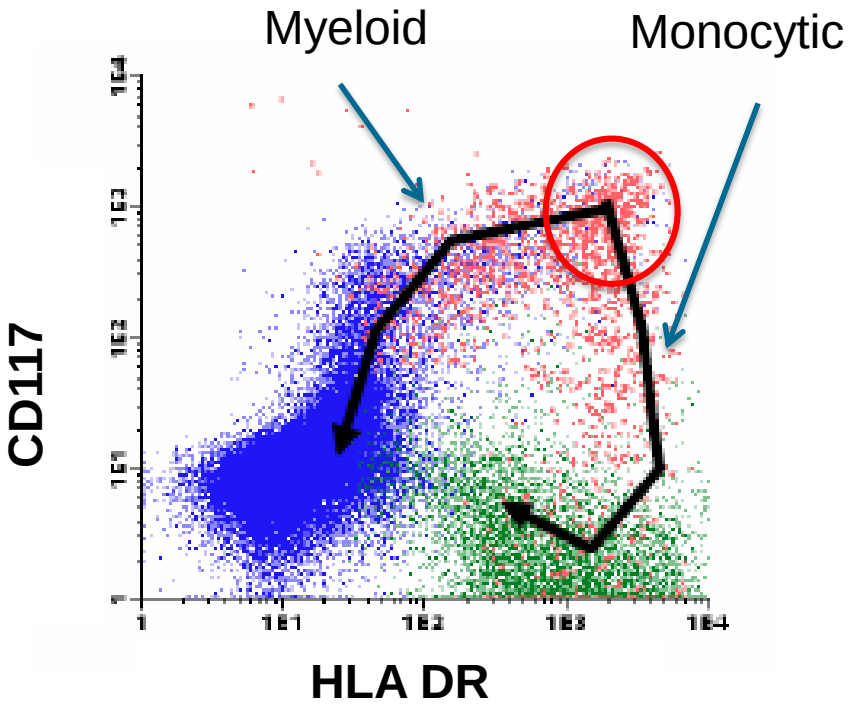
1. Expression pattern of CD34 / CD117 in CD45+ population

Normal bone marrow





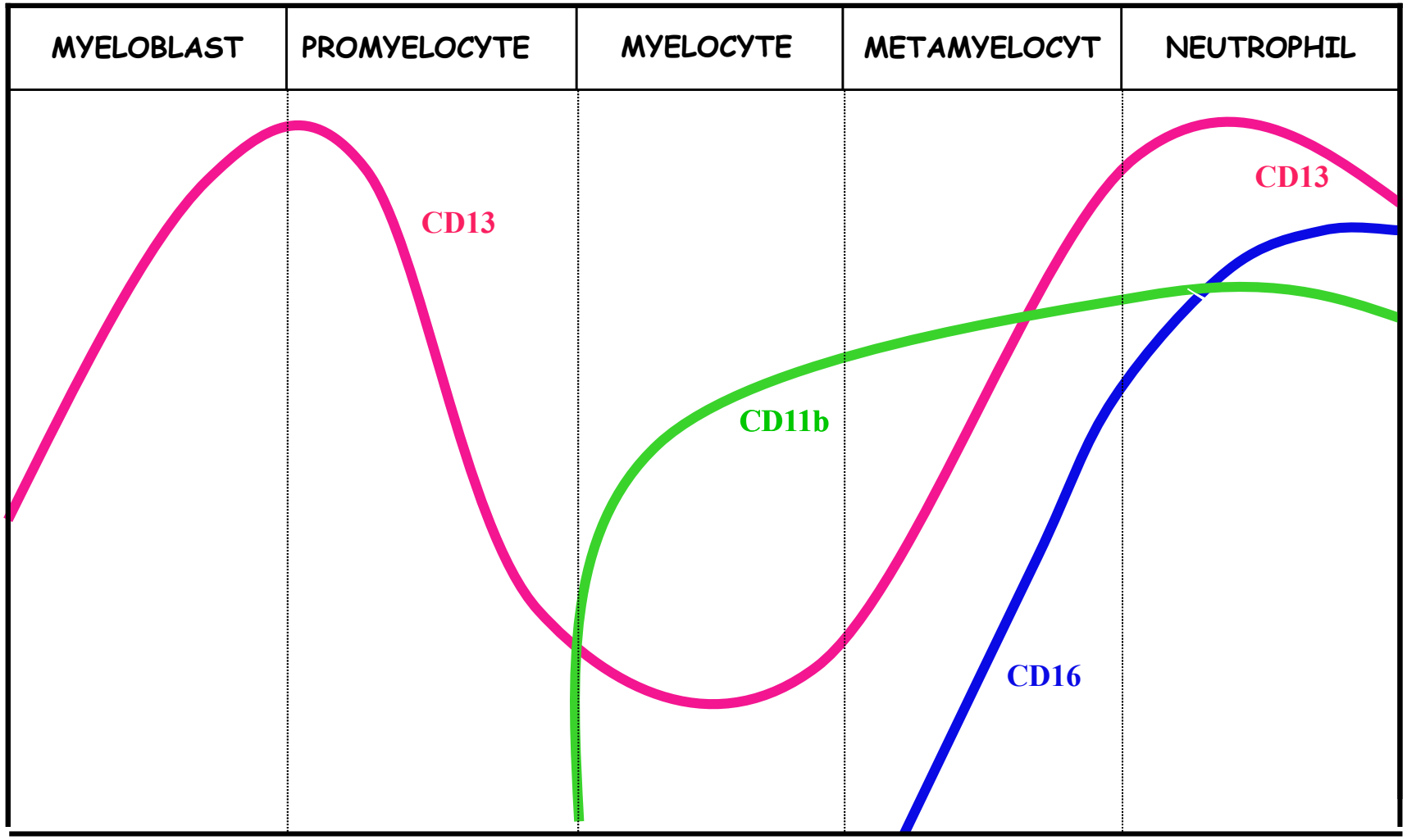
Myelomonocytic differentiation/maturation share the same progenitor cells!



Phenotyping of the Myeloid Lineages

CD11b / CD13 / CD16 / CD45

2. Expression pattern of
CD13, CD16, CD11b within CD45

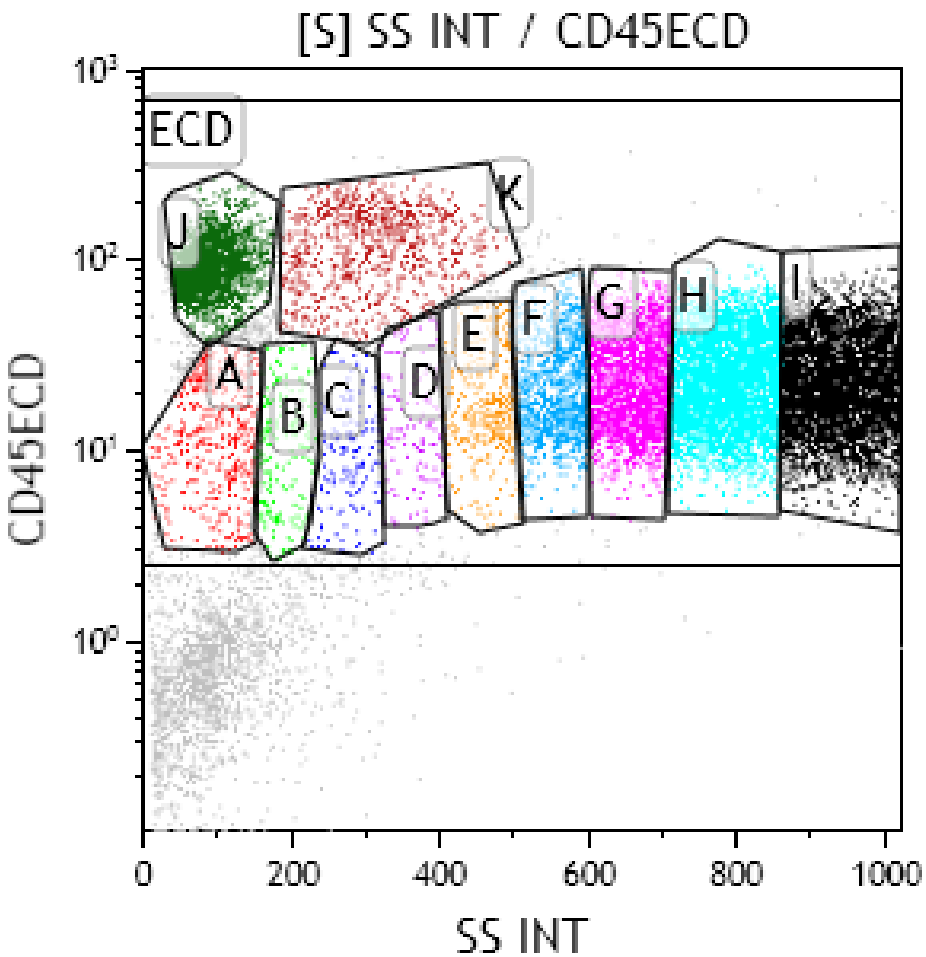


Ref. A. Orfao et al

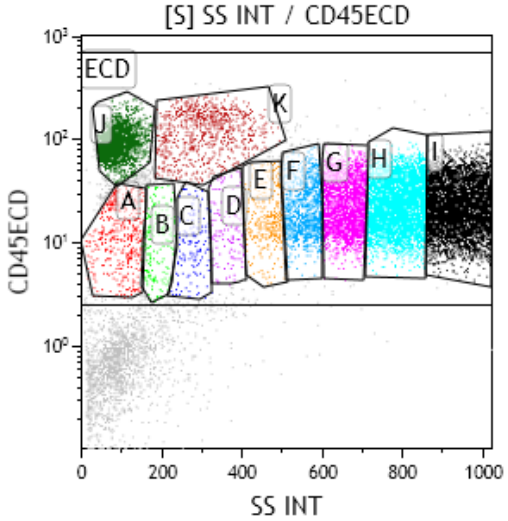
2.I. Expression pattern CD13/CD16/CD11b (normal BM)

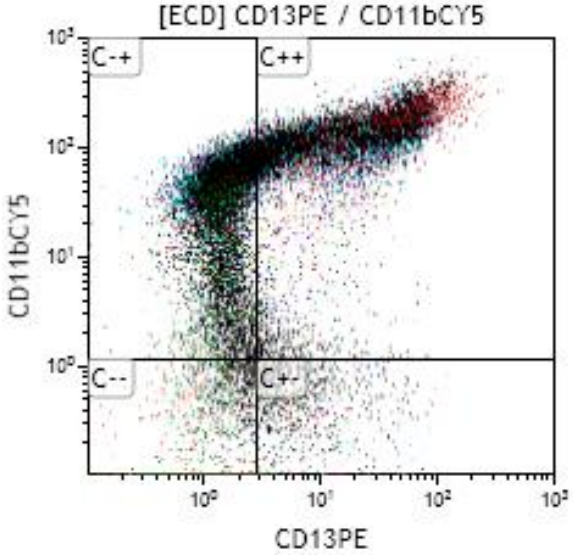
Task:

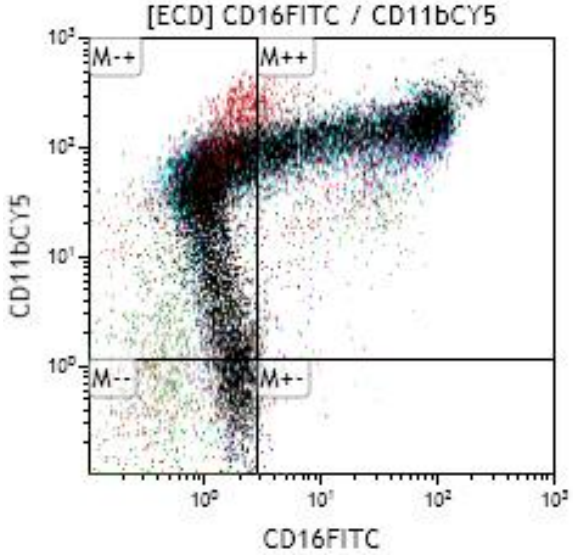
1. Where can we find the myeloid maturation stages?
2. Where are the mono-myeloid progenitors?

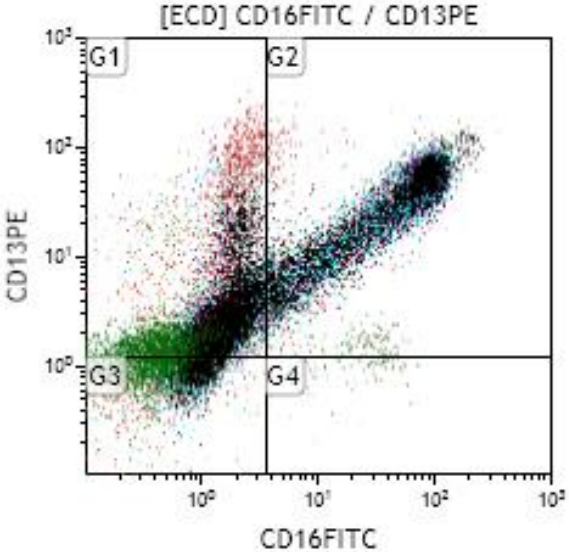


2.I. Expression pattern CD13/CD11b/CD16 (normal BM)









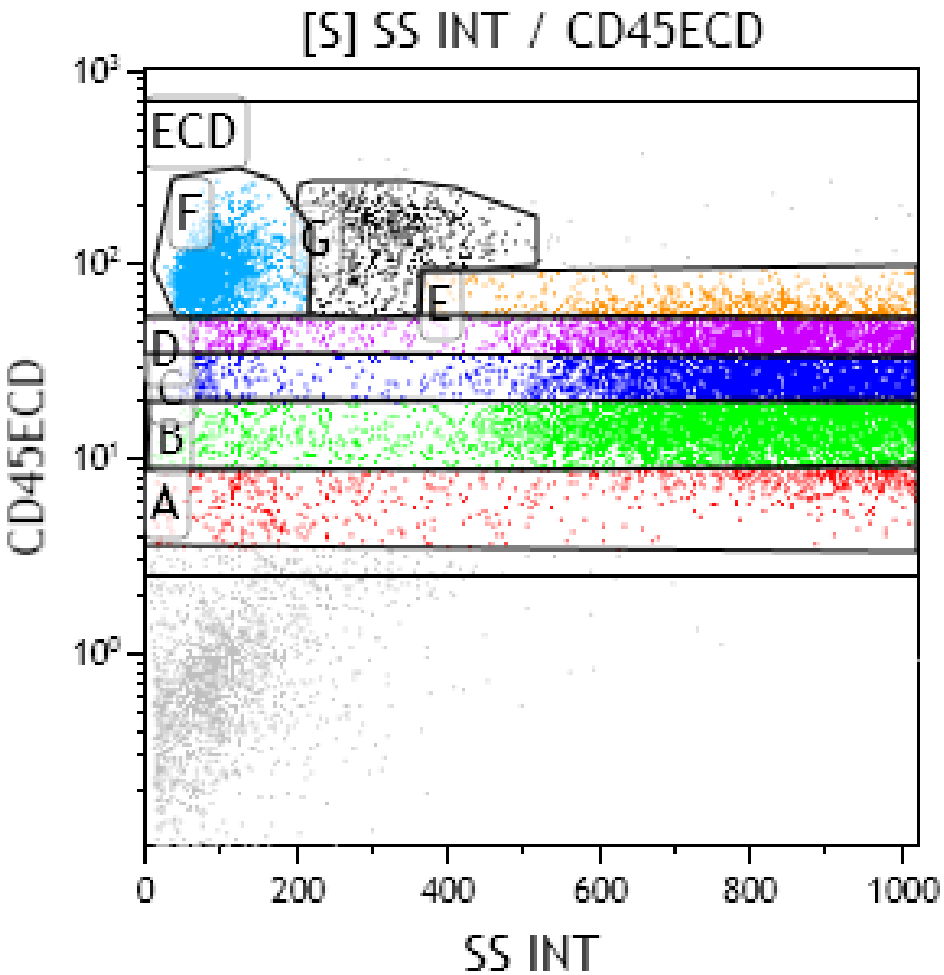
2.II. Expression pattern CD13/CD16/CD11b (normal BM)

Horizontal gates:

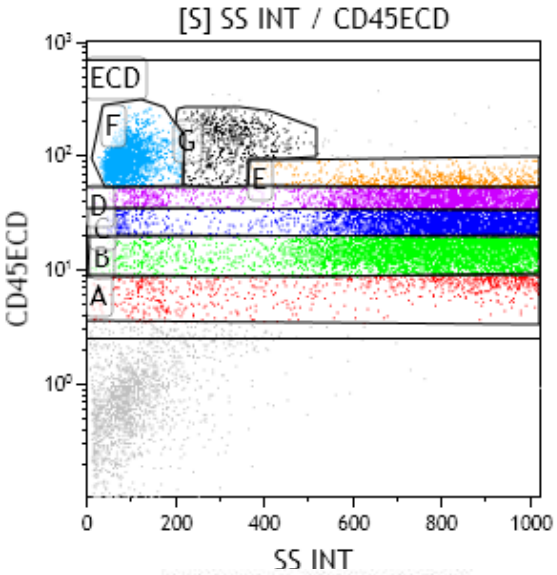
Better gating option???

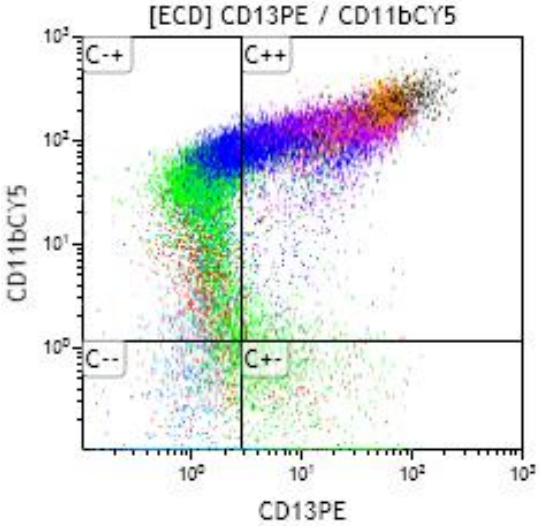
Task:

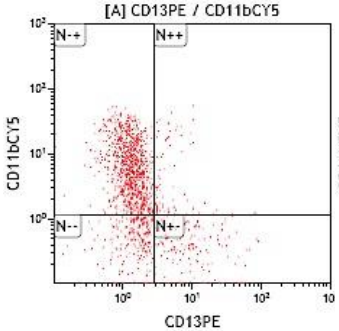
1. Where can we find the myeloid maturation stages?
2. Where are the mono-myeloid progenitors?

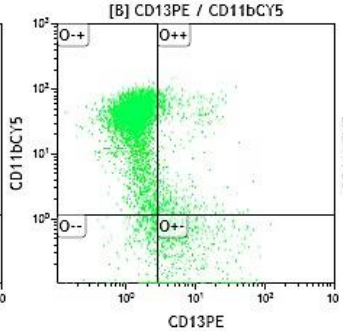


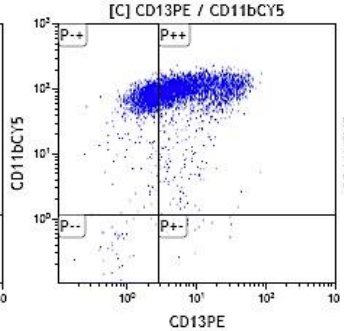
2.II.a. Expression pattern CD13/CD11b (normal BM)

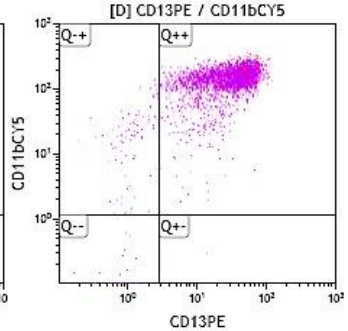


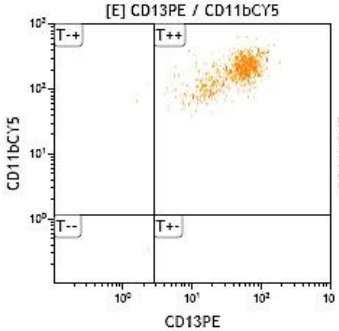


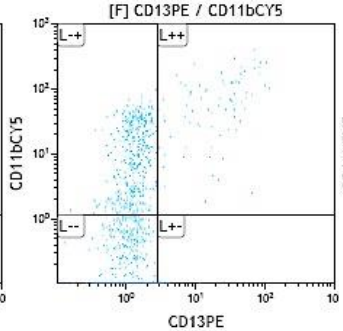


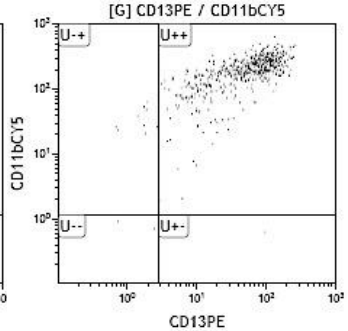




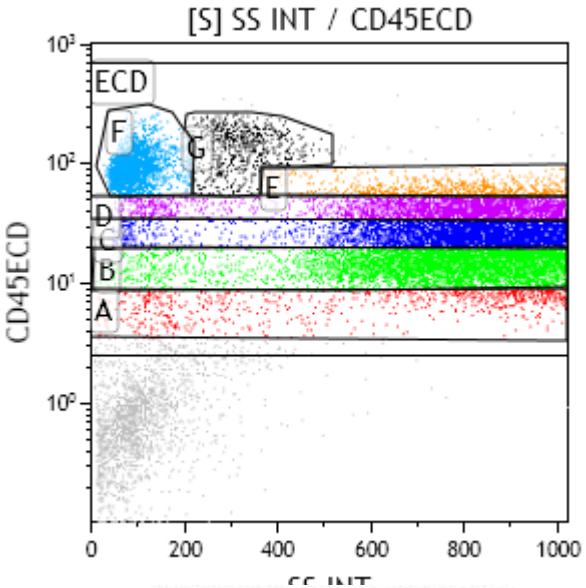


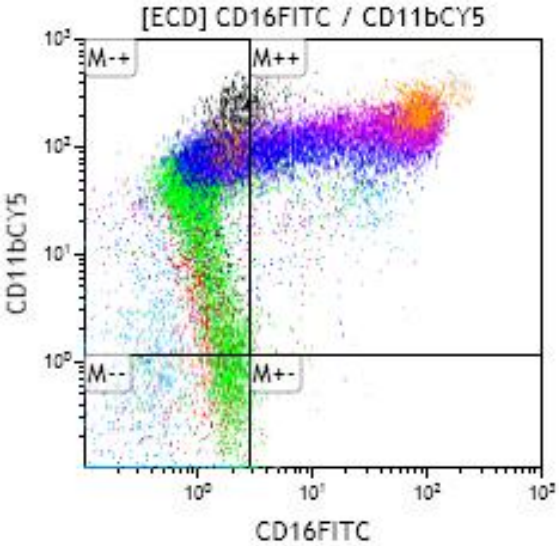


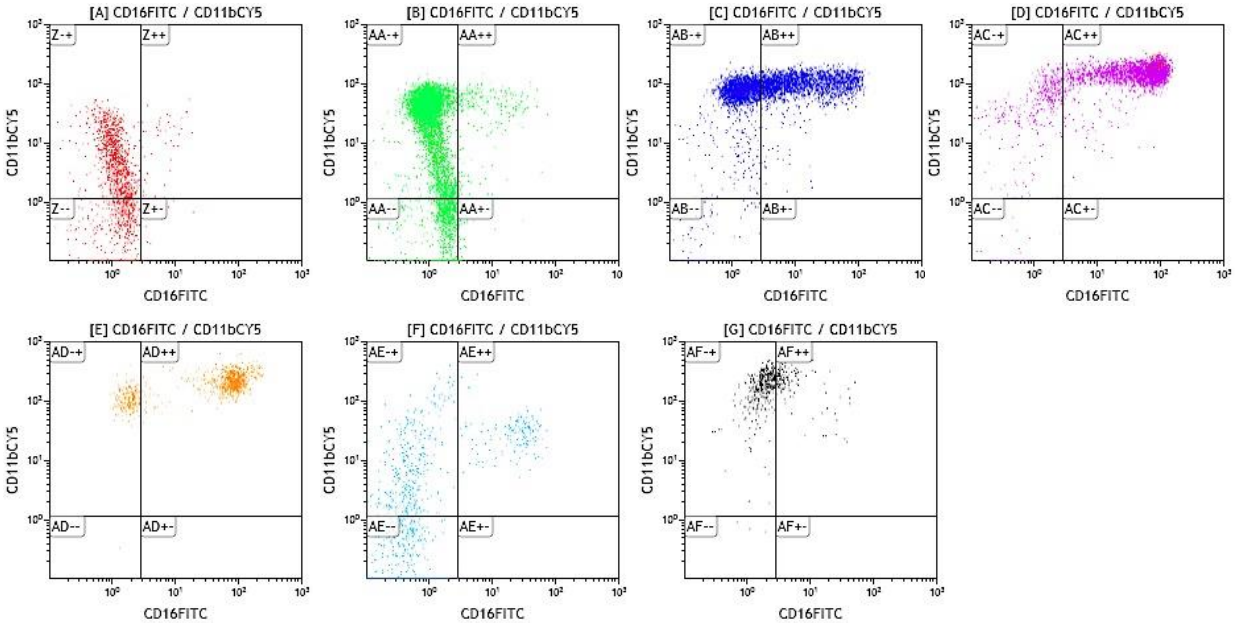




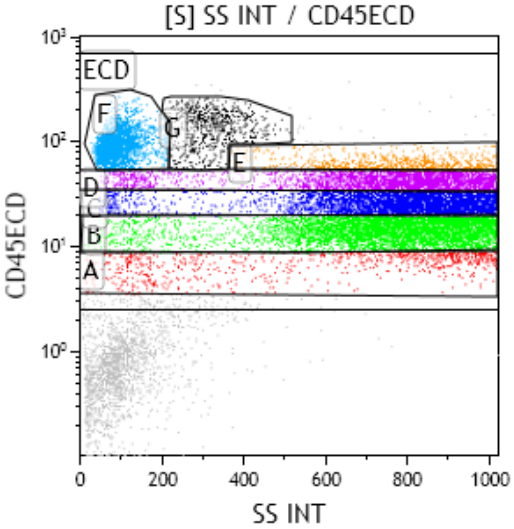
2.II.b. Expression pattern CD16/CD11b (normal BM)

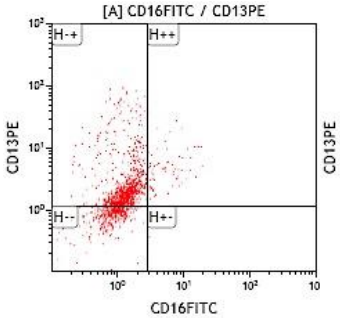


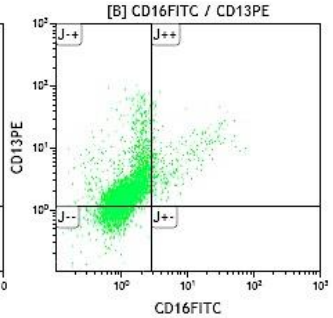


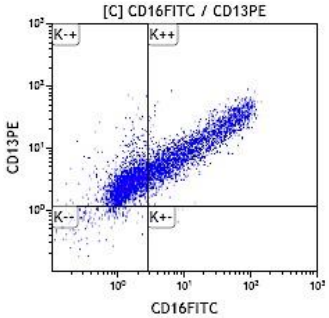


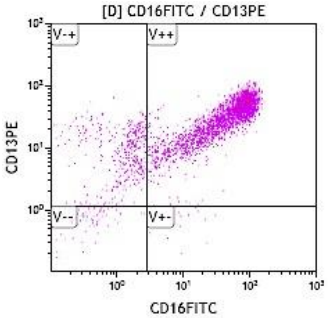
2.II.c. Expression pattern CD13/CD16 (normal BM)

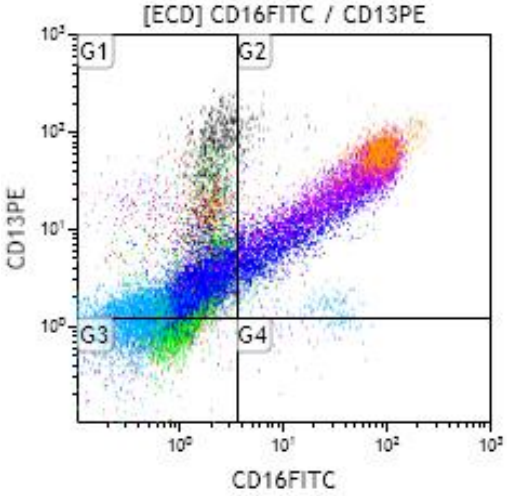


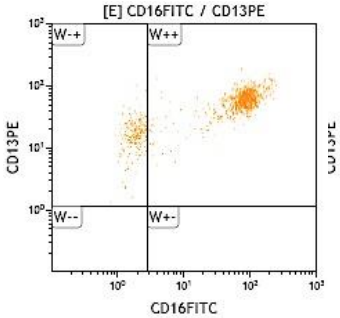


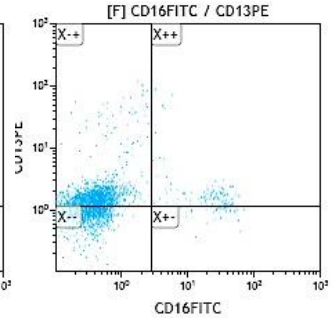


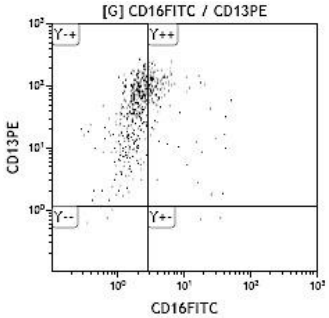




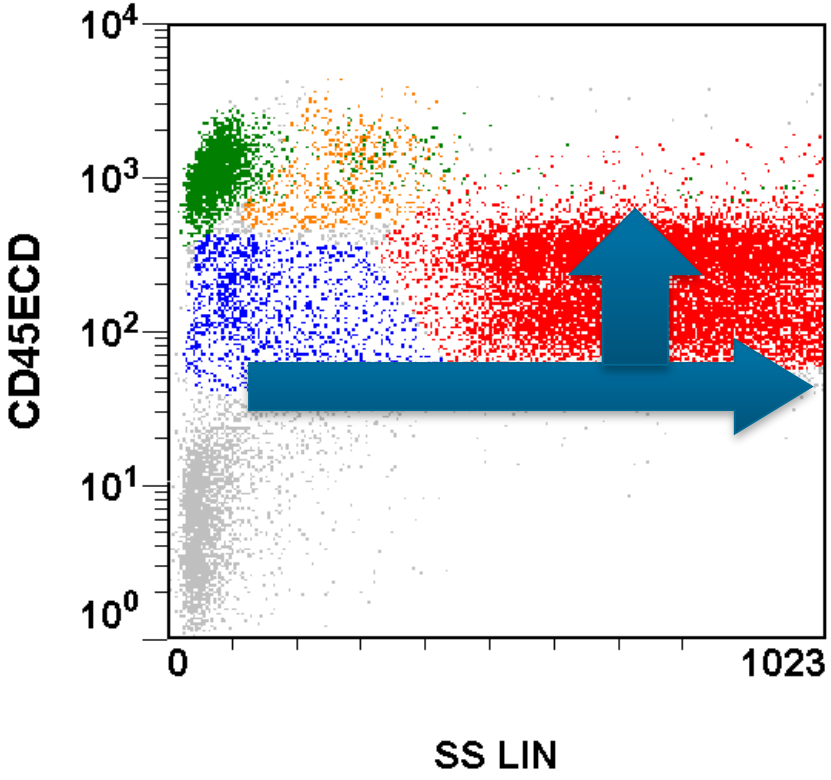








Conclusion
(myeloid differentiation pattern in CD45)

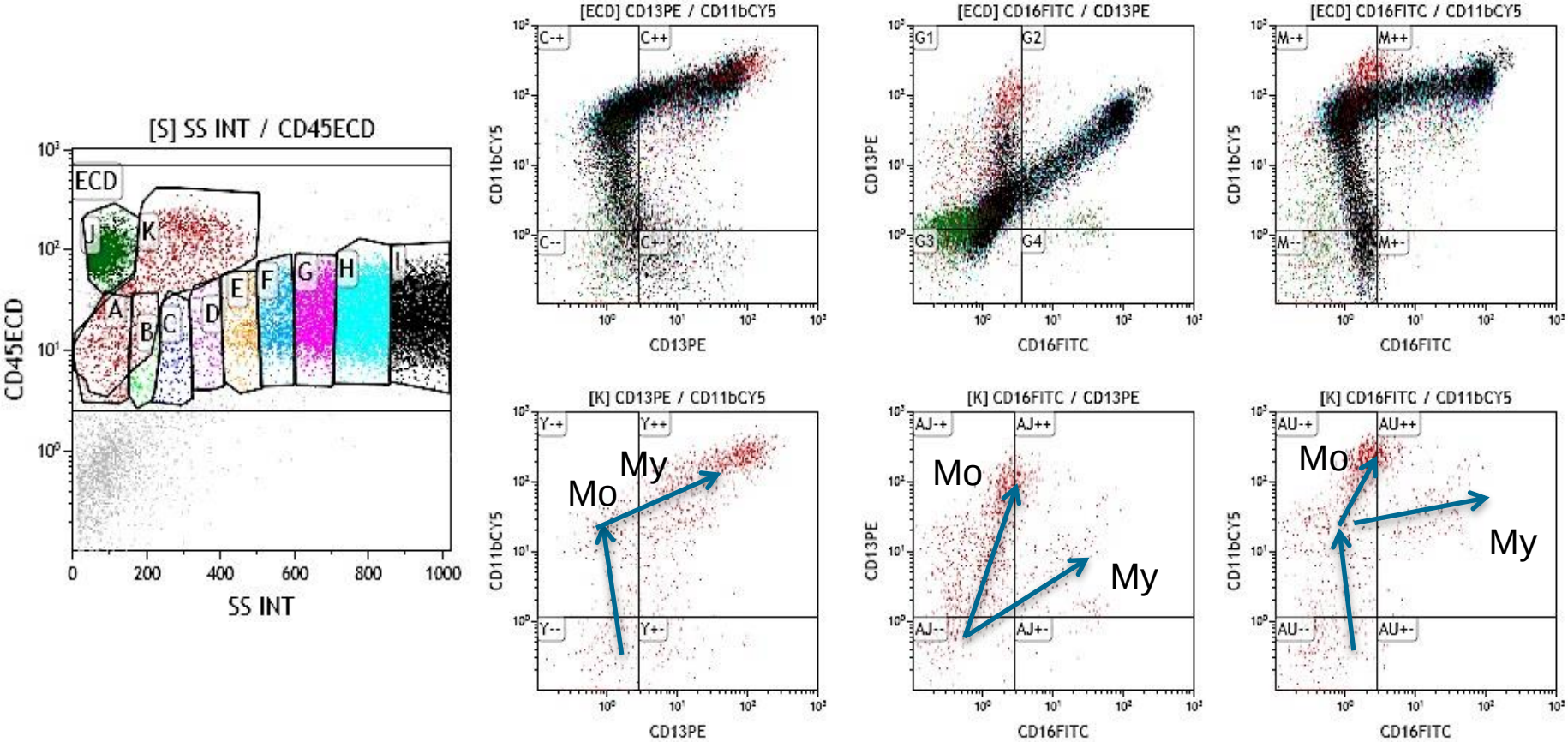


Phenotyping of the Monocytic Lineages CD11b / CD13 / CD16 / CD45

Searching for the myelo-monocytic progenitor cells

2.III. Monocyte + precursors gating: normal bone marrow

One gate: monocytes and precursors

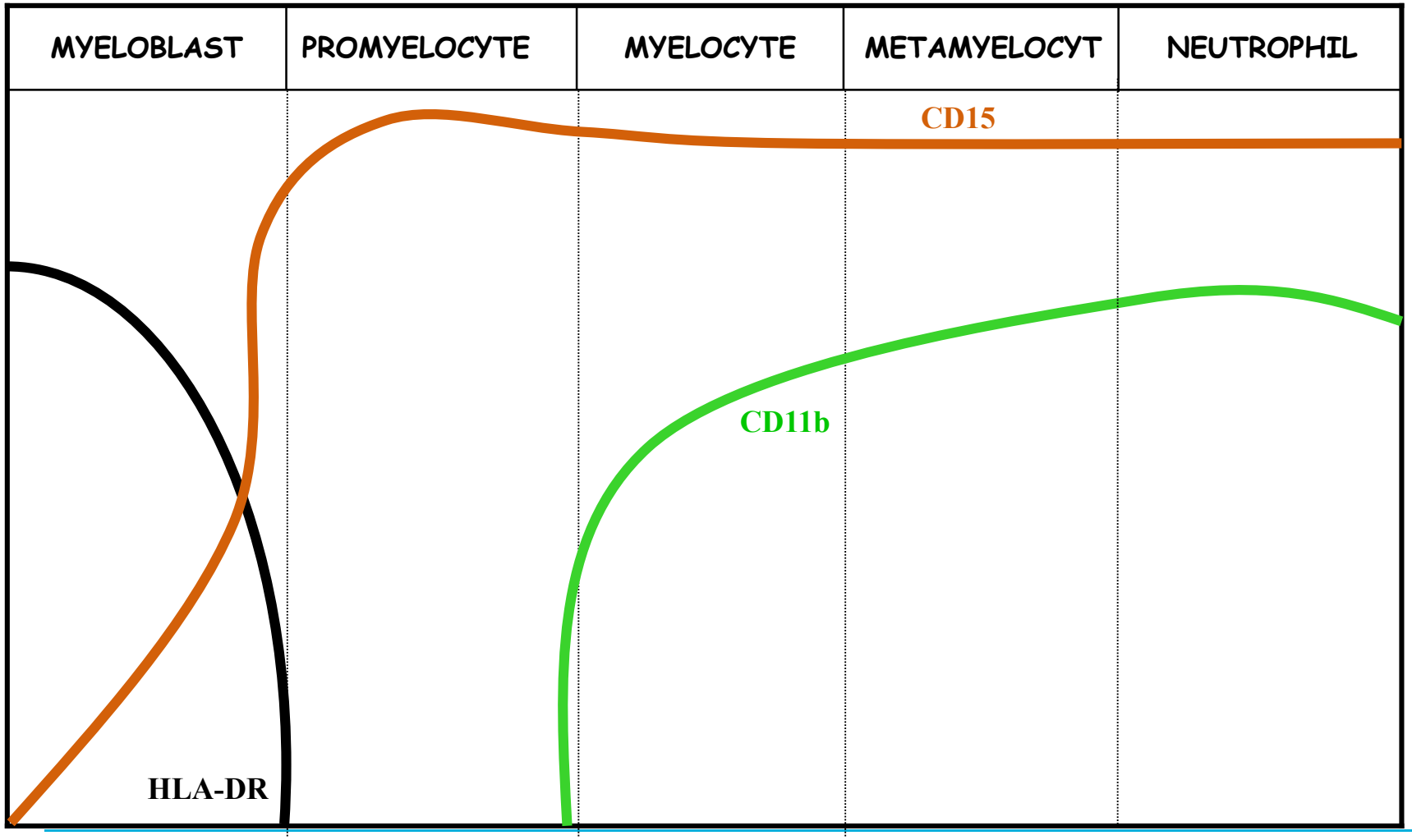


Conclusion: Mature monocyte stages are clear but where we can found the separation between mono- myeloid lineage??

More detailed phenotyping of the Myeloid and Monocytic Lineages By CD11b / CD15 / HLA-DR / CD45

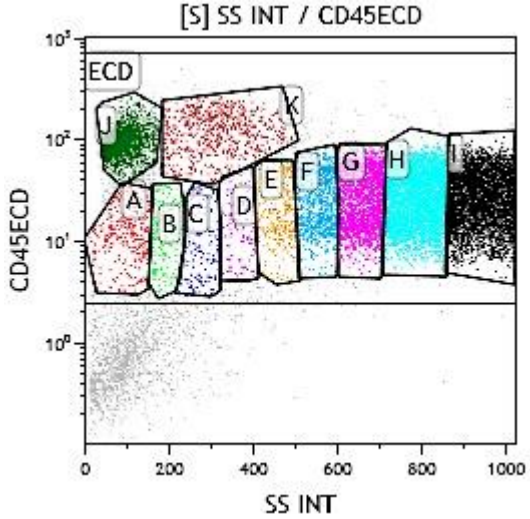
With focus on the separation point by gating at lineage markers and back to CD45

3. Expression pattern
CD11b, CD15, HLA-DR



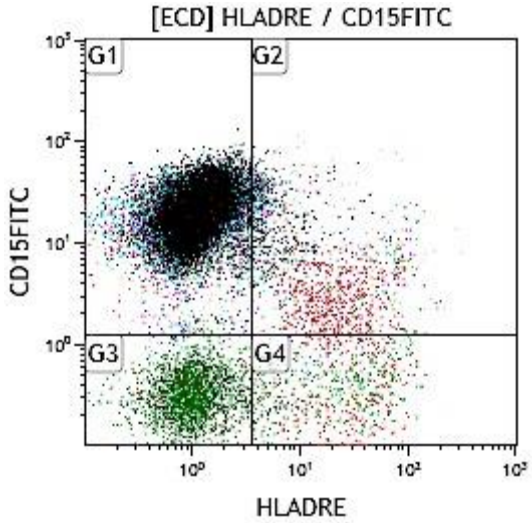
Ref.: A. Orfao et al

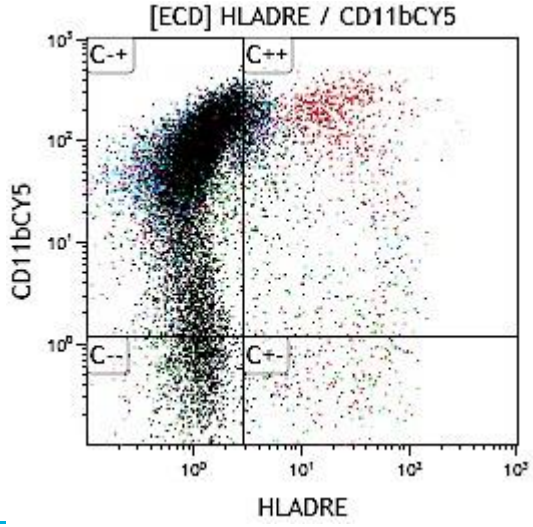
3.a. Expression pattern CD11b/CD15/HLA-DR in CD45+ population (normal BM)

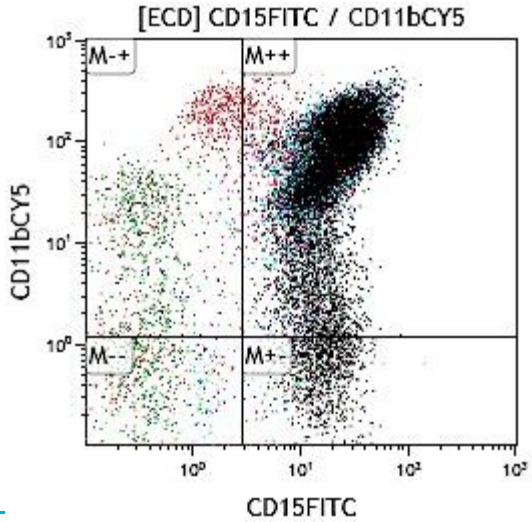


Focus on monocytic and myeloid lineage

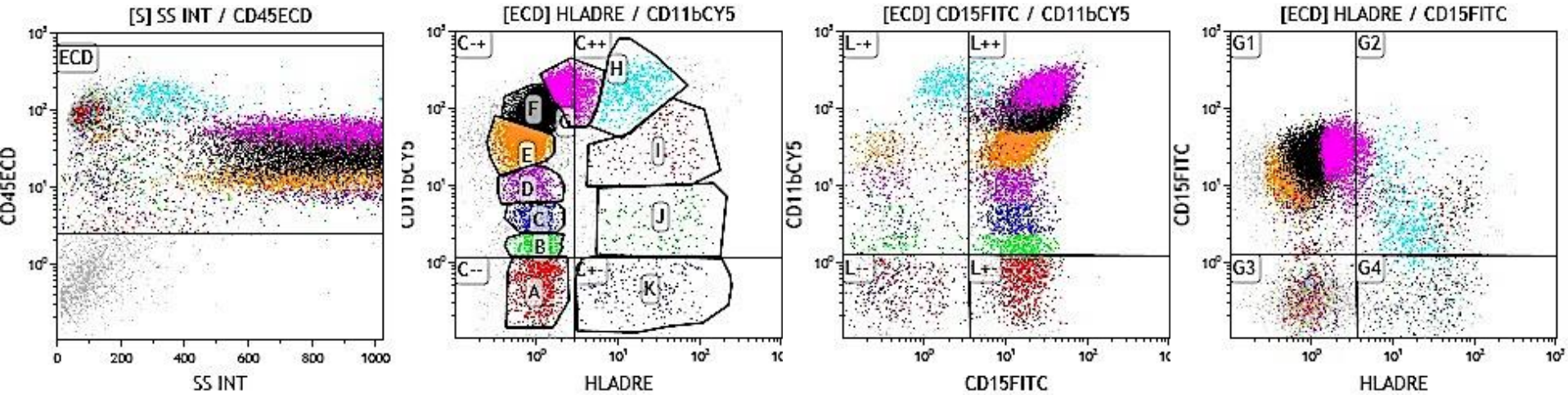
lineage gates A, (B, C), K



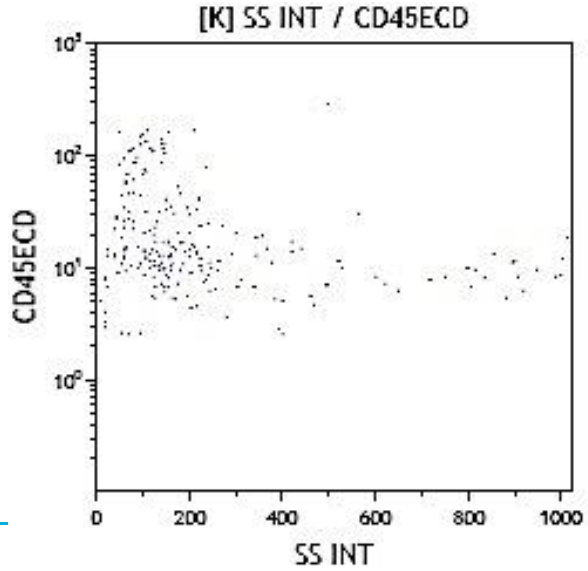




3.b. Expression pattern of CD11b/CD15/HLA-DR in CD45+ population (normal BM)

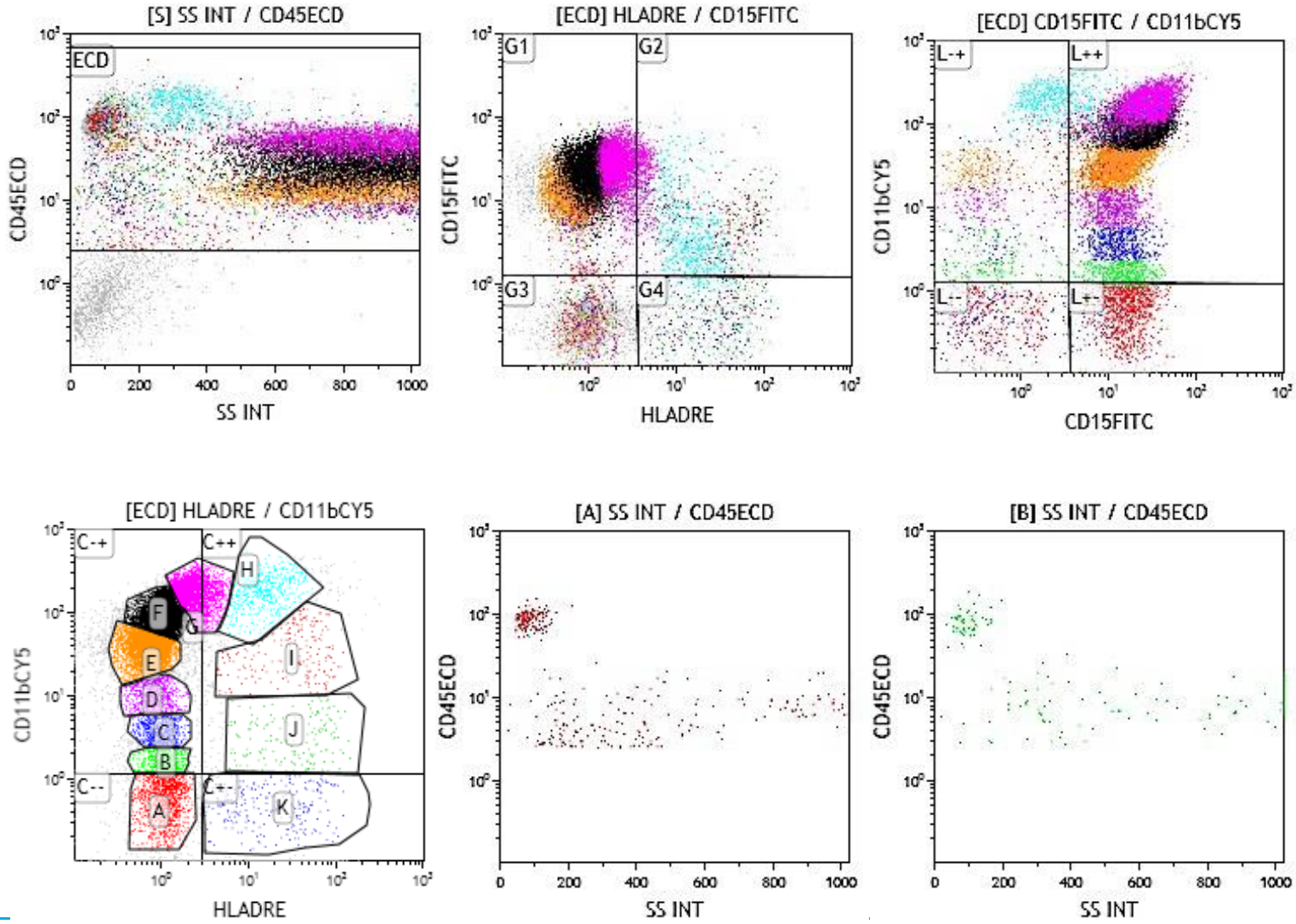


Focus on Myeloid and Monocytic



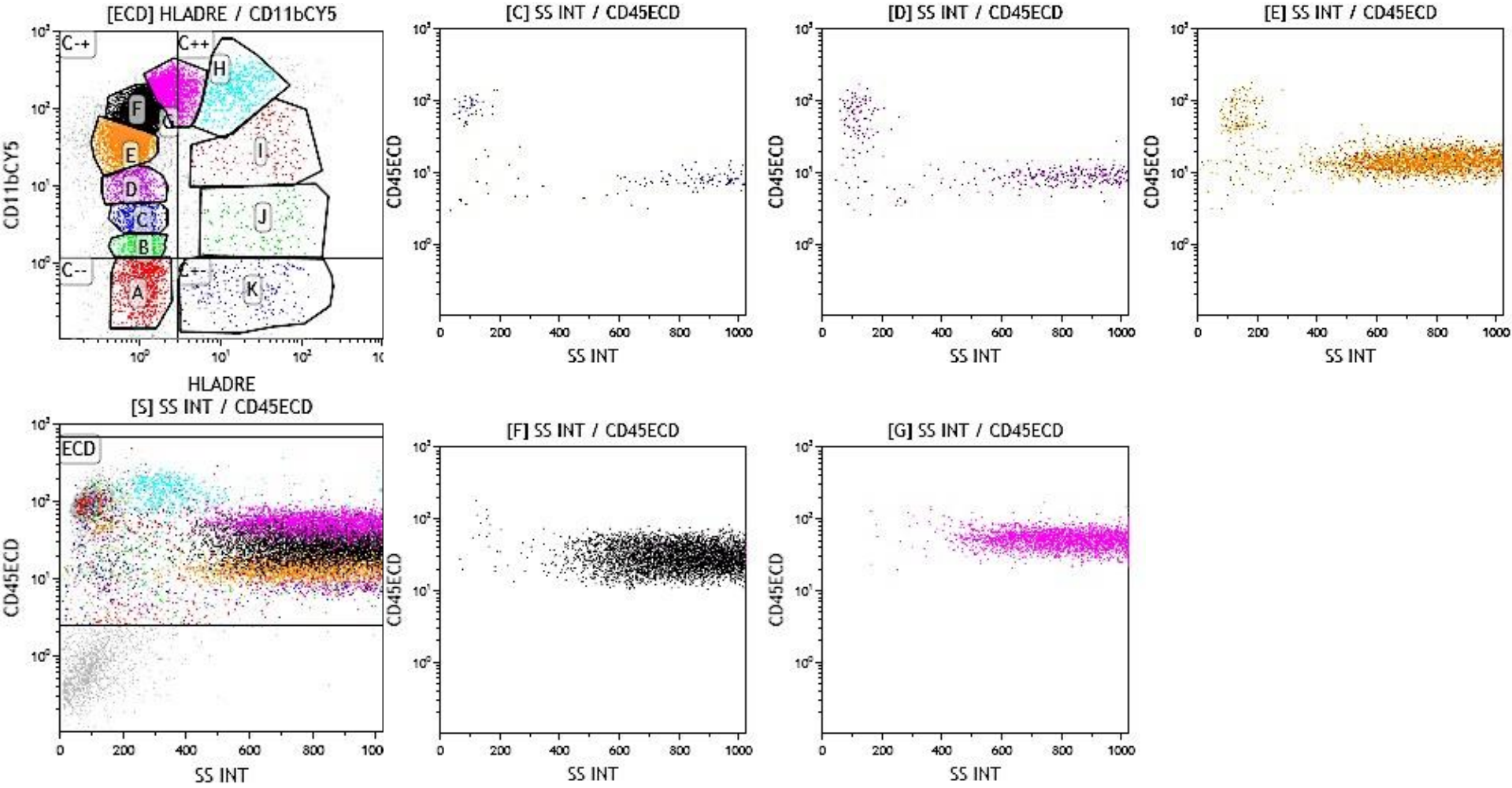
3.c. Expression pattern: myeloid

CD11b/CD15/HLA-DR



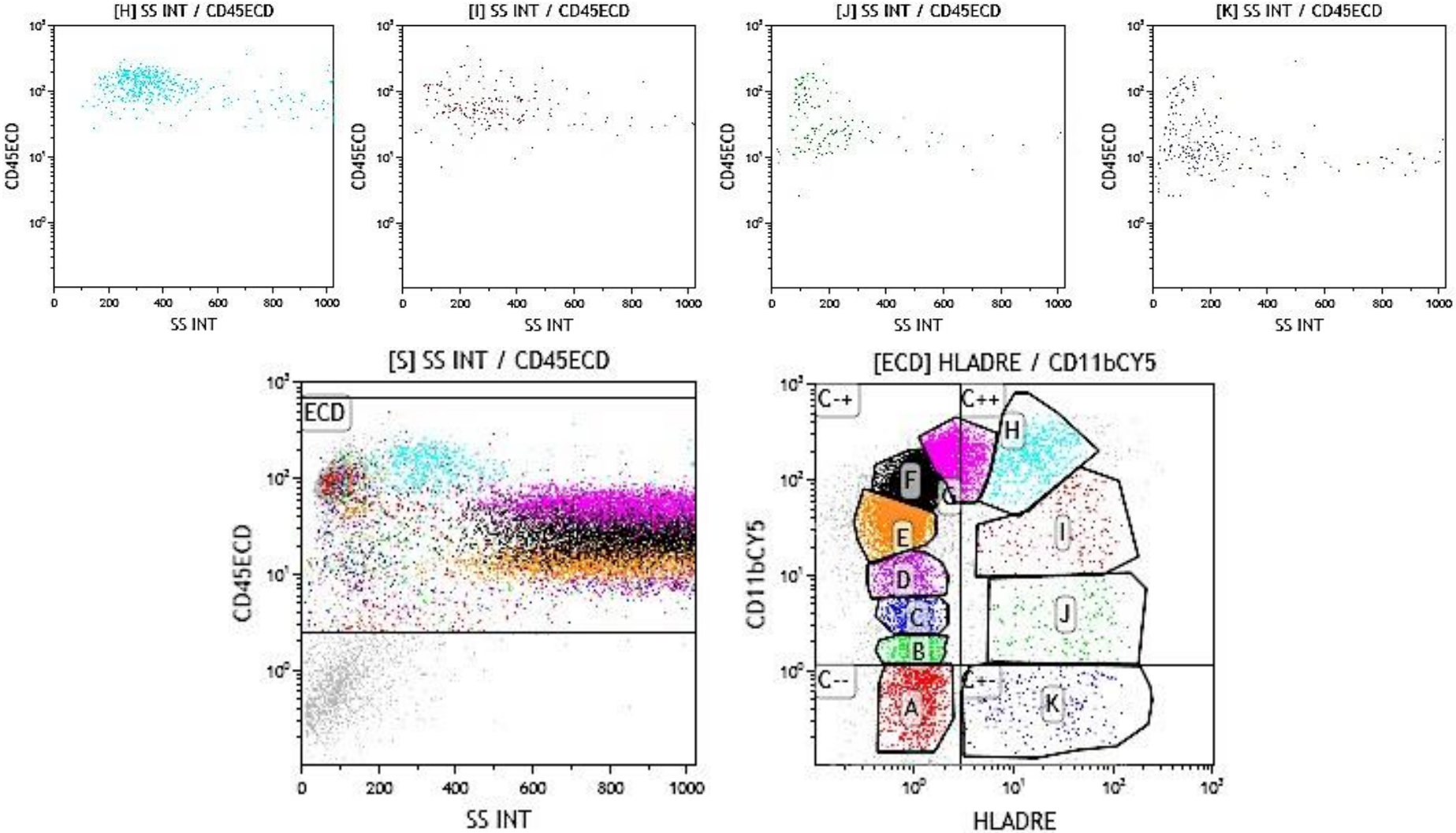
3.d. Expression pattern: myeloid

CD11b/CD15/HLA-DR

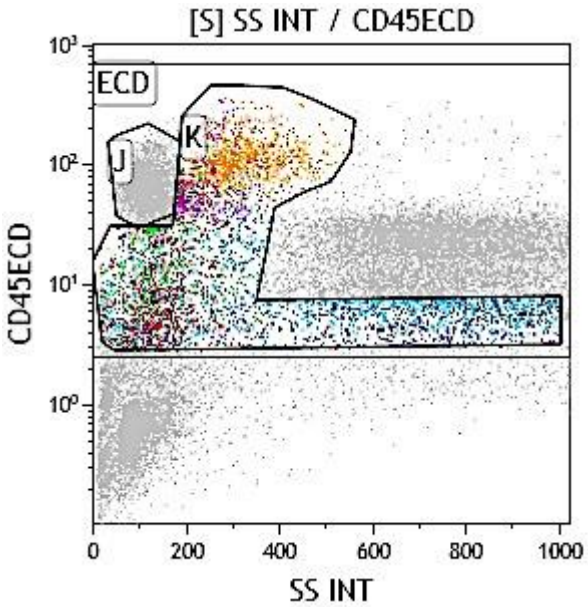


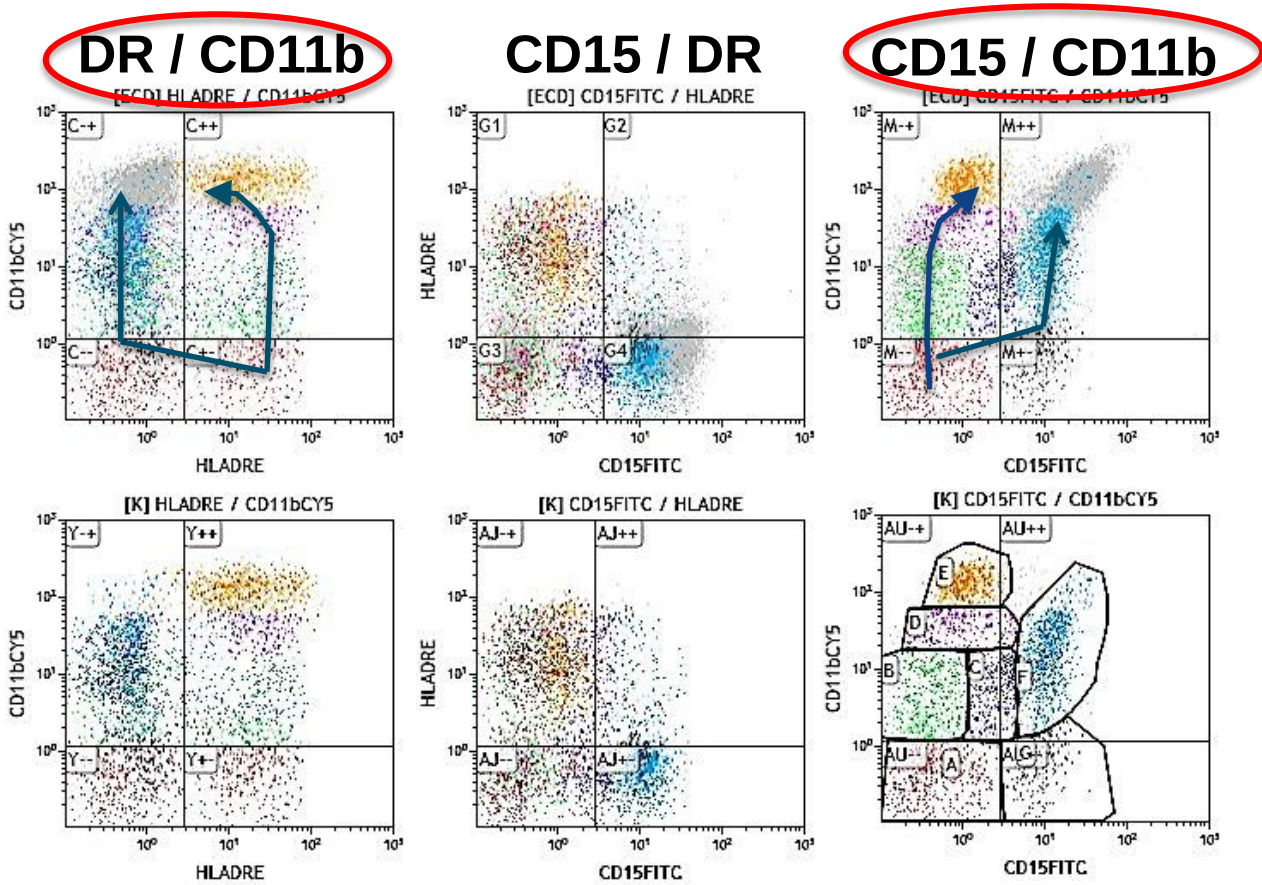
3.e. Expression pattern: monocytic

CD11b/CD15/HLA-DR



3.f. Details: Focus on separation point of both lineages

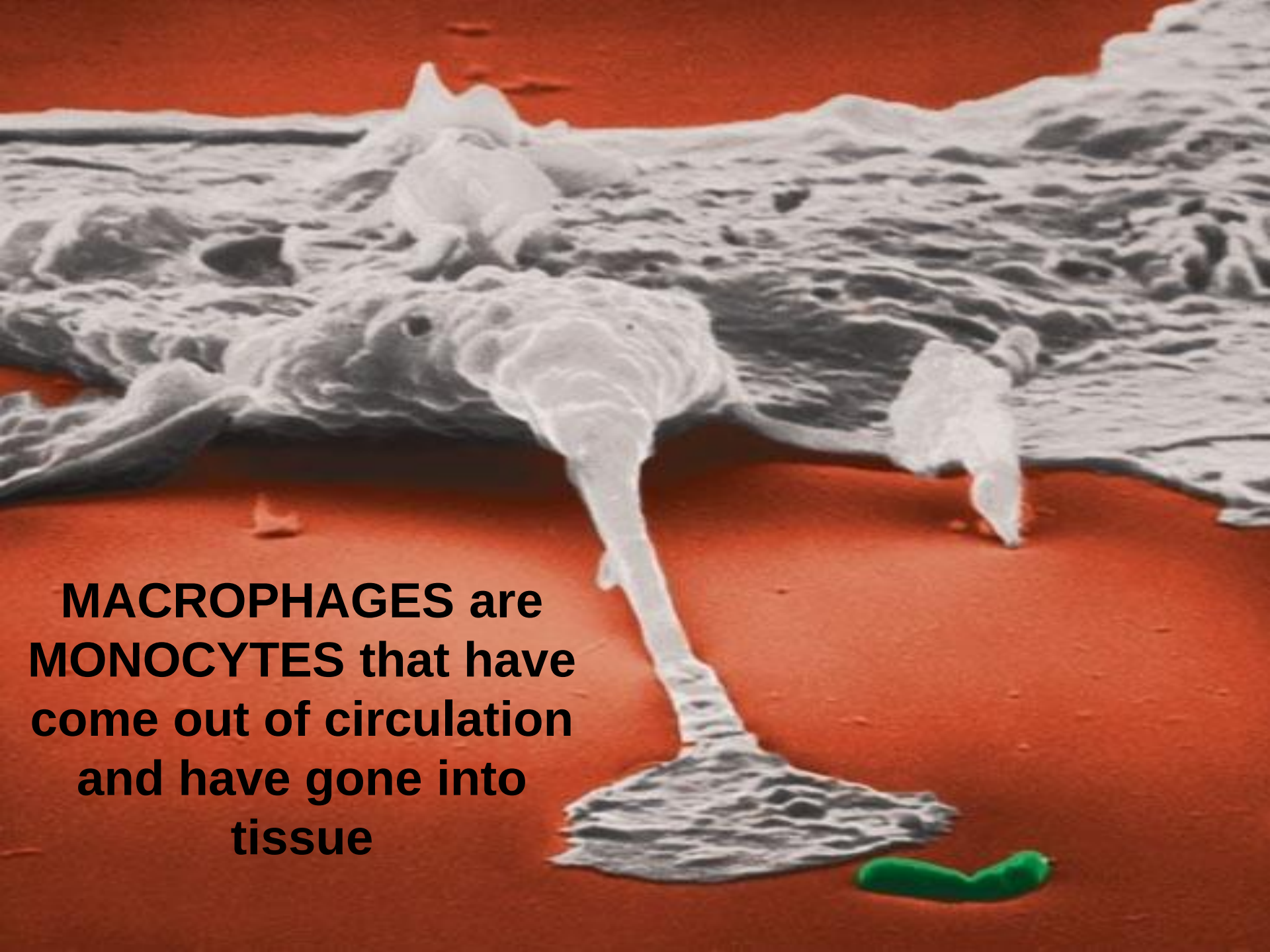




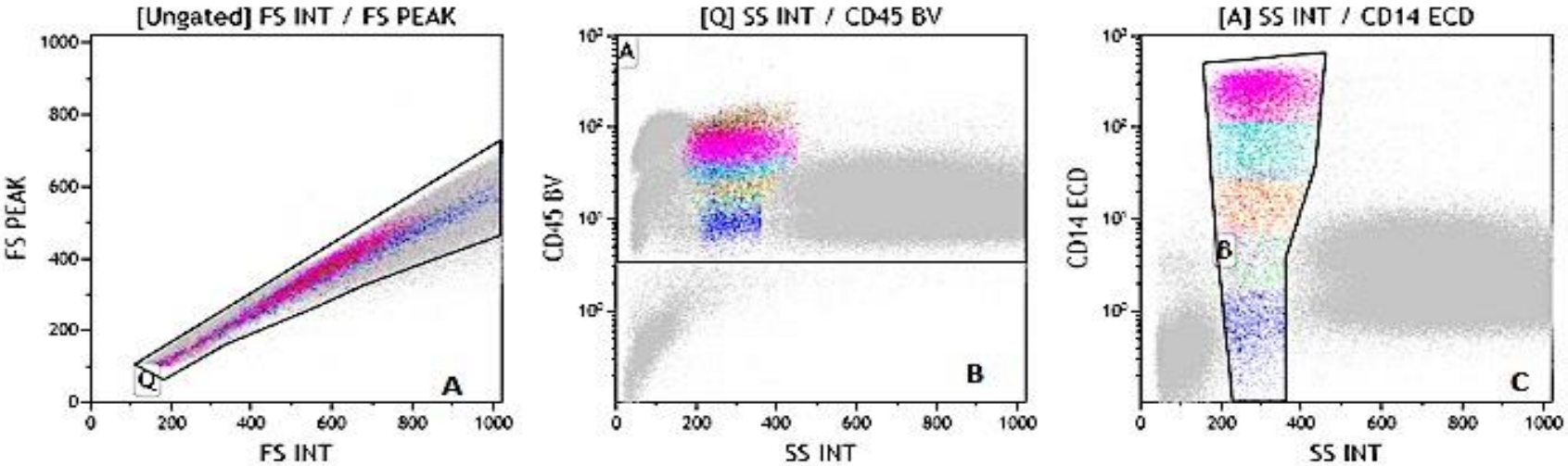
….BUT THERE IS MORE!....

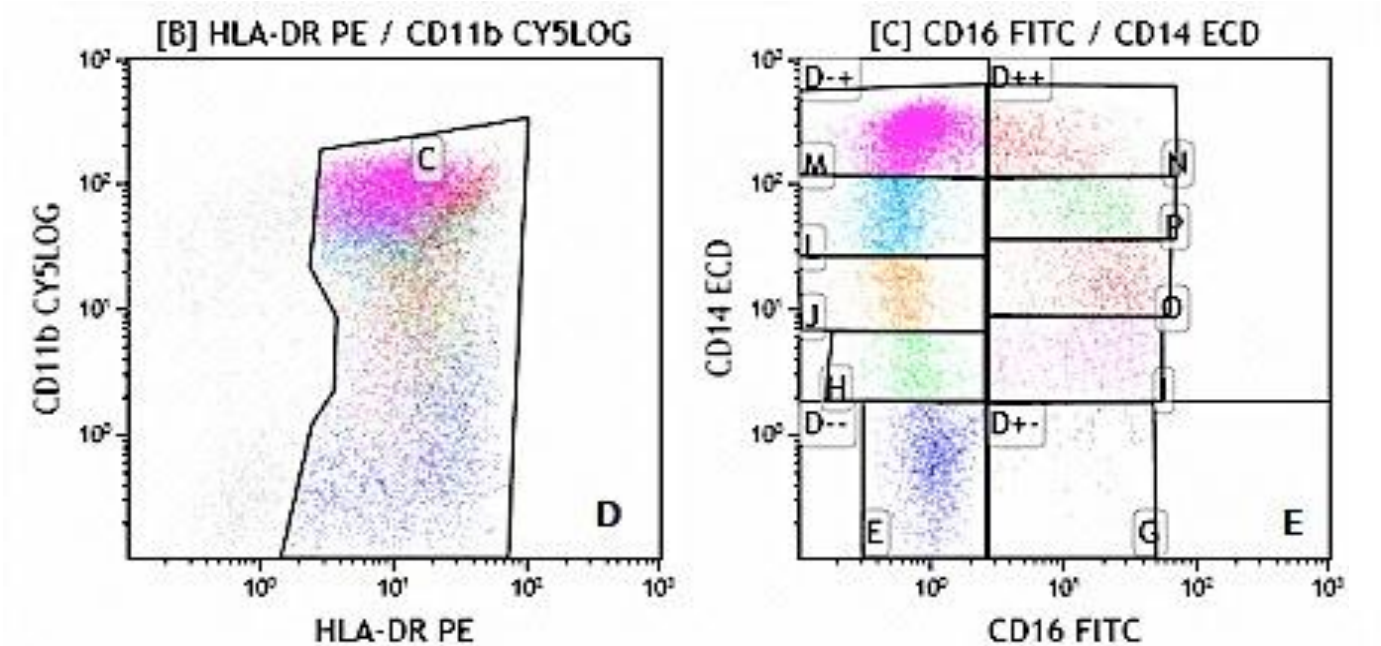
Macrophages? Erytroid cells?

Radboudumc


MACROPHAGES are MONOCYTES that have come out of circulation and have gone into tissue

4.a. Where are the Macrophages?
Maturation of the monocytes based on CD14 and CD16





CD14/SS

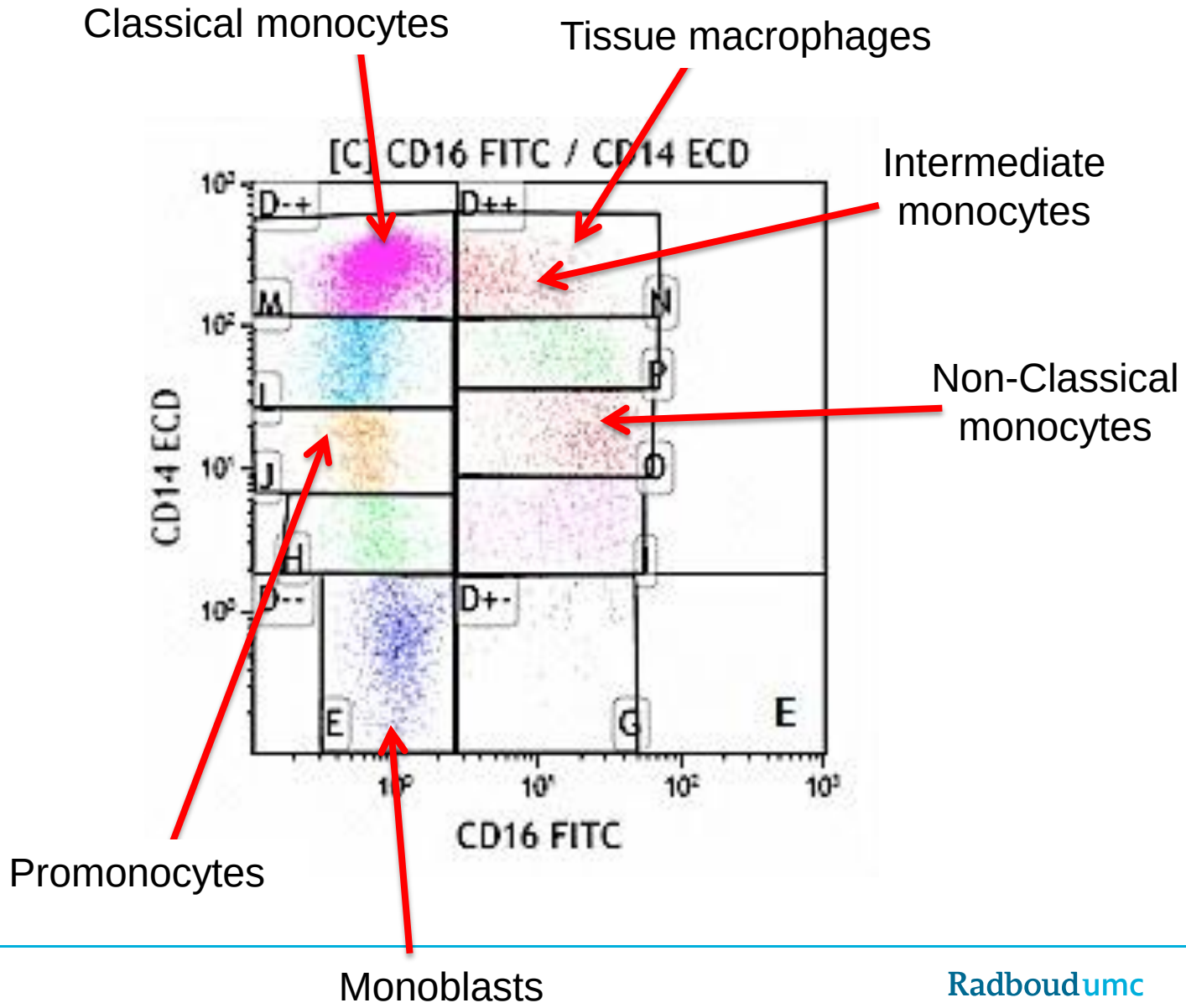
↓

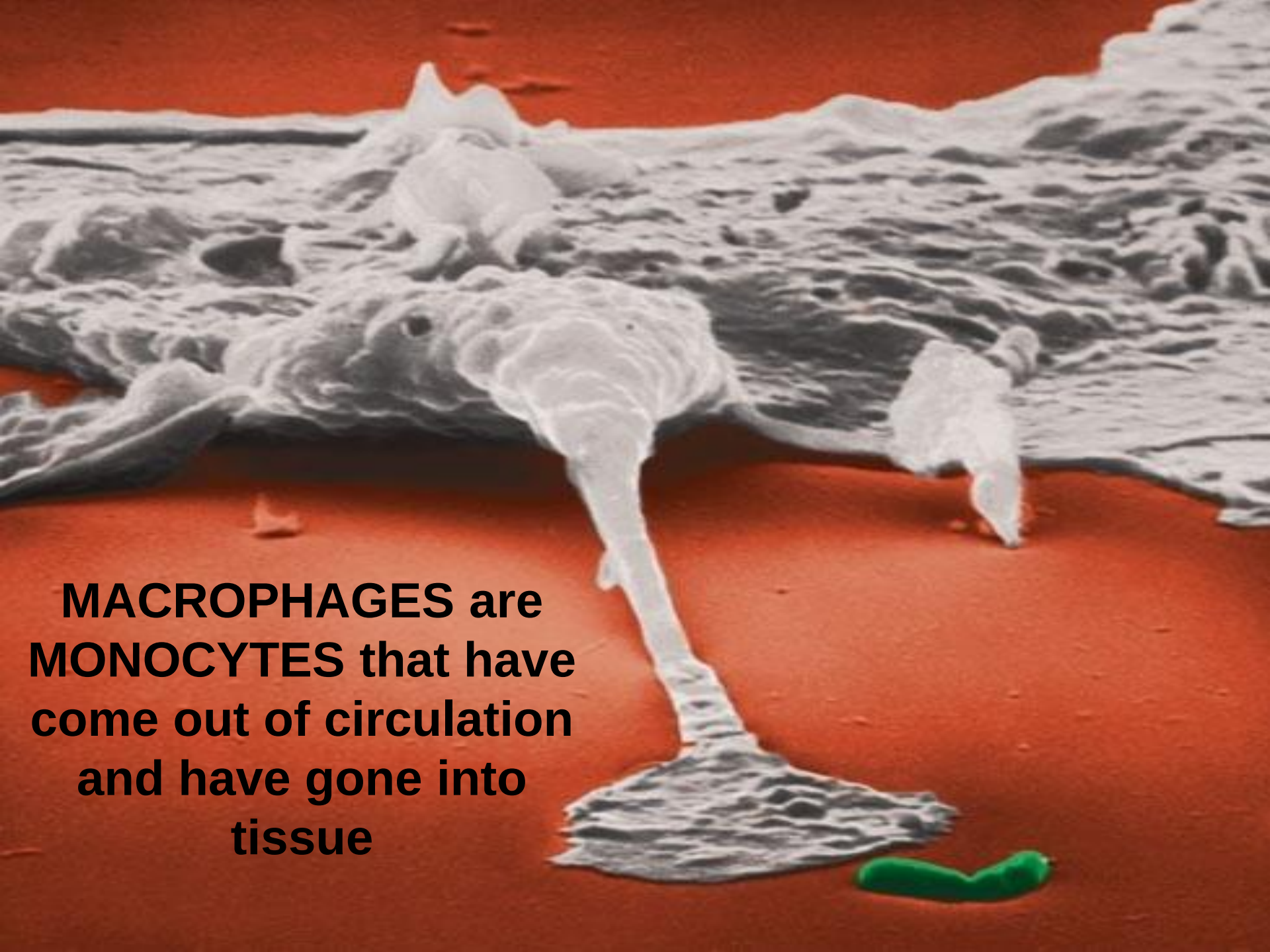
CD11b/DR

↓

CD14/CD16

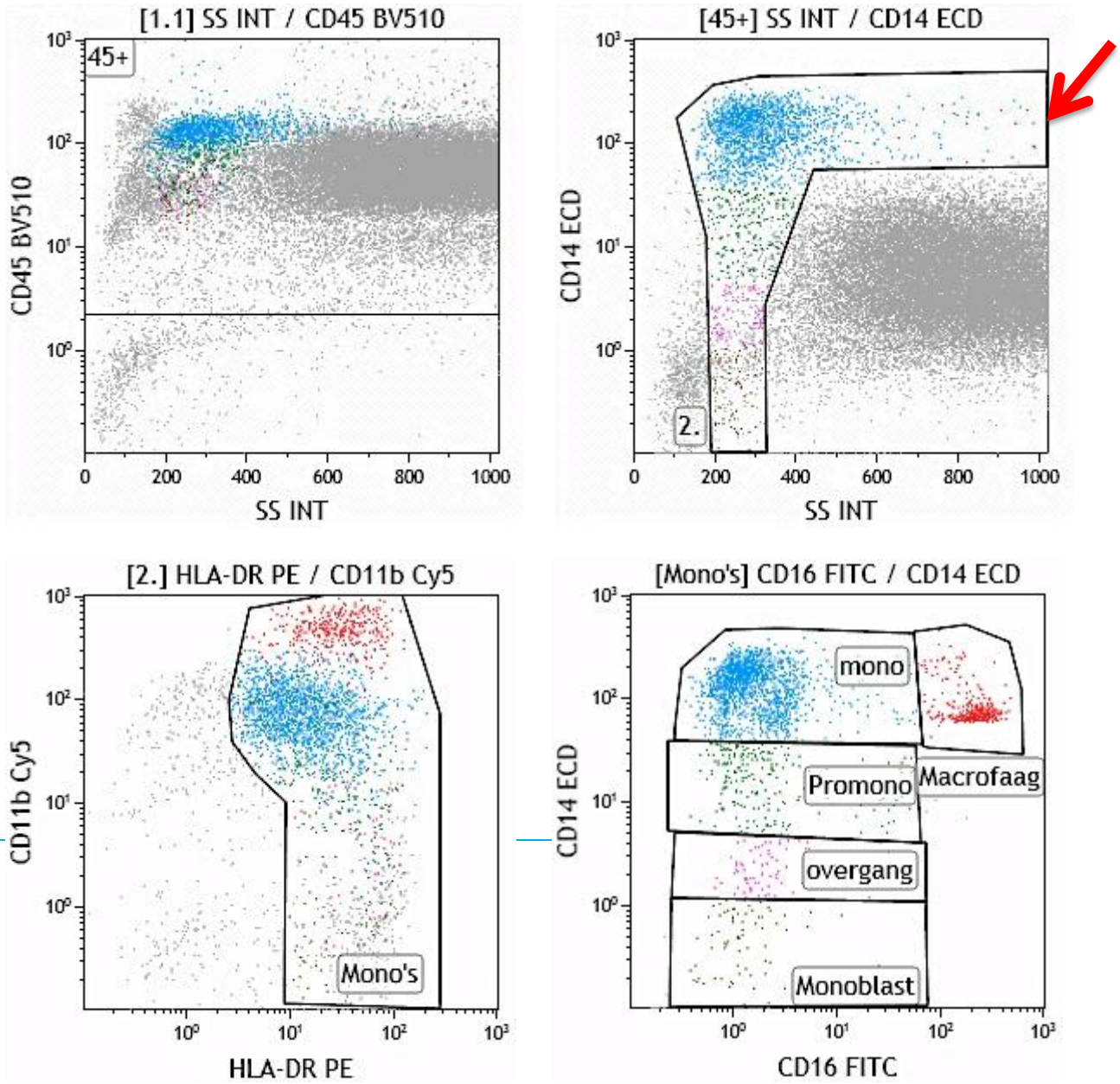
Maturation from monocytes to macrophage



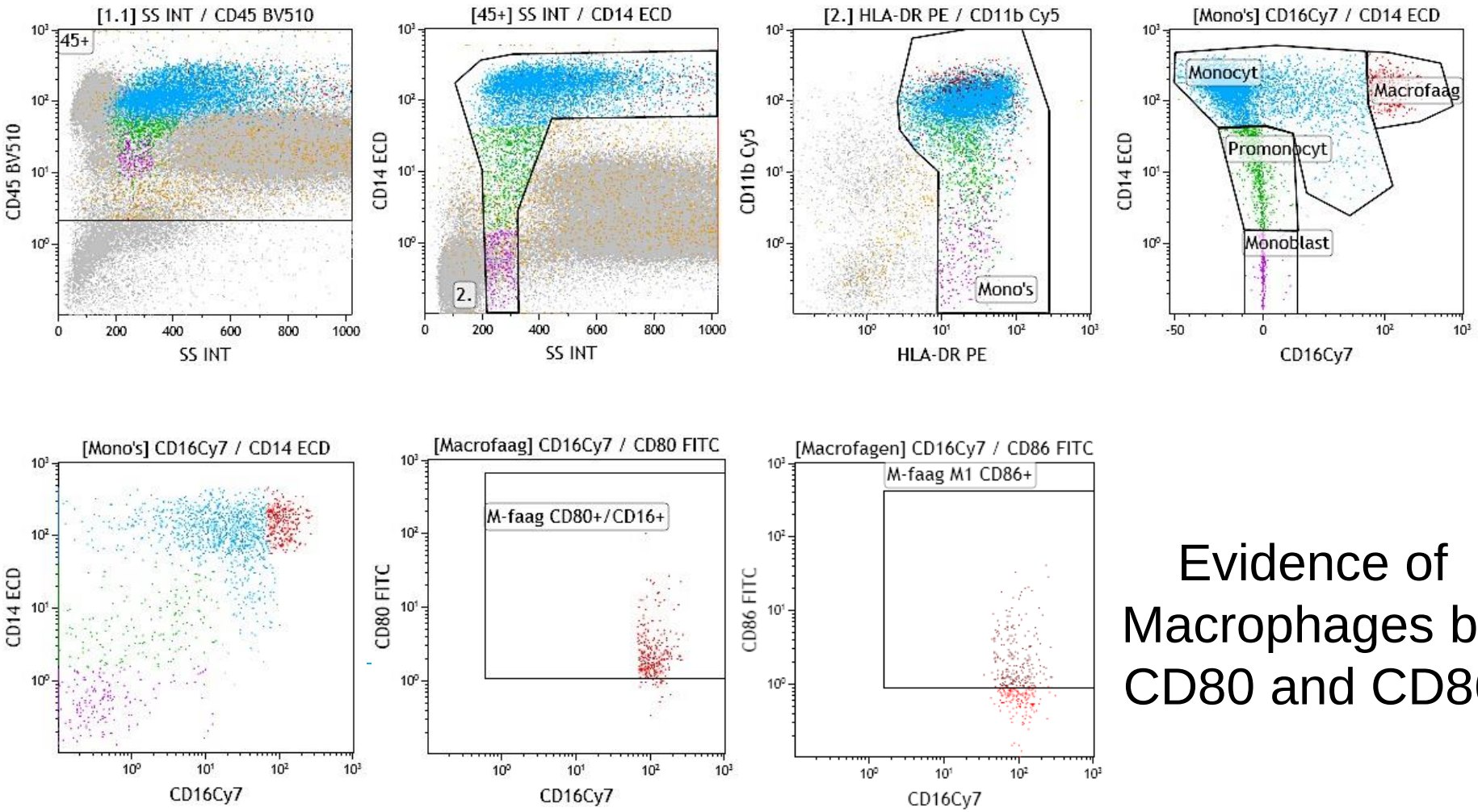

MACROPHAGES are MONOCYTES that have come out of circulation and have gone into tissue

4.b. Maturation from monocytes to macrophages (details)

Macrophages
are
SS++ CD11b++
HLA-DR++
CD14+ CD16+



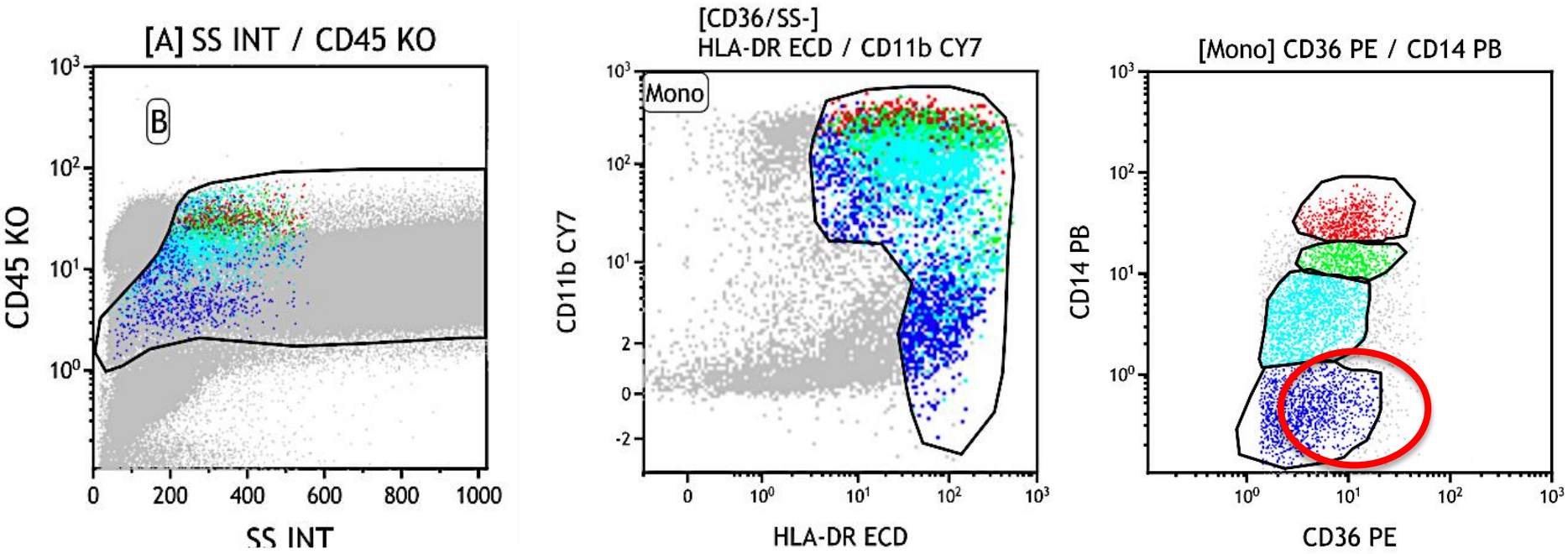
4.c. Maturation from monocytes to macrophages (CD80 and CD86)



Evidence of Macrophages by CD80 and CD86

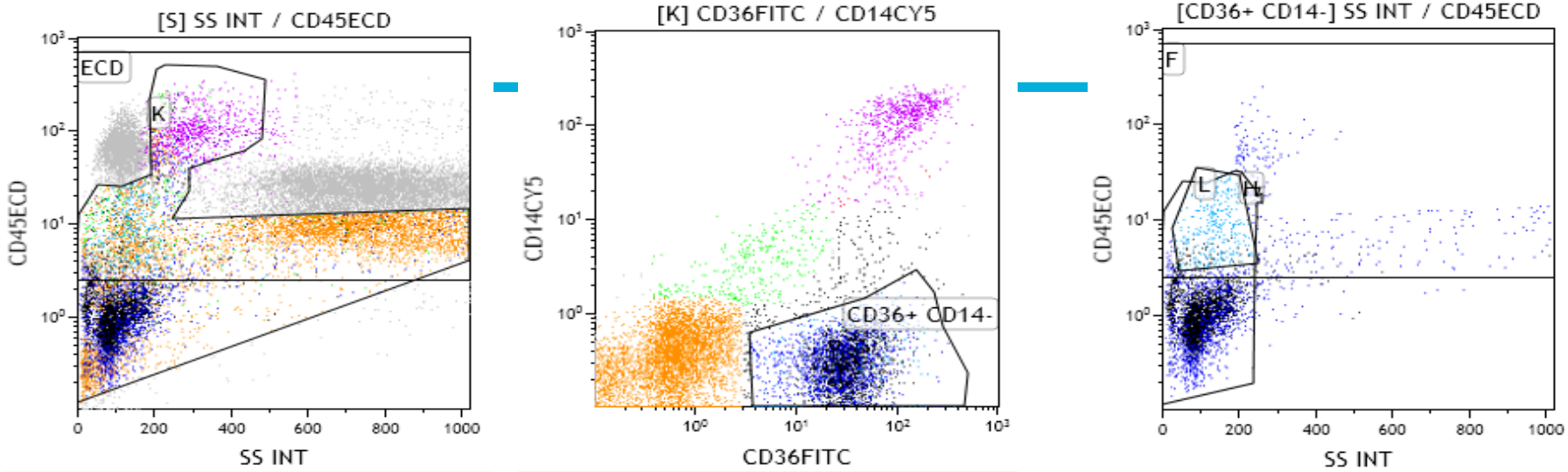
Radboudumc

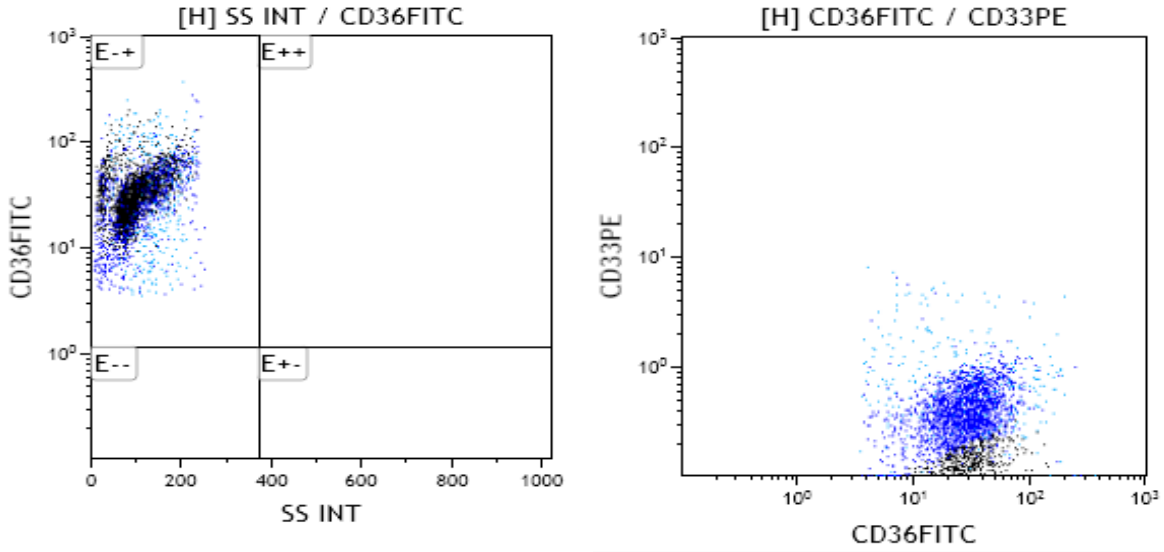
5. Maturation from monocytes. But what about CD36+CD14-



CD36: **monocytes**
nucleated erythroid
megakaryocytes

Radboudumc



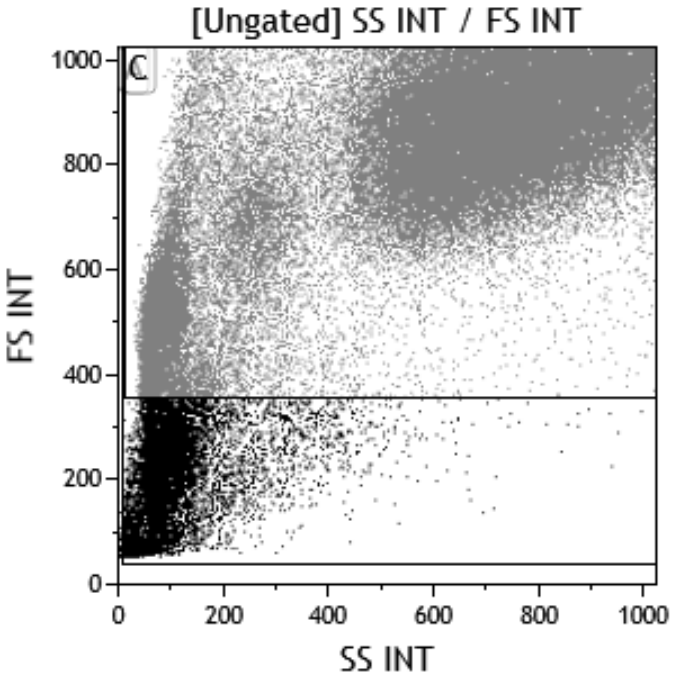


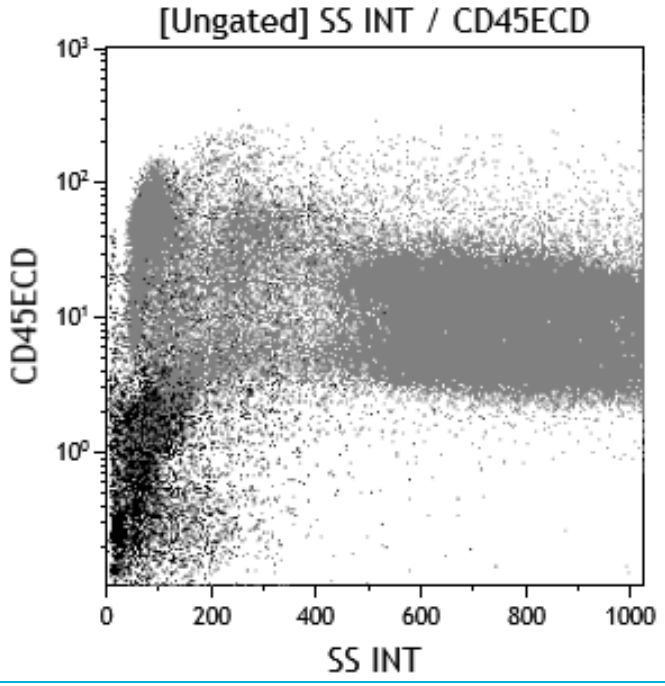
5.a. Erythroid pathway

Using CD36/CD14/CD3

In routine diagnostics we gate out debris

But what is lost?

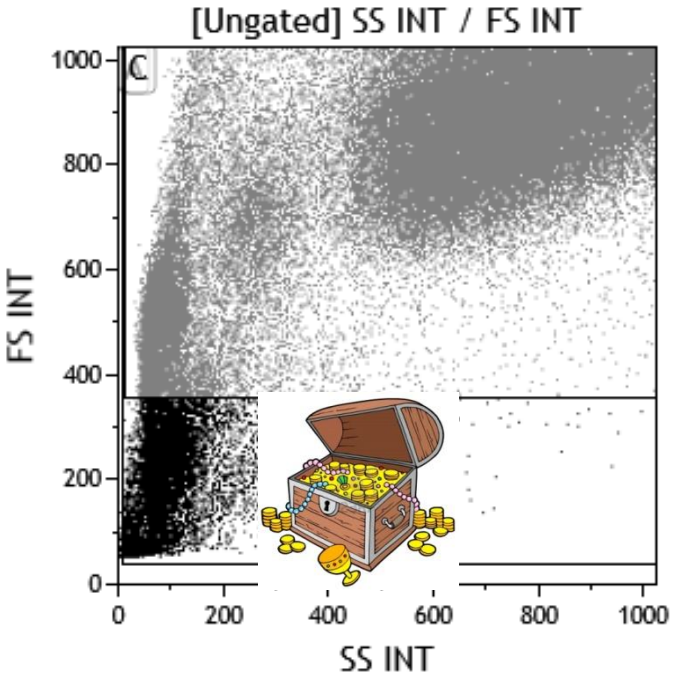


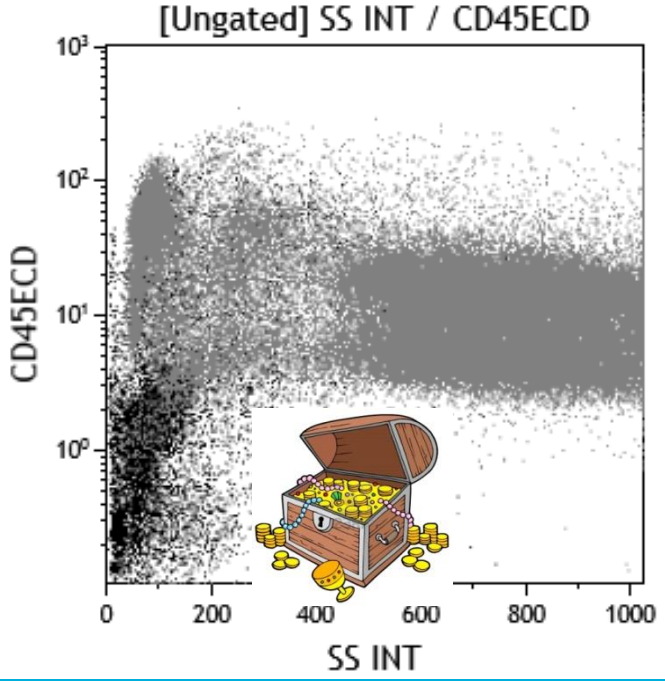


Radboudumc

In routine diagnostics we gate out debris

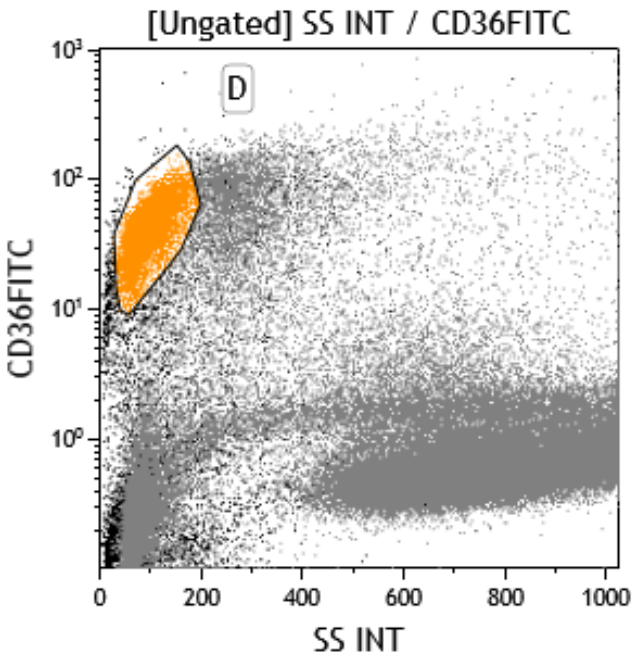
But what is lost?

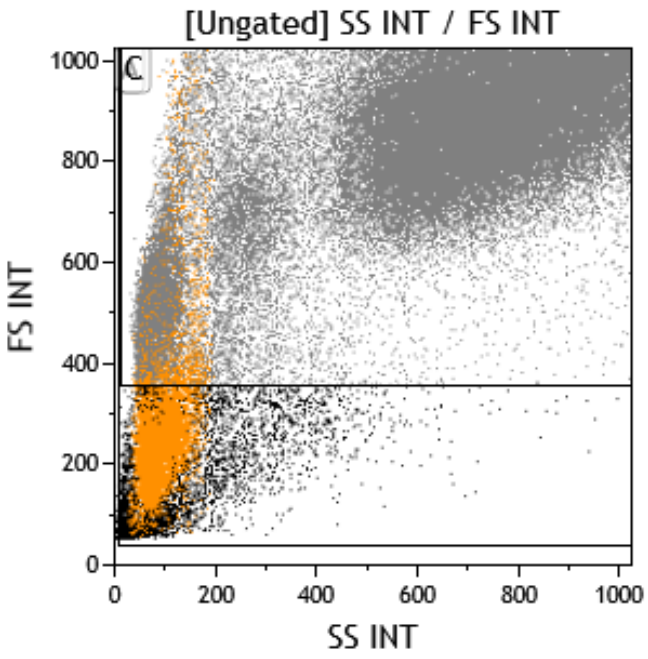


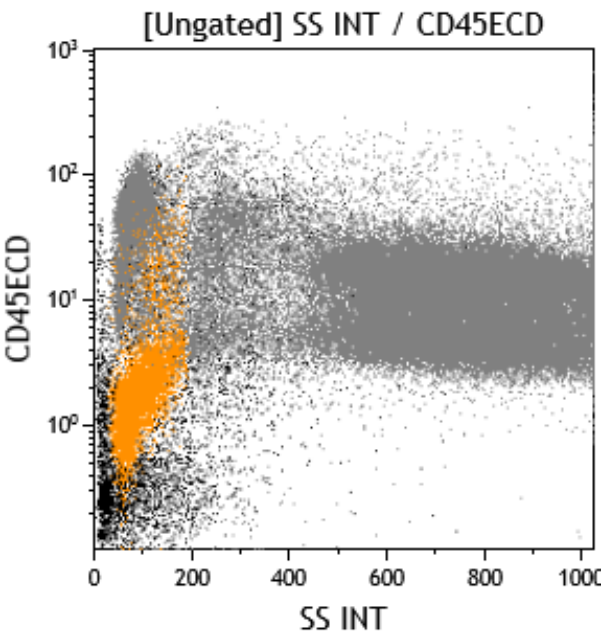


Radboudumc

But how can we characterize these CD36+ cells



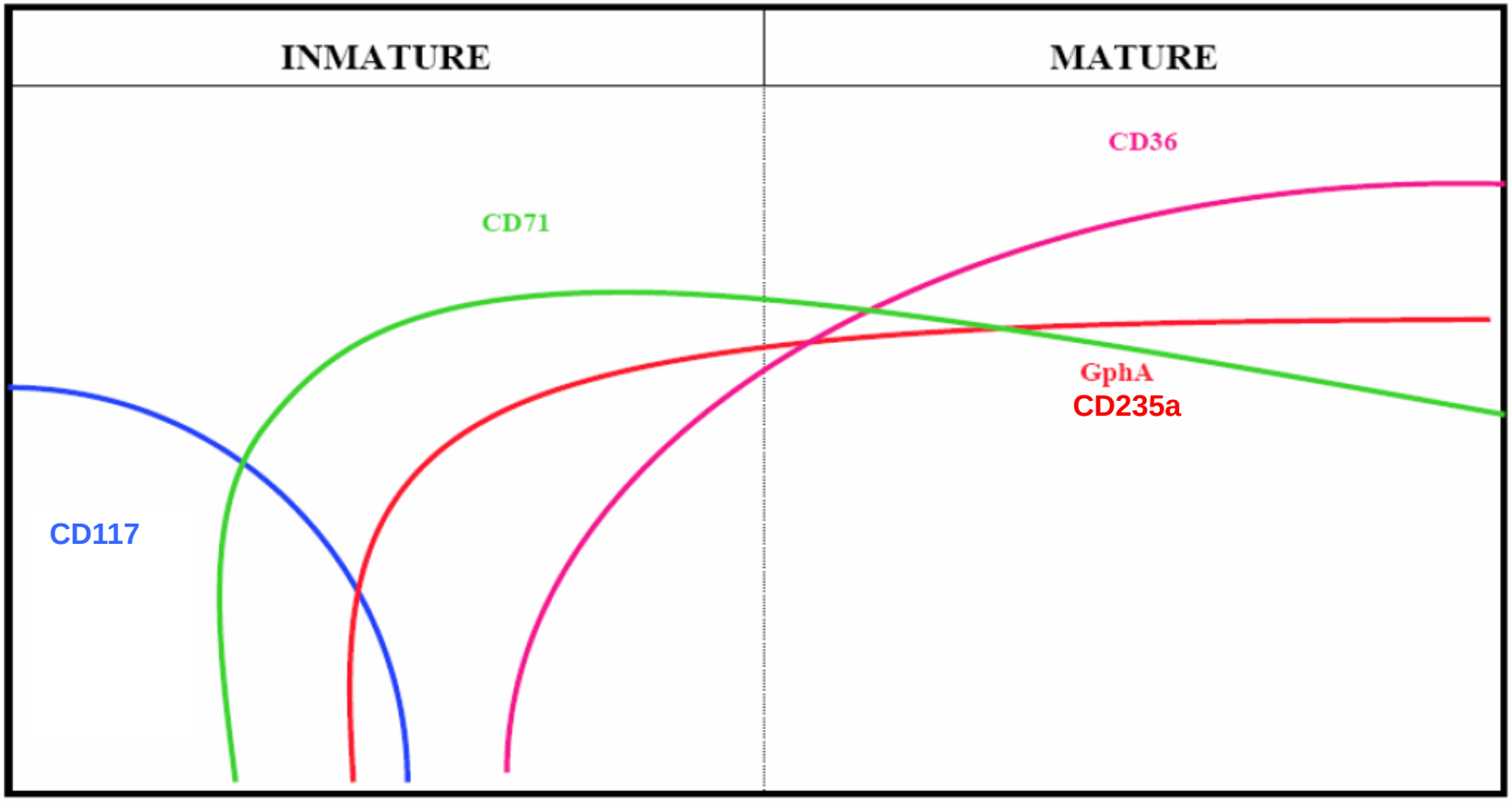




Radboudumc

Normal erythroid maturation

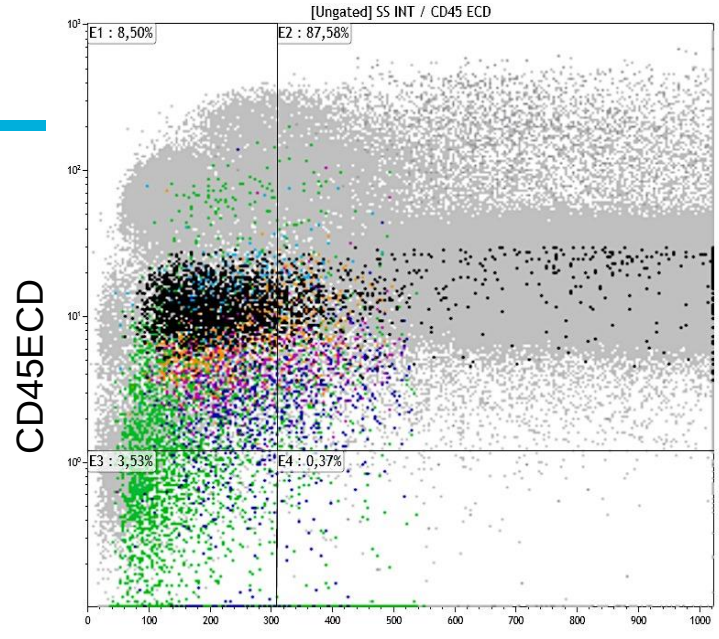
CD117 / CD71 / CD235a / CD36



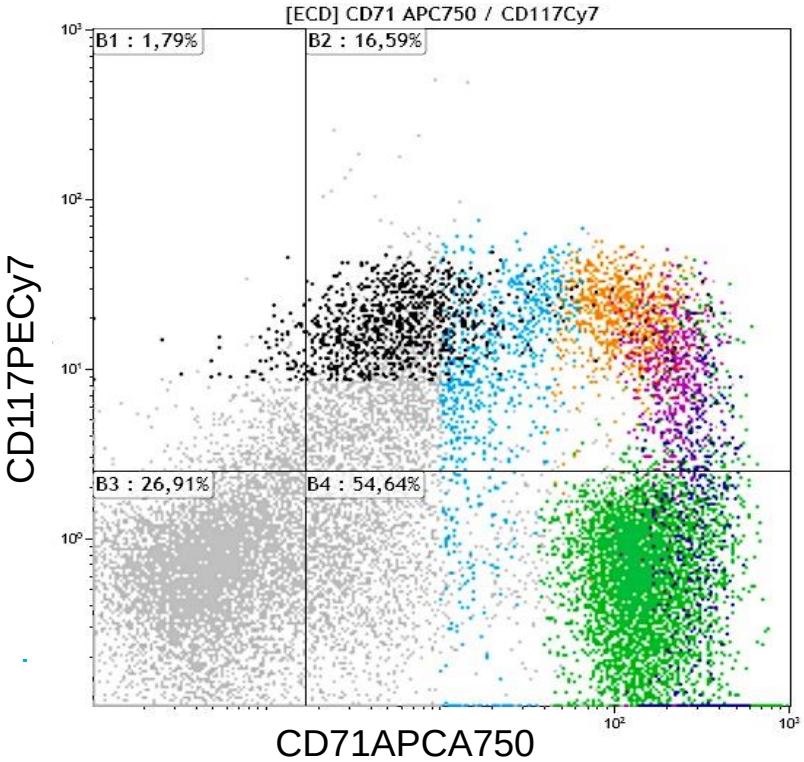
Radboudumc

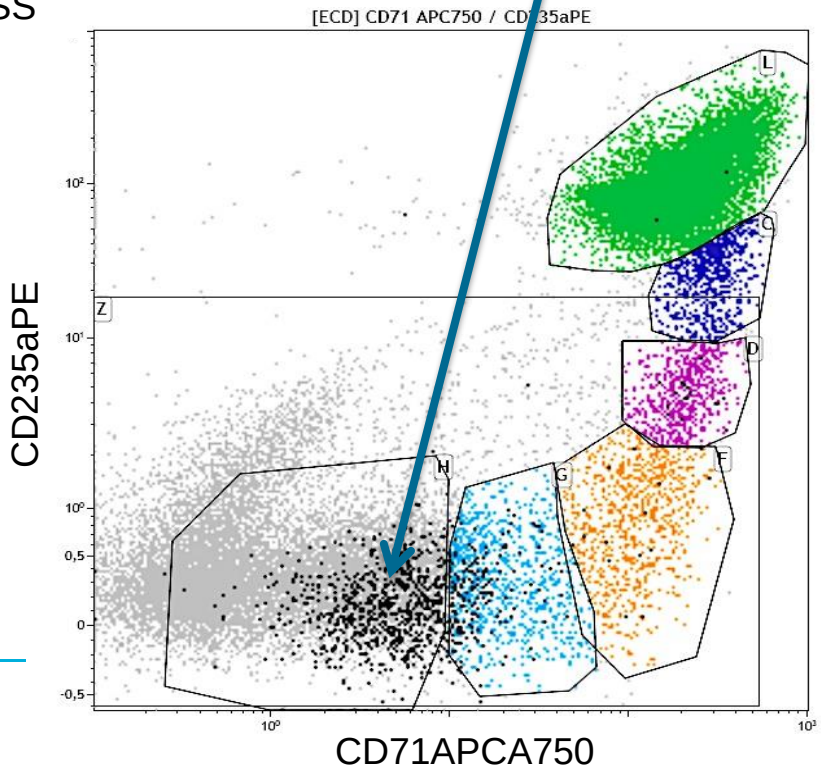
5.b. Erythroid pathway

Using CD235a/CD117/CD71

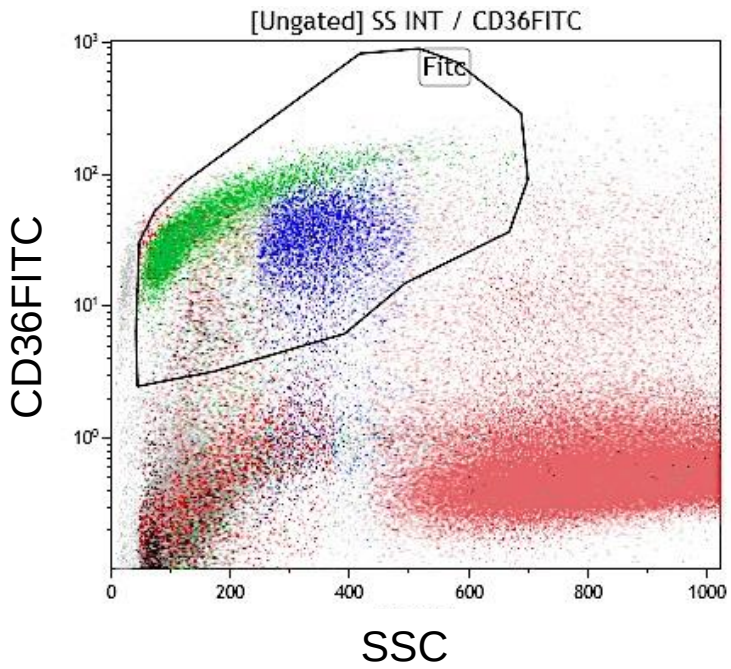


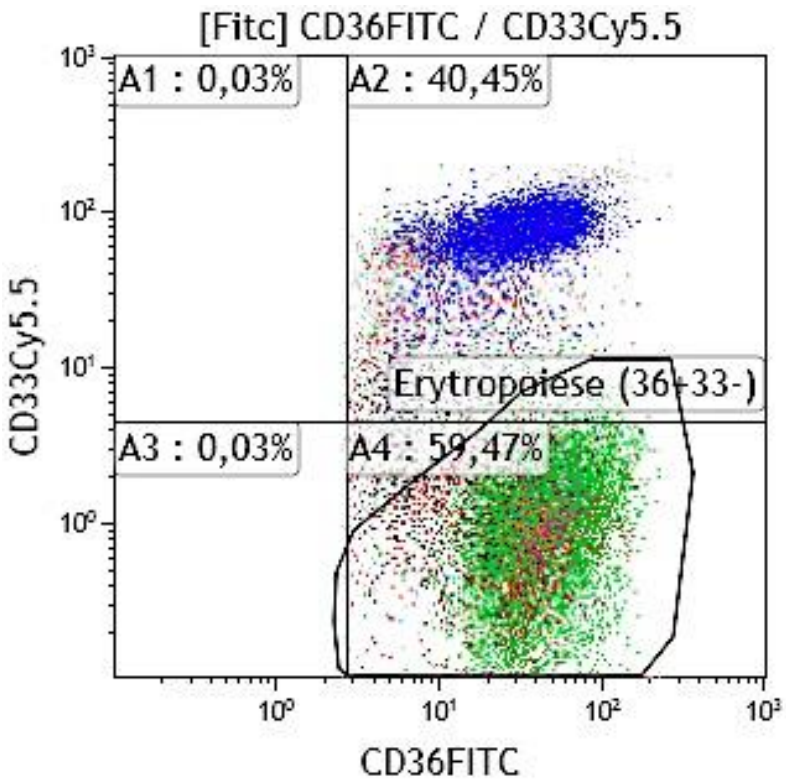
Which lineage represent in the black population?





Elimination of myeloid cells from the erythroid lineage (CD36+CD33-)

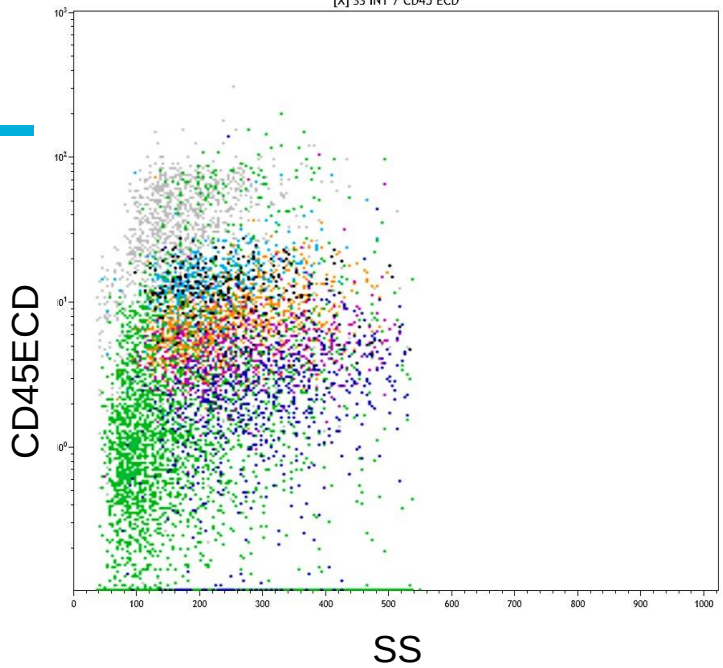


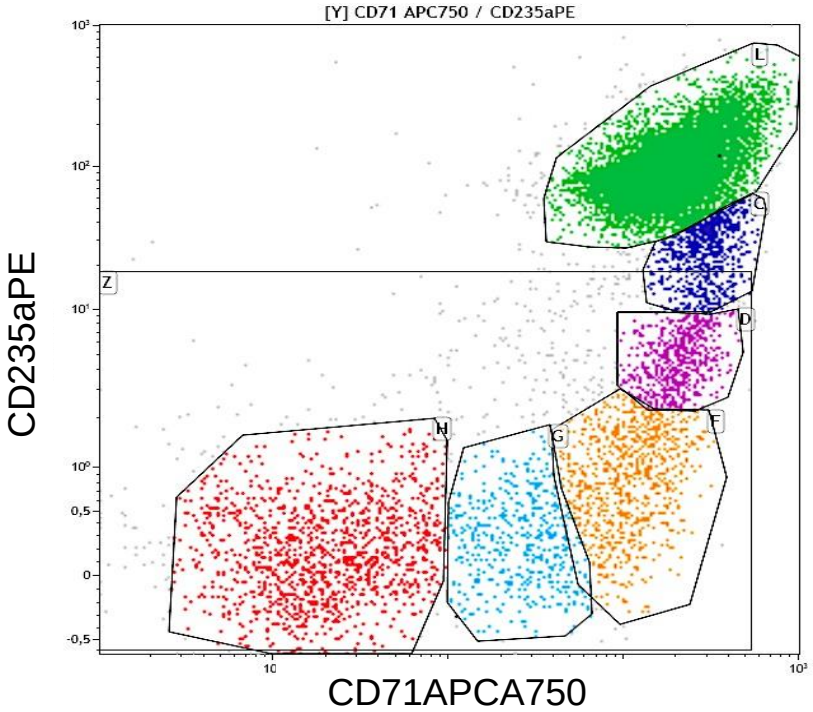


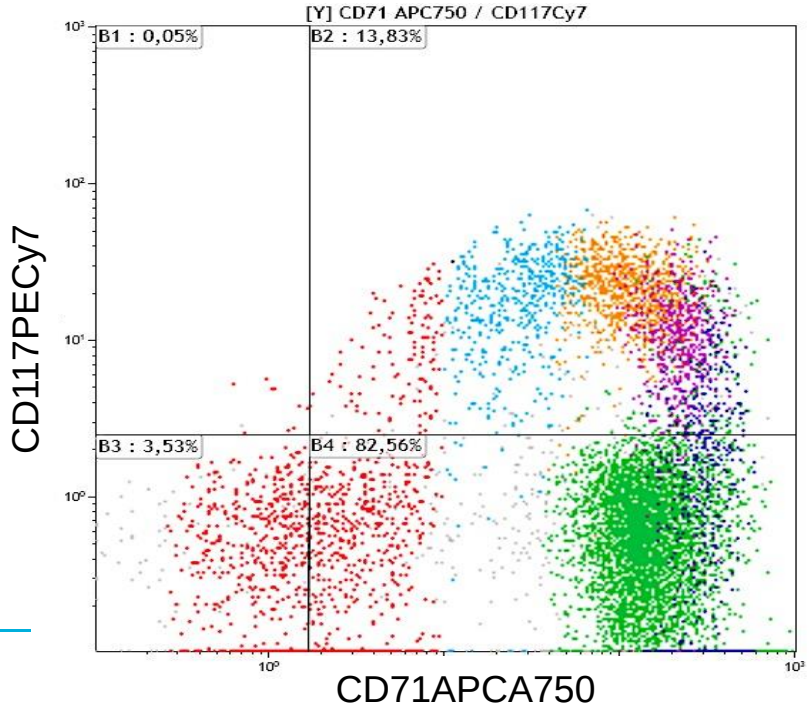
Radboudumc

5.c. Erythroid pathway (focus on erythroid only)

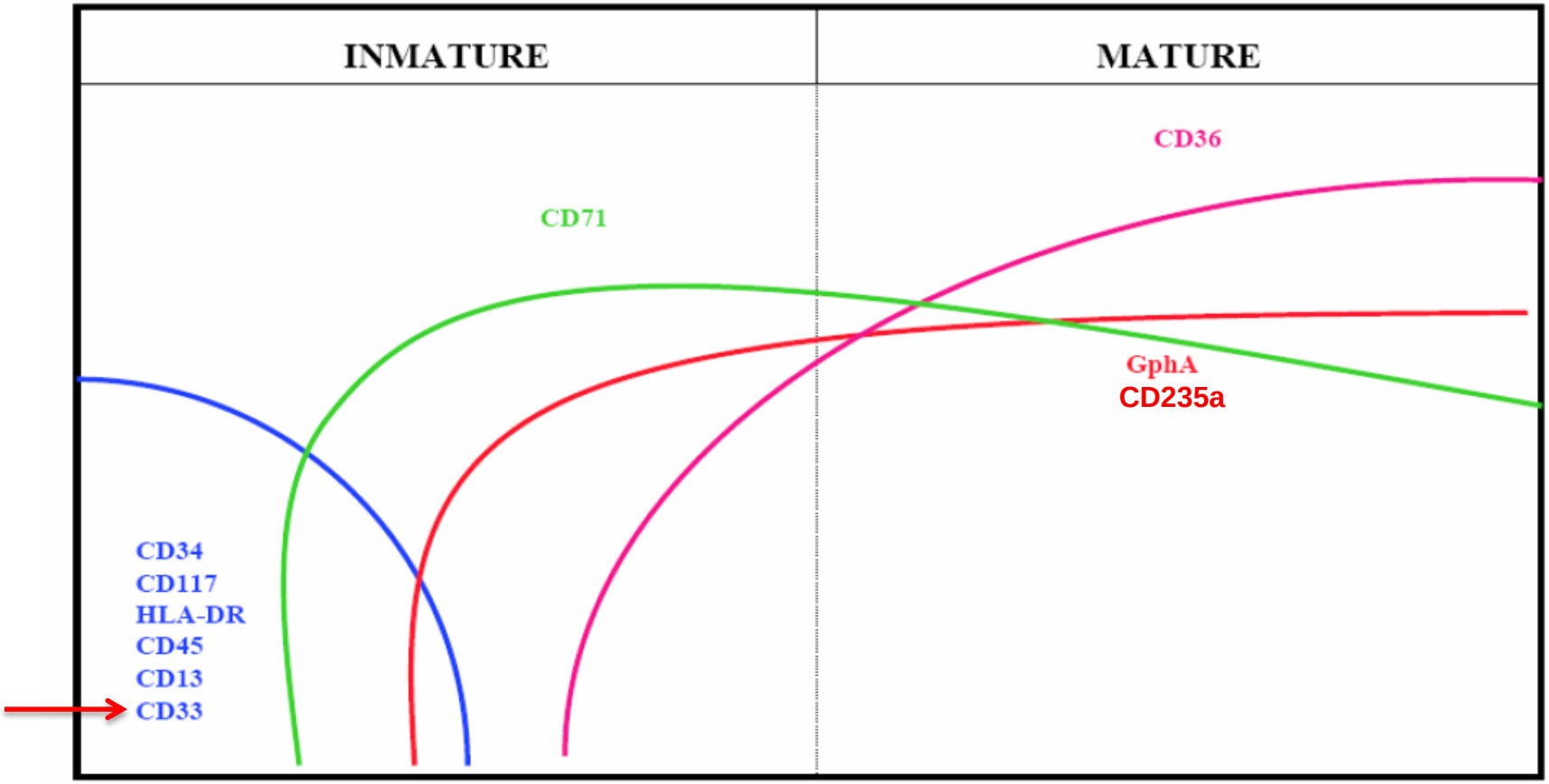
Using CD235a/CD117/CD71







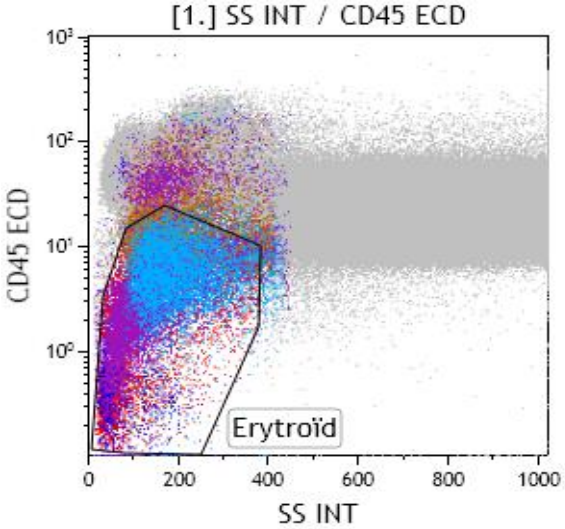
Normal erythroid maturation

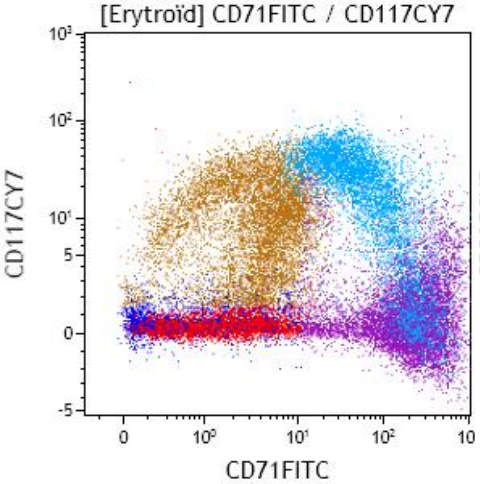


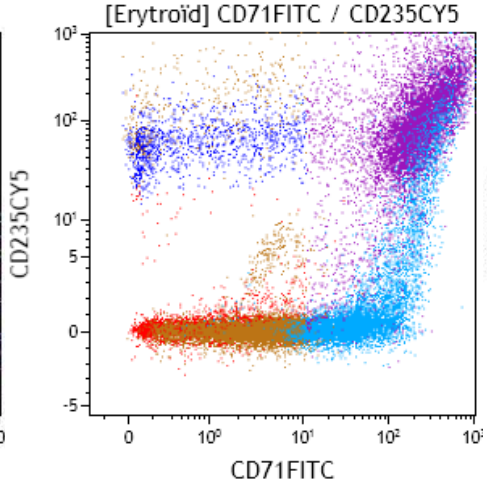
Radboudumc

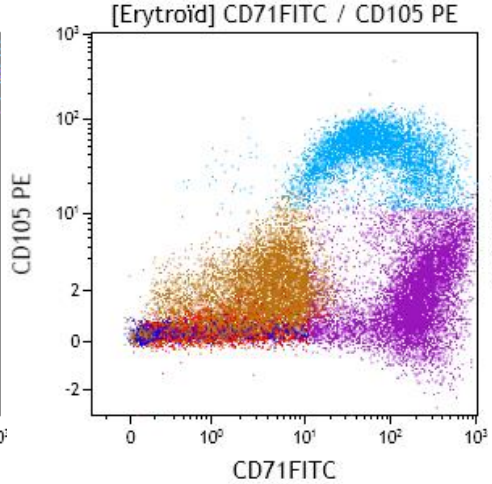
5.d. CD33 is also expressed on erythroid prcursors:

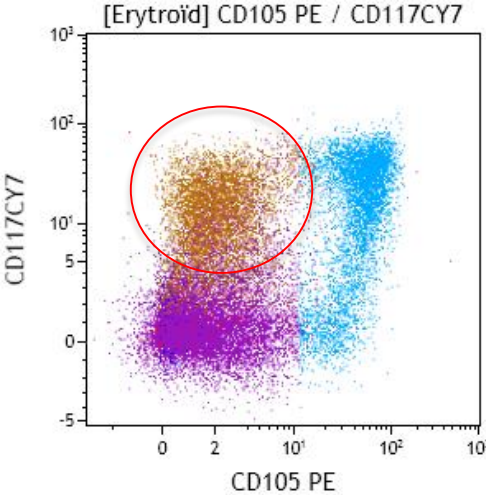
Selection of the erythroid lineage by CD105











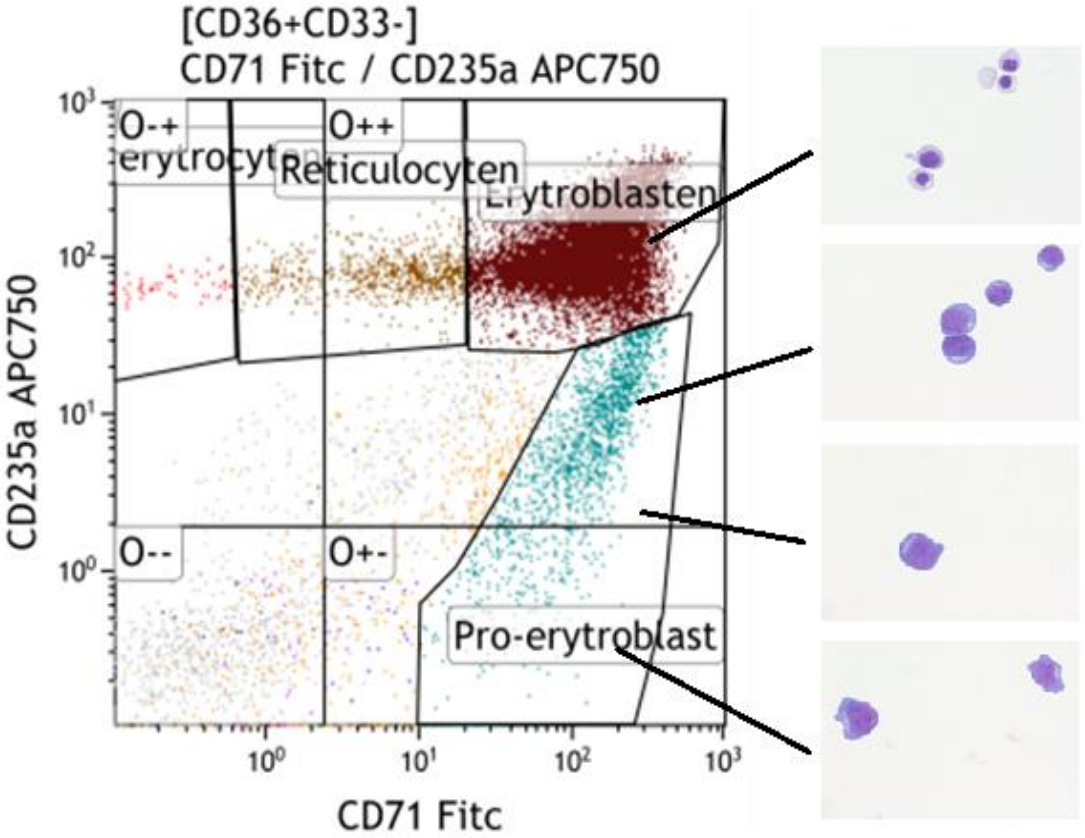
Radboudumc

Can we find a relation between FCM and microscopy?

?

Radboudumc

6. Cell sorting of various erythroblast populations



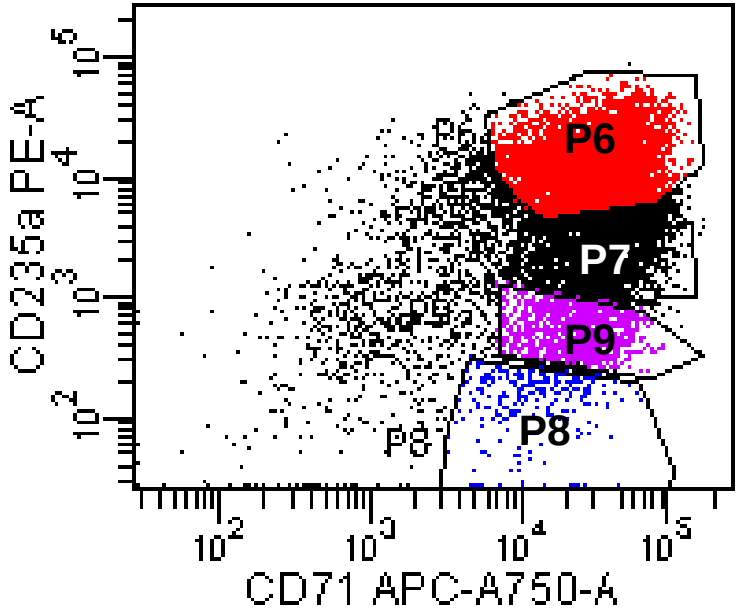
Polychrom. erytroblast

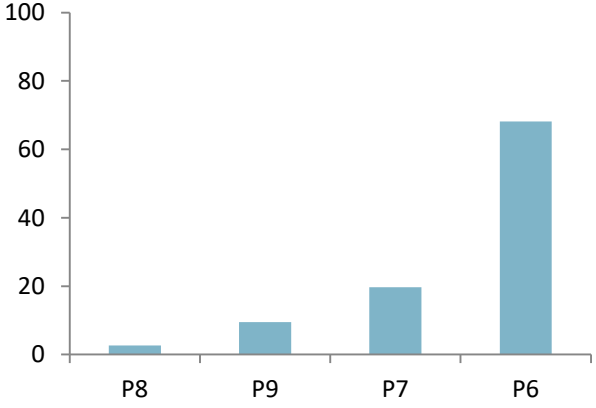
“Mature” pro-erytroblast

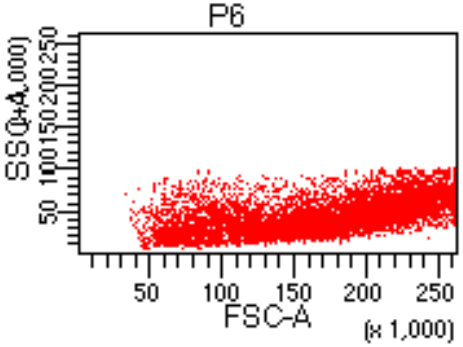
Pro-erytroblast

Immature pro-erytroblast

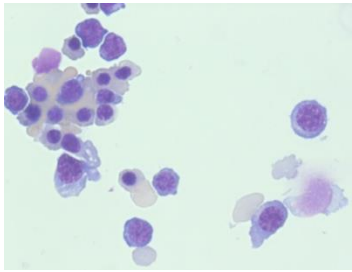
Ficoll separation



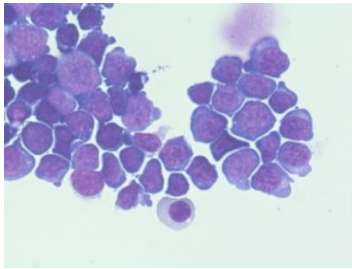




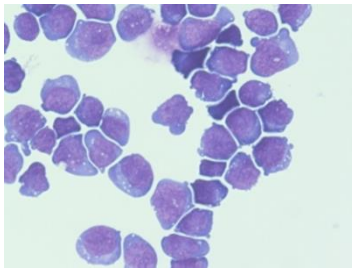
P6



P7



P9



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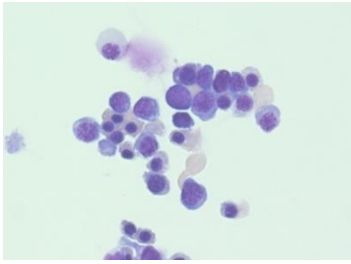
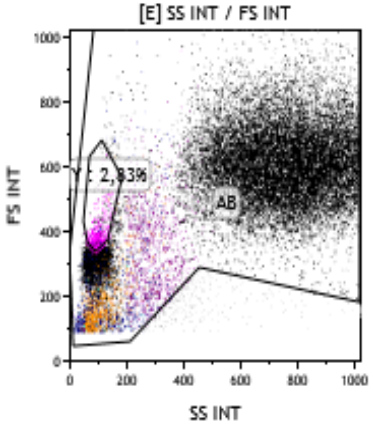
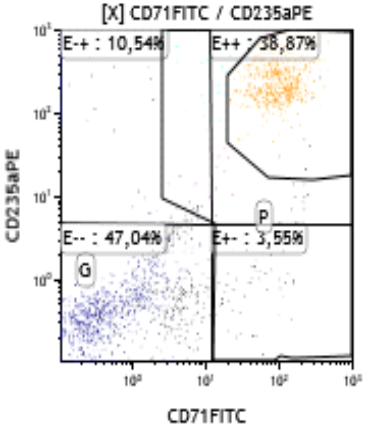


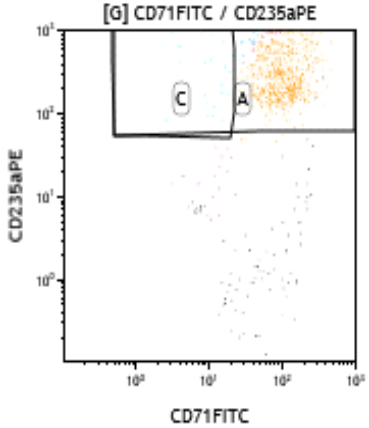
Use of FCM in MDS diagnostics



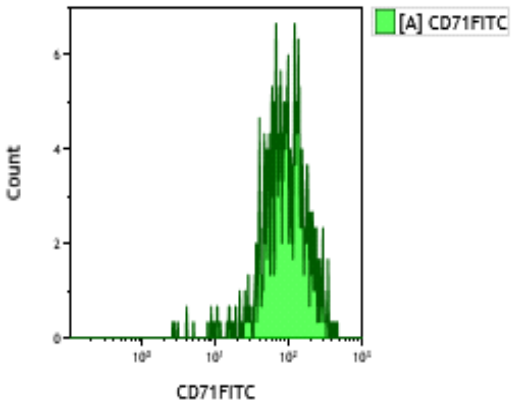
Gate	Number	%Total	%Gated
All	34.000	97,14	100,00
AB	33.555	95,87	98,69
Y	963	2,75	2,83



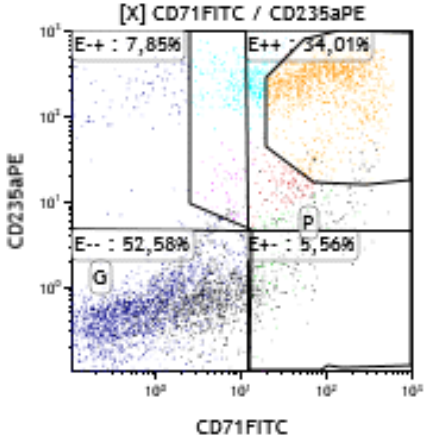
Gate	Number	%Total	%Gated
All	2.058	5,88	100,00
E--	968	2,77	47,04
E-+	217	0,62	10,54



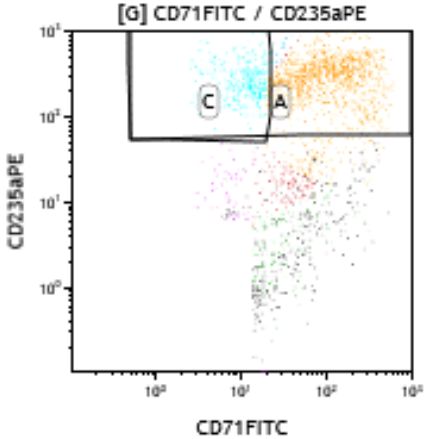
Gate	Number	%Total	%Gated	X-CV
All	894	2,55	100,00	69,33
A	785	2,24	87,81	65,03
C	29	0,08	3,24	49,67



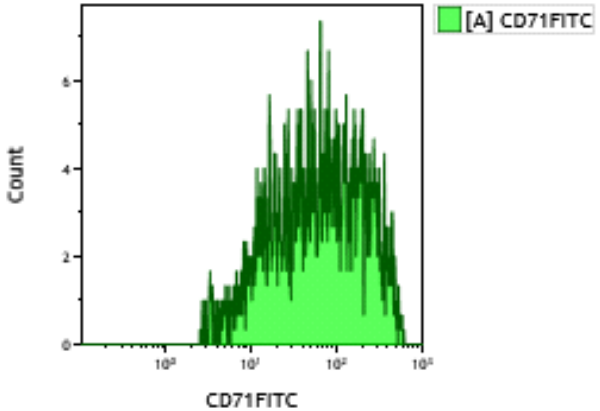
Marker	Number	%Total	%Gated	X-Med	X-AMean	X-CV
All	785	2,24	100,00	90,96	108,97	65,03



Gate	Number	%Total	%Gated
All	5.234	14,95	100,00
E--	2.752	7,86	52,58
E-+	411	1,17	7,85

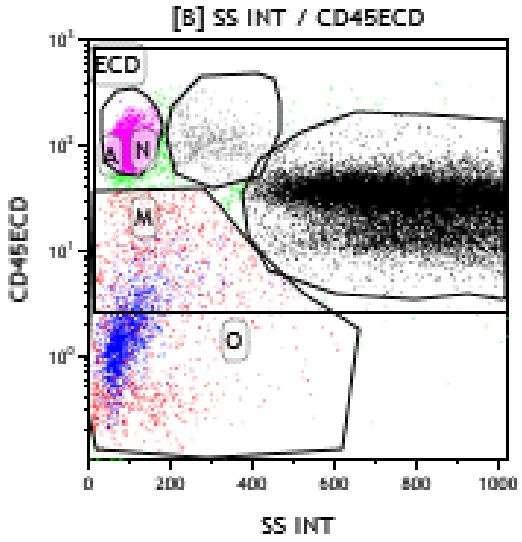


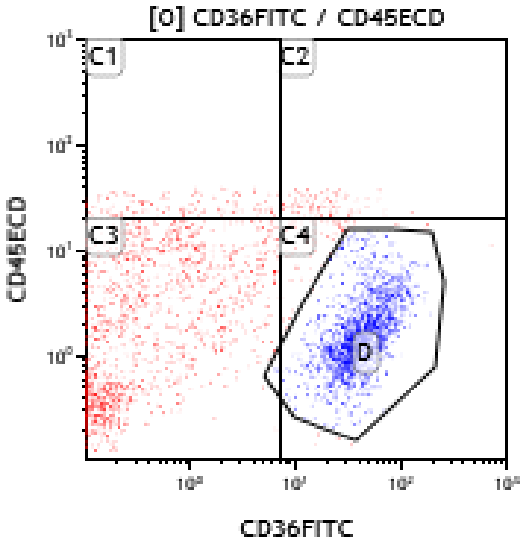
Gate	Number	%Total	%Gated	X-CV
All	2.237	6,39	100,00	112,91
A	1.528	4,37	68,31	106,73
C	381	1,09	17,03	43,84

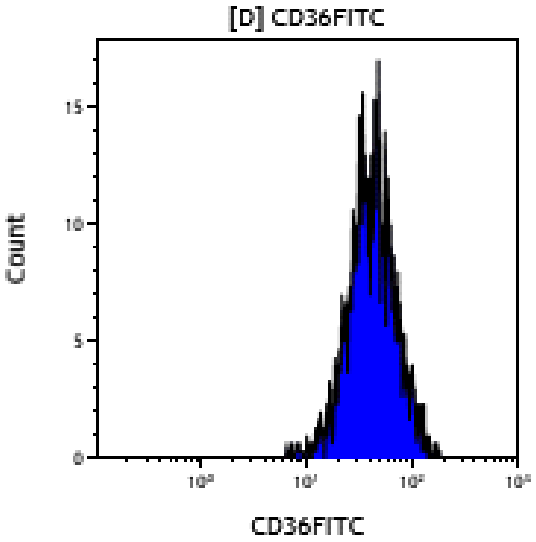


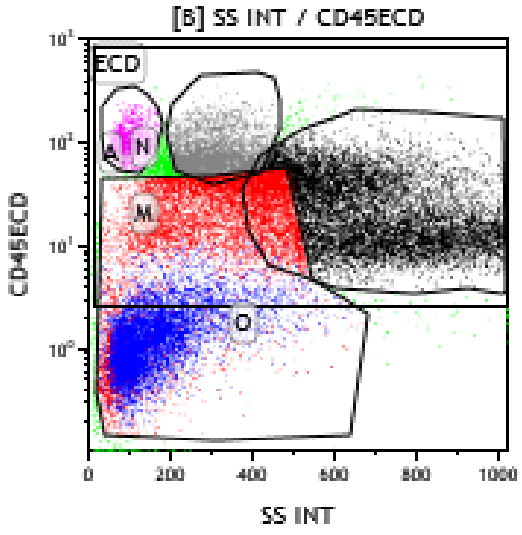
Marker	Number	%Total	%Gated	X-Med	X-AMean	X-CV
All	1.528	4,37	100,00	64,22	110,15	106,73

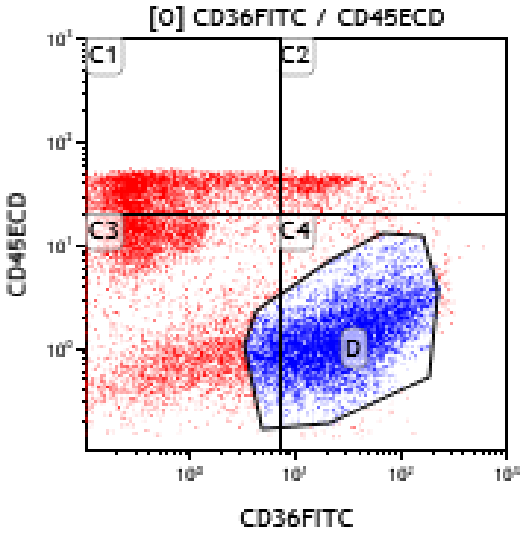
Radboudumc

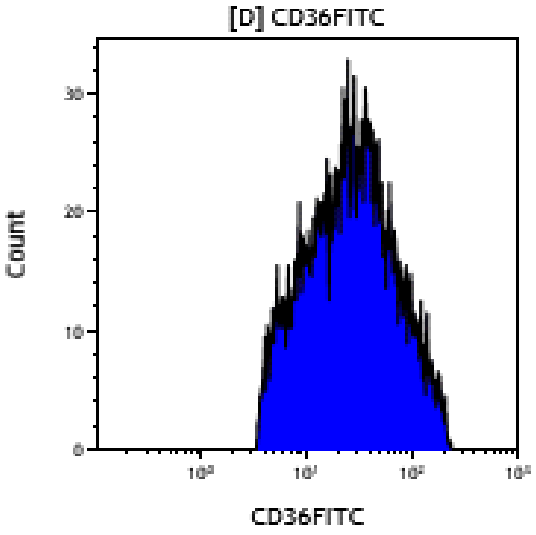












Gate	Number	%Total	%Gated
All	33.270	95,06	100,00
A	950	2,71	2,86
ECD	24.814	70,90	74,58
M	11.885	33,96	35,72
N	4.244	12,13	12,76
O	16.037	45,82	48,20

Gate	Number	%Total	%Gated	X-Med
All	16.037	45,82	100,00	5,35
C1	3.163	9,04	19,72	0,39
C2	787	2,25	4,91	16,43
C3	5.378	15,37	33,53	0,67
C4	6.709	19,17	41,83	30,22
D	7.223	20,64	45,04	26,18

Gate	X-Med	X-CV
All	26,18	98,53

Summary

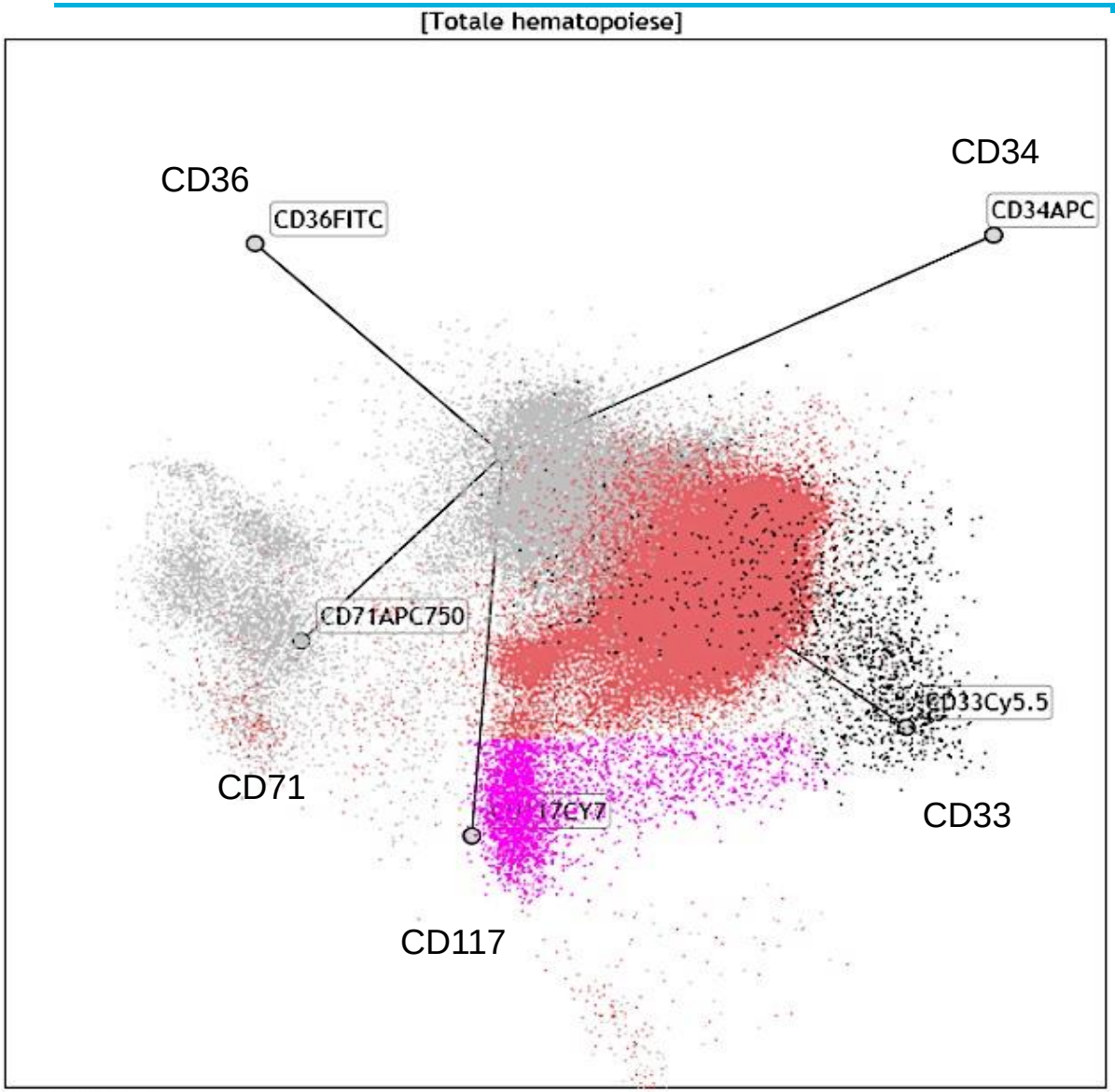
1. Myeloid differentiation
2. Monocytic differentiation
3. Separation point of myeloid and monocytic
4. Erythroid differentiation

How to show all the lineages in one plot??

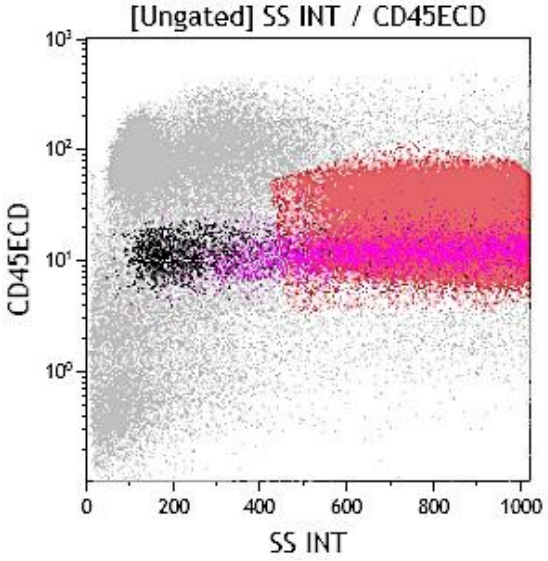
The Radar plots

Myelopoiese

CD45+-/CD34+ precursors,
Promyelocytes + Mature myeloid

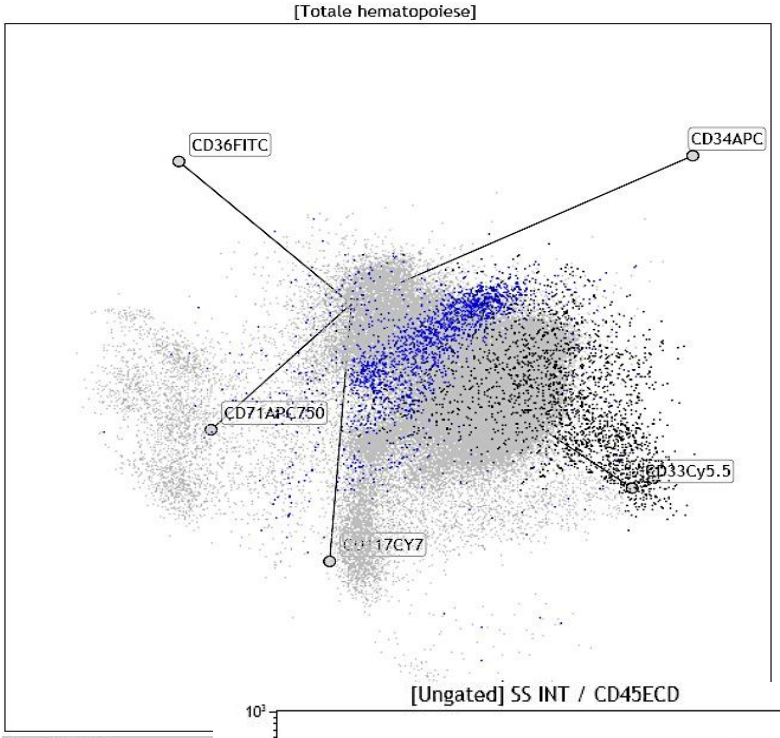


Gate	%Total
All	50,77

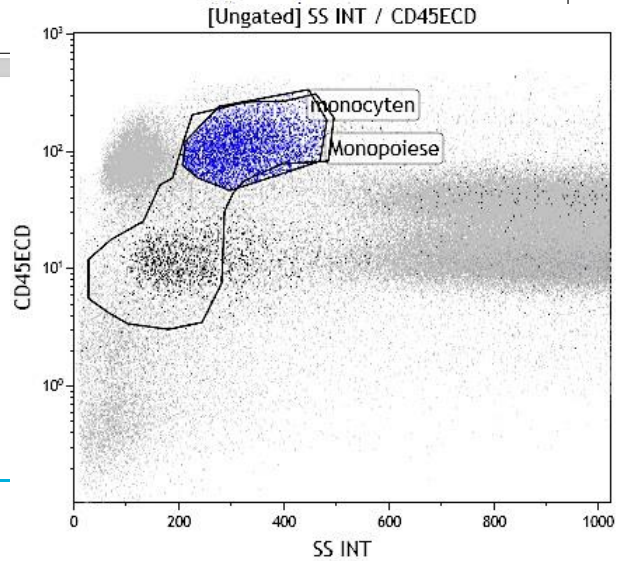


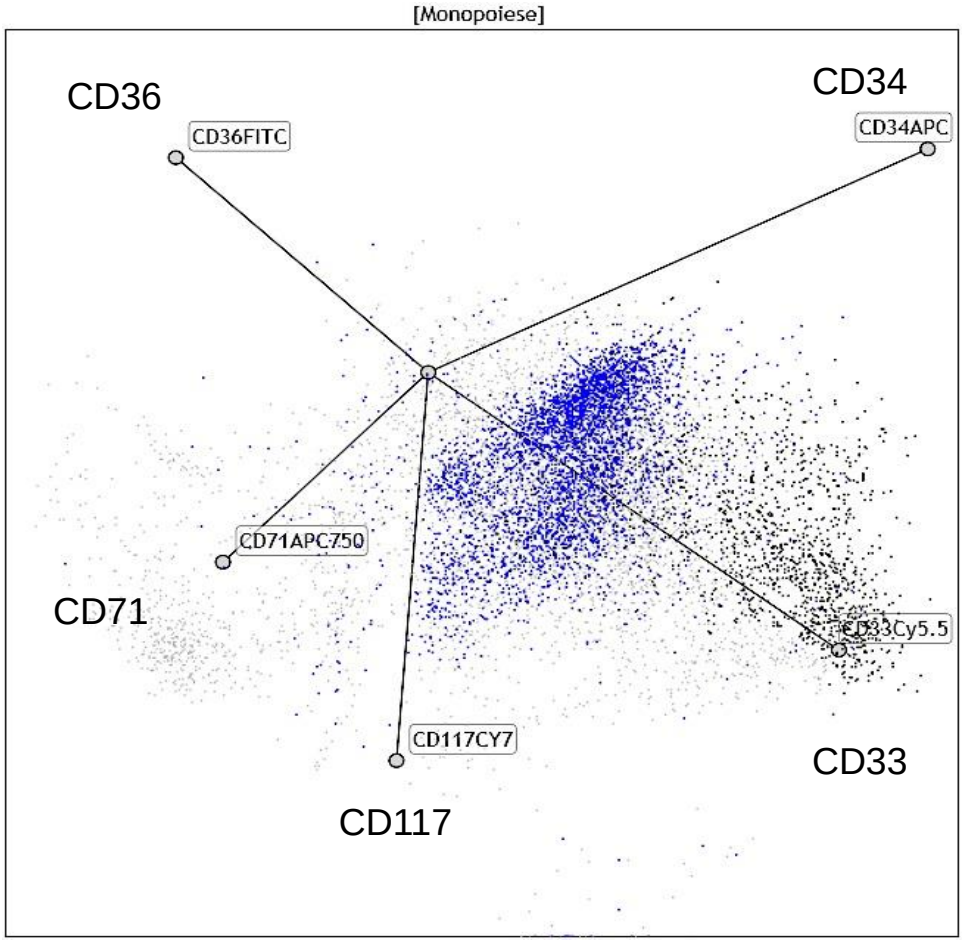
Monopoiese

Mature monocytes and CD45+-/CD34+ precursors

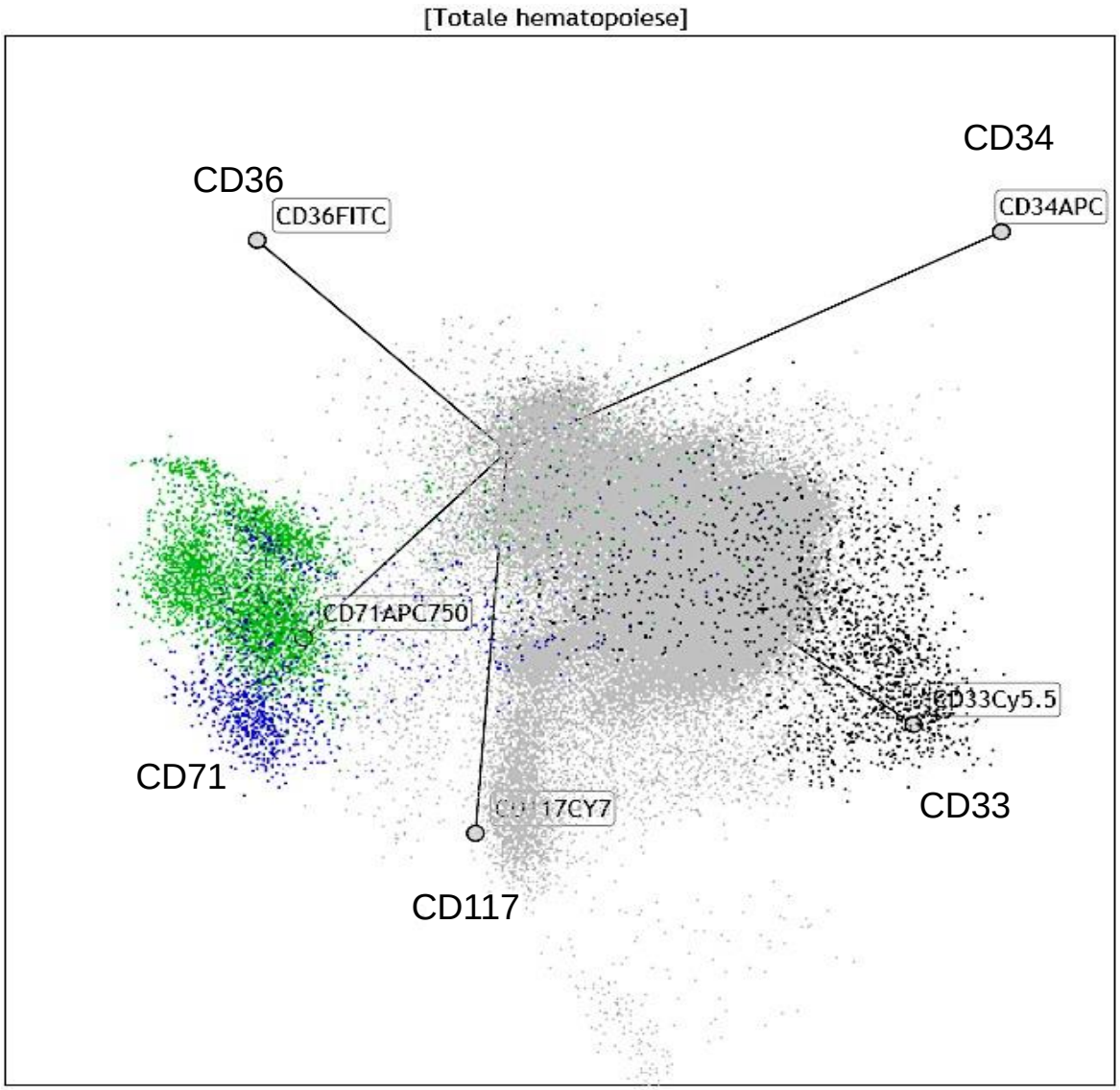


Gate	%Total
All	48,80

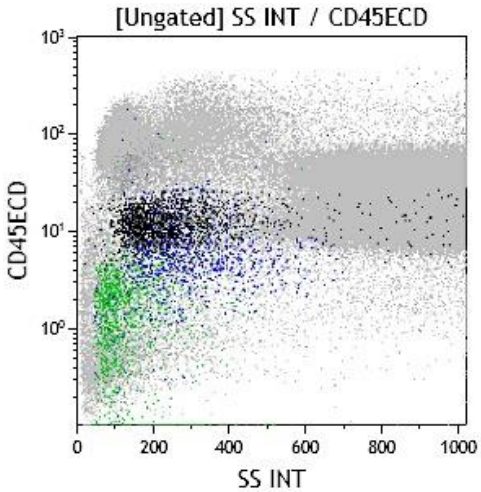


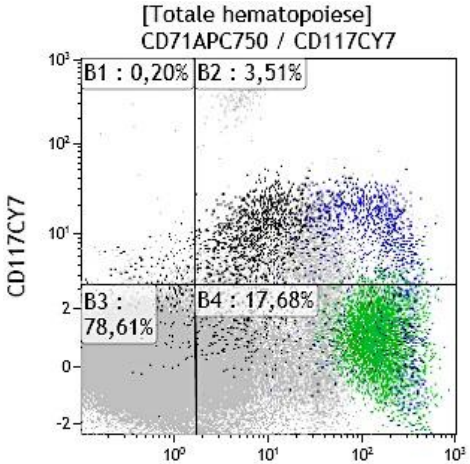


Erythropoiese

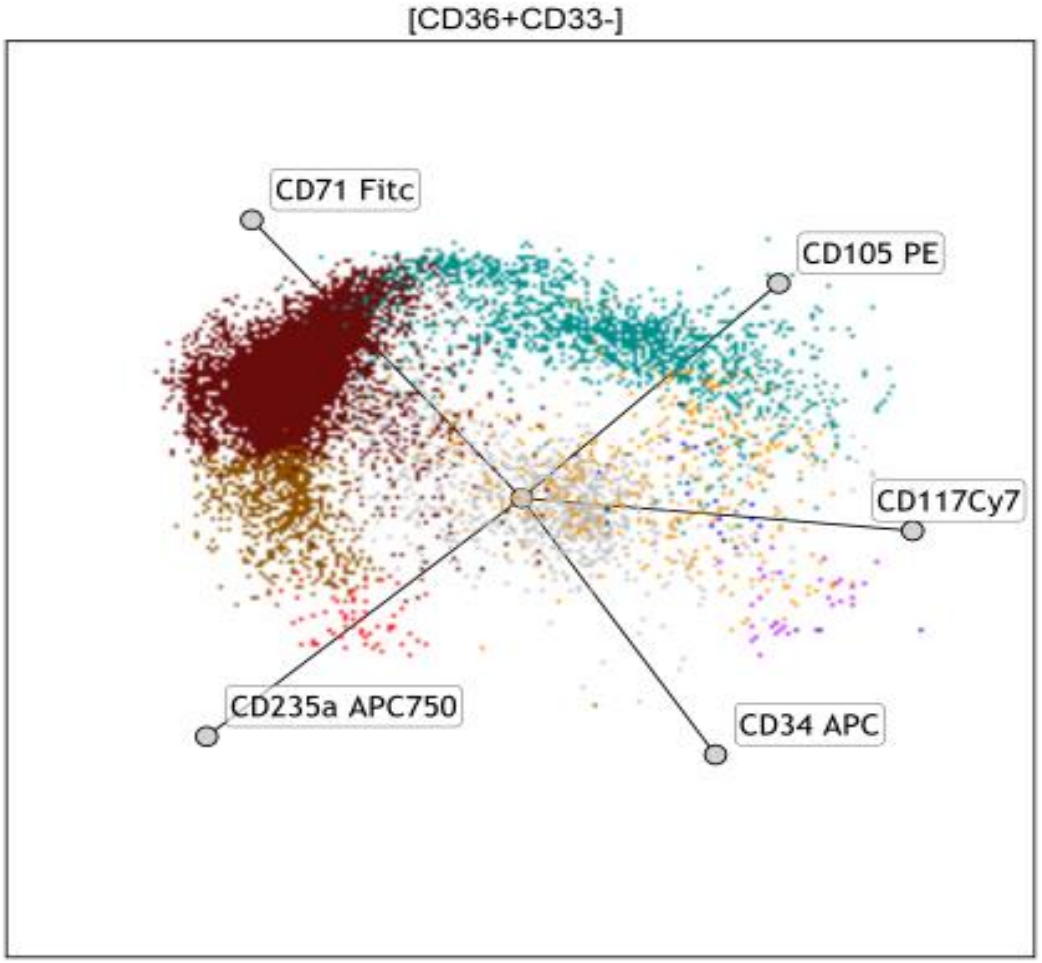


Gate	%Total
All	50,01

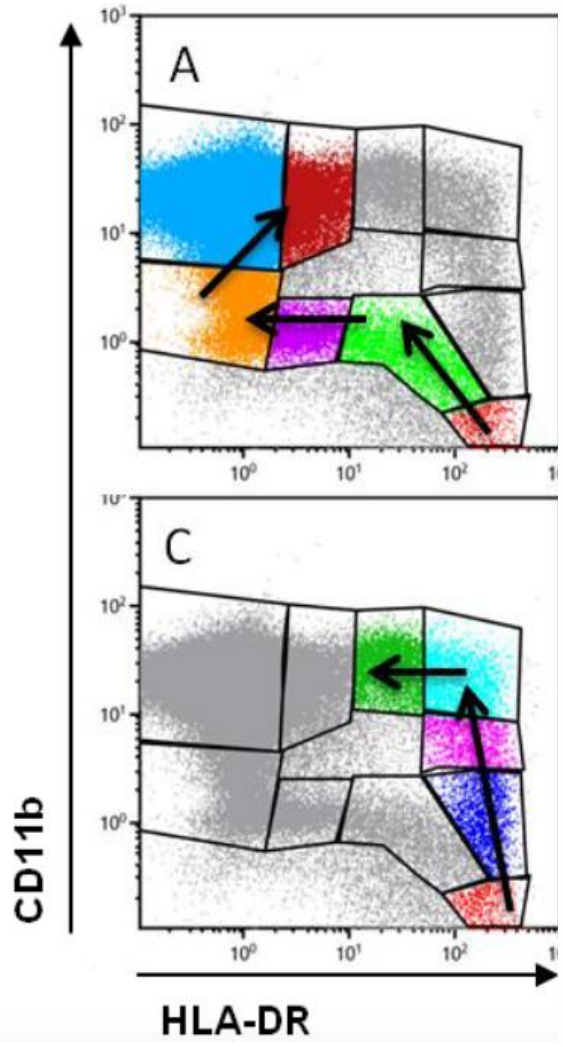


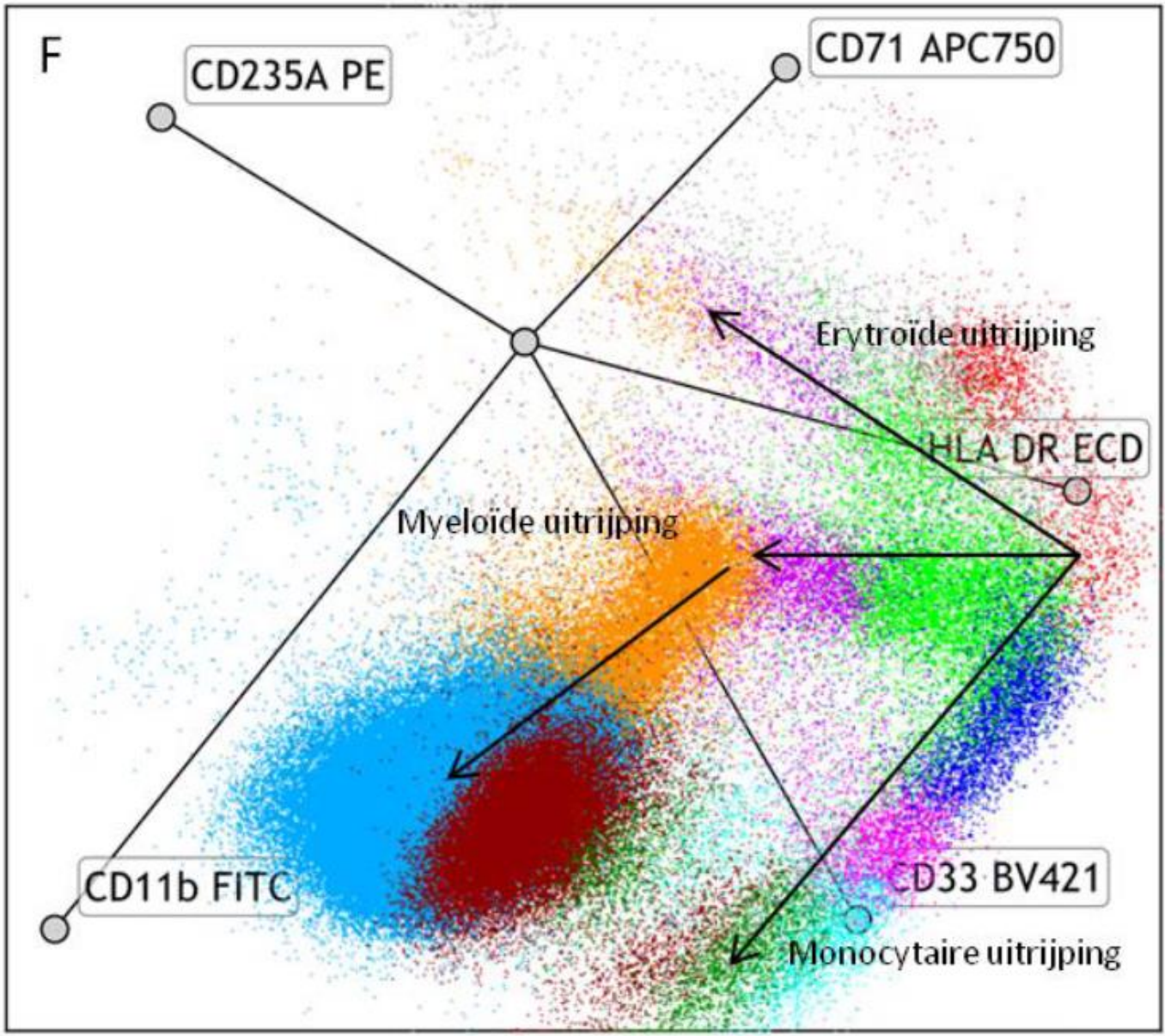


Erythropoiese (CD36+CD33- and CD105/CD117)

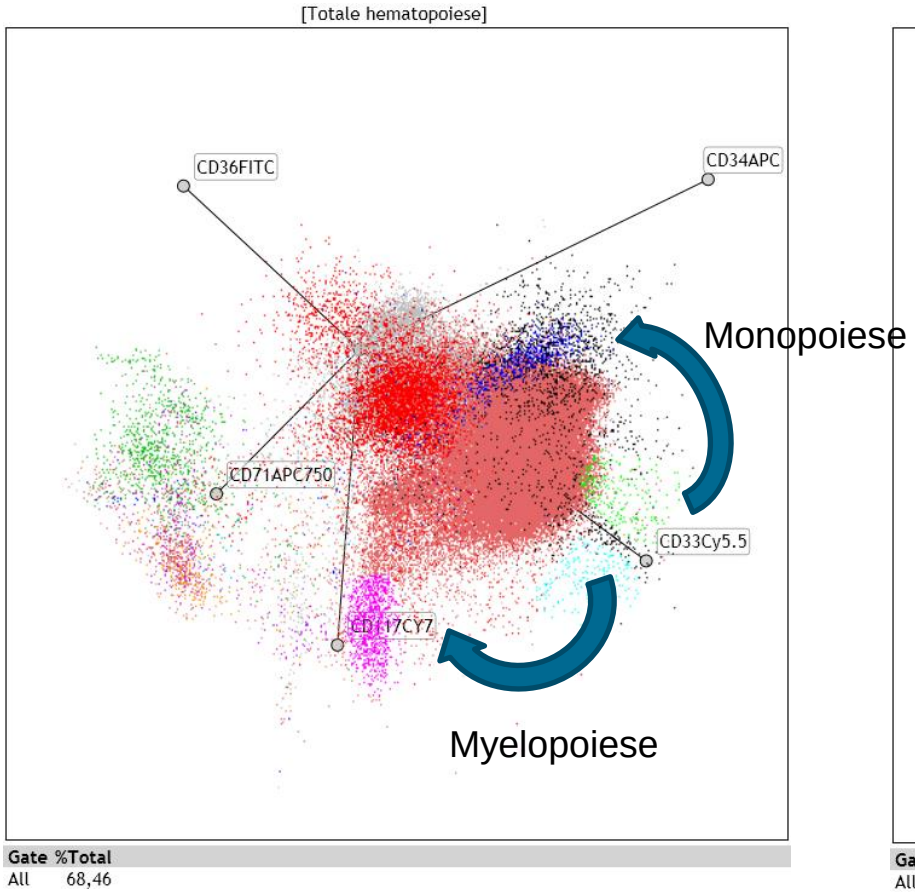


Hematopoiese (Myeloid - Monocytic - Erythroid)

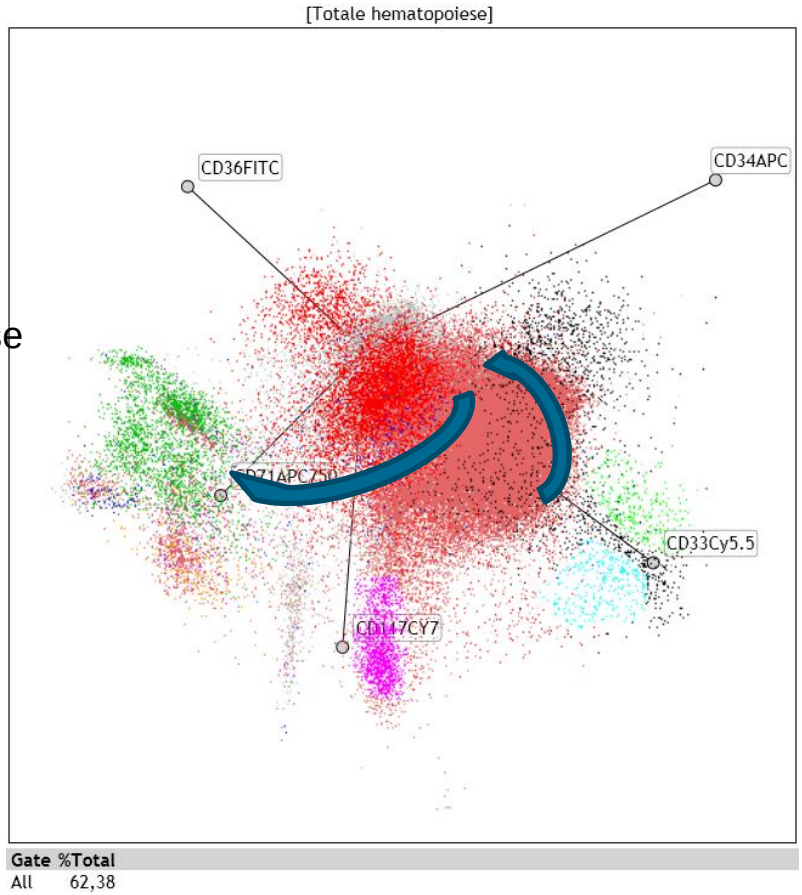




Normal hematopoiese

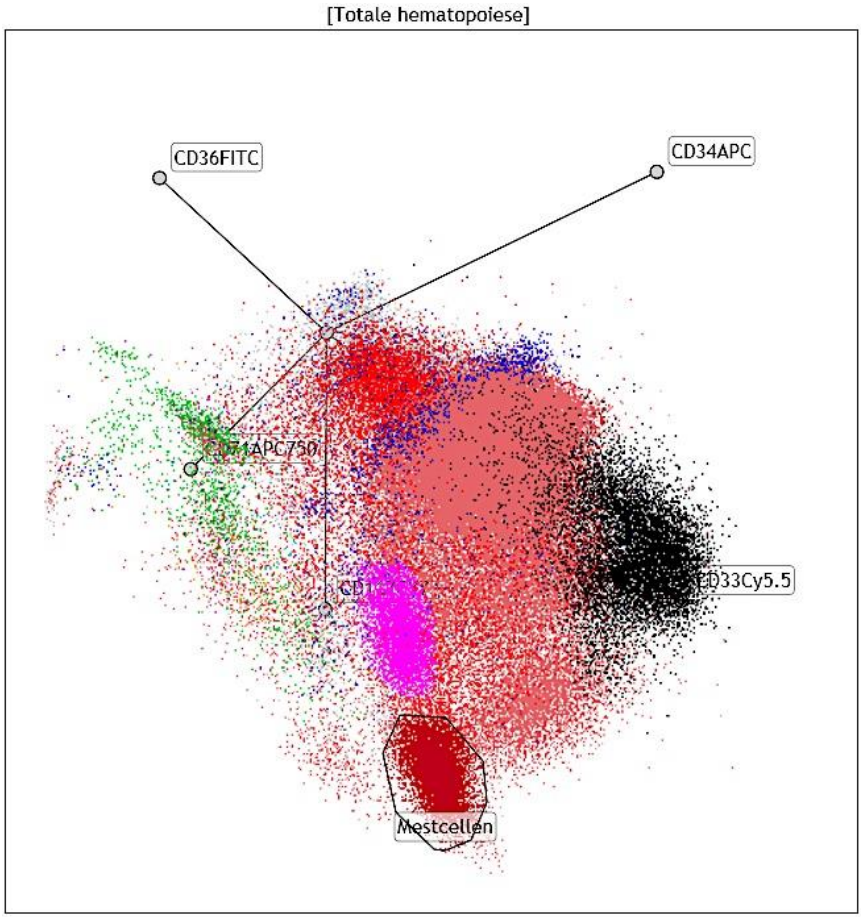


Myeloid and monocytic hematopoiese

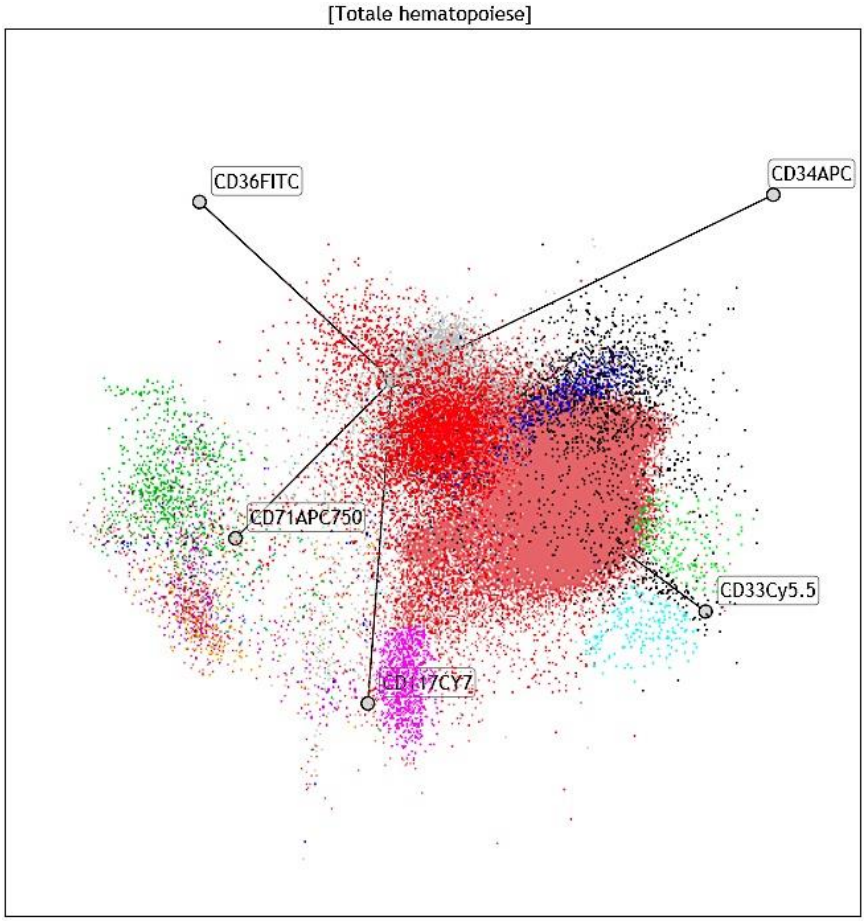


Erythroid hematopoiese

CMML and mastocytosis



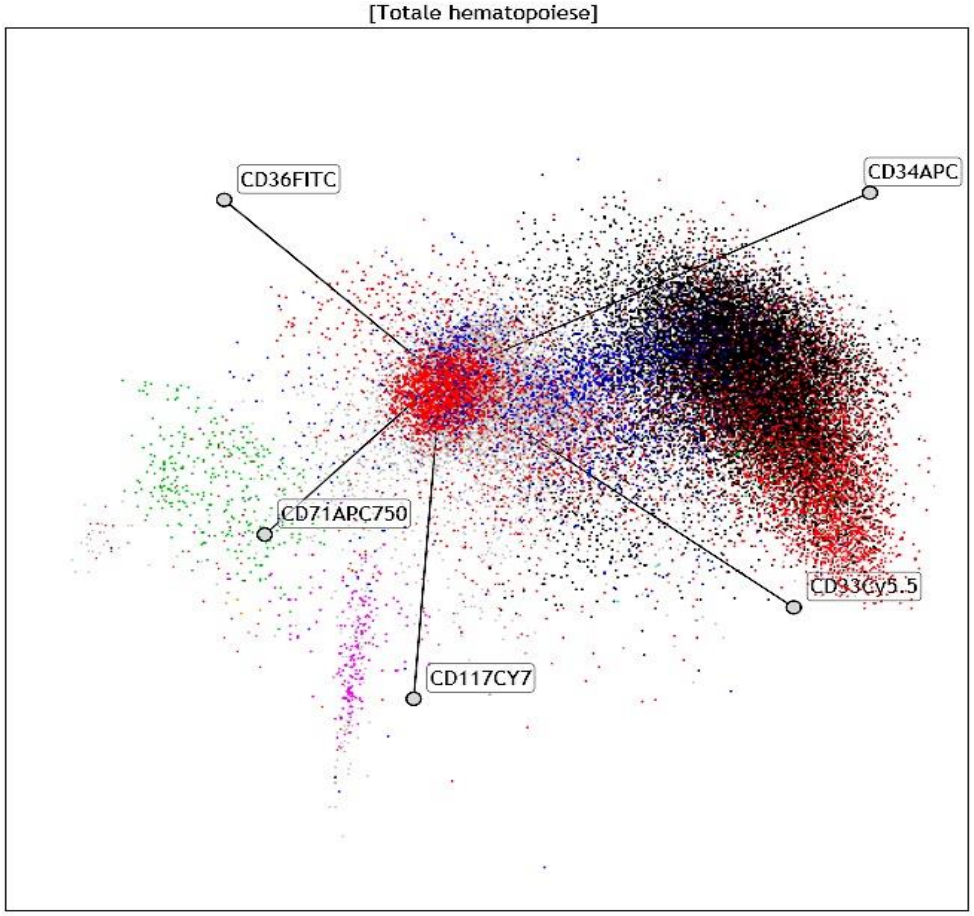
Gate	%Total
All	52,80
Mestcellen	2,59



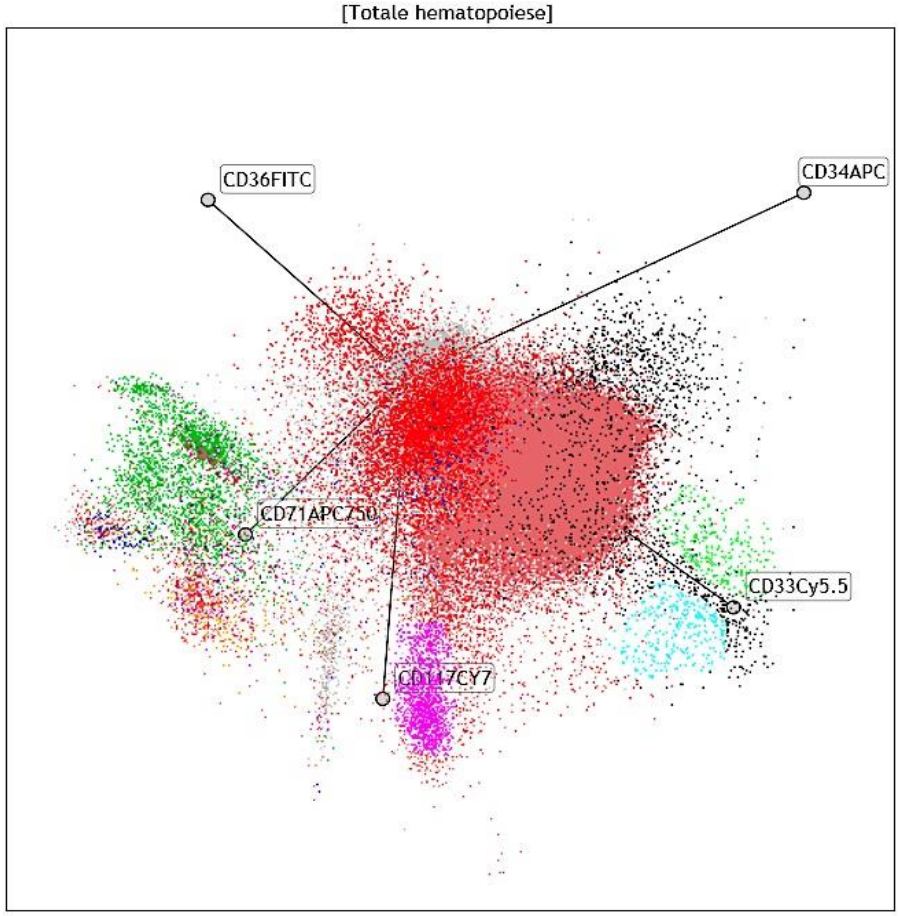
Gate	%Total
All	68,46

Normal control

AML without differentiation and monocytic characteristics



Gate	%Total
All	92,83

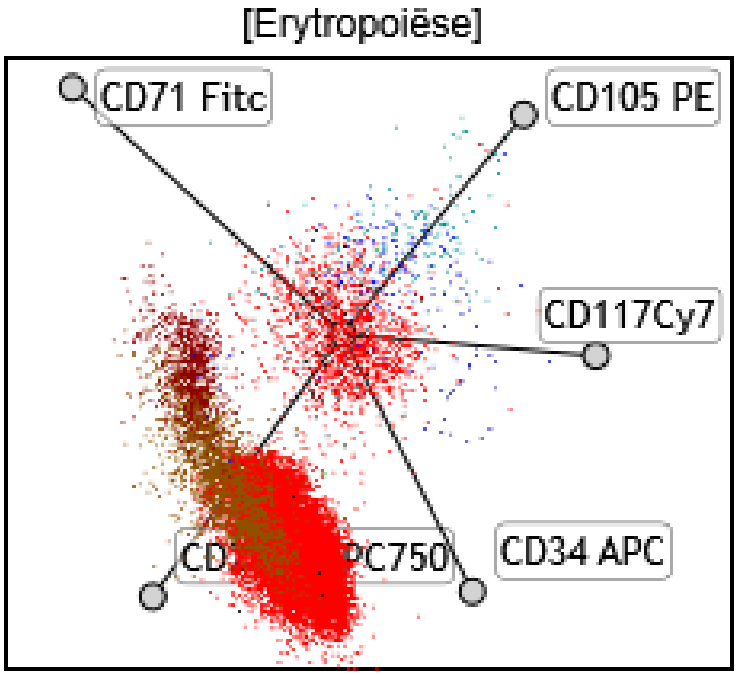


Gate	%Total
All	62,38

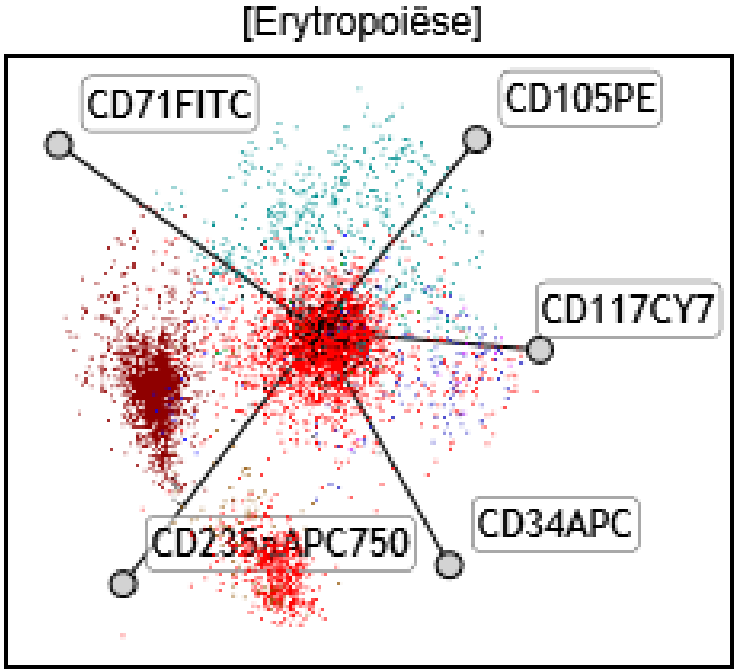
Normal control

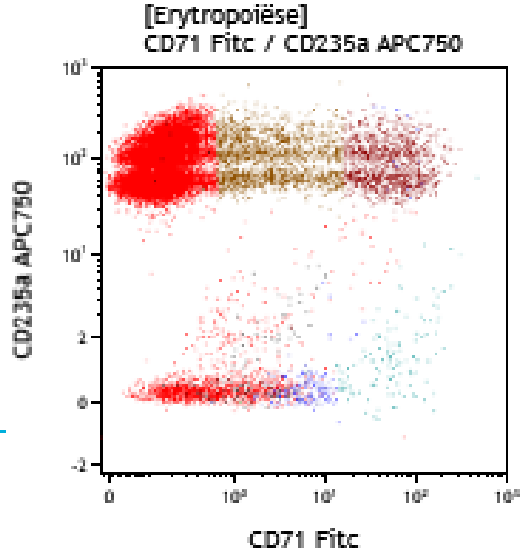
MDS with dyserythropoiesis (increase of CD71dim/CD71CV)

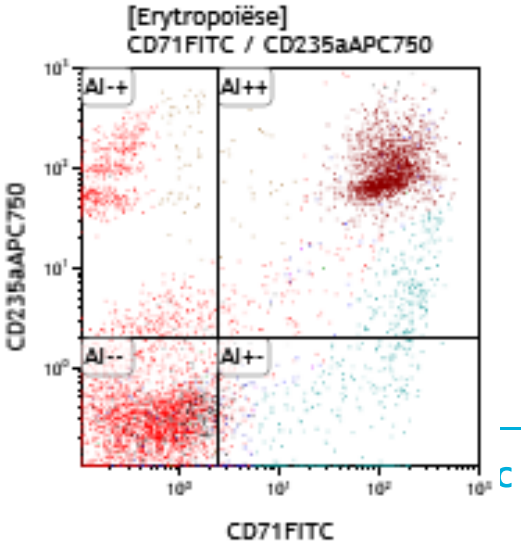
MDS



Normal control

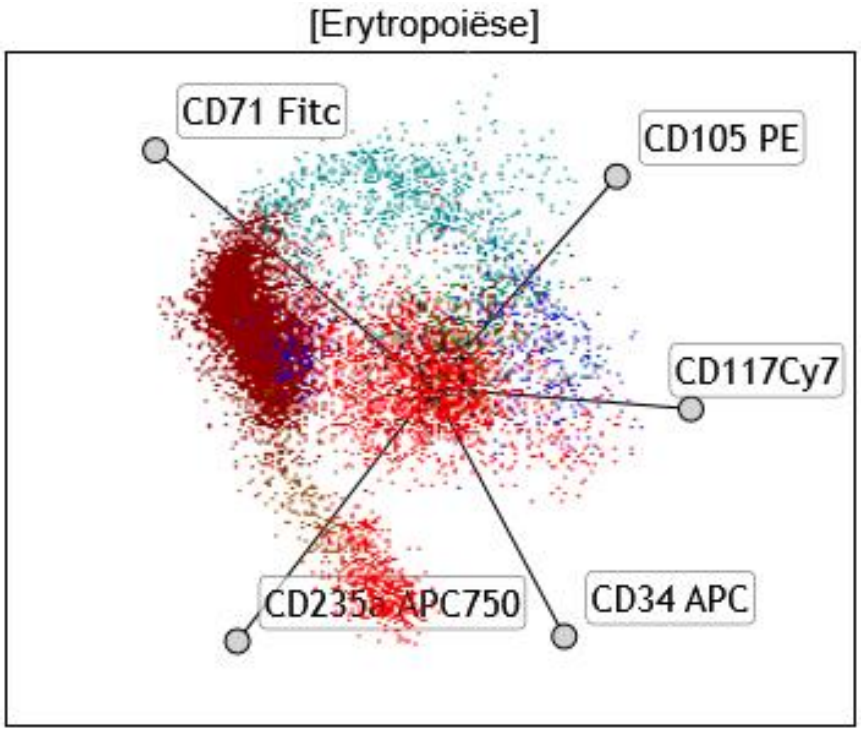




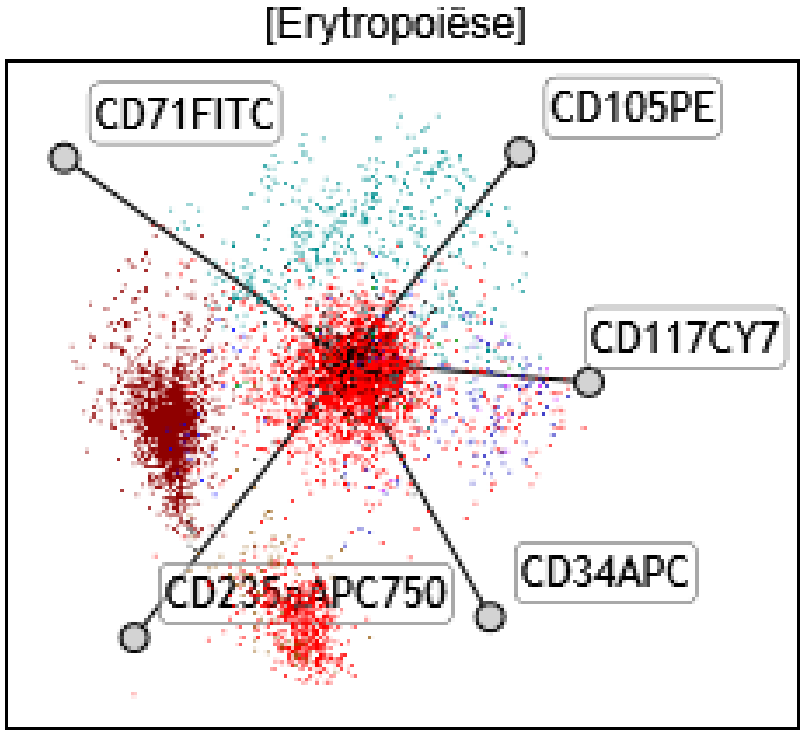


MDS with dyserythropoiesis (increase CD71 CV)

MDS

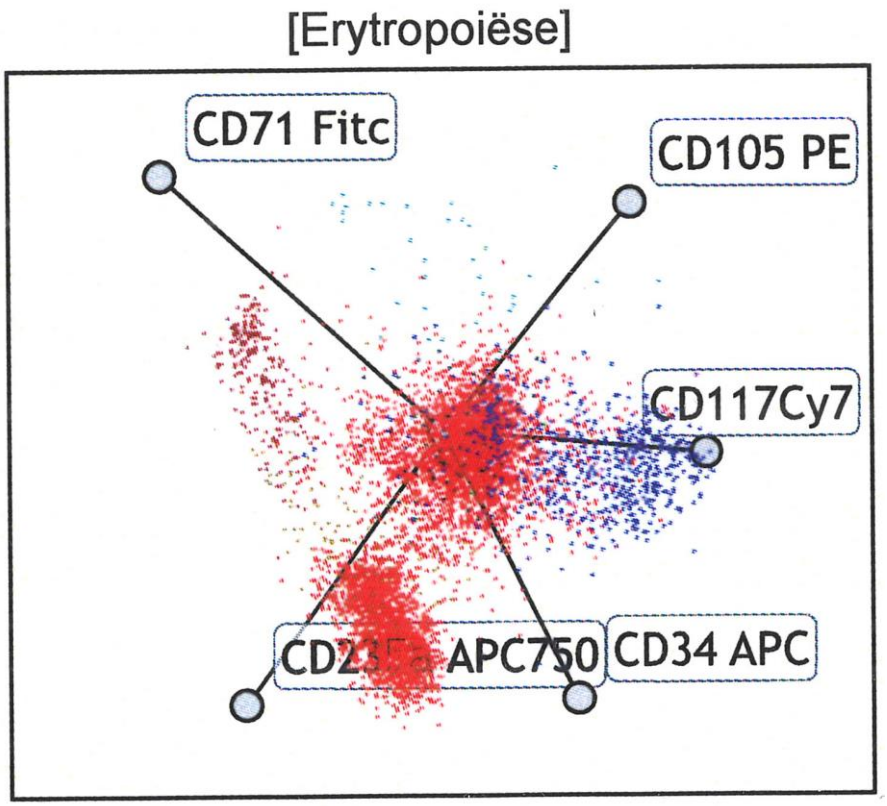


Normal control

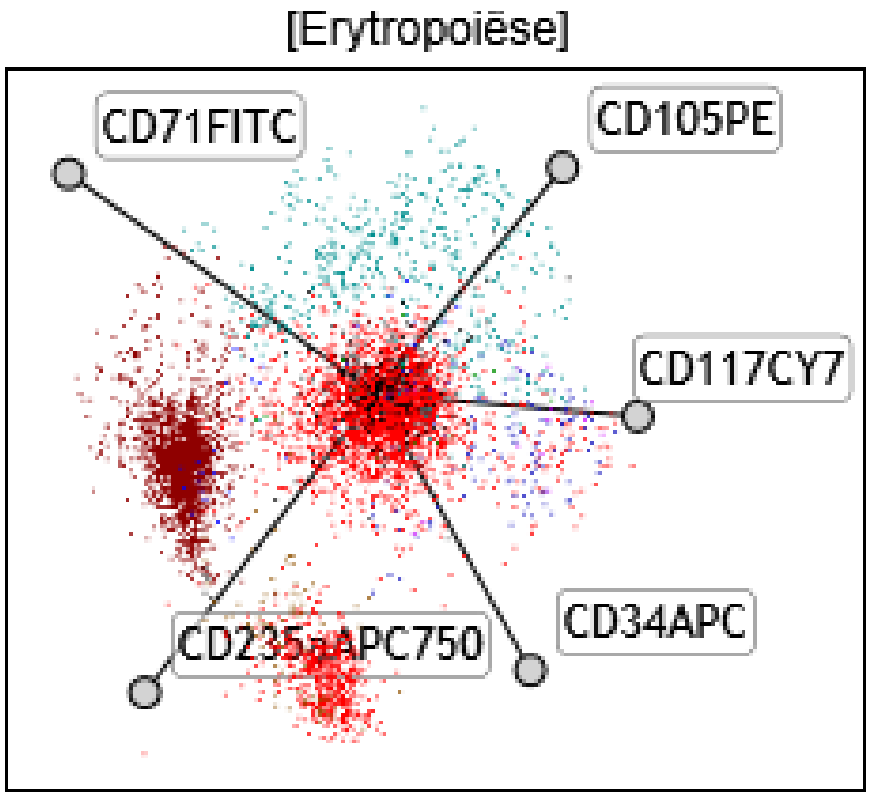


MDS with dyserythropoiesis (decrease immature)

MDS



Normal control



Conclusions

- CD11b/CD13/CD16 and CD11b/CD15/HLADR are essential marker combinations to study the myelo/monocytic maturation pathways
- In relatively mature myeloid stage, the SSC is expressed from dim to bright with increase in CD45 expression
- CD14/CD36/CD16 are essential marker combinations to study the monocytes and macrophages
- CD36/CD235a/CD117/CD71/CD105 are essential marker combinations to study the erythroid differentiation
- **Radar plots are very useful to study**
  - **The differentiation pathways of the different lineages**
  - **The differentiate between normal and aberrant**

He who is blind to the view of our plots, will not enjoy and see maturation as it is.

Thanks for your attention