

# Analyse van stam cel tot bloedcel: Flowcytometrie van de differentiatie

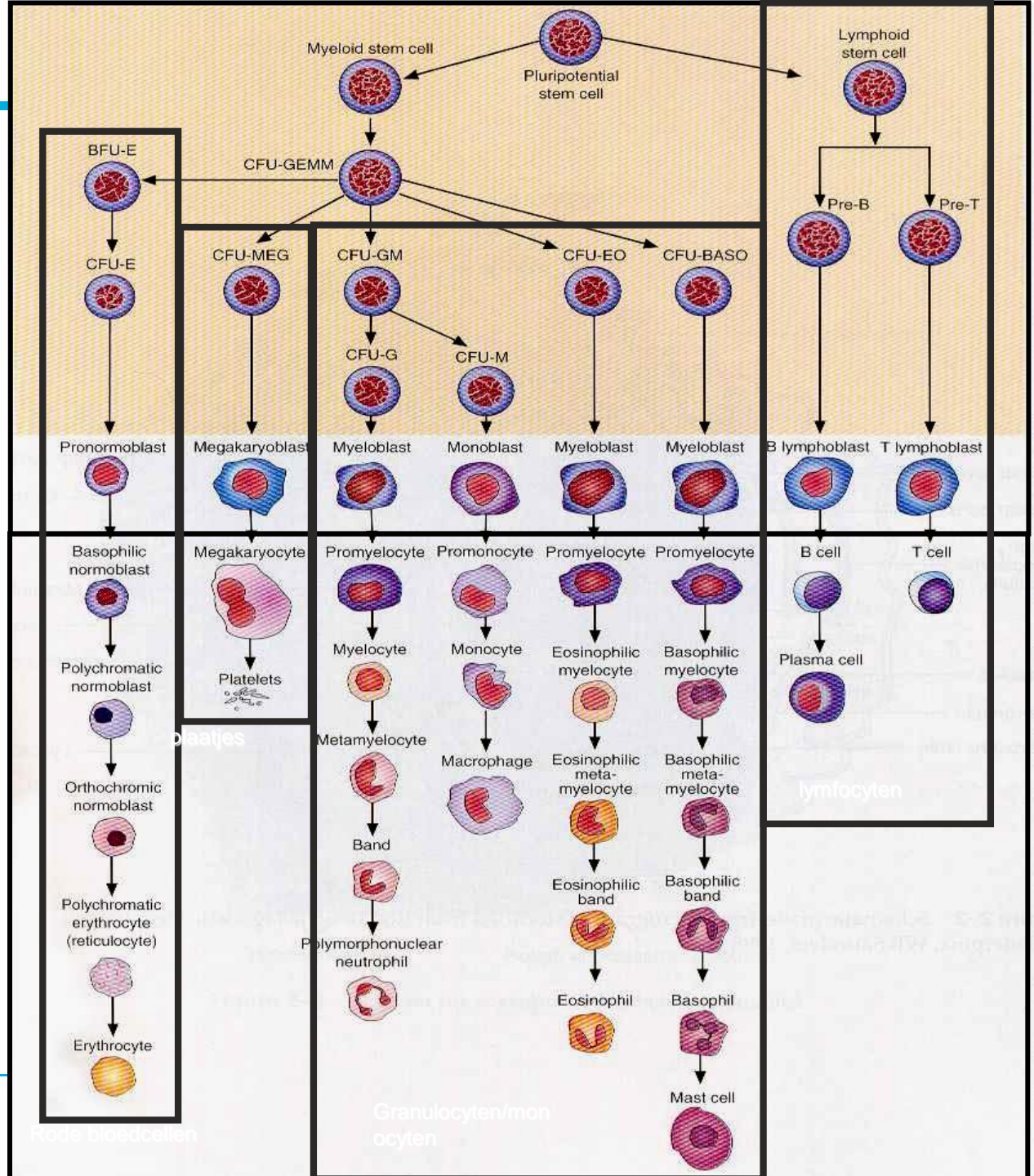
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Dept. Laboratory Medicine – Laboratory for Hematology  
Radboud University Medical Center  
Nijmegen, The Netherlands*



Radboudumc

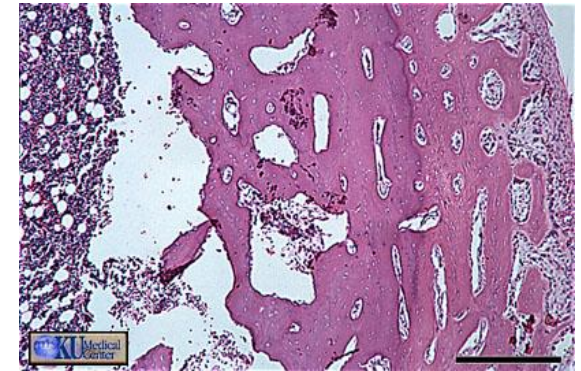
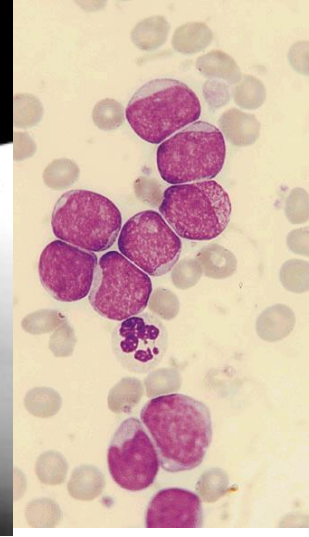


# Maturatie van Bloedcellen

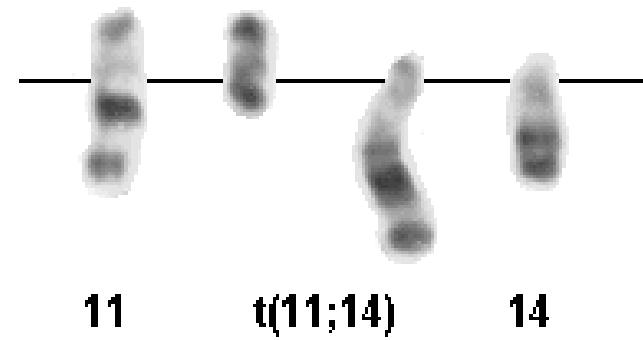
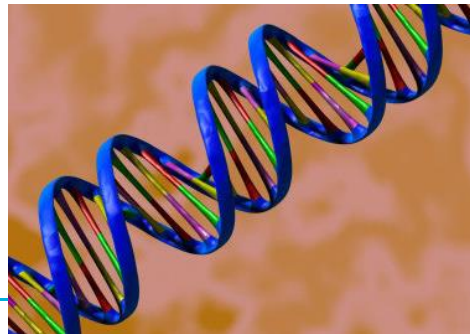
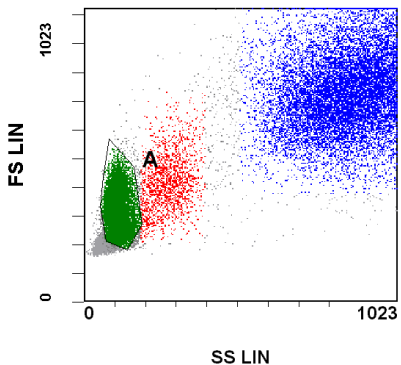


# Diagnostiek voor identificatie van cellen

- Morfologie
- Cytohistochemie
- Cytogenetica
- Moleculaire biologie
- **Immunofenotypering**



[Ungated] SS LIN/FS LIN



# Flow Analyse Today

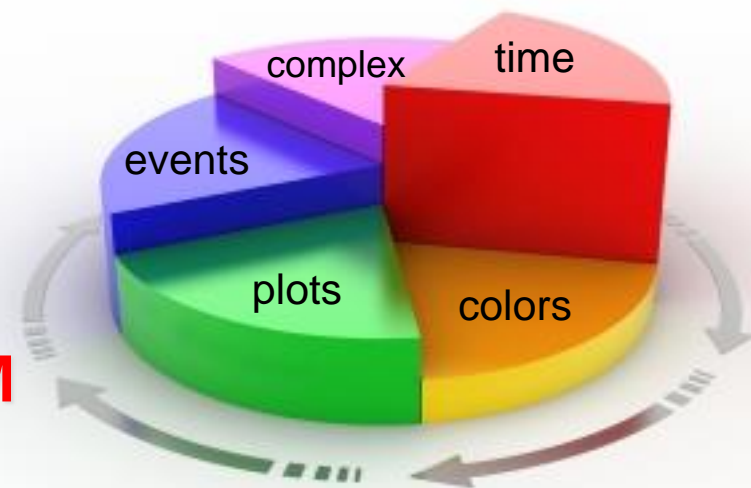
Flowcytometrische  
immunofenotypering anno 2019

- Geavanceerde multi-kleur FCM
- Sterke intensiteit fluorochromen
- Hoog-specifieke MoAbs

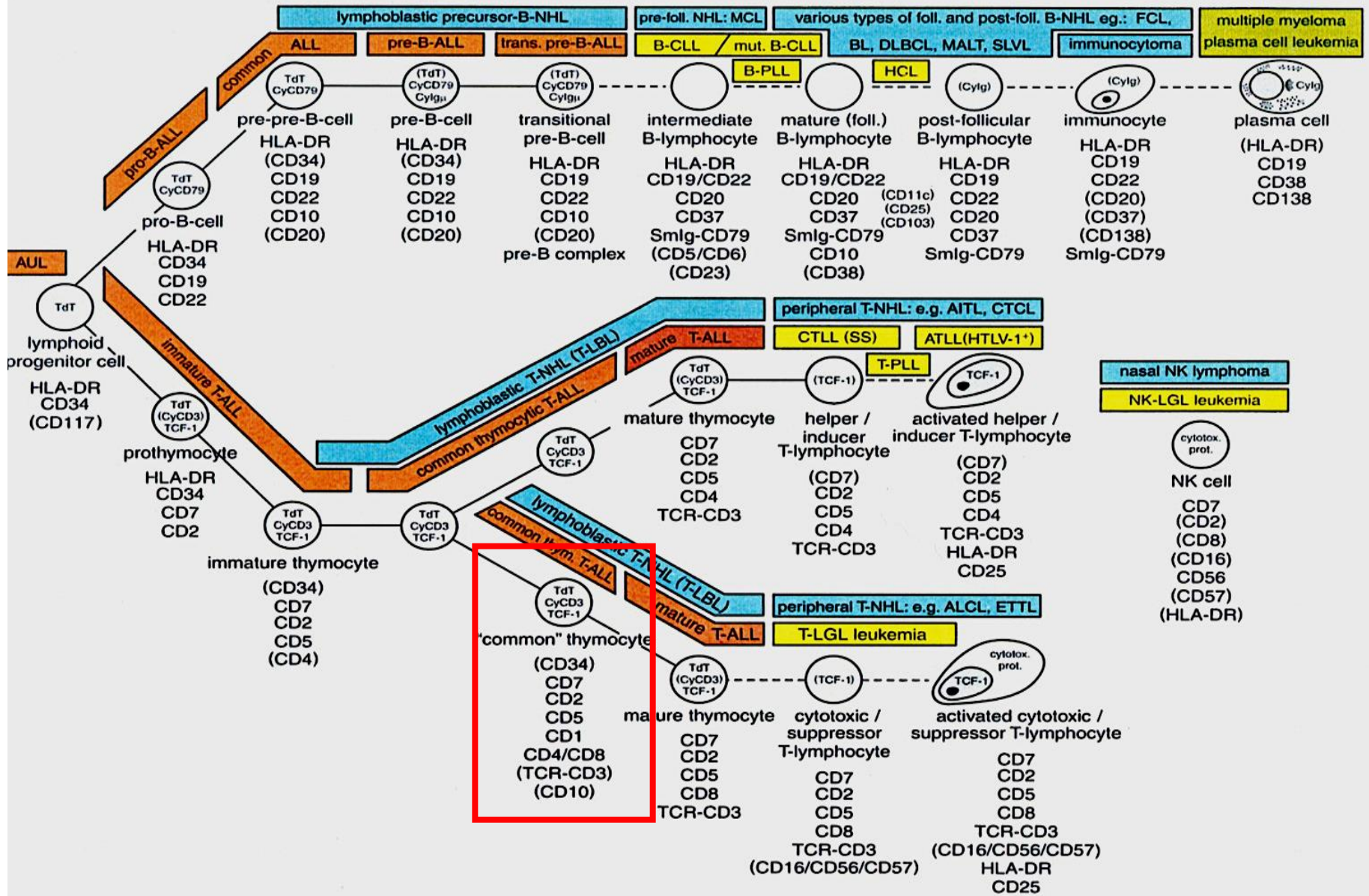


Meer kleuren vraagt meer plots,  
events, protocol complexiteit en  
uiteindelijk toename analyse tijd

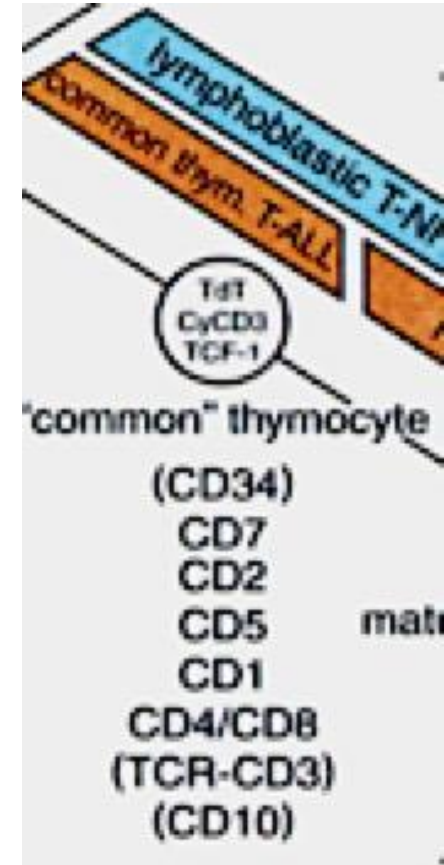
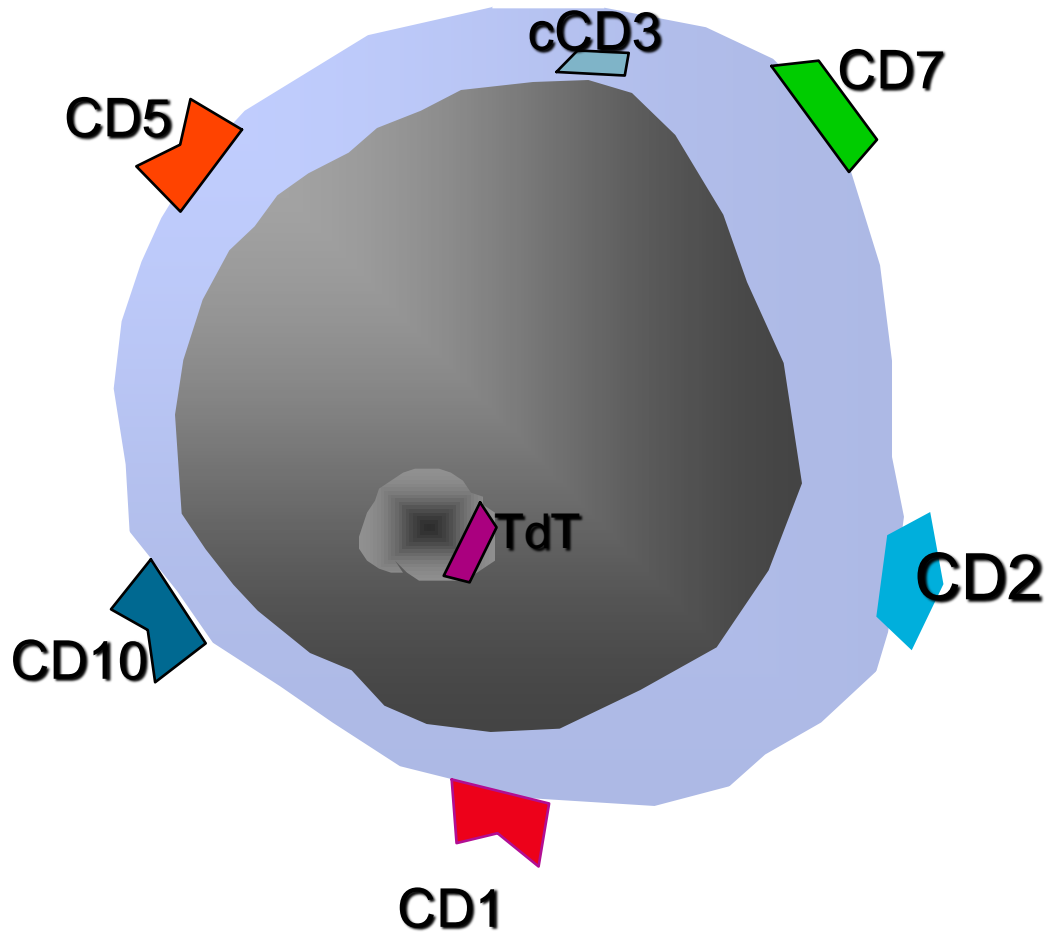
**Hoe kun je deze multi-kleur FCM  
controleren en toepassen??**



# Lymfoïde differentiatie

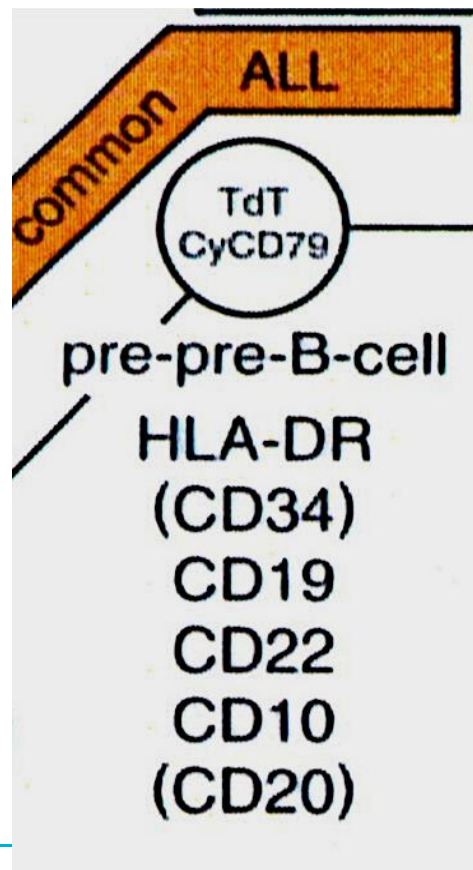


# T-ALL cel (7-kleuren)



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# Hoe maak je onderscheid tussen normale en maligne cellen



# Voorwaarden voor Immunofenotypering van leukemie en lymfoom

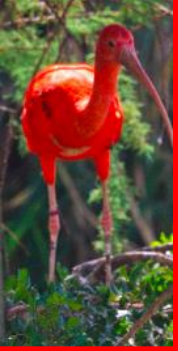
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## Hoe kun je differentiëren tussen normaal en malignant

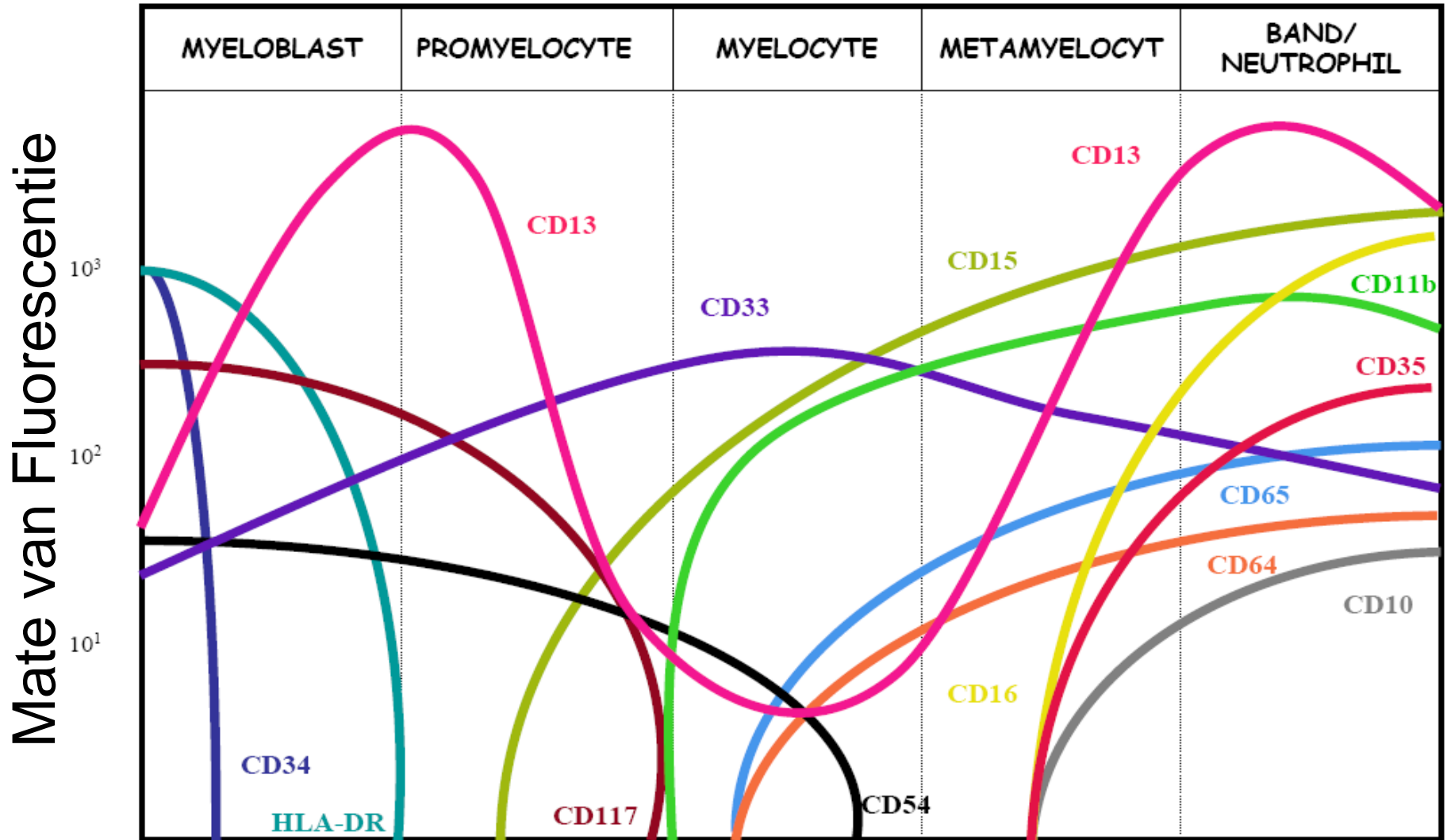
- **Patroon herkenning:**
  - Wat is het normale patroon van expressie?
  - Wat is het aberrante patroon van expressie?



Ook de cellen met laagste intensiteit moeten detecteerd worden

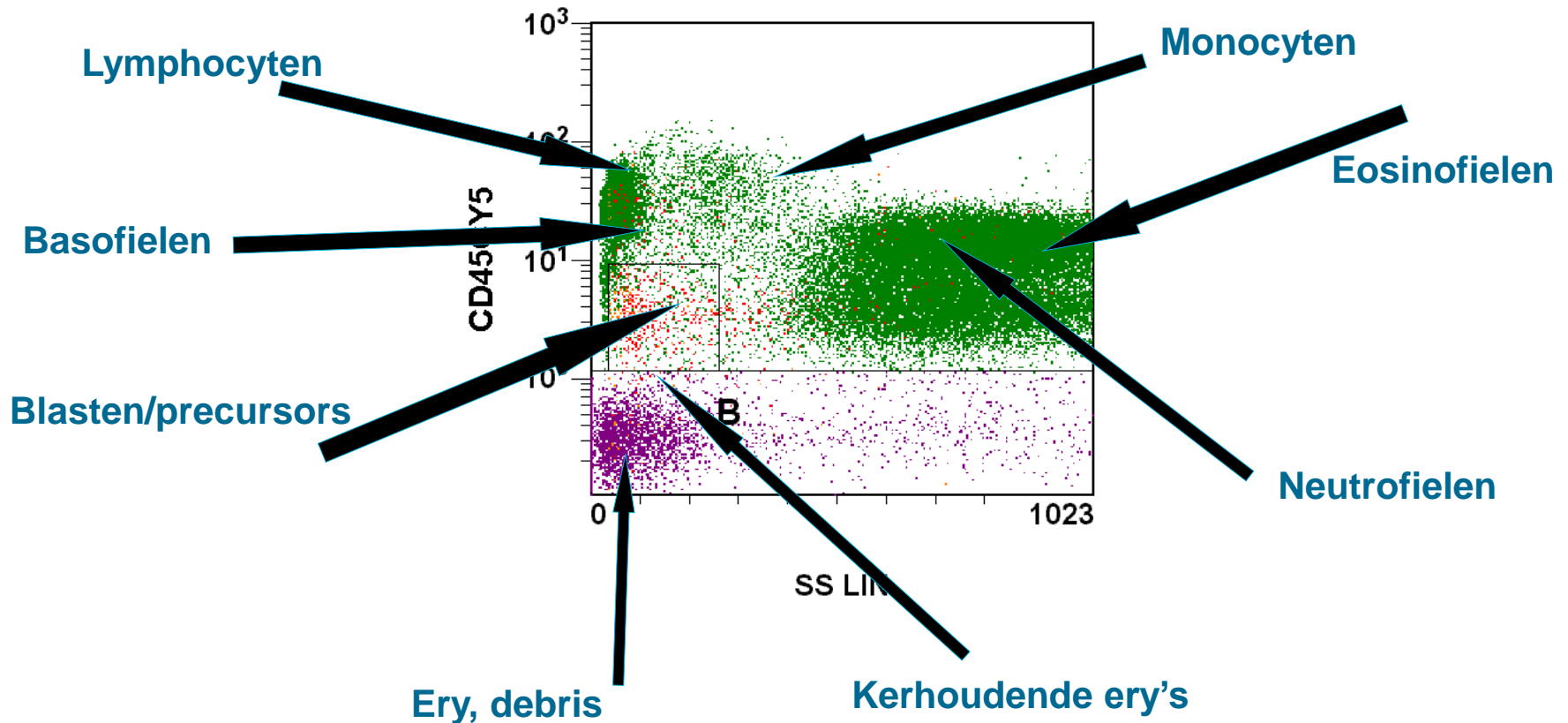


# Fenotype veranderingen in de neutrofielen differentiatie pathway



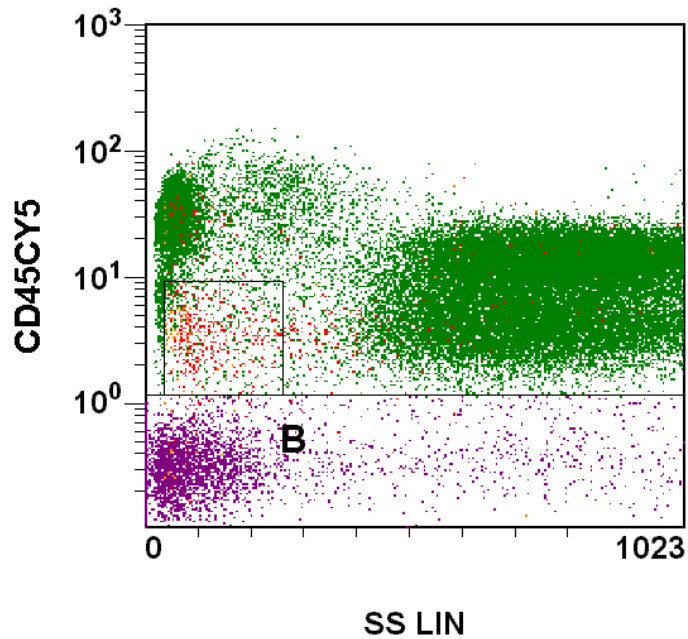
# Celpopulaties in CD45/SS plot van beenmerg

CD45: medium tot hoog Ag dichtheid  
CD45: beschikbaar in meeste kleuren

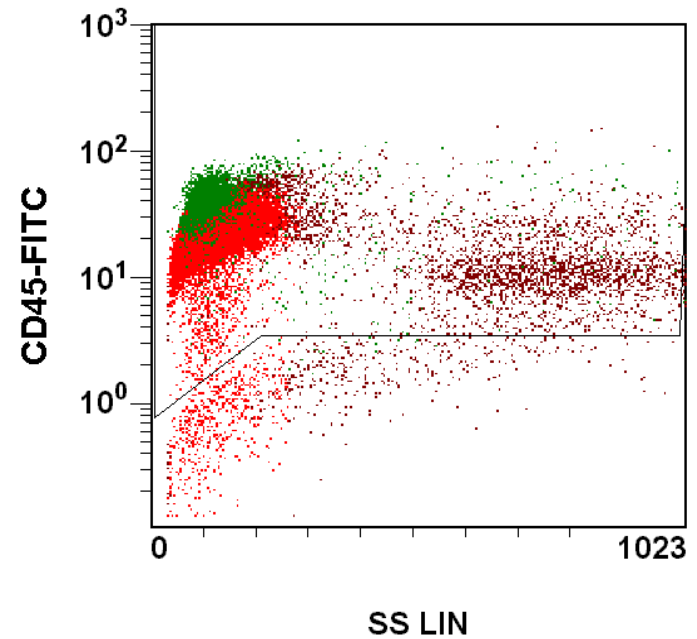


# CD45 patroon

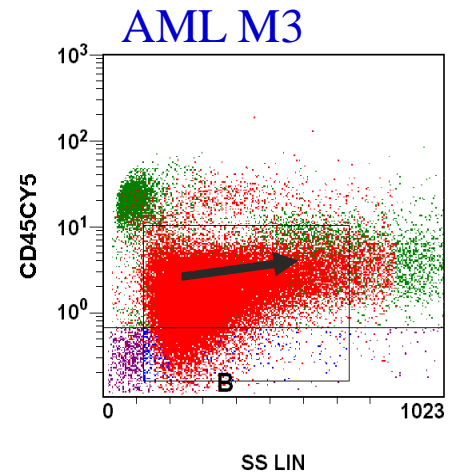
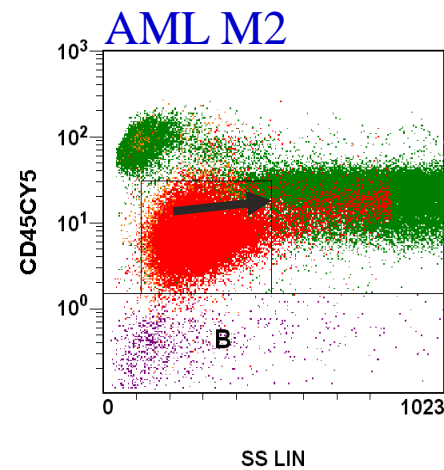
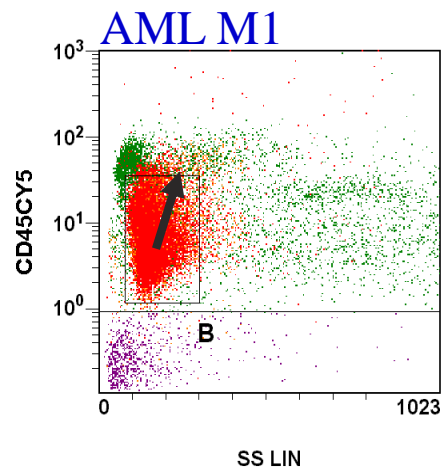
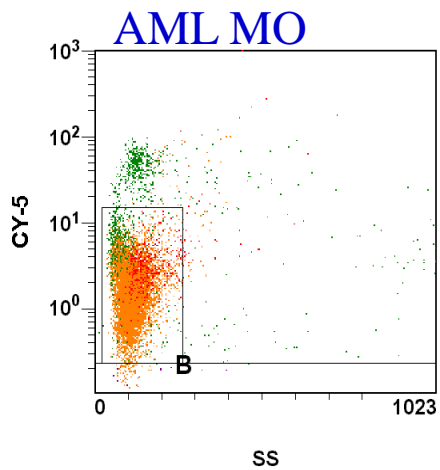
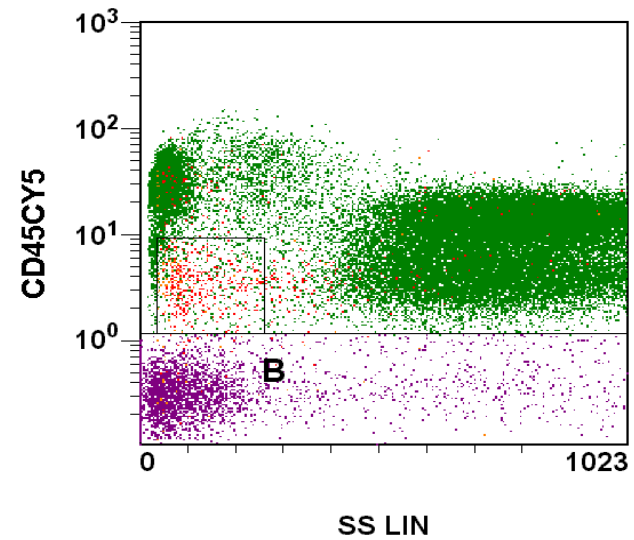
Normaal



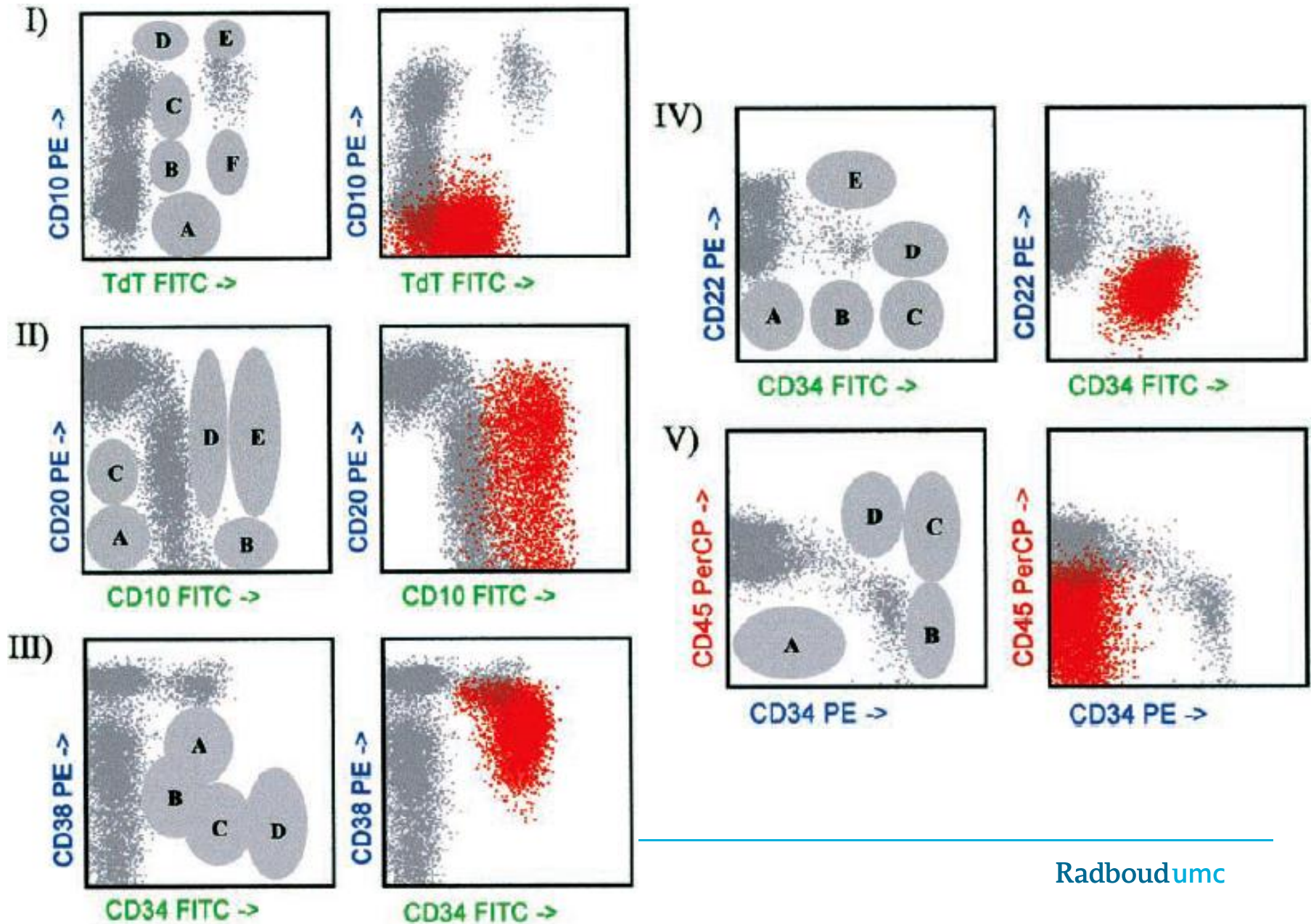
B-CLL



# Immunologische differentiatie op basis van CD45

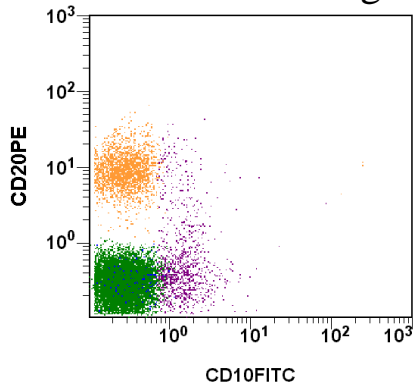


# Precursor B maturatie

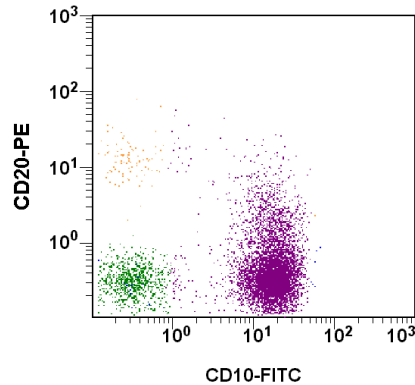


# CD10-C20-CD19 patronen

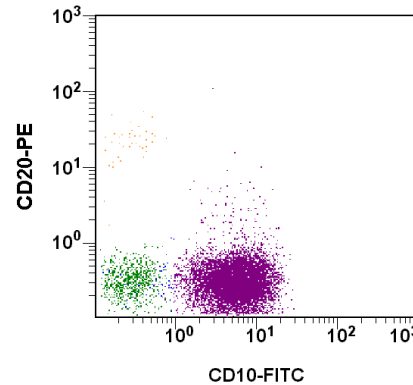
## Normaal beenmerg



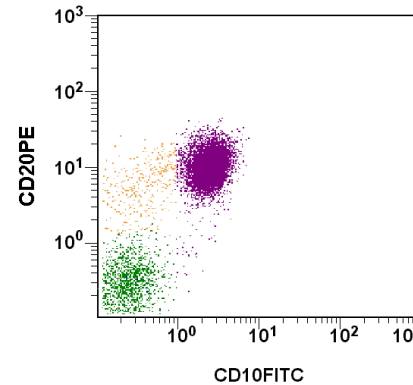
## Pre B-ALL



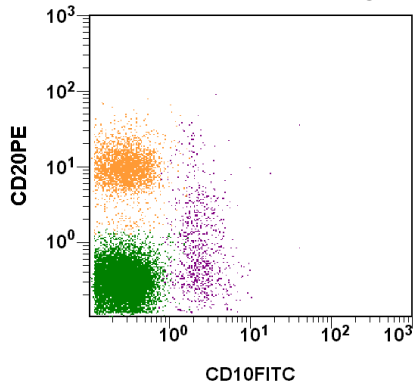
## Common ALL



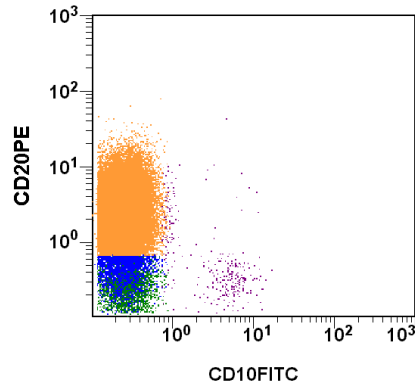
## B-ALL Burkitt



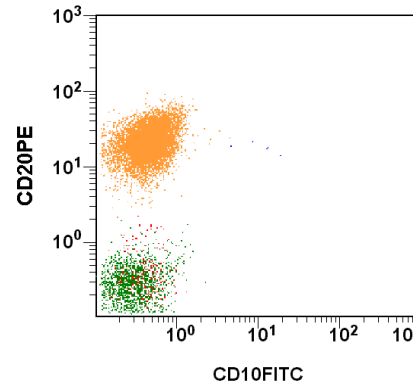
## Normaal beenmerg



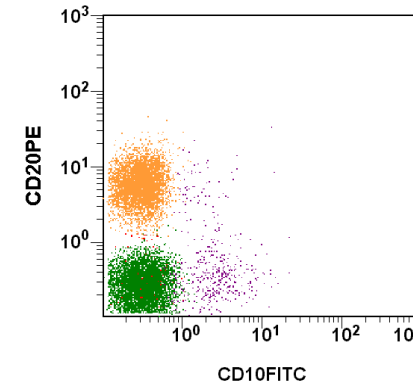
## B-CLL



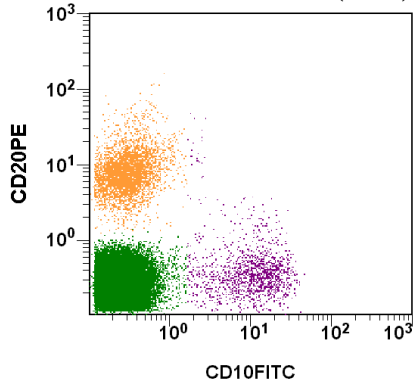
## MCL



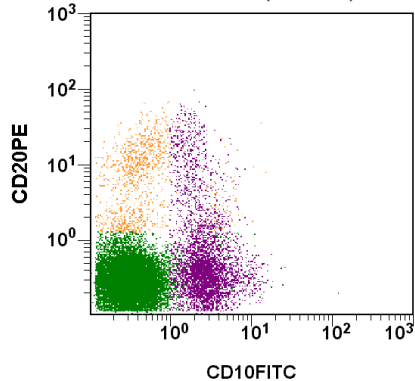
## B-NHL



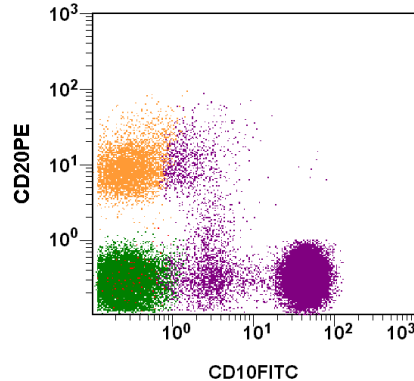
## Recidief C-ALL (3%)



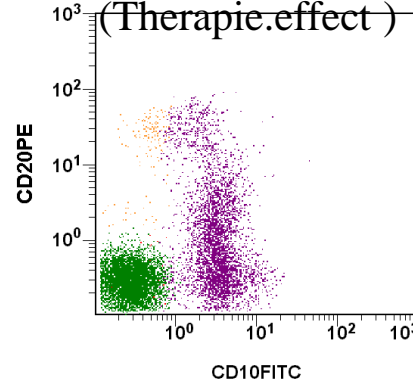
## Rec.NHL (FCL)



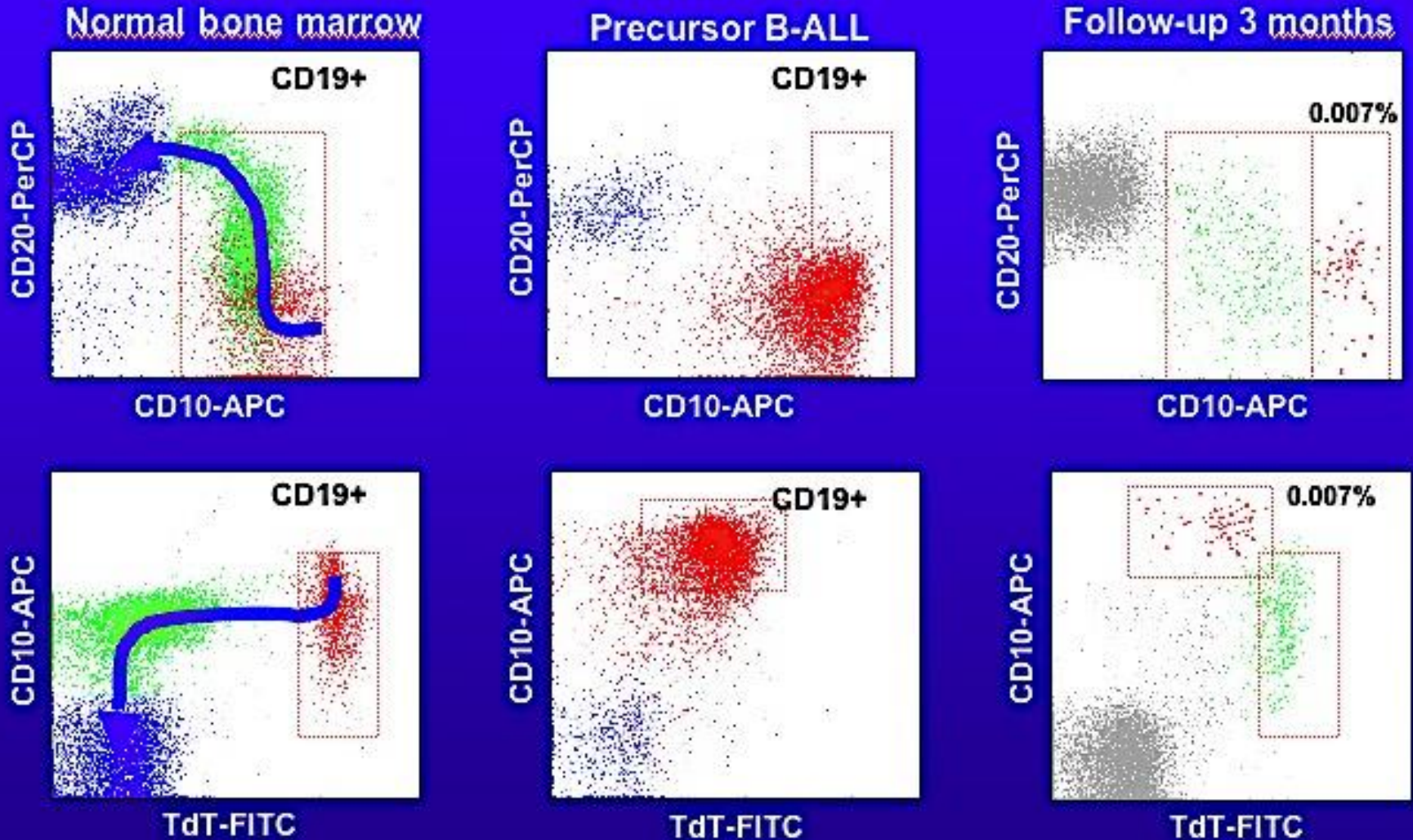
## Recidief Pre B-ALL



## Precursor B-cellen (Therapie.effect)



# MRD analyse in ALL

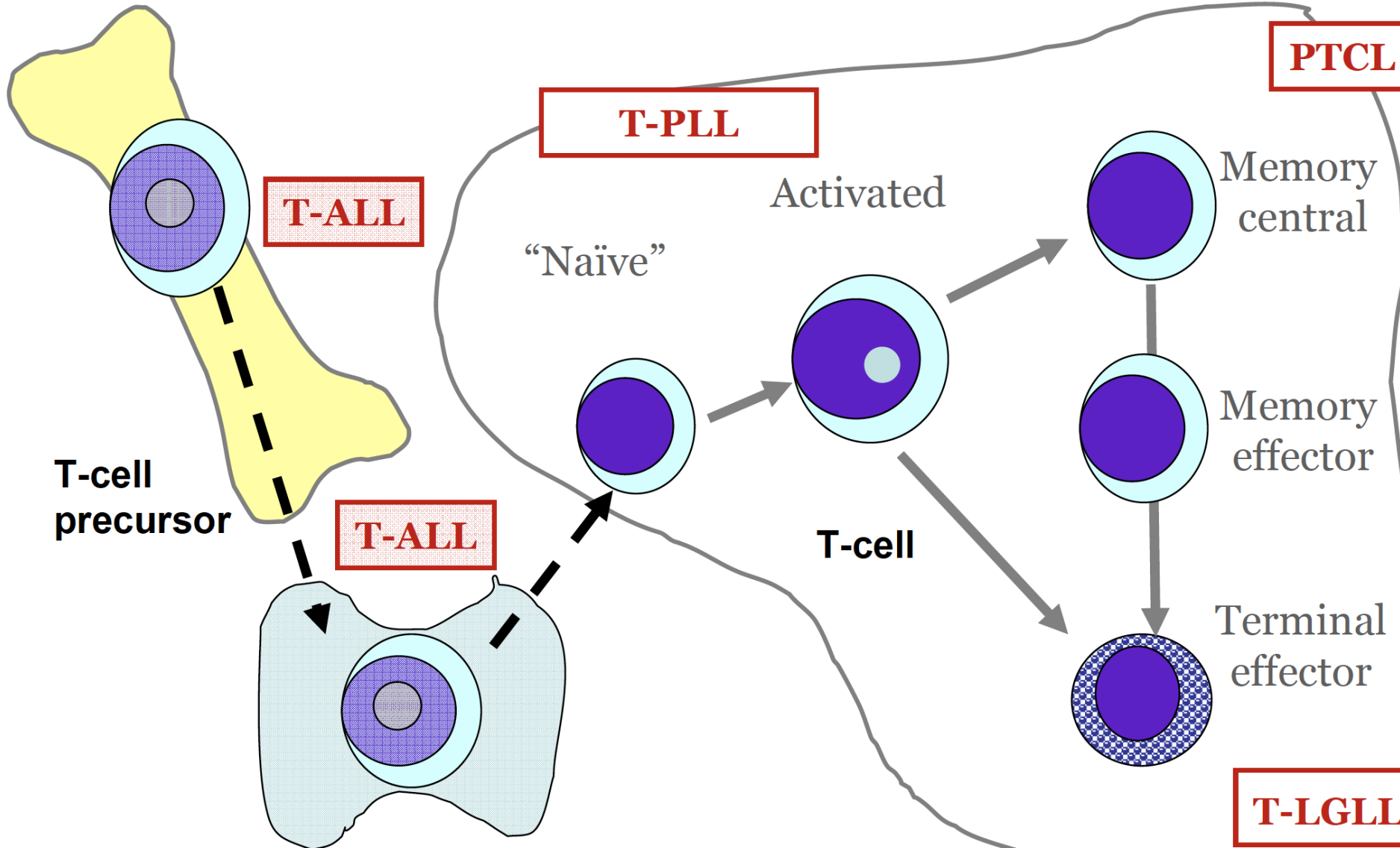




# T-celontwikkeling and maligne ontaarding

**T cells precursors**

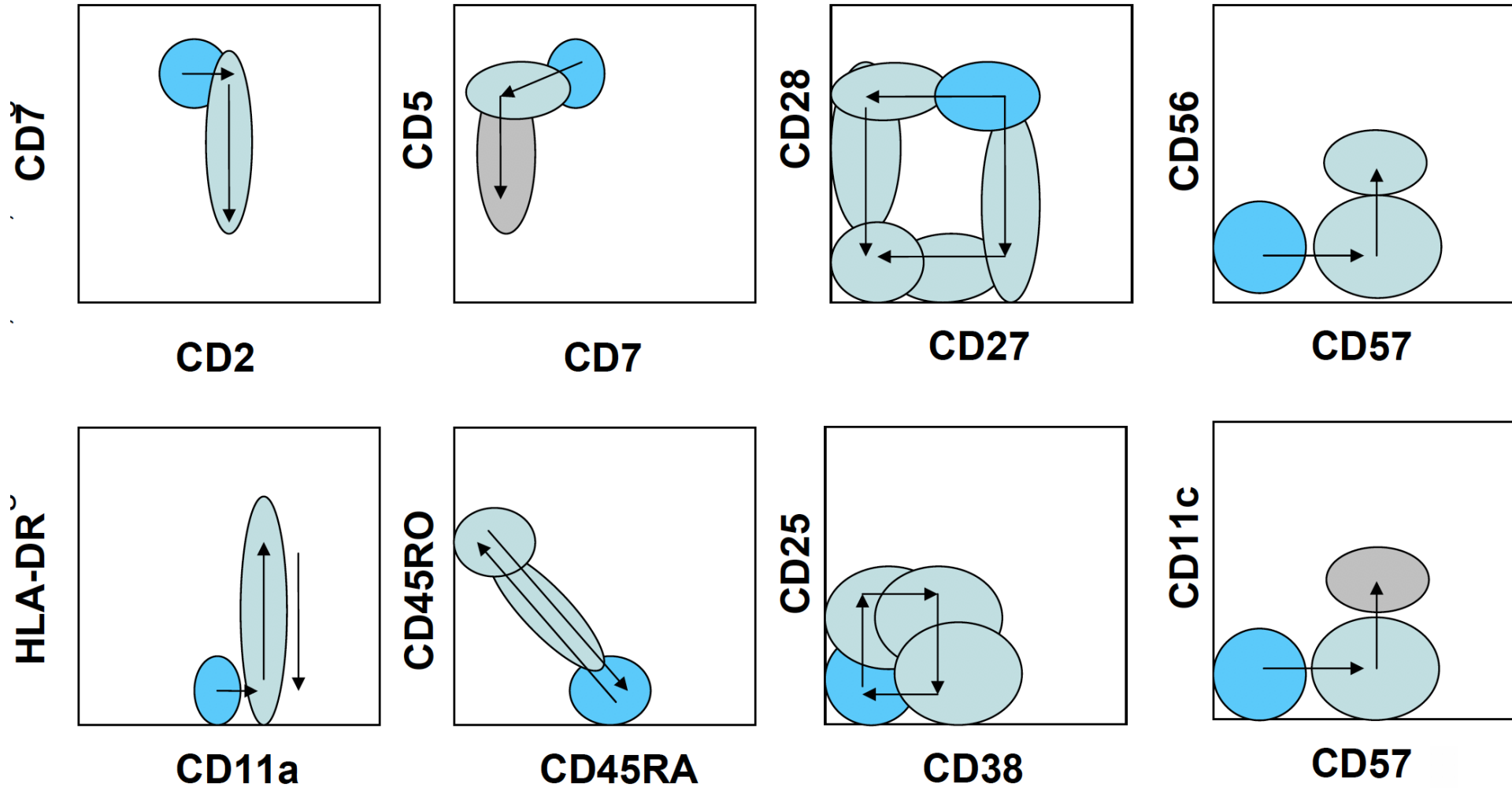
**Peripheral / mature T cells**



# T-cell activation / differentiation stages

## Phenotypic changes

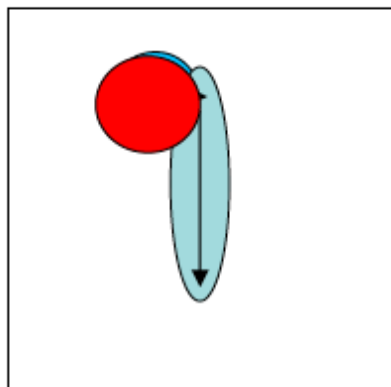
1 – Naive; 2 – Activated (recently); 3 – Activated (late); 4 – Differentiated (terminally)



# T-cell activation / differentiation stages

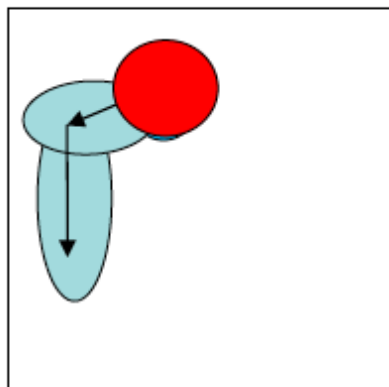
## Naive T cells (T-PLL)

CD7



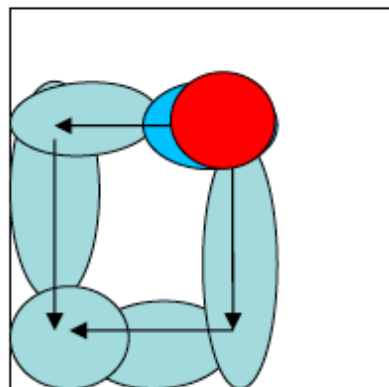
CD2

CD5



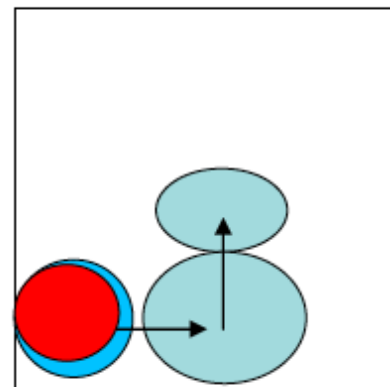
CD7

CD28



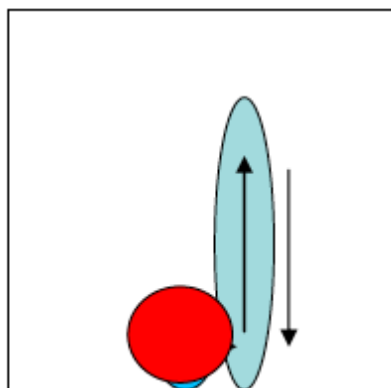
CD27

CD56



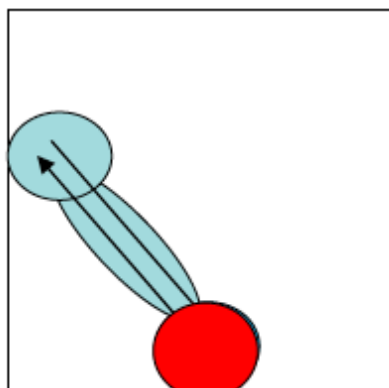
CD57

HLA-DR



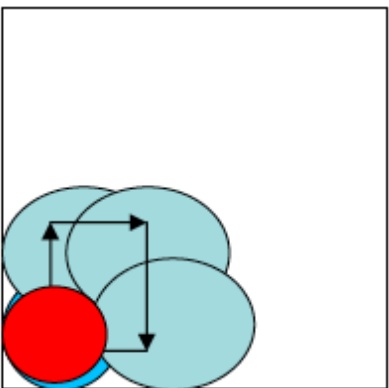
CD11a

CD45RO



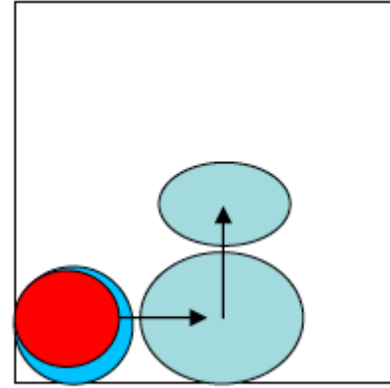
CD45RA

CD25



CD38

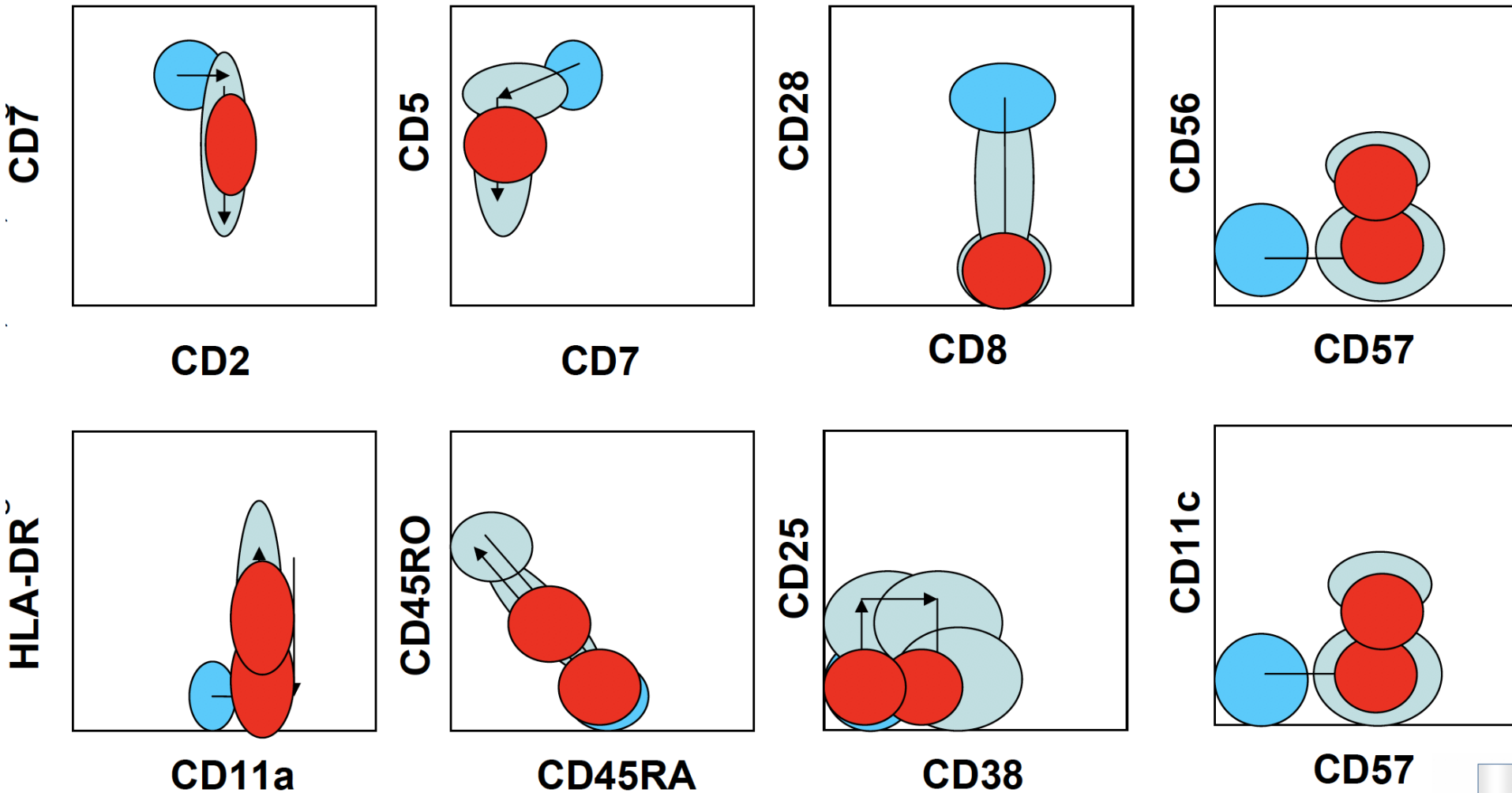
CD11c



CD57

# T-cell activation / differentiation stages

## Late activated / Terminally differentiated T cells (LGL)

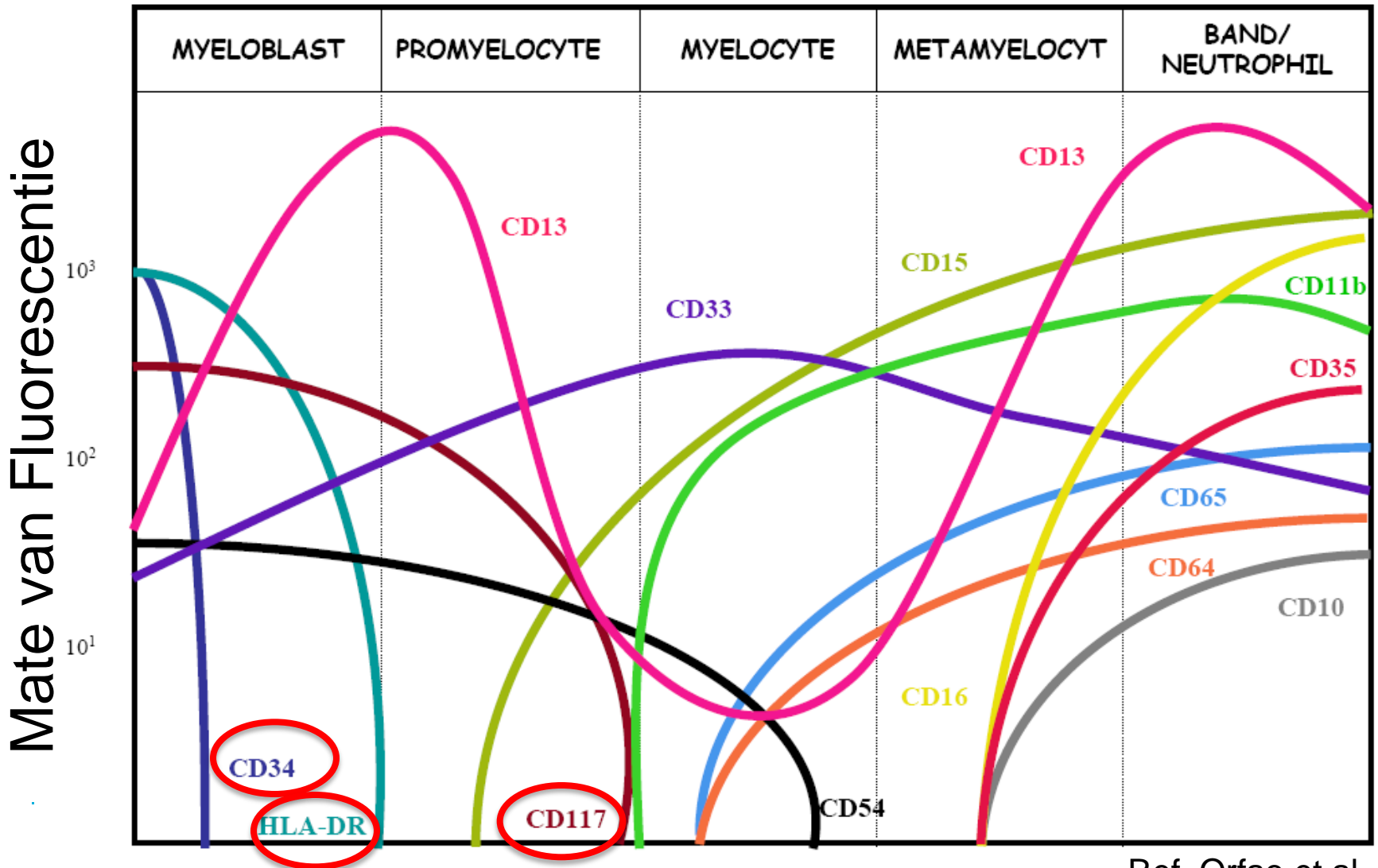


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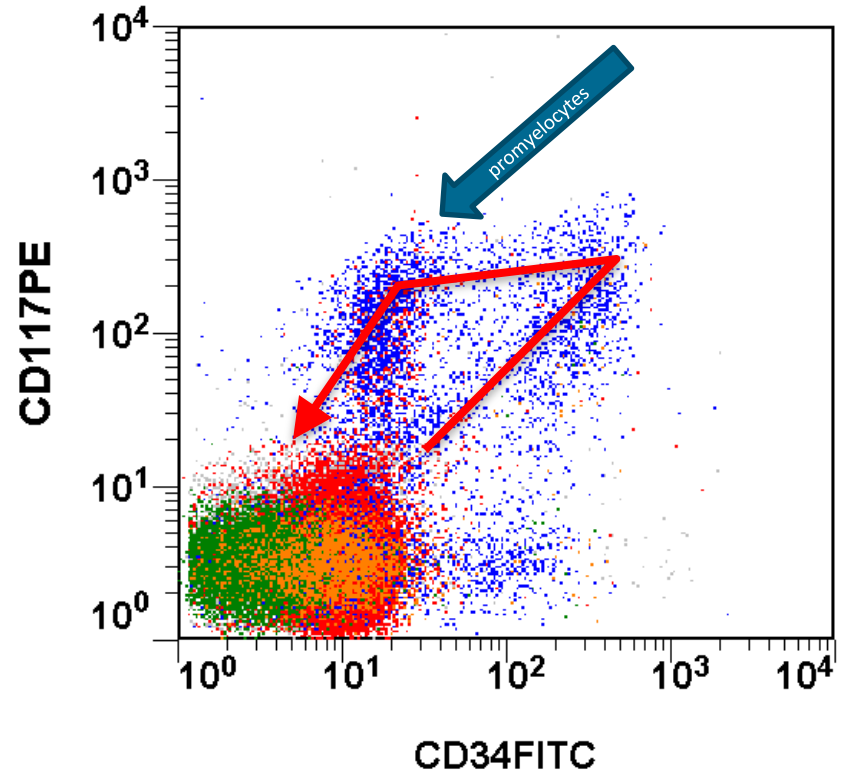
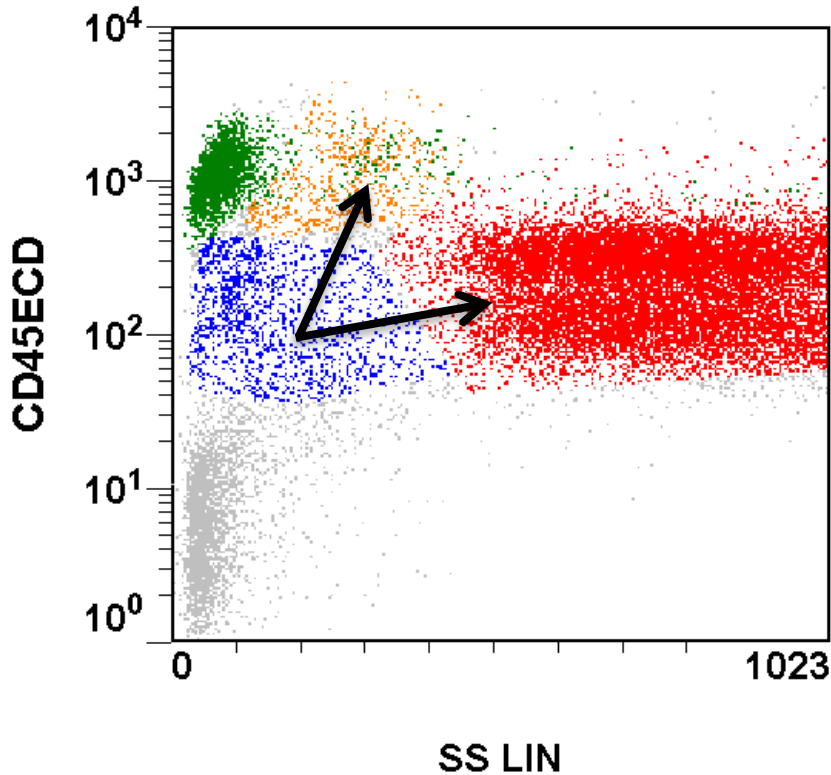
# Fenotypering van de Myeloïde lijnen om de verschillende maturatie stadia te identificeren

*Zoektocht naar de myelo-monocyttaire  
progenitorcellen*

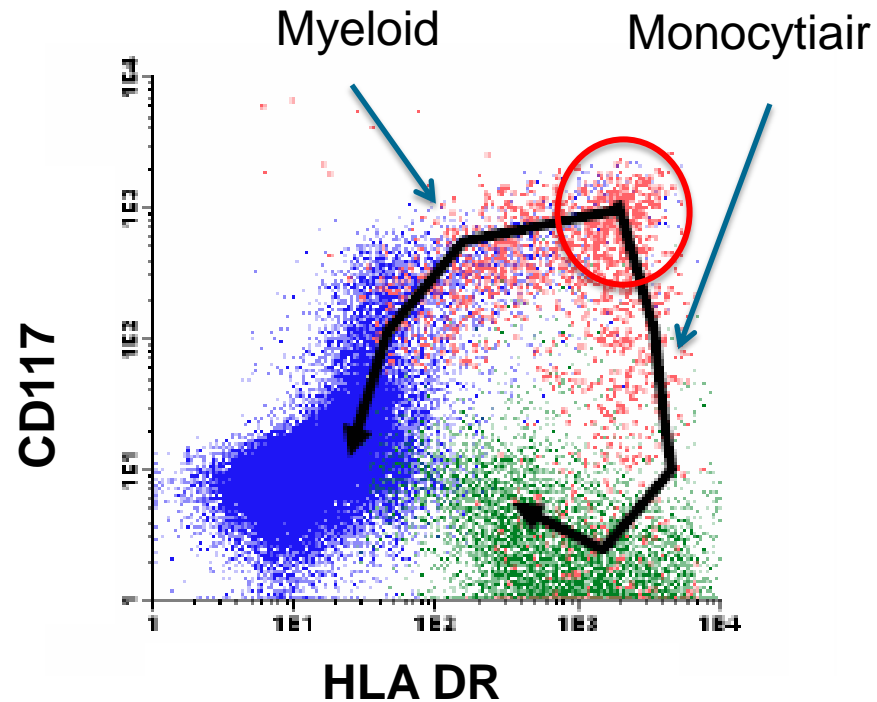
# Fenotype veranderingen in de neutrofielen differentiatie pathway



# 1. Expressie patroon van CD34 / CD117 in CD45+ populatie Normaal beenmerg



# Myelomonocyttaire differentiatie/maturatie delen dezelfde progenitor cellen!



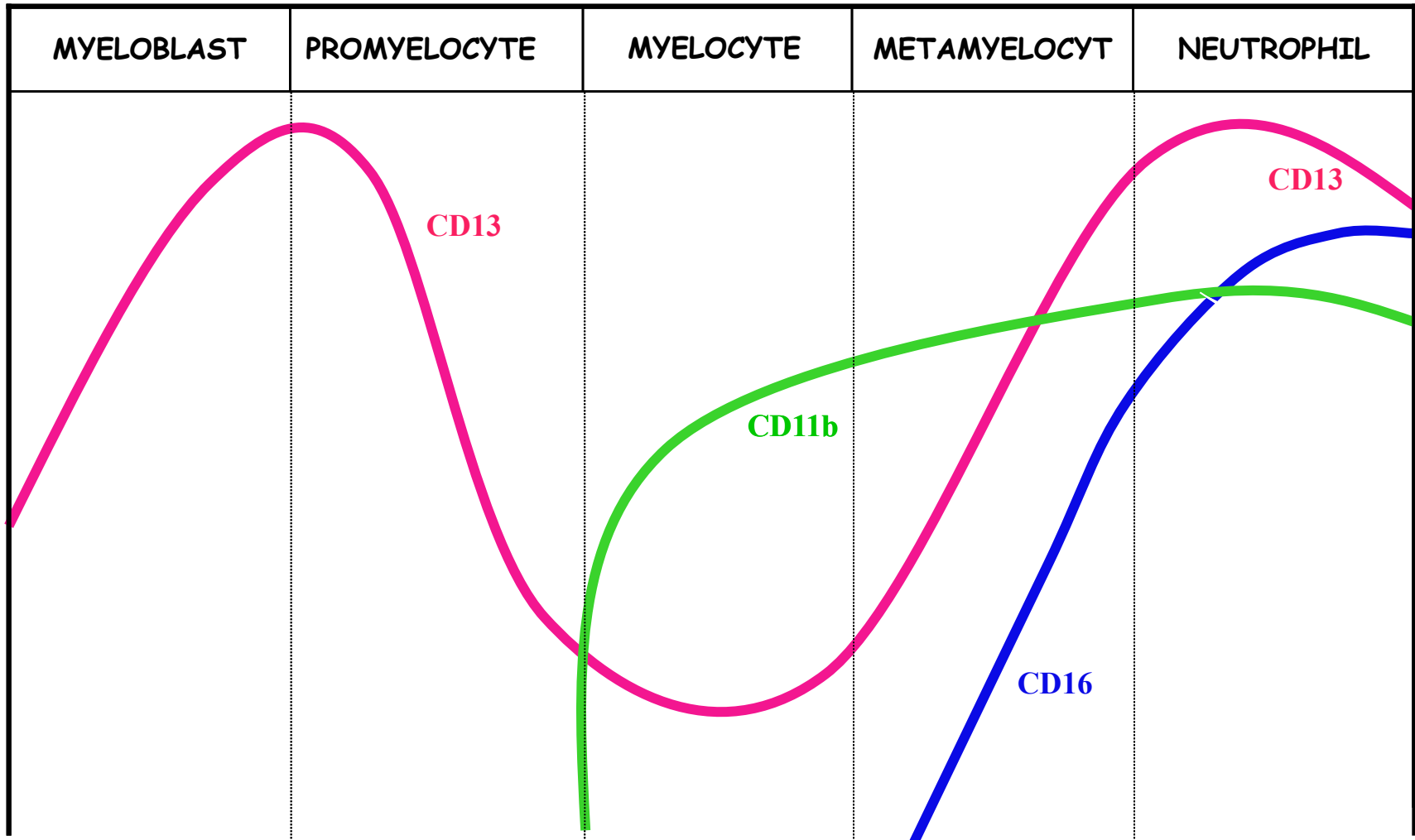


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# **Fenotypering van de myeloïde lijnen**

## **CD11b / CD13 / CD16 / CD45**

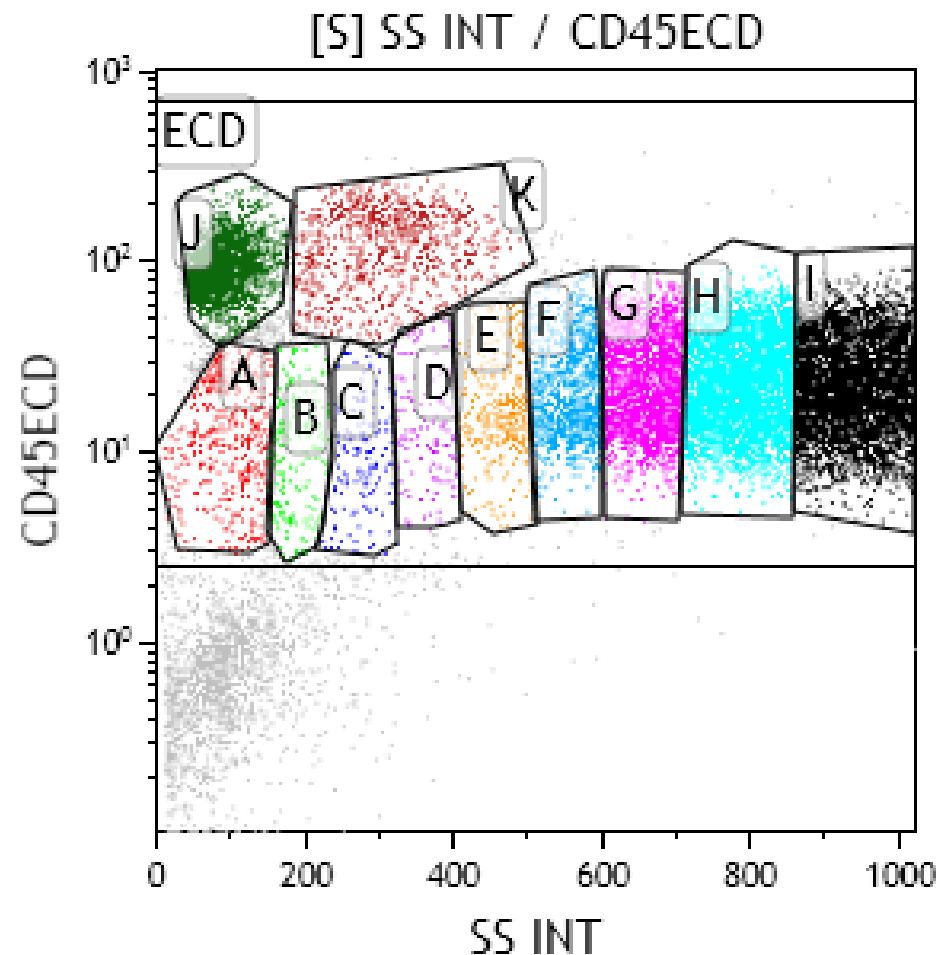
## 2. Expressie patroon van CD13, CD16, CD11b binnen CD45



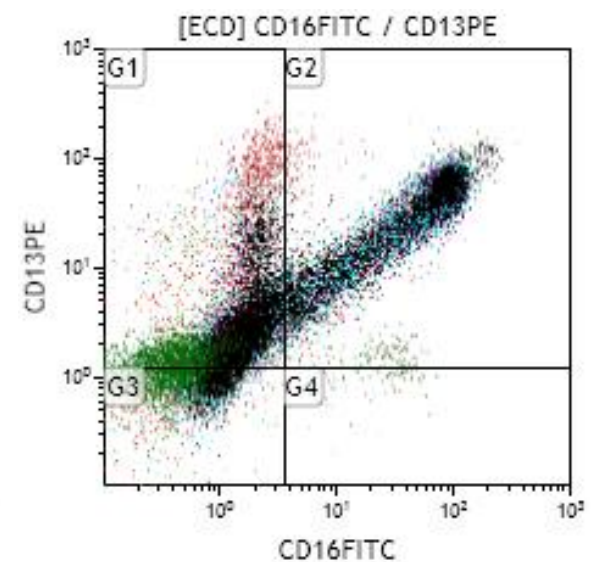
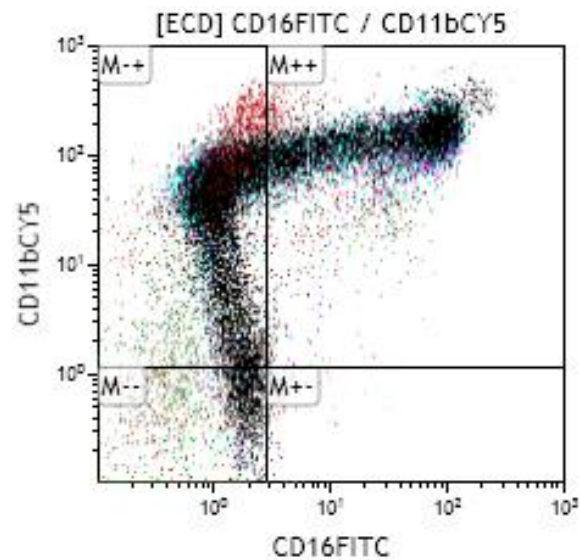
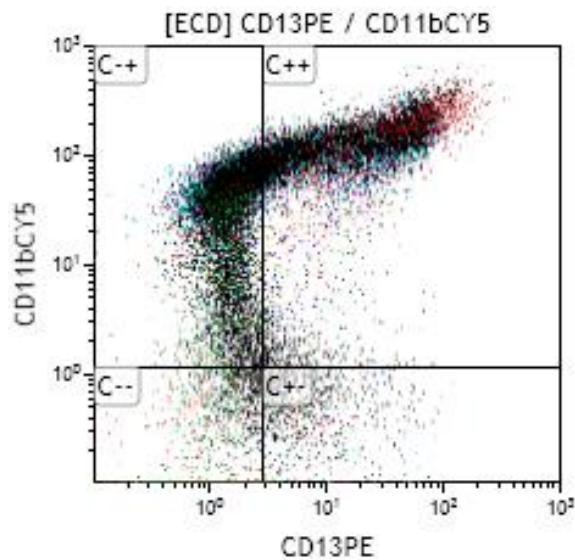
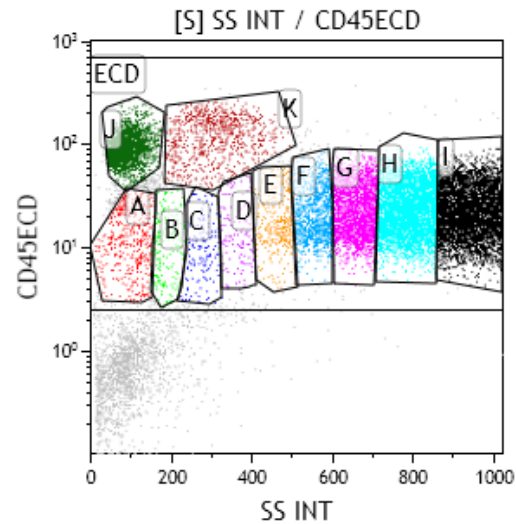
## 2.I. Expressie patroon CD13/CD16/CD11b (normaal BM)

Doel:

1. Waar bevinden zich de myeloïde maturatie stadia?
2. Waar bevinden zich myeloïd-mono progenitors?



## 2.I. Expressie patroon CD13/CD16/CD11b (normaal BM)



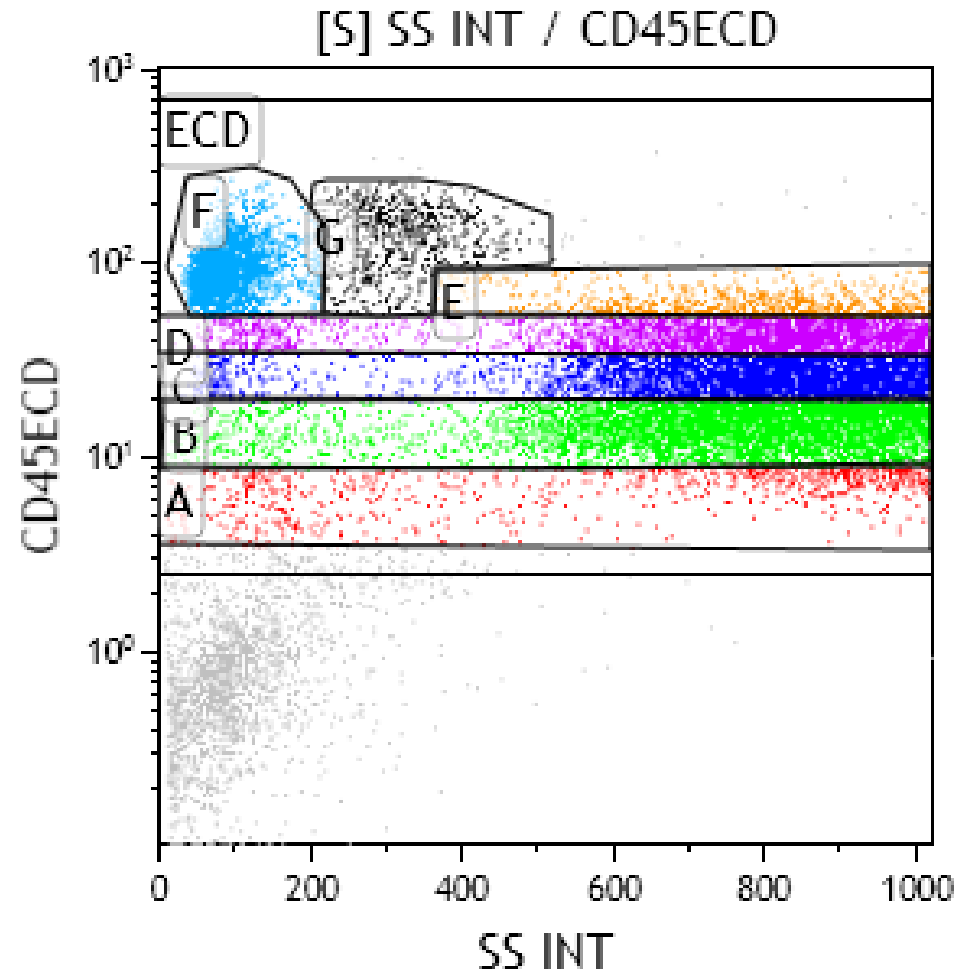
## 2.II. Expressie patroon CD13/CD16/CD11b (normaal BM)

Horizontale gates:

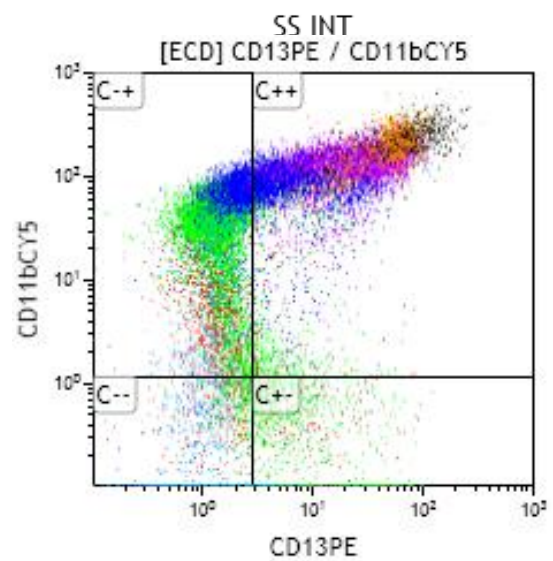
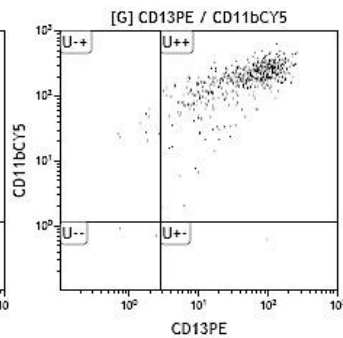
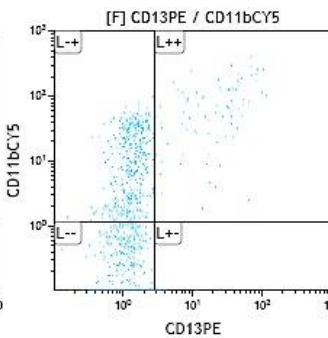
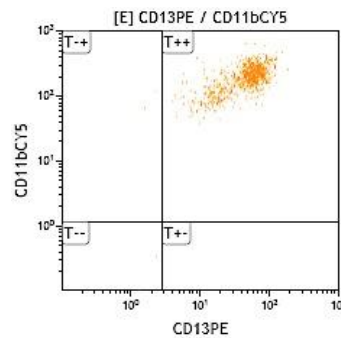
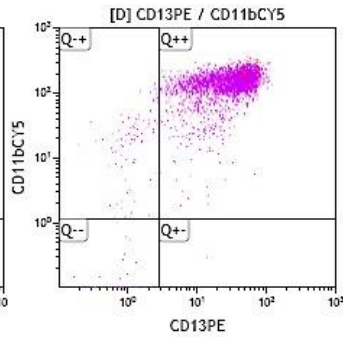
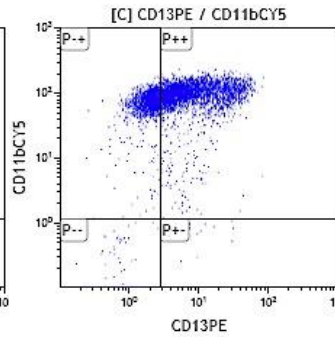
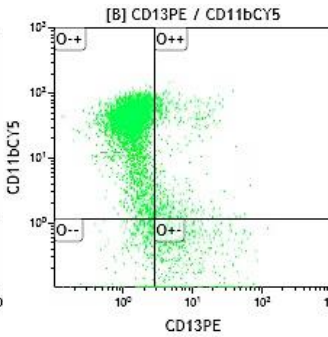
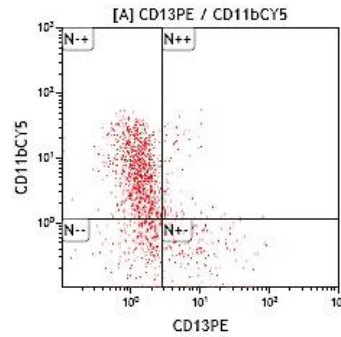
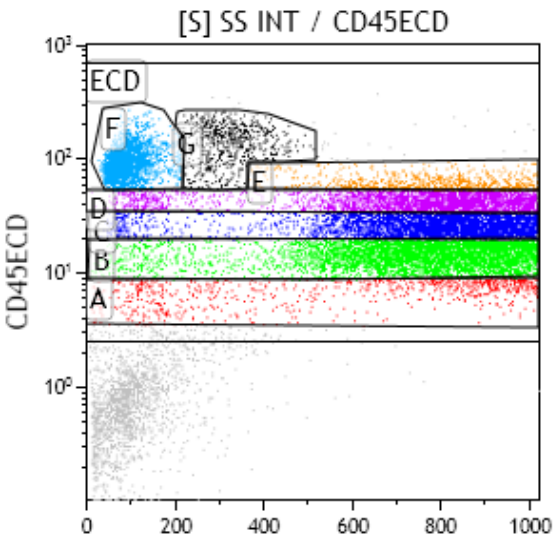
Betere gating optie??

Doel:

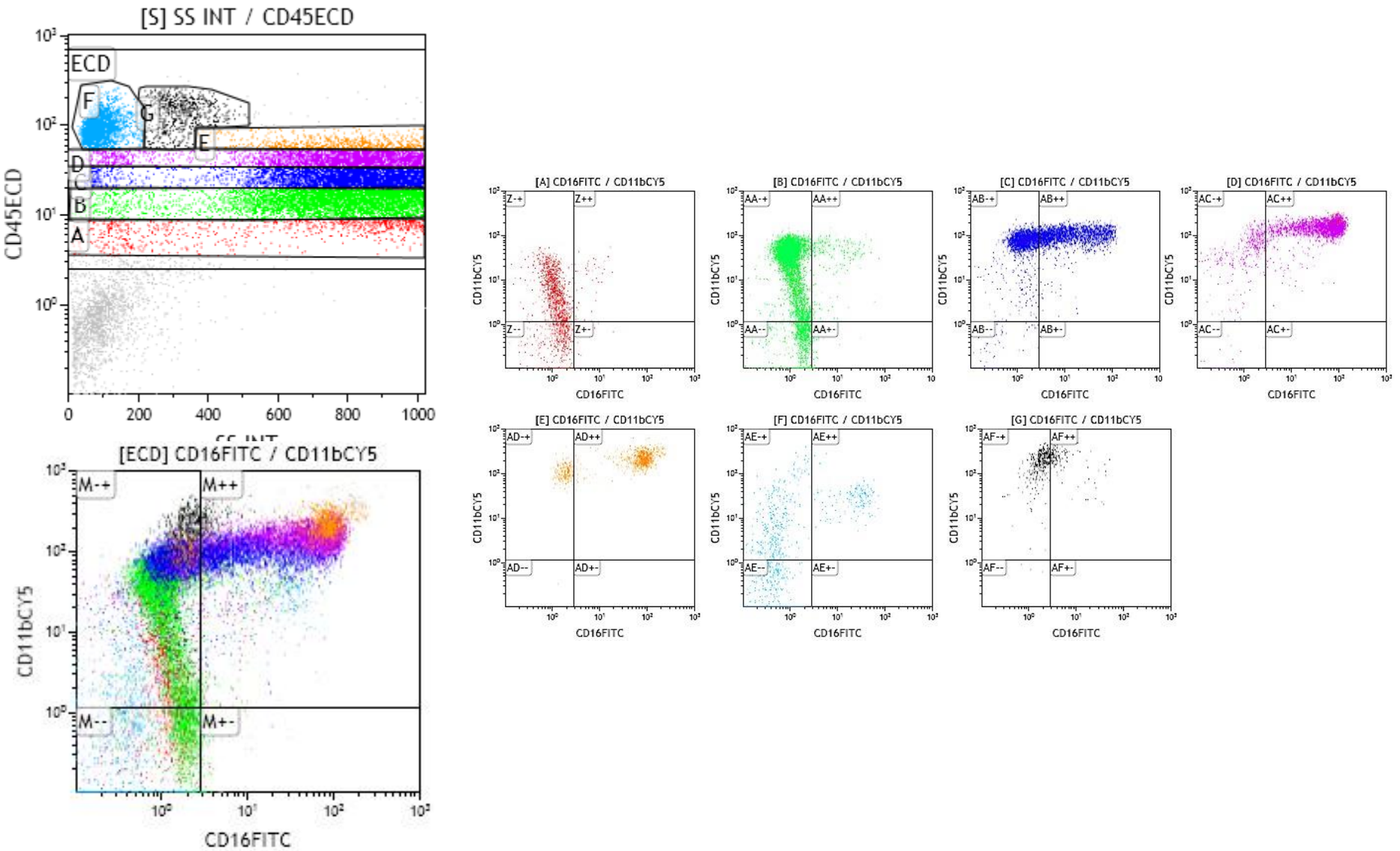
1. Waar bevinden zich de myeloïde maturatie stadia?
2. Waar bevinden zich myeloïd -mono progenitors?



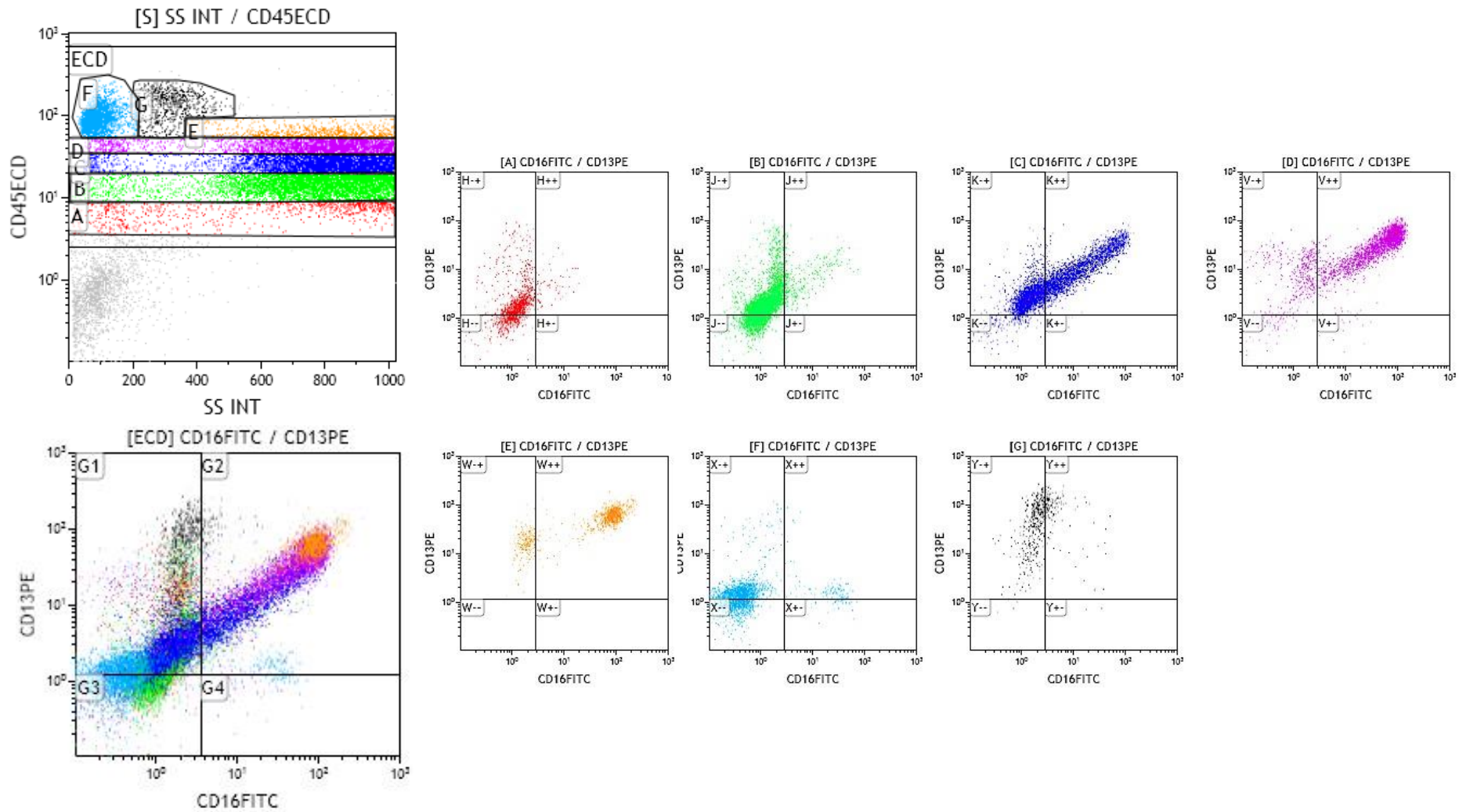
## 2.II. Expressie patroon CD13/CD16/CD11b (normaal BM)



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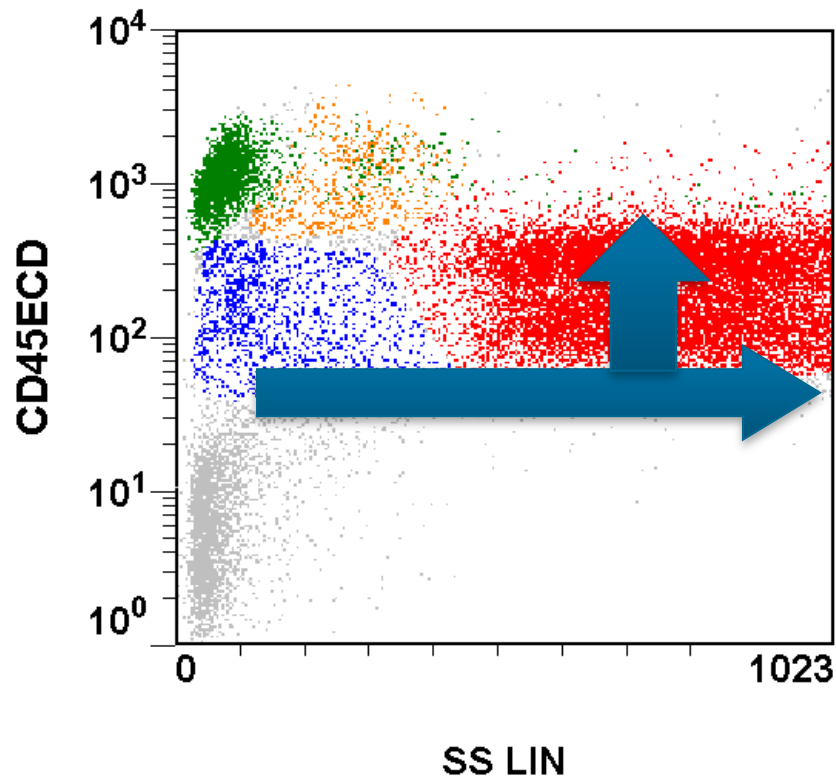




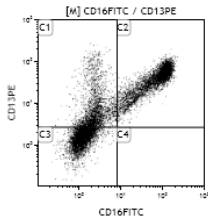
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# Conclusie

(myeloïde differentiatie patroon in CD45)



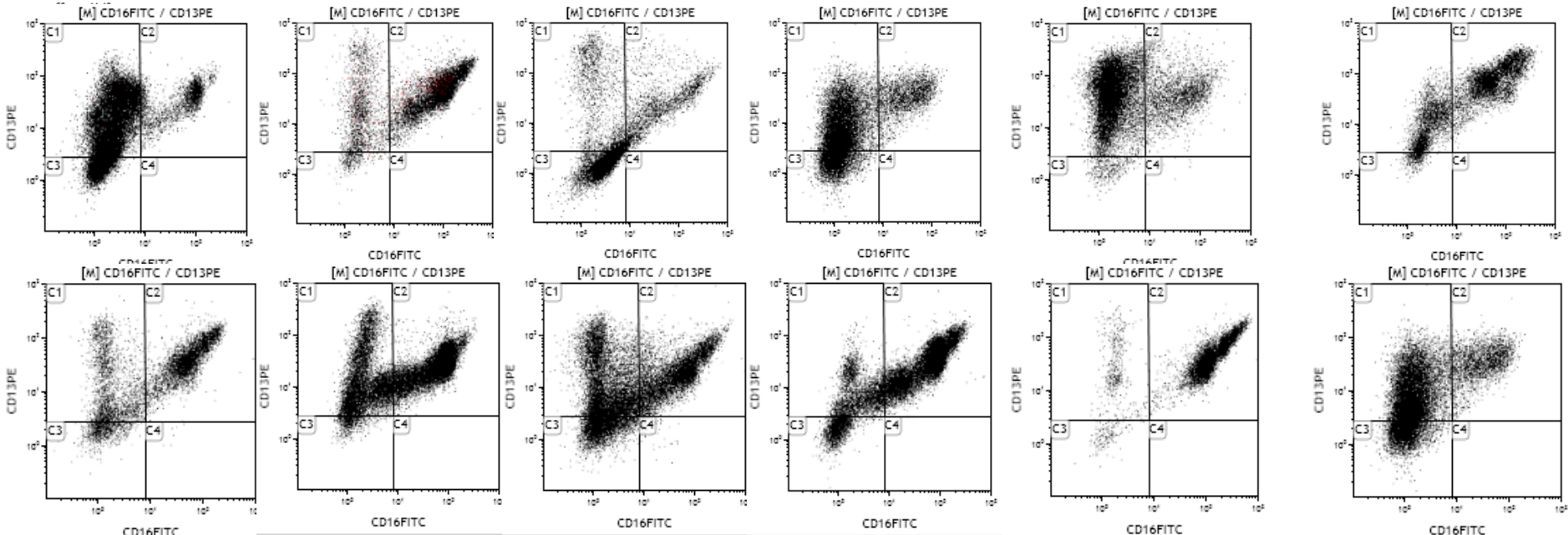
# Afwijkende patronen CD13/CD16 bij MDS op granulocyten



normaal

Gate	Number	%Total	%Gated	X-Mean
All	13.011	37,58	100,00	35,92
C1	2.608	7,53	20,04	2,73
C2	5.303	15,32	40,76	85,51
C3	5.097	14,72	39,17	1,33
C4	3	0,01	0,02	10,48

Gate	Y-Mean
All	21,74
C1	15,20



Gate	Number	%Total	%Gated	X-A-Mean
All	8.145	23,27	100,00	40,52
C1	2.412	6,89	29,61	2,31
C2	4.709	13,45	57,81	68,55
C3	1.017	2,91	12,49	1,51
C4	7	0,02	0,09	25,64

Gate	Number	%Total	%Gated	X-A-Mean
All	29.055	83,01	100,00	48,23
C1	9.495	27,13	32,68	2,76
C2	18.641	53,26	64,16	73,70
C3	915	2,61	3,15	1,31
C4	4	0,01	0,01	10,22

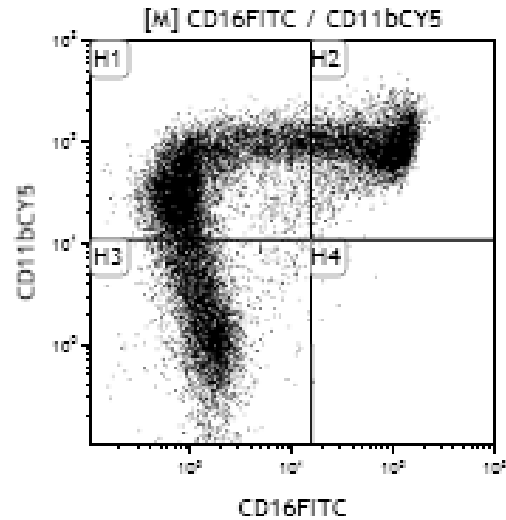
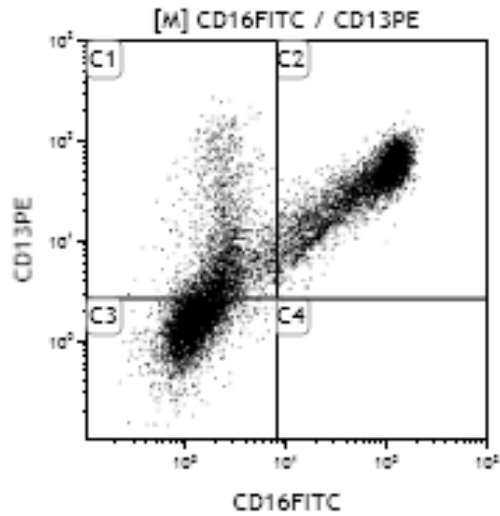
Gate	Number	%Total	%Gated	X-A-Mean
All	23.585	67,39	100,00	25,07
C1	10.121	28,92	42,91	2,42
C2	8.376	23,93	35,51	66,38
C3	4.952	14,15	21,00	1,84
C4	136	0,39	0,58	13,27

Gate	Number	%Total	%Gated	X-A-Mean
All	27.360	78,17	100,00	61,43
C1	3.853	11,01	14,08	3,38
C2	20.964	59,90	76,62	79,42
C3	2.540	7,26	9,28	1,08
C4	3	0,01	0,01	25,49

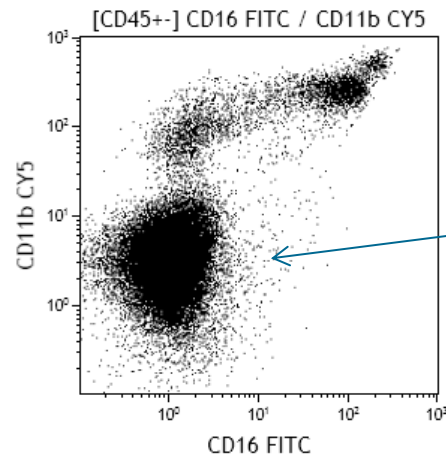
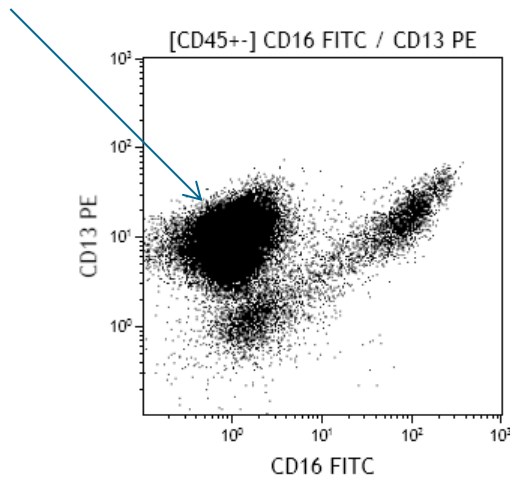
Gate	Number	%Total	%Gated	X-A-Mean
All	8.640	30,47	100,00	141,53
C1	521	1,84	6,03	2,16
C2	7.948	28,03	91,99	153,68
C3	170	0,60	1,97	1,42
C4	1	0,00	0,01	9,20

Gate	Number	%Total	%Gated	X-A-Mean
All	21.592	61,69	100,00	6,36
C1	13.274	37,93	61,48	1,71
C2	2.814	8,04	13,03	38,34
C3	5.502	15,72	25,48	1,20
C4	2	0,01	0,01	10,69

# Afwijkende patronen CD13/CD16 bij AML op granulocyten

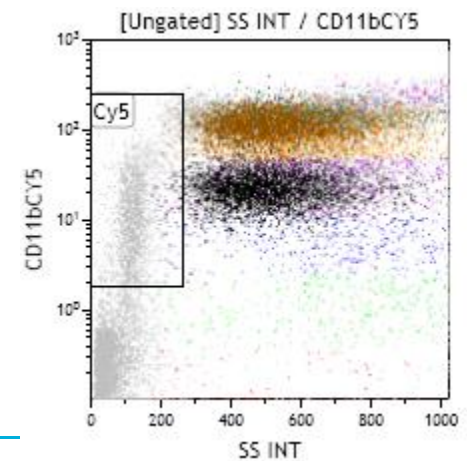
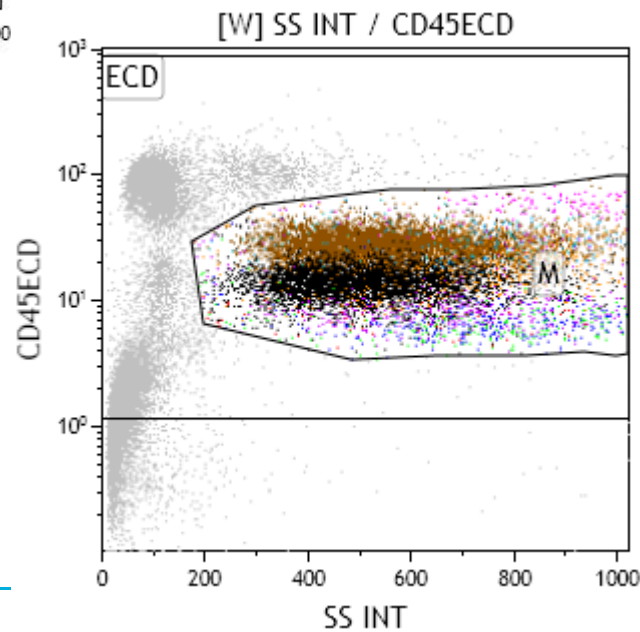
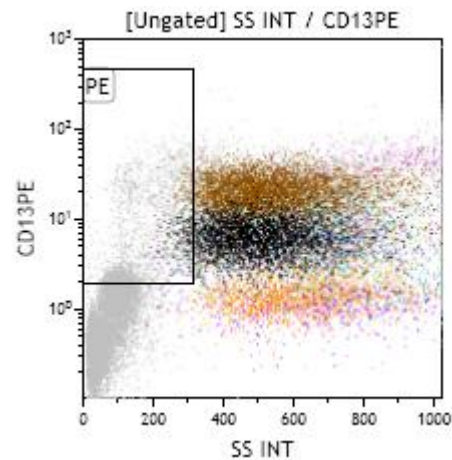
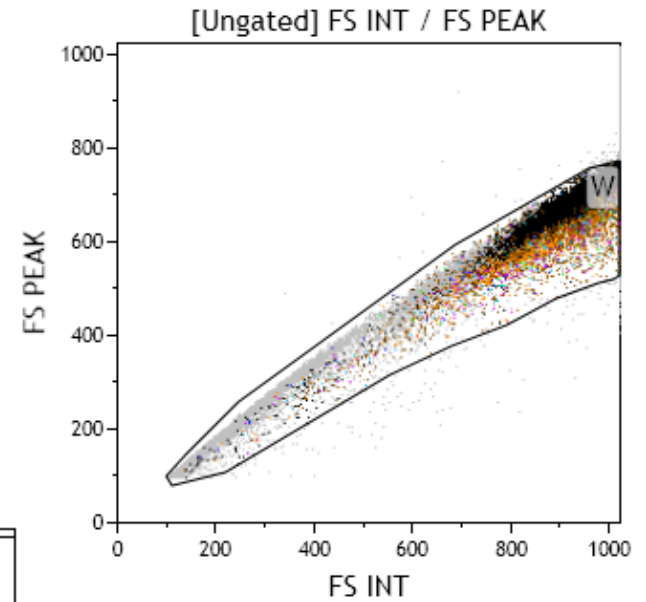
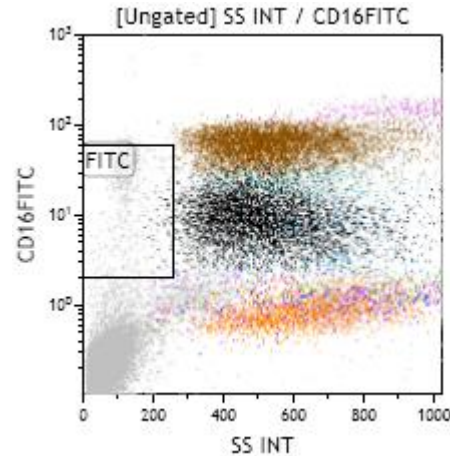
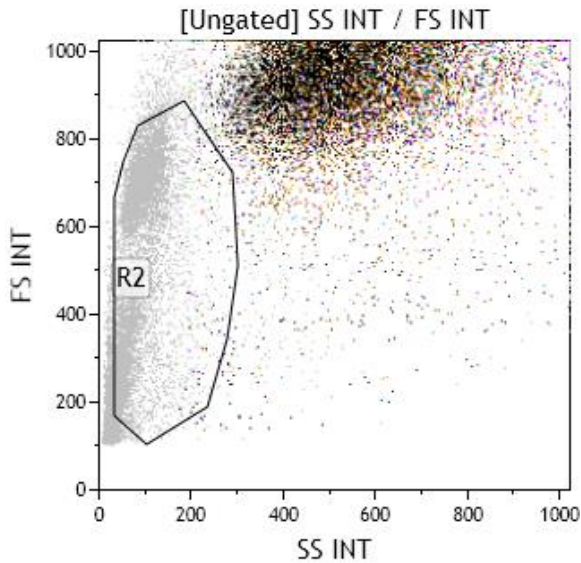


normaal

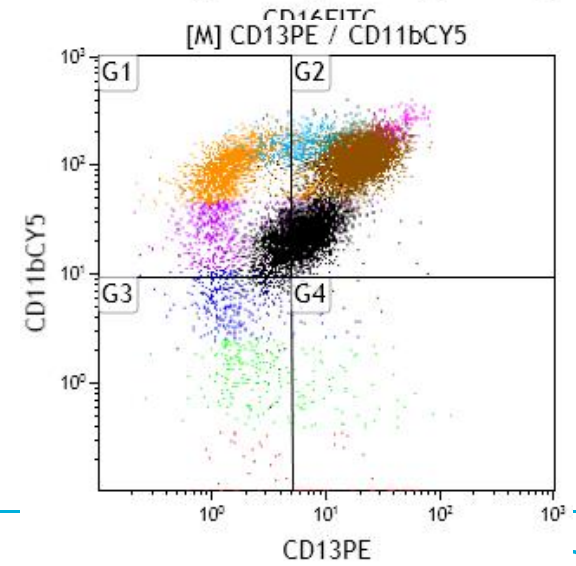
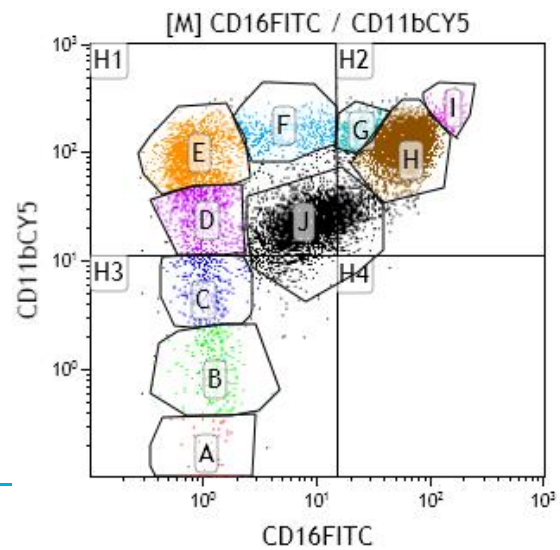
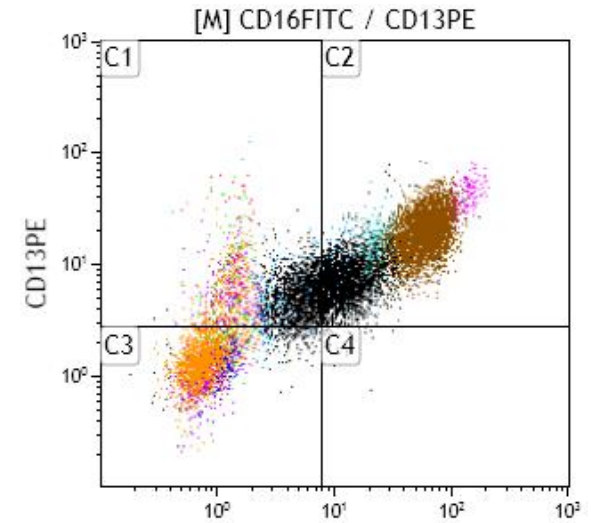
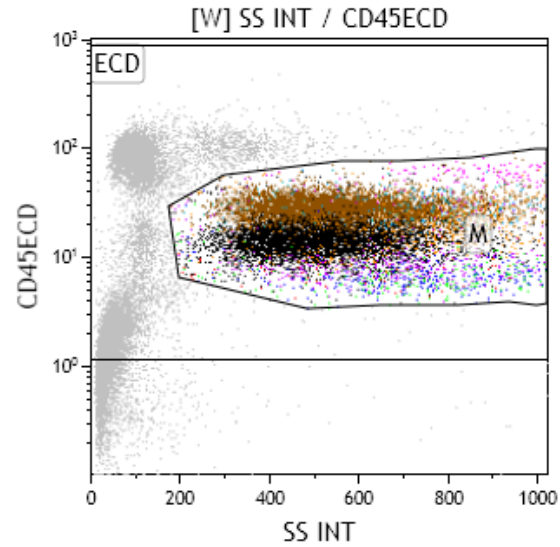


AML

# Patroon bij eosinofilie



# Patroon met eosinofilie



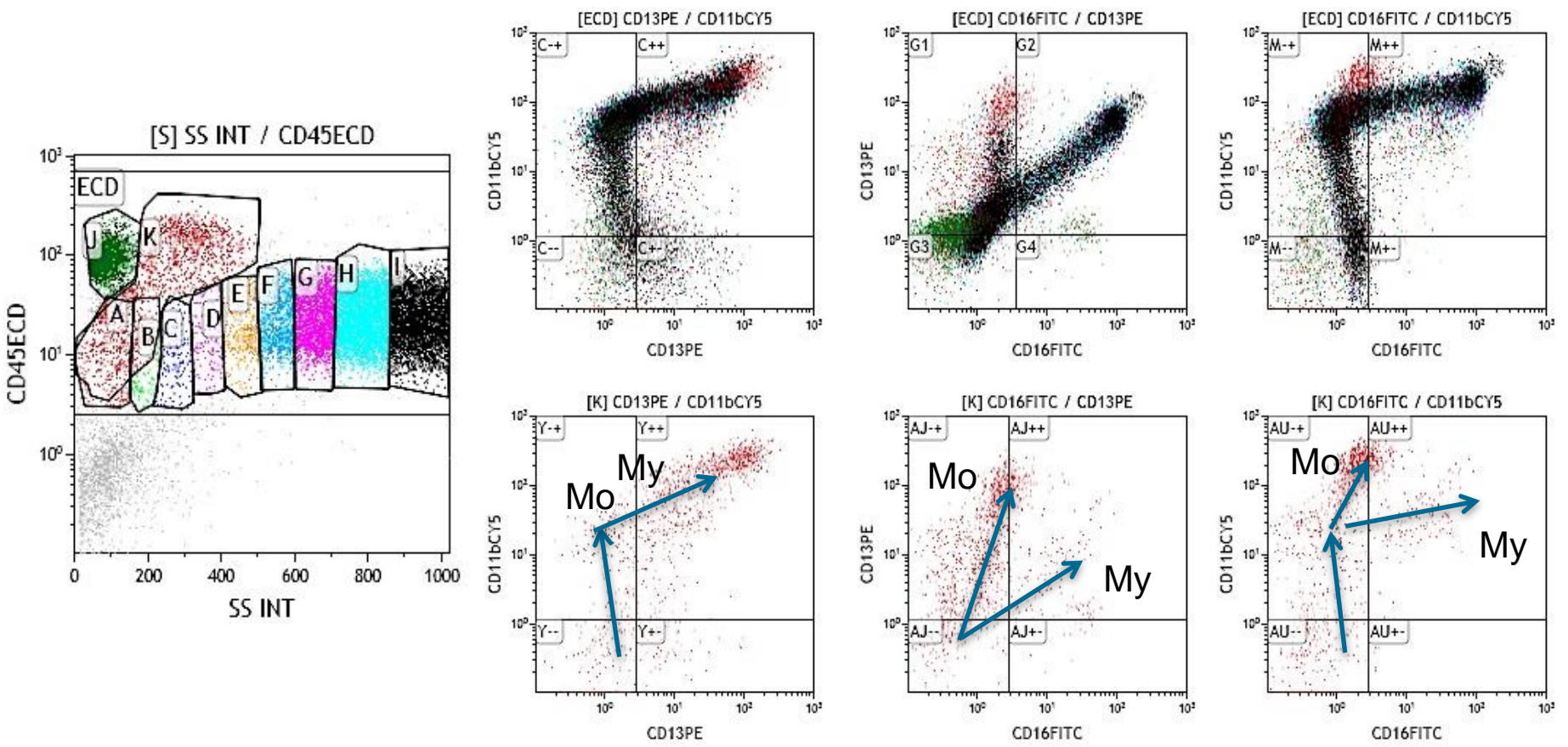
---

# **Fenotypering van de Monocytaire lijnen CD11b / CD13 / CD16 / CD45**

***Zoektocht naar de myelo-monocytaire  
progenitorcellen***

# 2.III. Monocyt + precursors gating: normaal beenmerg

## Eén gate: monocyt en precursors



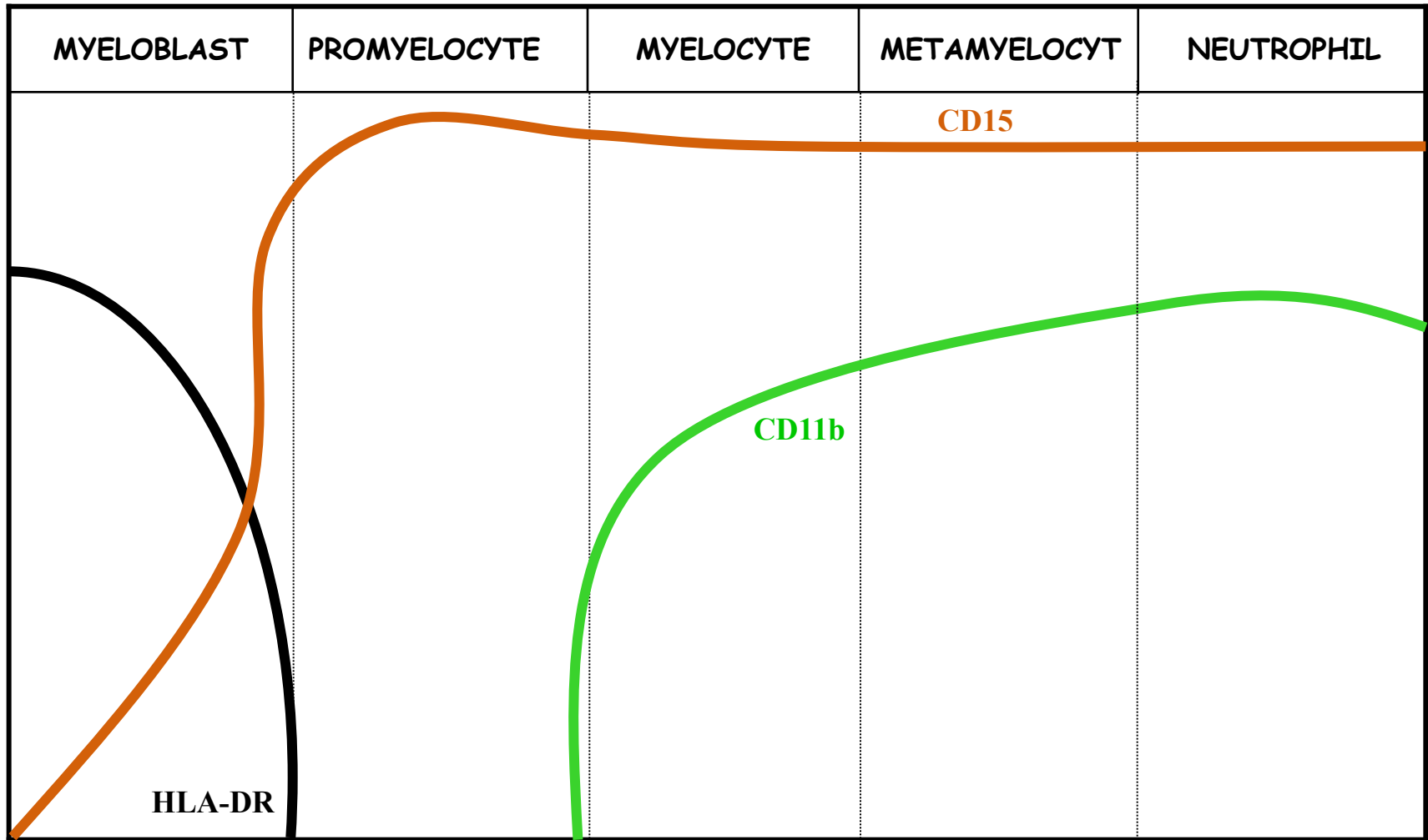
Conclusie: Mature monocyt stadia zijn duidelijk maar waar kunnen we de scheiding tussen myeloid-mono lijnen vinden??

---

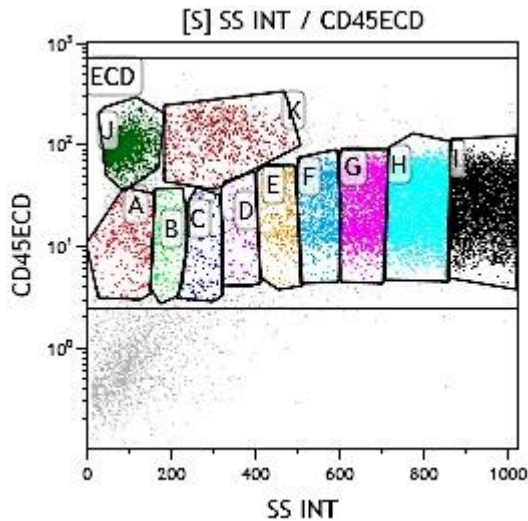
**Meer gedetailleerde fenotypering van  
De Myeloïde en Monocytaire lijnen  
met  
CD11b / CD15 / HLA-DR / CD45**



### 3. Expressie patroon CD11b, CD15, HLA-DR

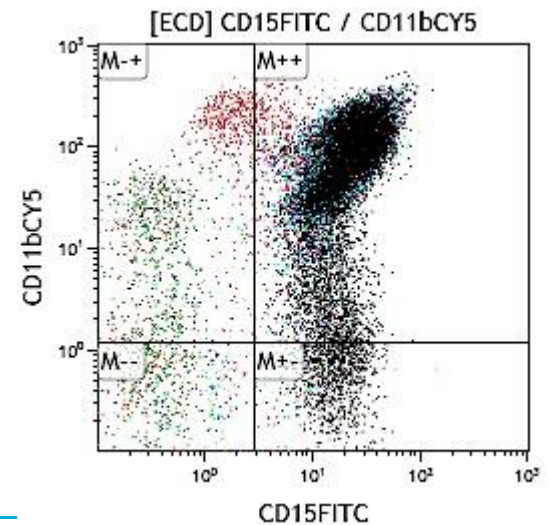
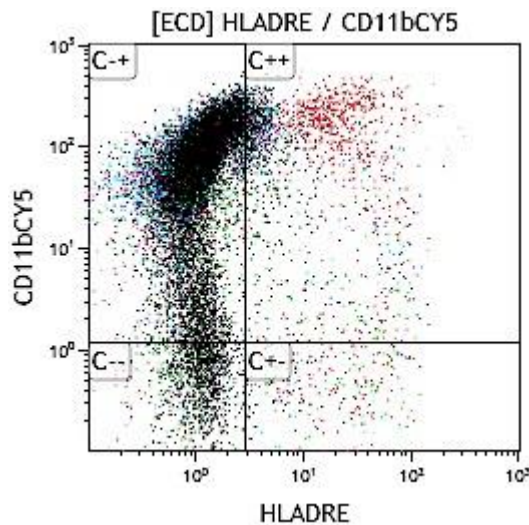
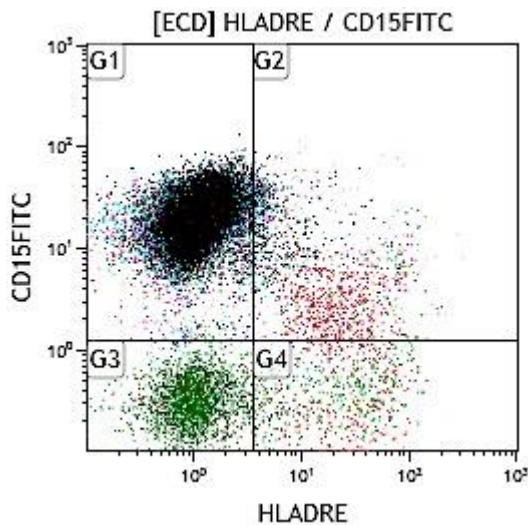


# 3.a. Expressie patroon CD11b/CD15/HLA-DR in CD45+ populatie (normaal BM)



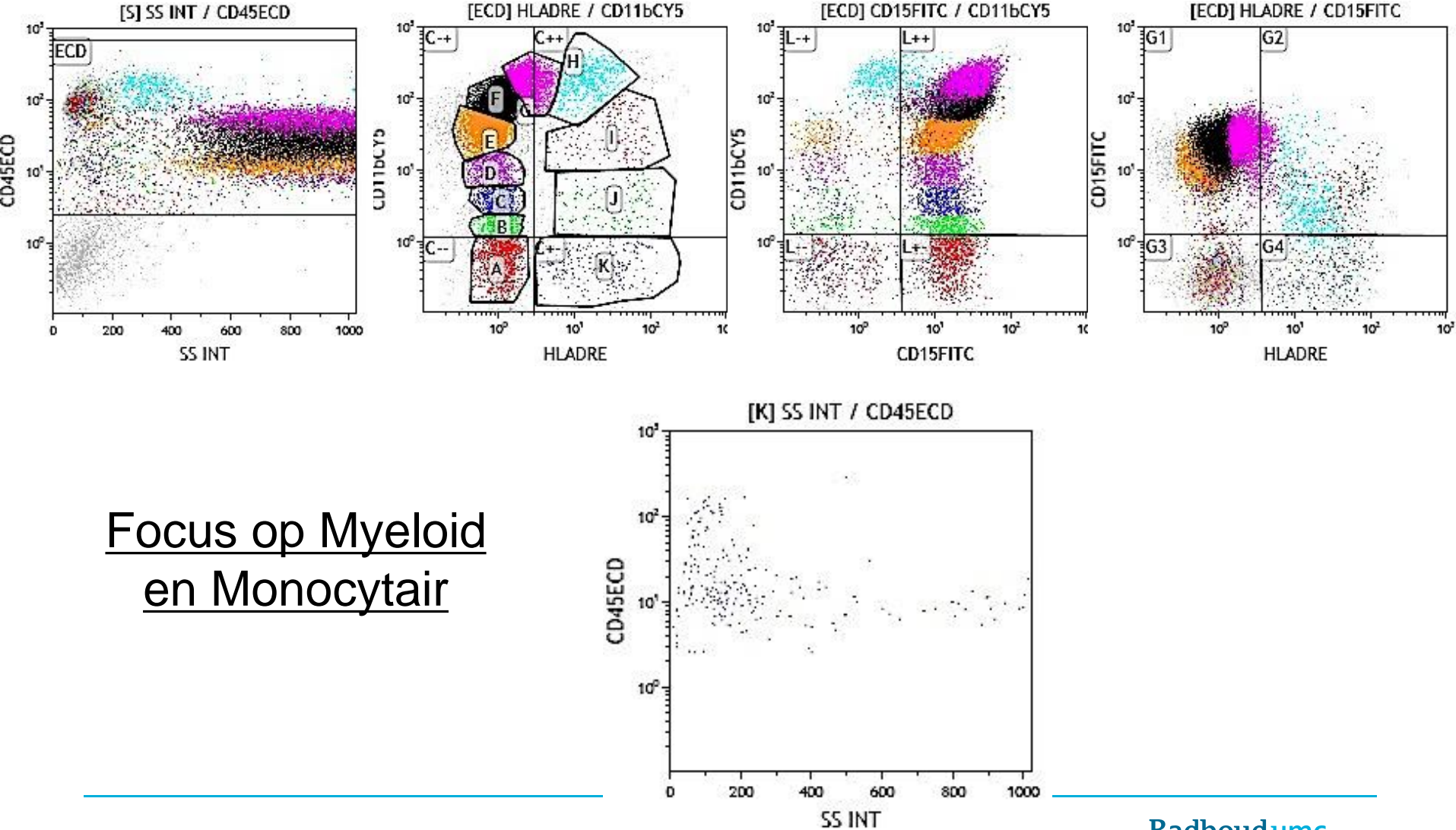
Focus op monocyttaire en  
myeloïde lijn

lineage gates A, (B, C), K



# 3.b. Expressie patroon van CD11b/CD15/HLA-DR

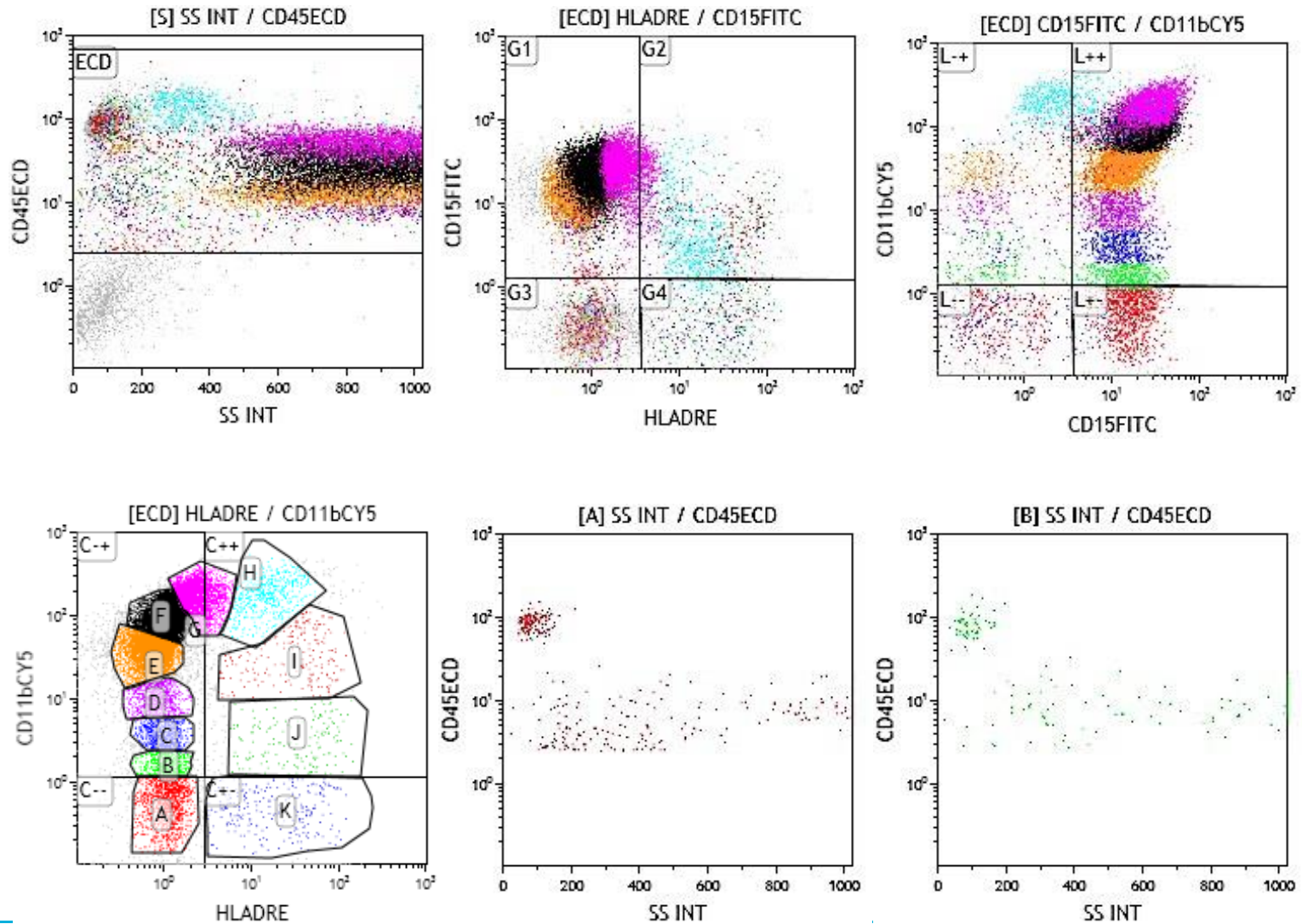
## in CD45+ populatie (normaal BM)



Focus op Myeloid  
en Monocytair

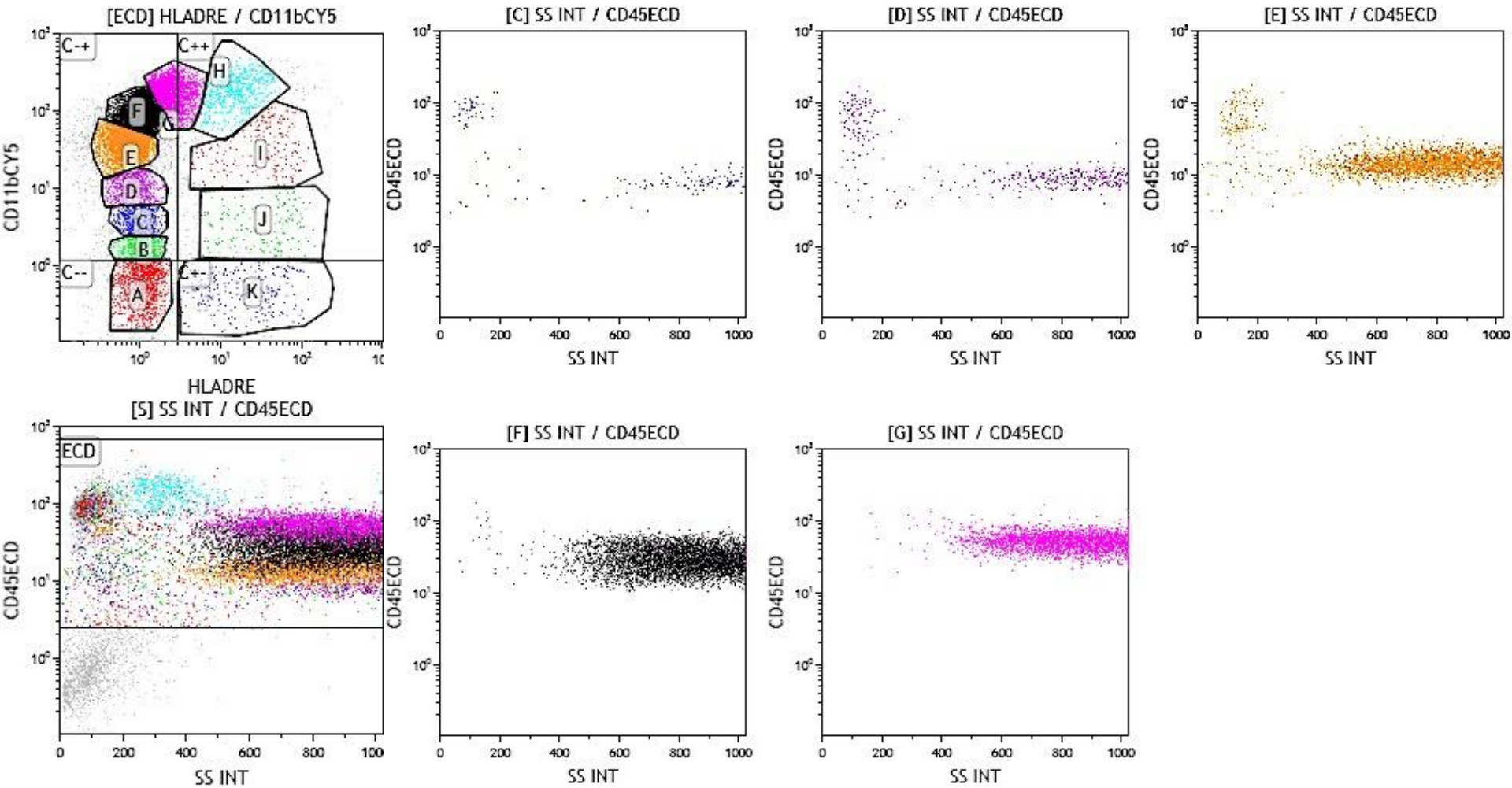
# 3.c. Expressie patroon: myeloid

## CD11b/CD15/HLA-DR



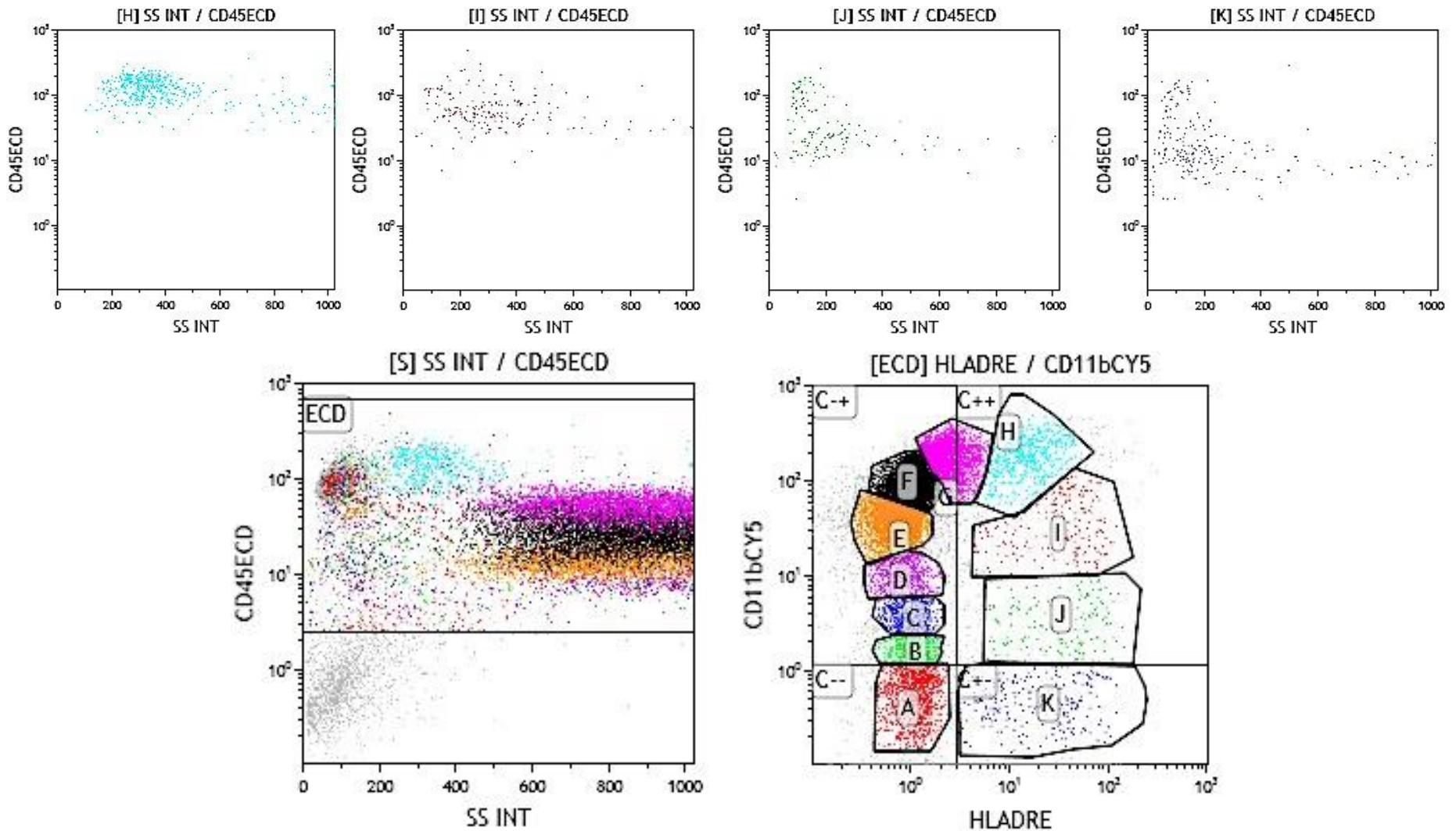
# 3.d. Expressie patroon: myeloid

## CD11b/CD15/HLA-DR

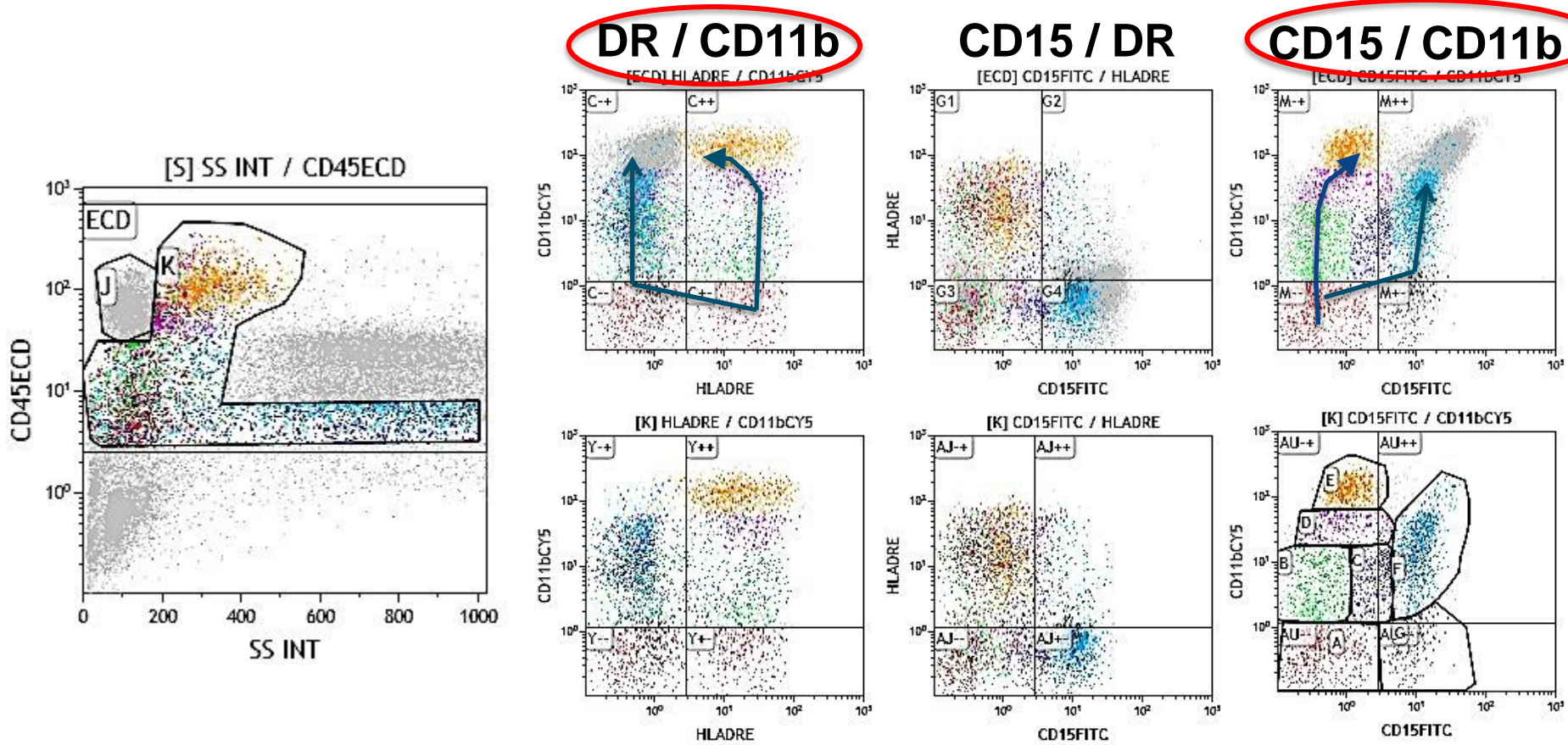


### 3.e. Expressie patroon: monocytair

## CD11b/CD15/HLA-DR



# 3.f. Details: Focus op scheiding van beide lijnen

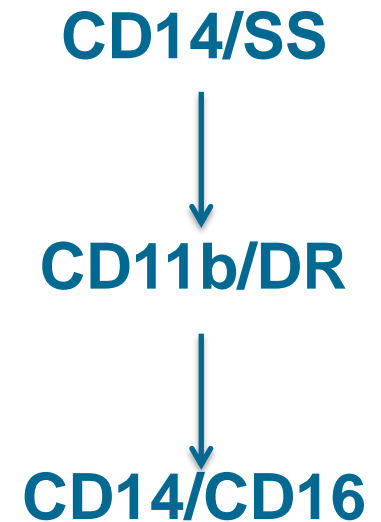
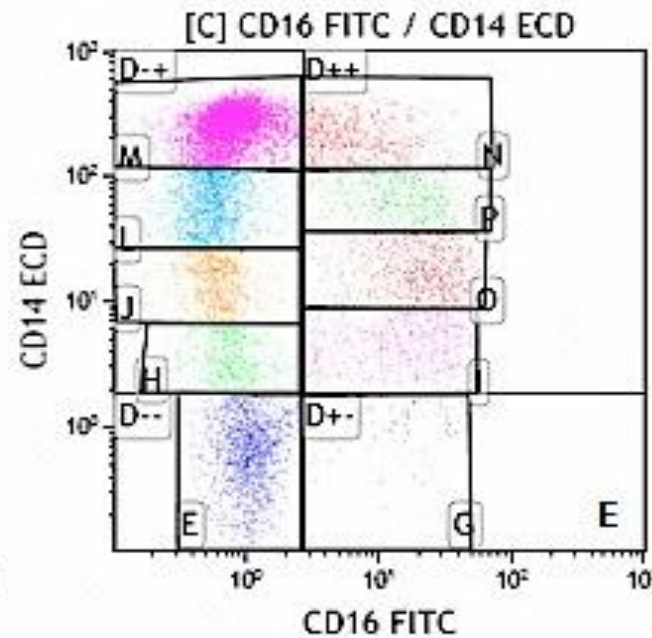
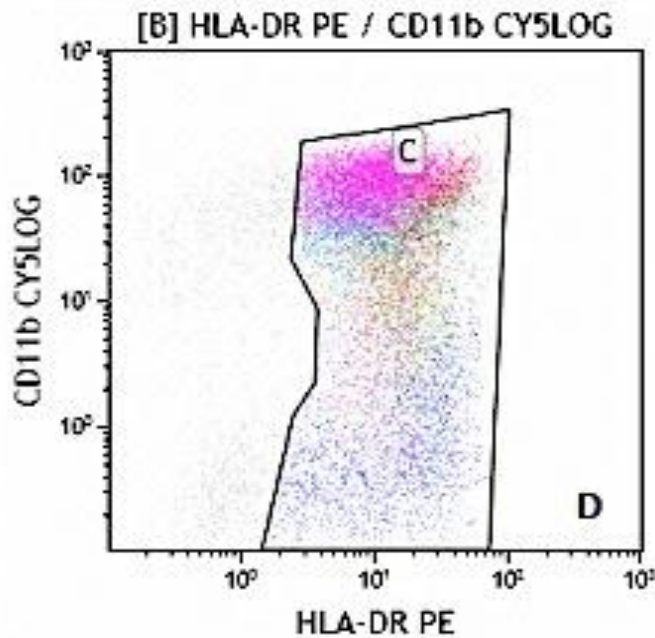
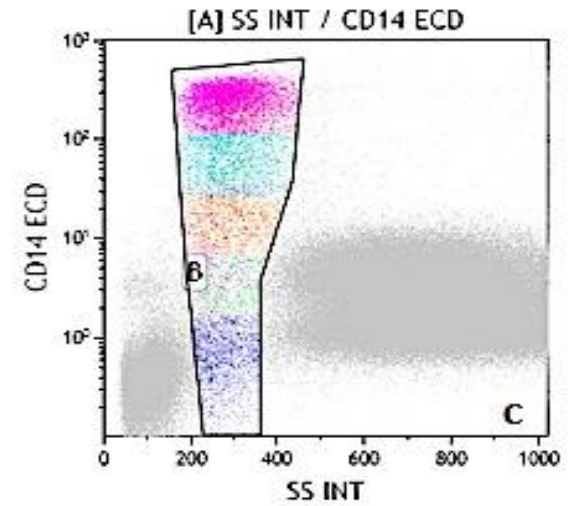
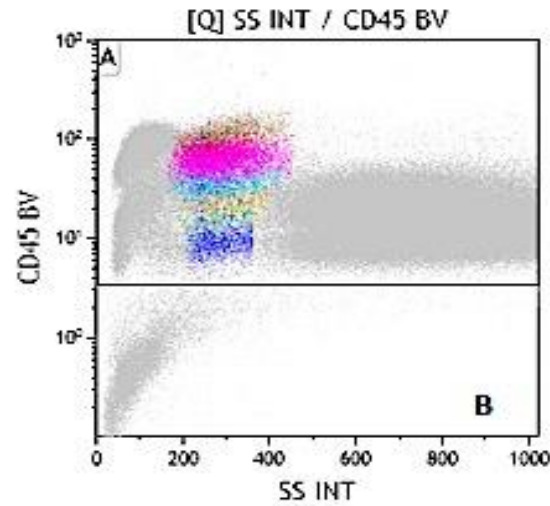
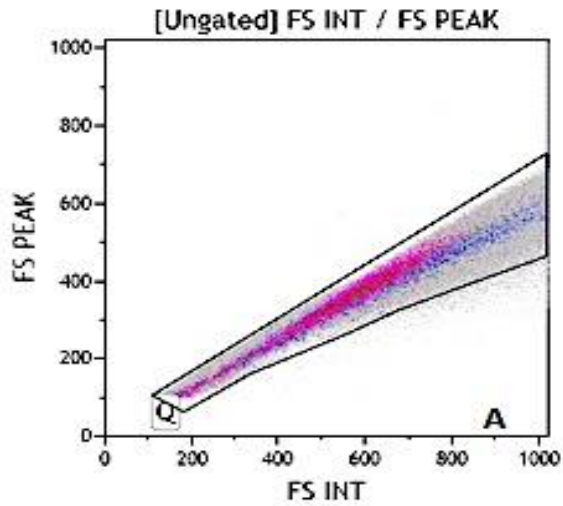


....MAAR er is MEER!....

Macrofagen? Erytroïde cellen?

# 4.a. Waar zijn de Macrofagen?

## Maturatie van de monocyten gebaseerd op CD14 en CD16

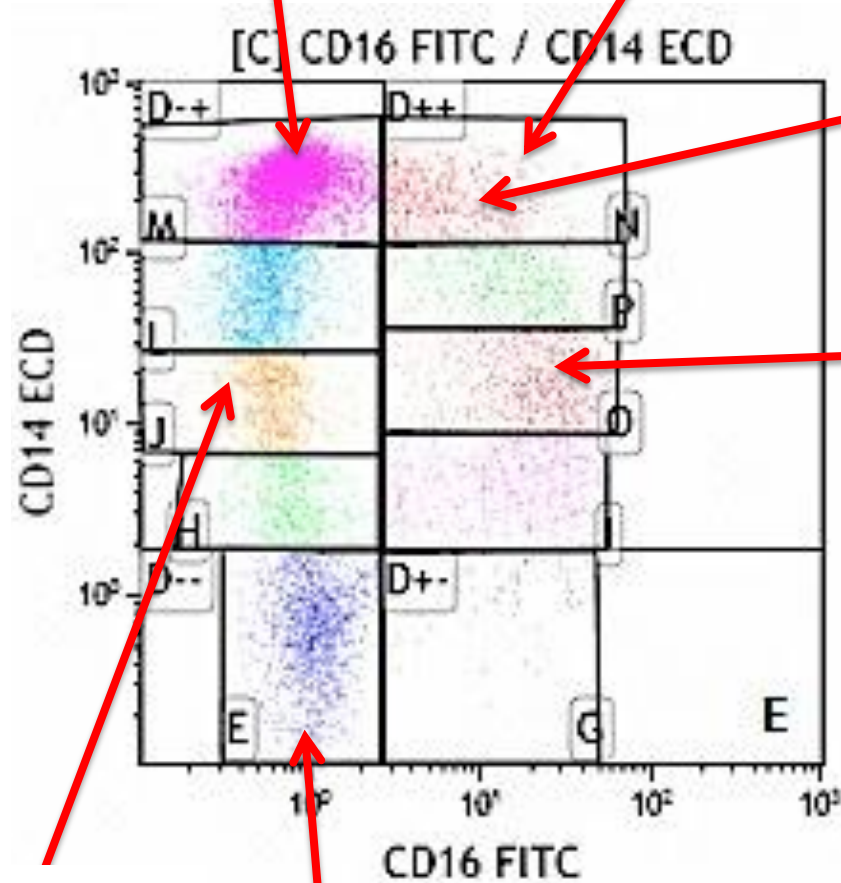




# Maturatie van monocyten tot macrofagen

Classical monocytes

Tissue macrophages



Intermediate monocytes

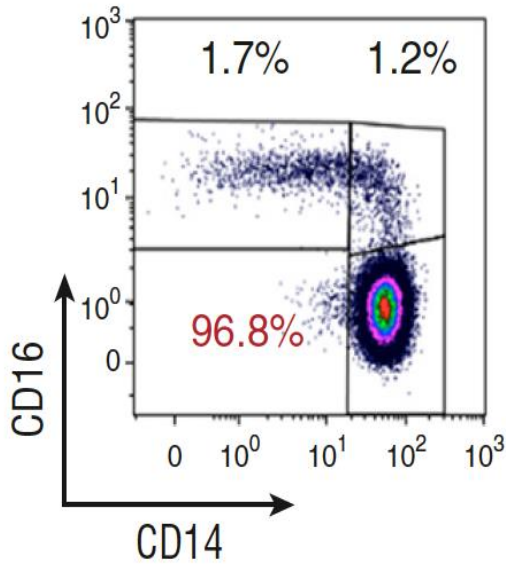
Non-Classical monocytes

Promonocytes

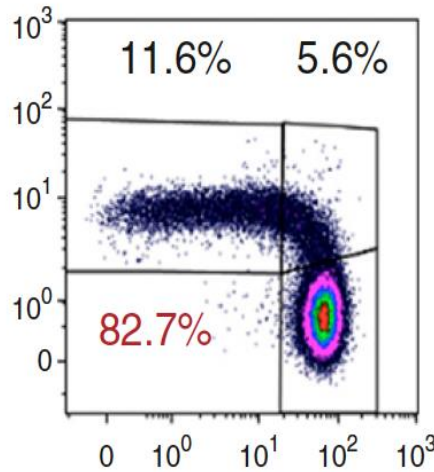
Monoblasts

# CD14/CD16 in CMML: CD14+CD16- >93%

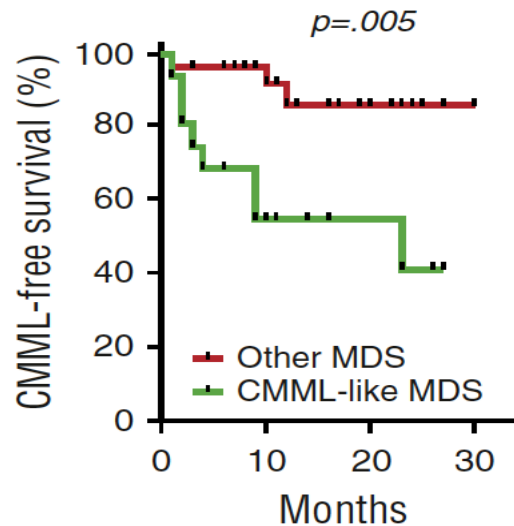
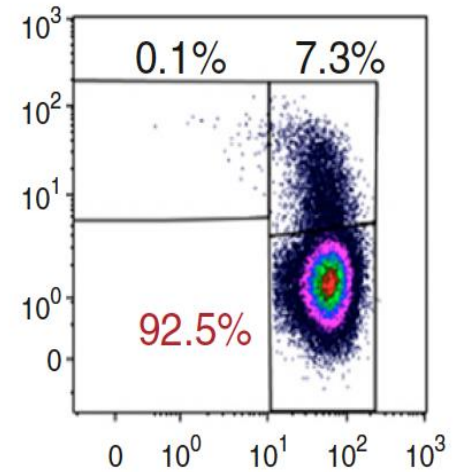
CMML



Normaal



CMML

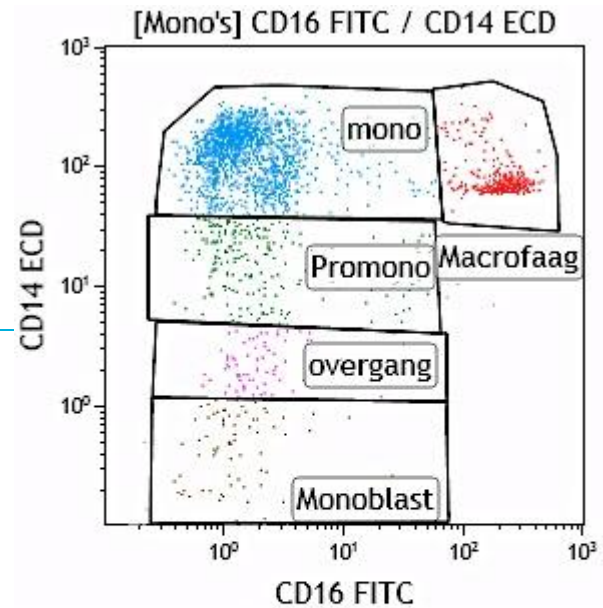
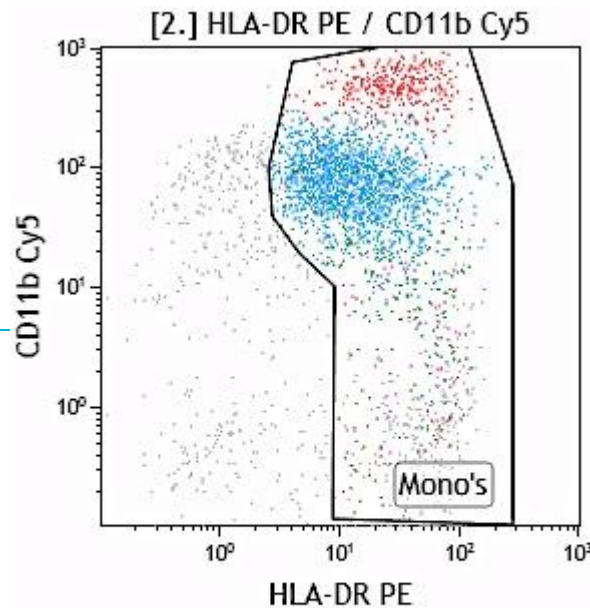
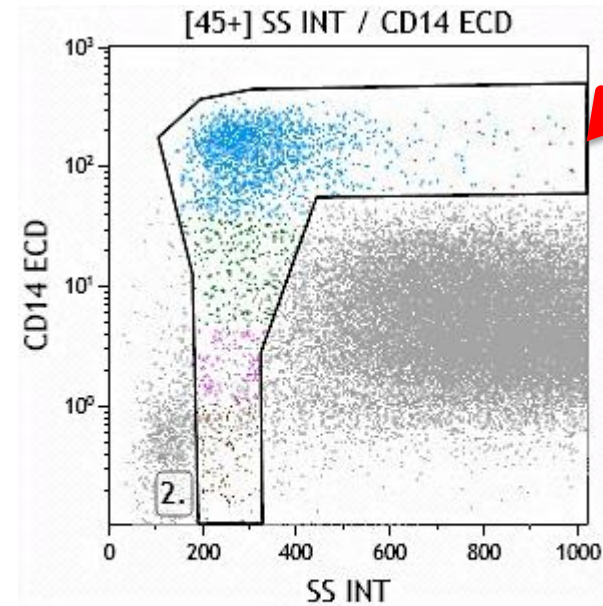
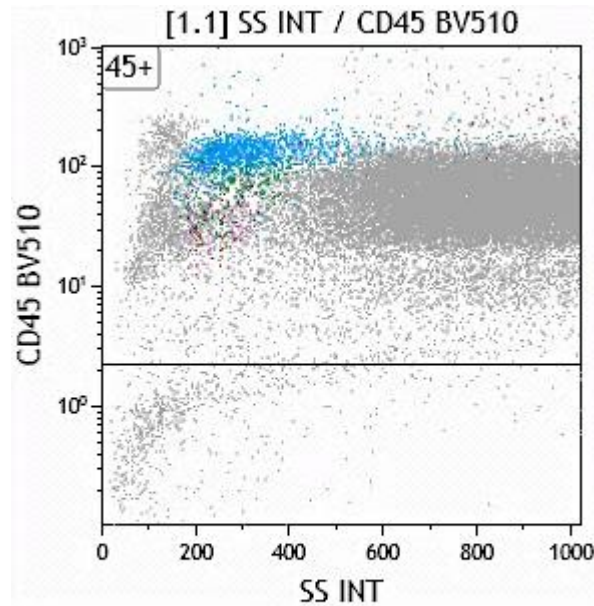




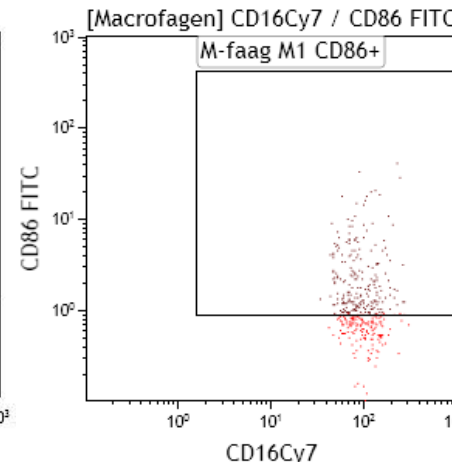
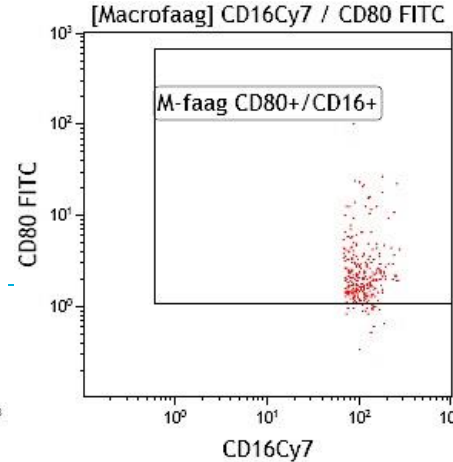
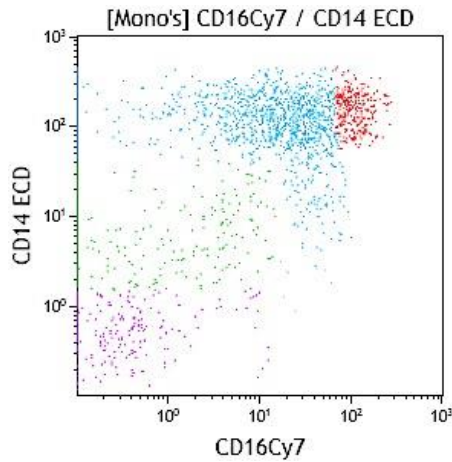
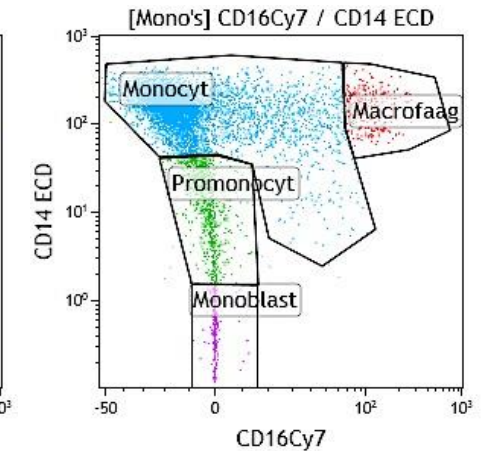
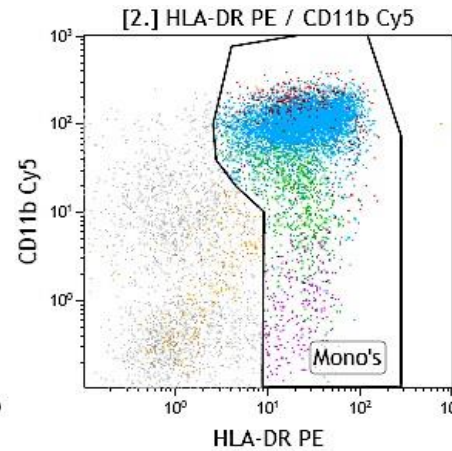
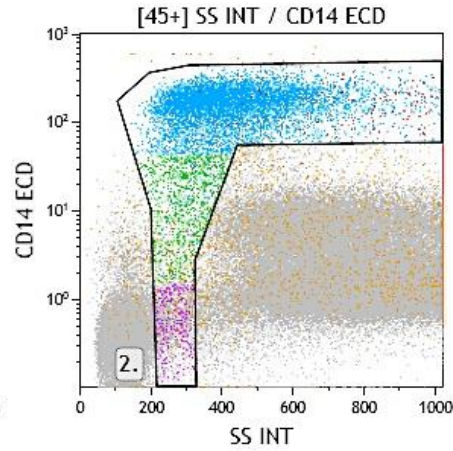
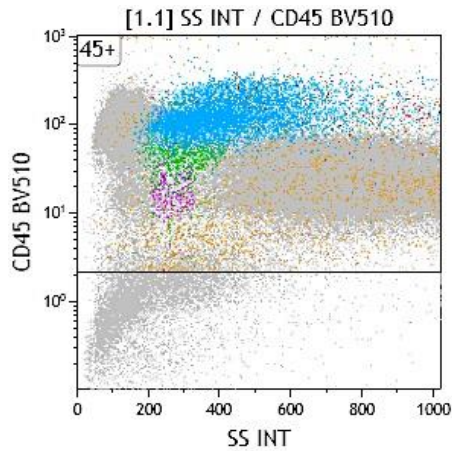
**MACROFAGEN** zijn  
**MONOCYTEN** die vanuit  
de circulatie in de  
weefsels zijn  
gemigreerd

## 4.b. Maturatie van monocyten tot macrofagen (details)

Macrofagen zijn  
SS++ CD11b++  
HLA-DR++  
CD14+ CD16+

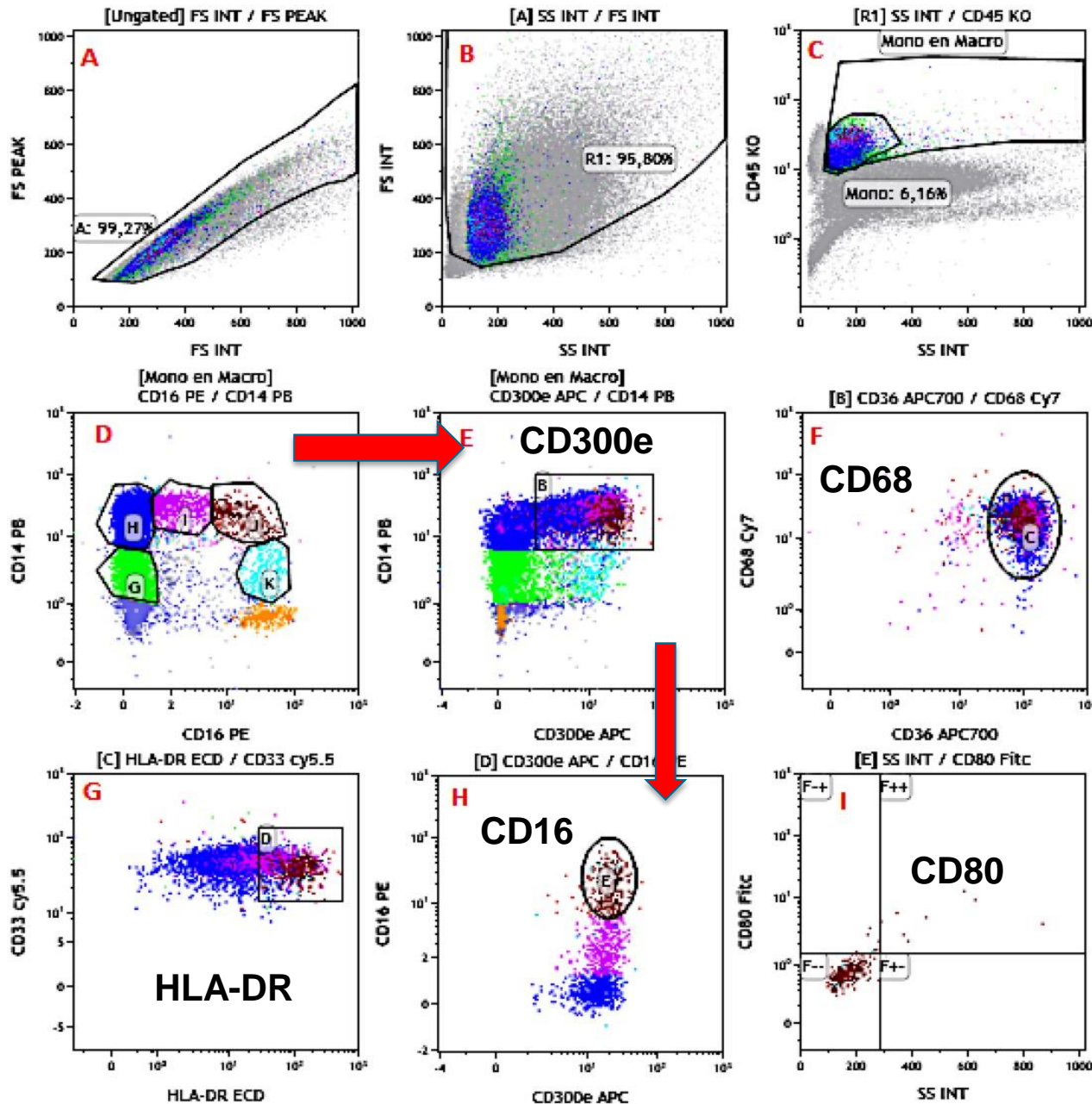


# 4.c. Maturatie van monocytten tot macrofagen (CD80 en CD86)



Bewijs van  
Macrofagen  
door  
CD80 en CD86

# 4.d. Maturatie van monocyten tot macrofagen



Differentiatie van  
Monocyt – Macrofaag

CD68  
Monocyten en  
Macrofagen

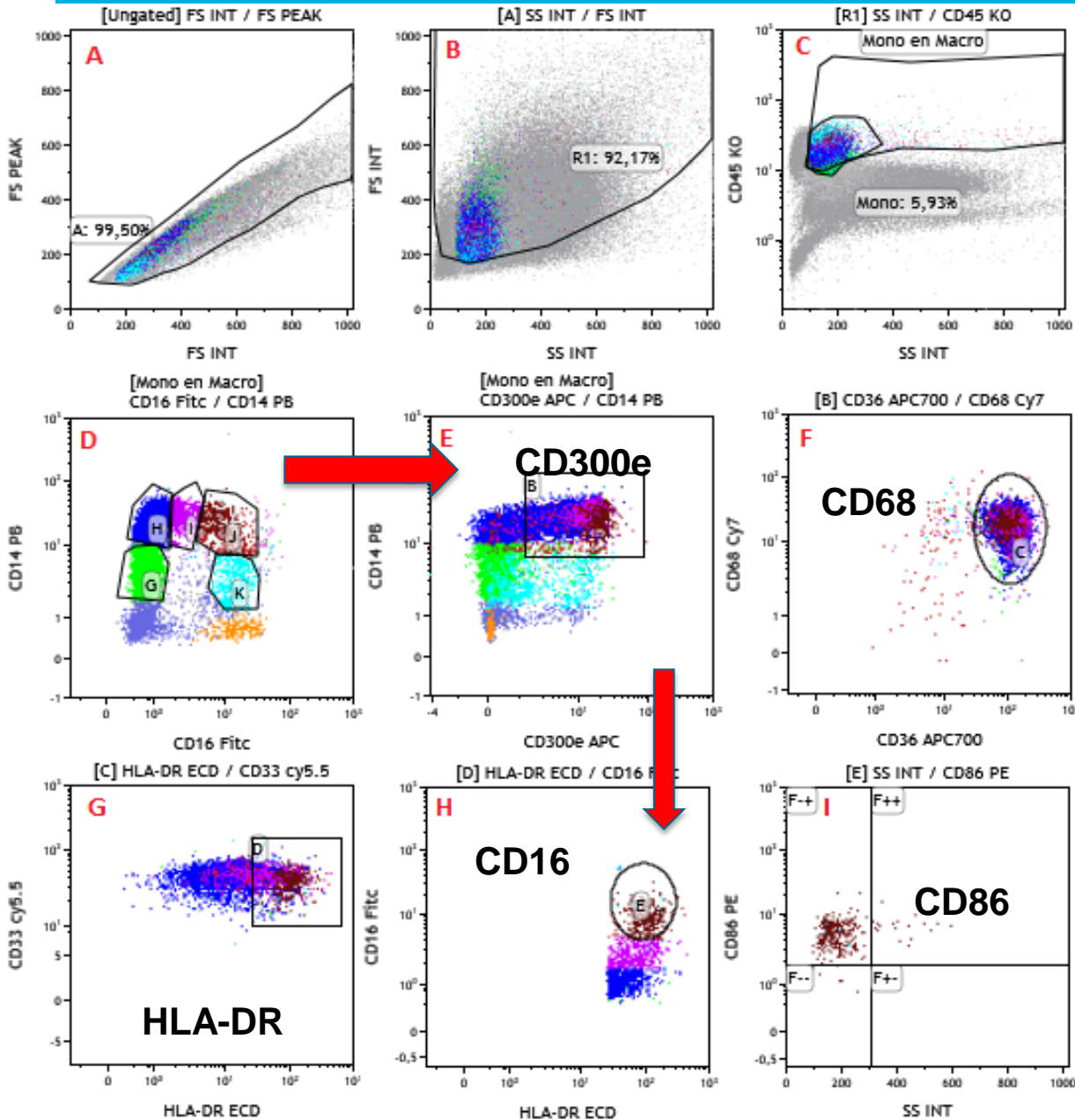
CD300e (IREM2)  
Separatie binnen  
classical monocyte

CD16  
Separatie binnen  
intermediate monocyte

CD80/CD86  
Separatie binnen  
Macrofagen

HLADR  
Separatie gebaseerd op  
activatie stadium

# 4.e. Maturatie van monocyten tot macrofagen



**Differentiatie van  
 Monocyt – Macrofaag**

**CD68  
 Monocyten en  
 Macrofagen**

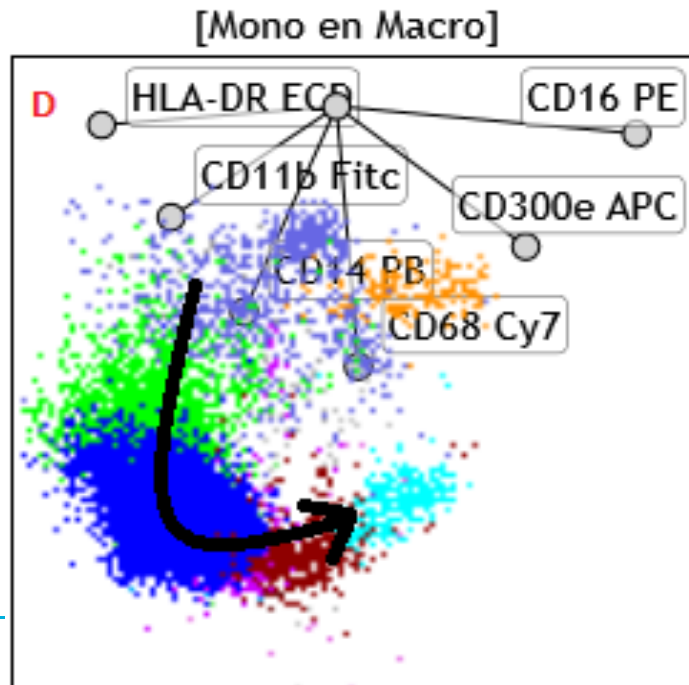
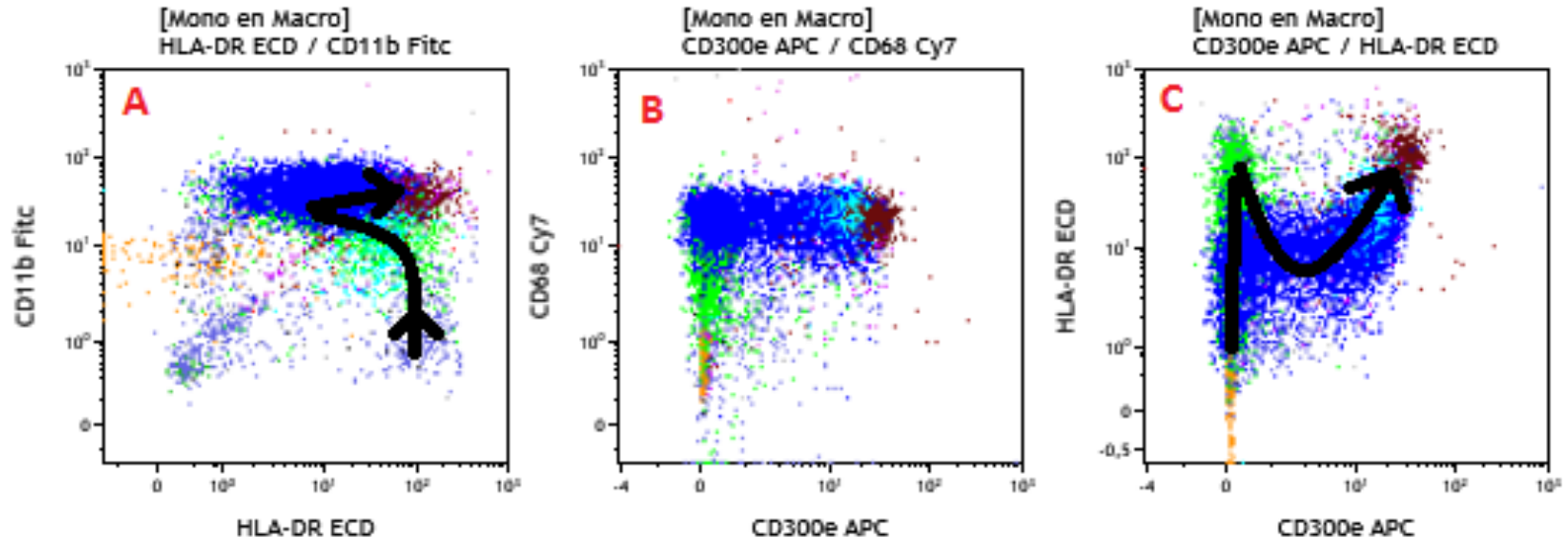
**CD300e (IREM2)  
 Separatie binnen  
 classical monocyte**

**CD16  
 Separatie binnen  
 intermediate monocyte**

**CD80/CD86  
 Separatie binnen  
 Macrofagen**

**HLADR  
 Separatie gebaseerd op  
 activatie stadium**

## 4.f. Maturatie van monocytten naar macrofagen



Activatie van monocytten

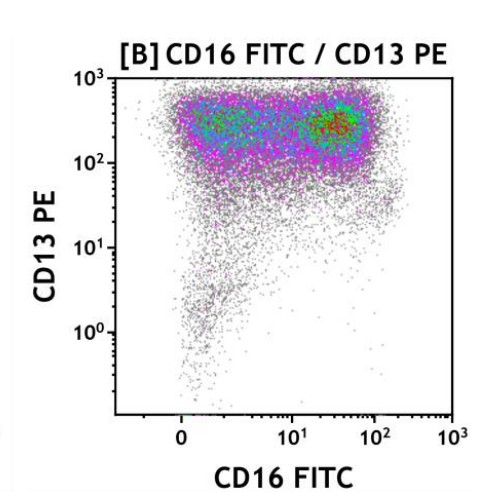
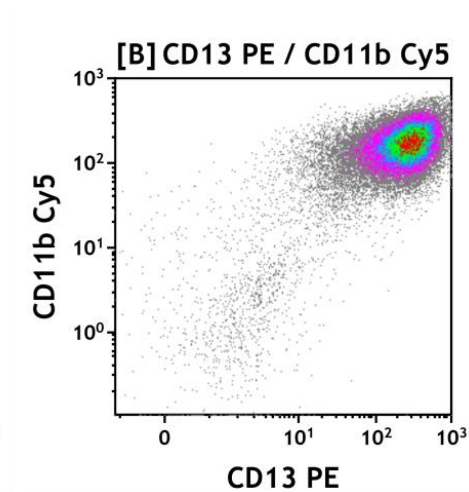
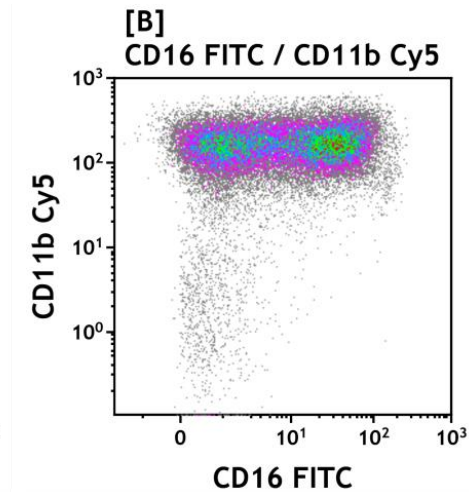
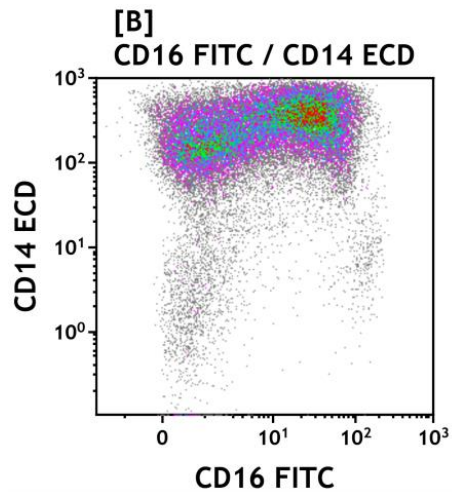
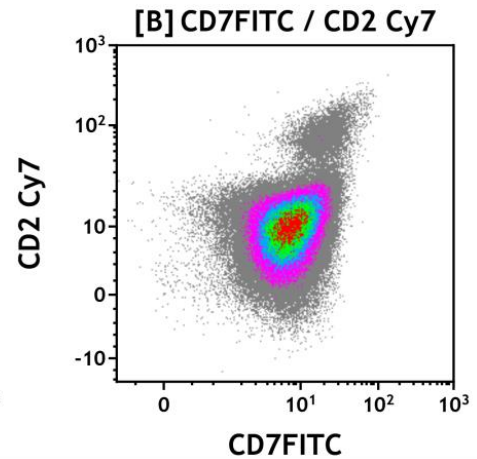
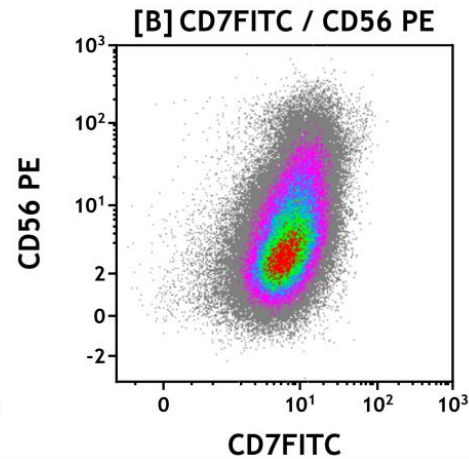
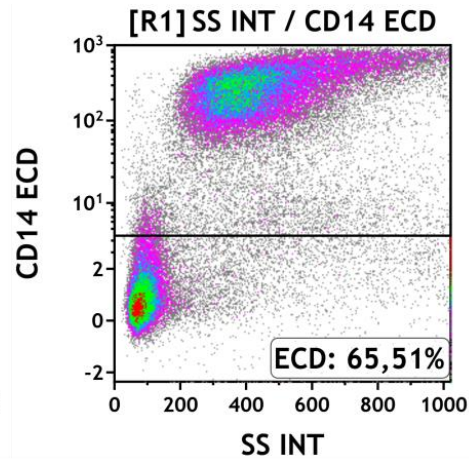
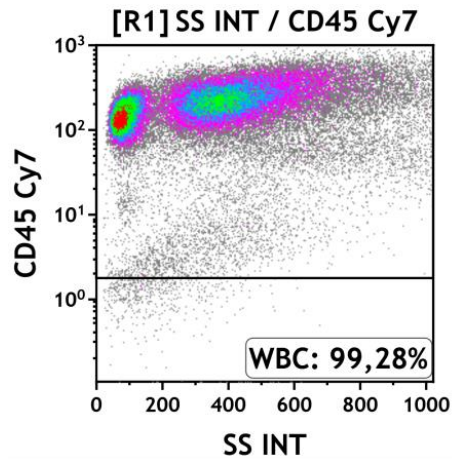
HLADR: van dim tot bright

CD300e: van dim tot bright

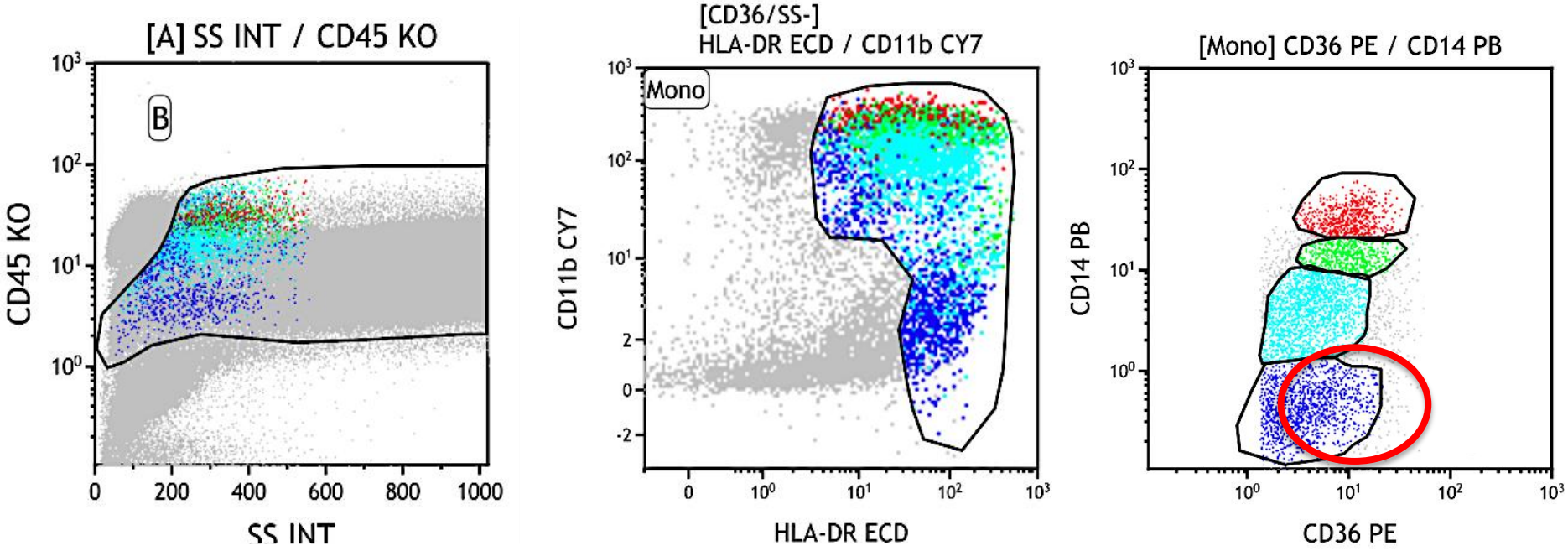
Rol van radar plot



# Monocytair leukemie: AML

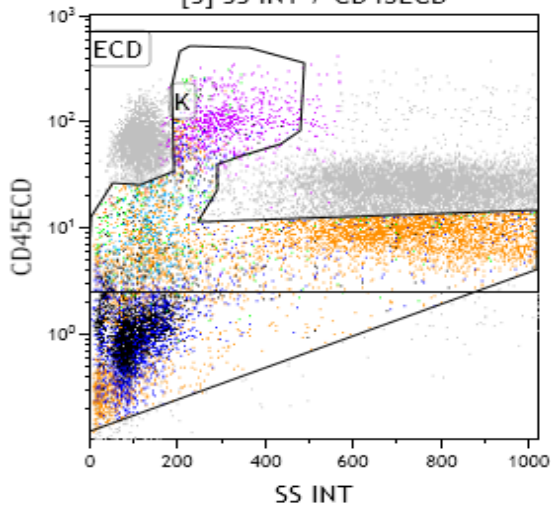


# 5. Maturatie van monocyten. Naar wat zijn CD36+CD14-

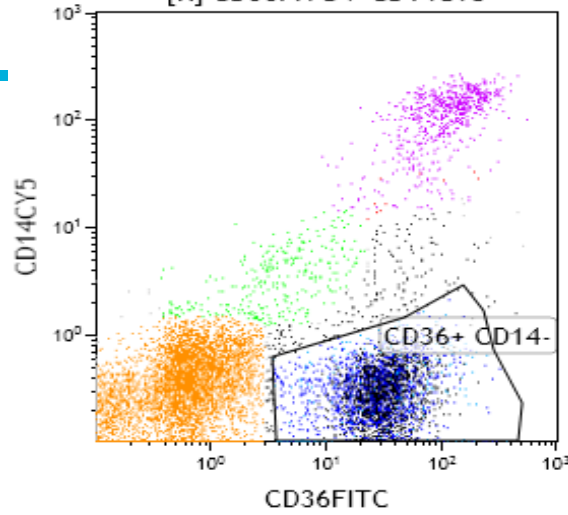


**CD36:** monocyten  
kernhoudend  
erythroid  
megakaryocyten

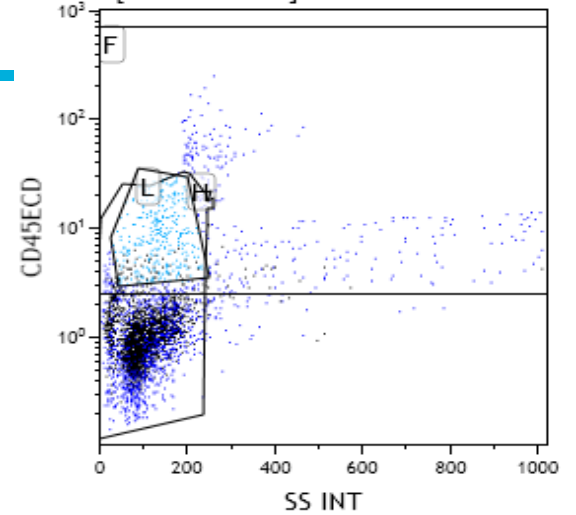
[S] SS INT / CD45ECD



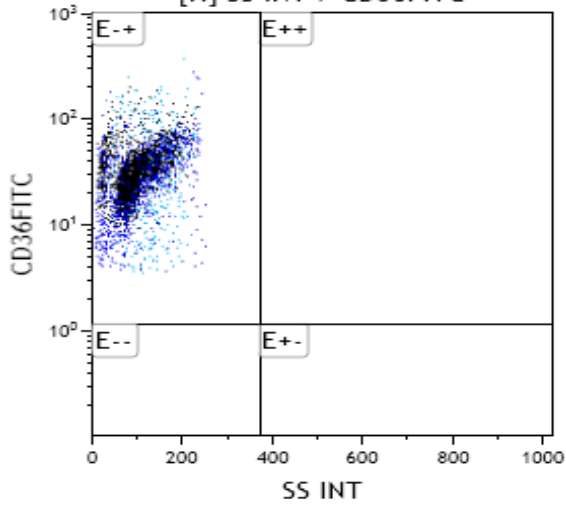
[K] CD36FITC / CD14CY5



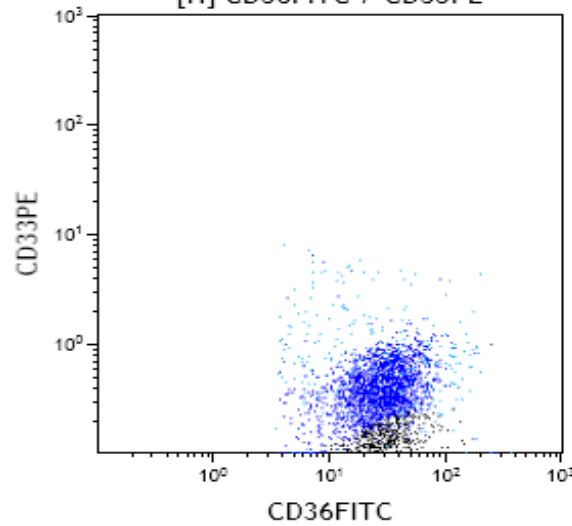
[CD36+ CD14-] SS INT / CD45ECD



[H] SS INT / CD36FITC



[H] CD36FITC / CD33PE

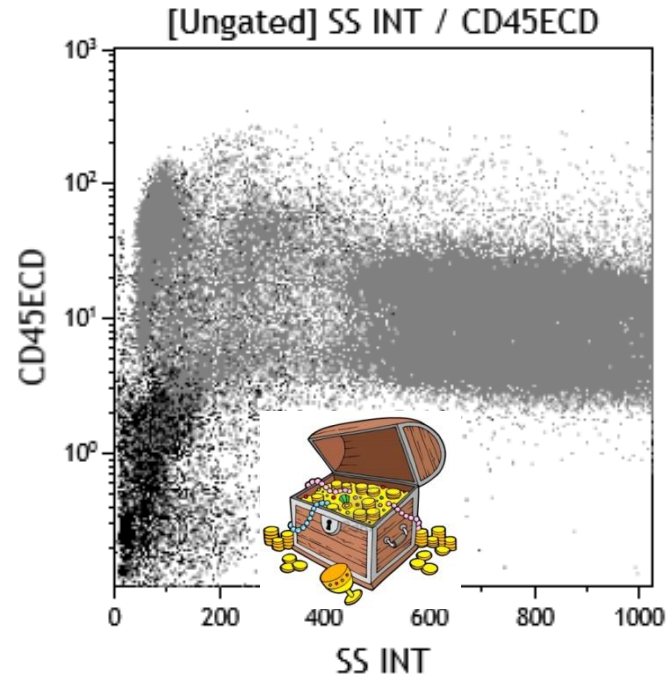
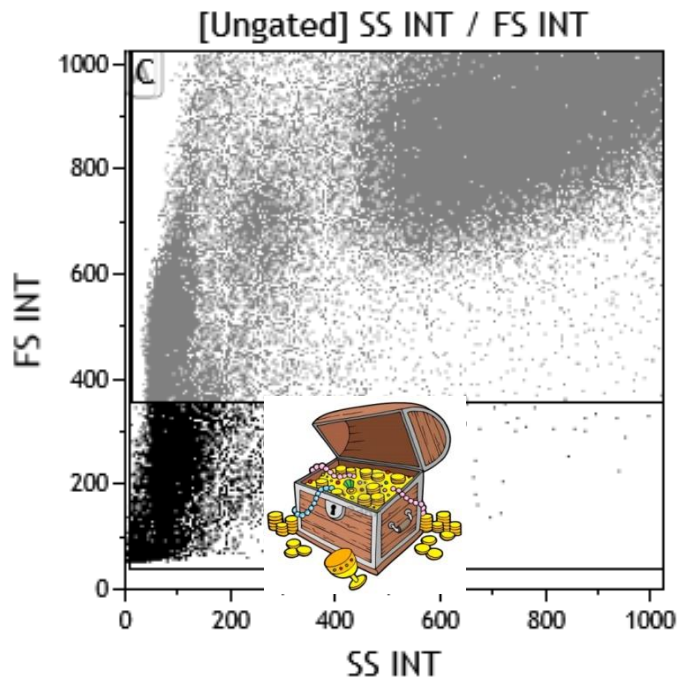


## 5.a. Erythroide pathway

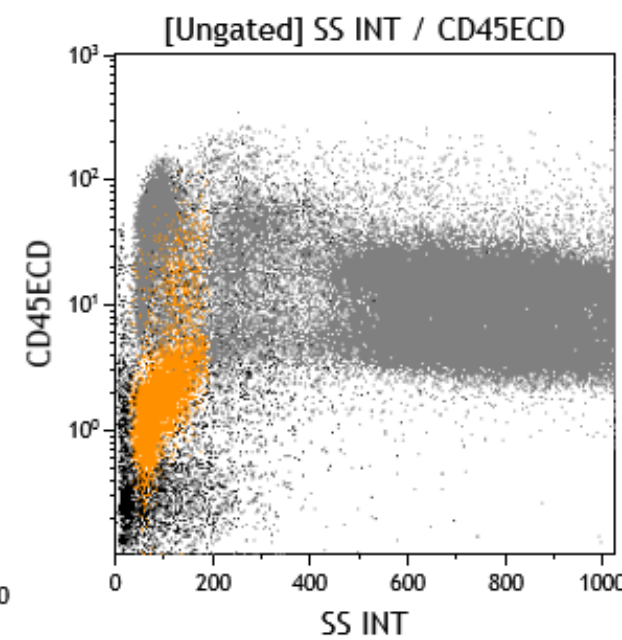
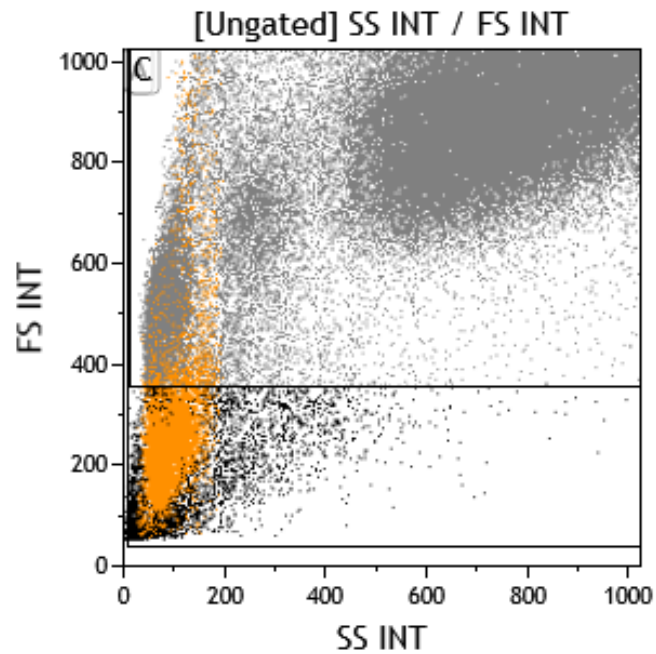
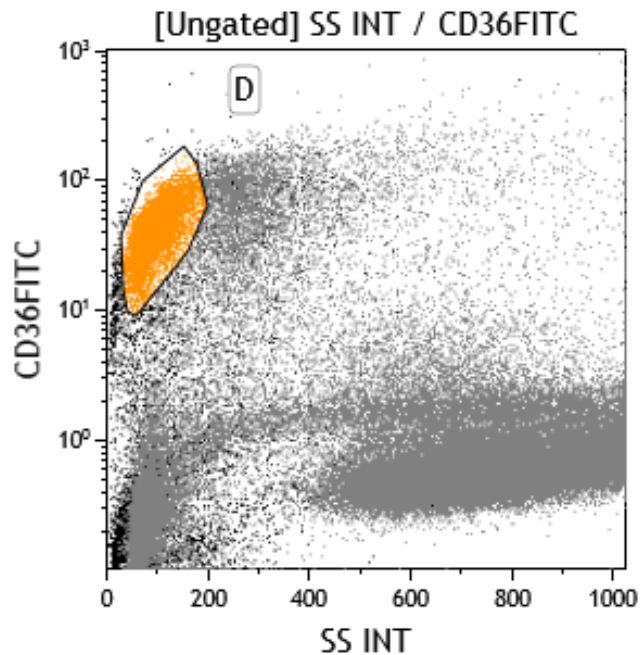
Door middel van CD36/CD14/CD33

# In routine diagnostiek wordt debris uit-gegate

## Maar wat gaat verloren?

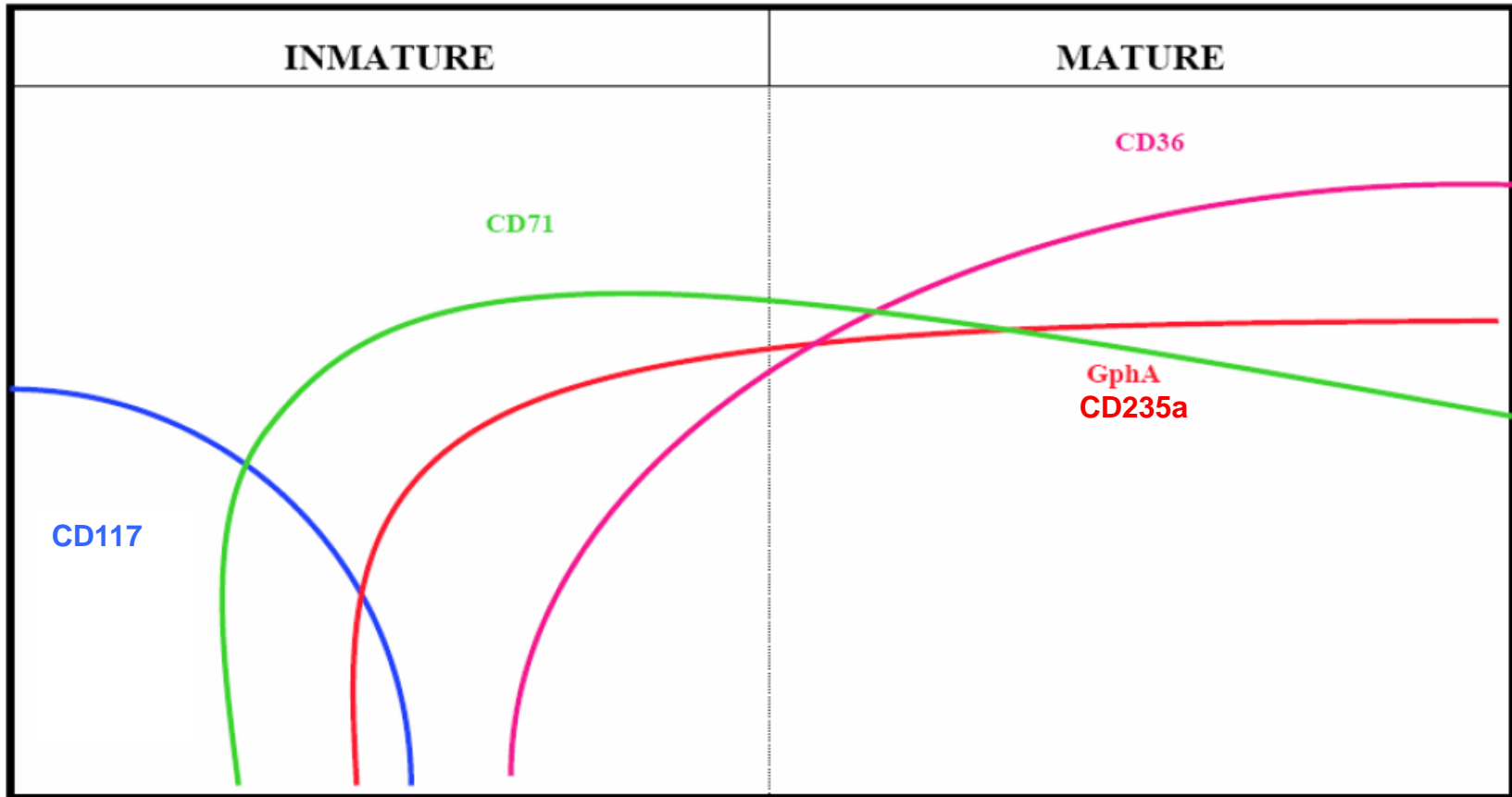


# Hoe moeten we deze CD36+ cellen karakteriseren?



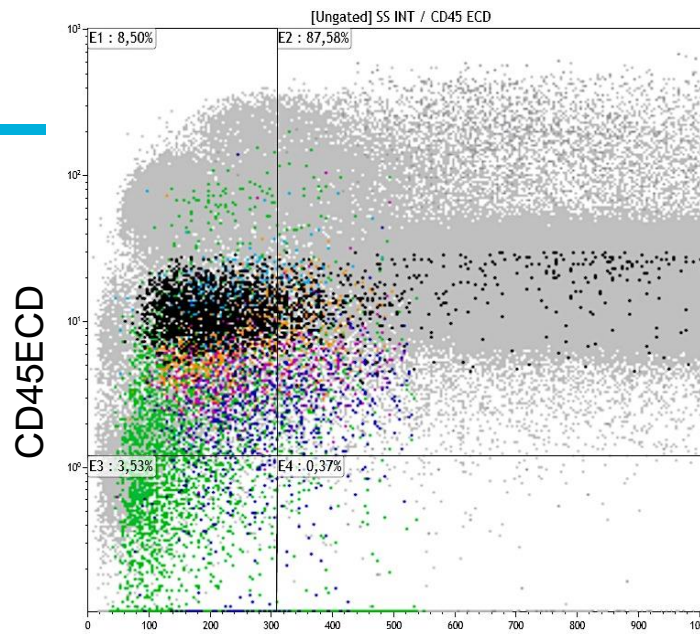
# Normale erythroïde maturatie

CD117 / CD71 / CD235a / CD36

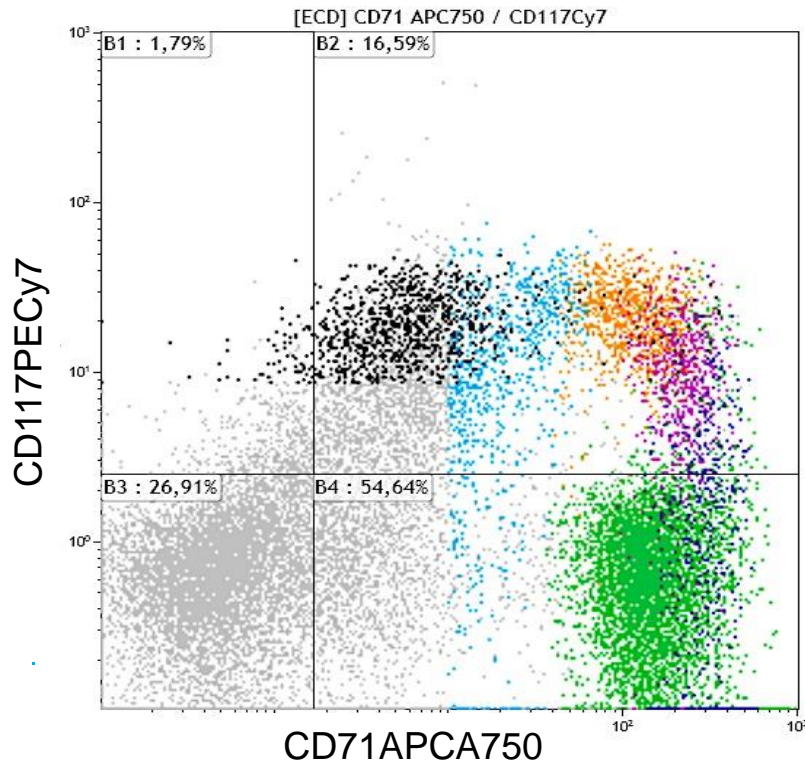


# 5.b. Erythroide pathway

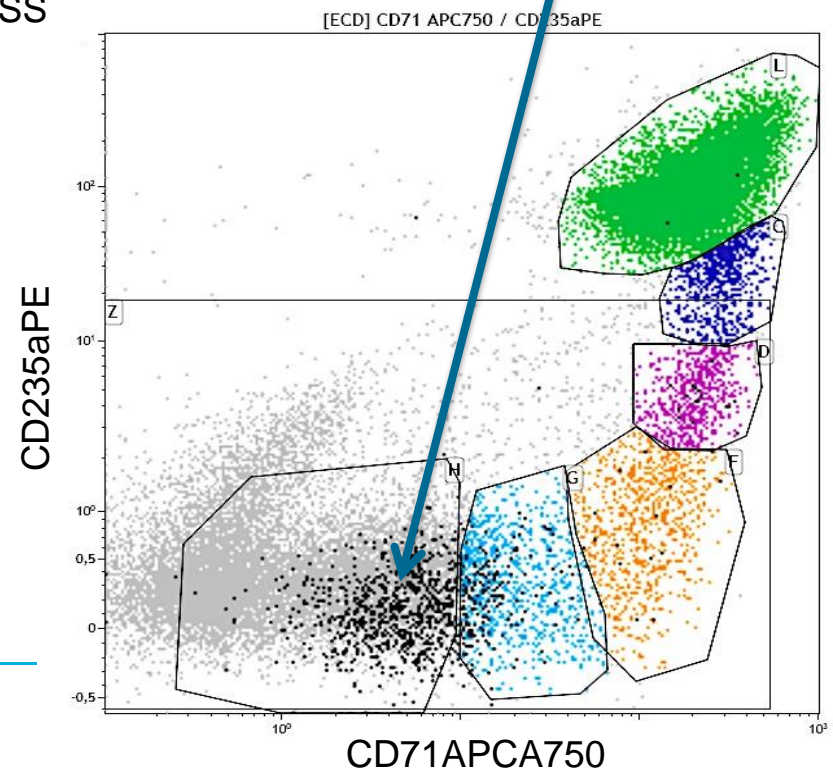
D.m.v.  
CD235a/CD117/  
CD71



Welke cellijn in zwart?

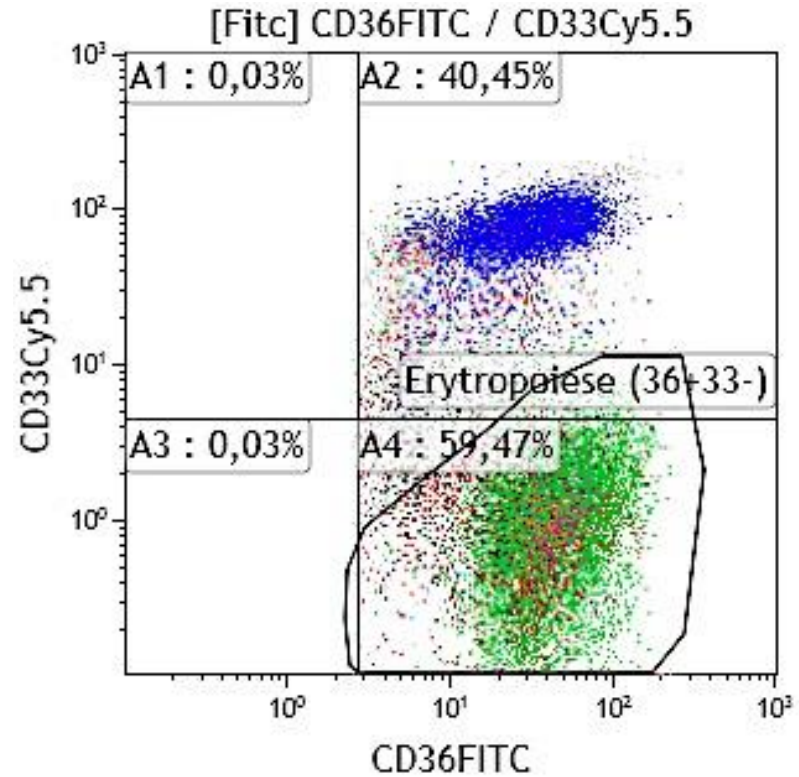
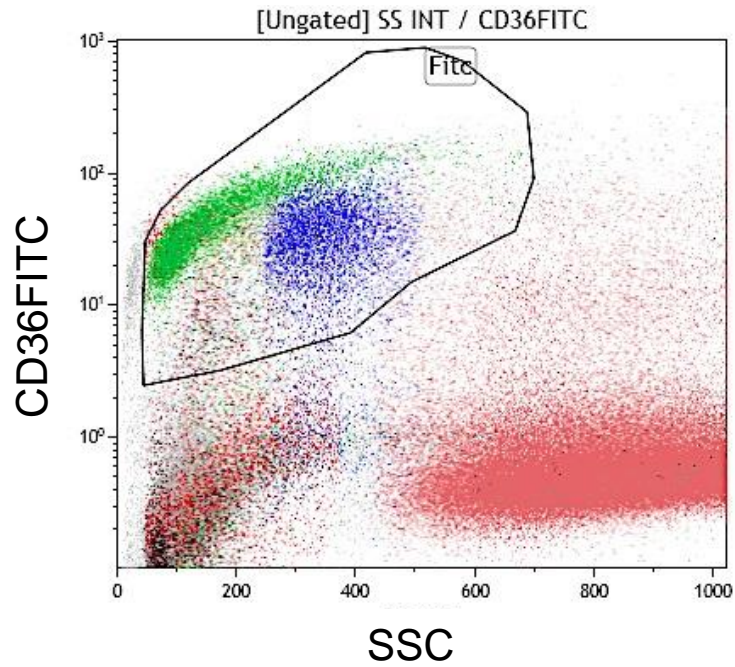


SS



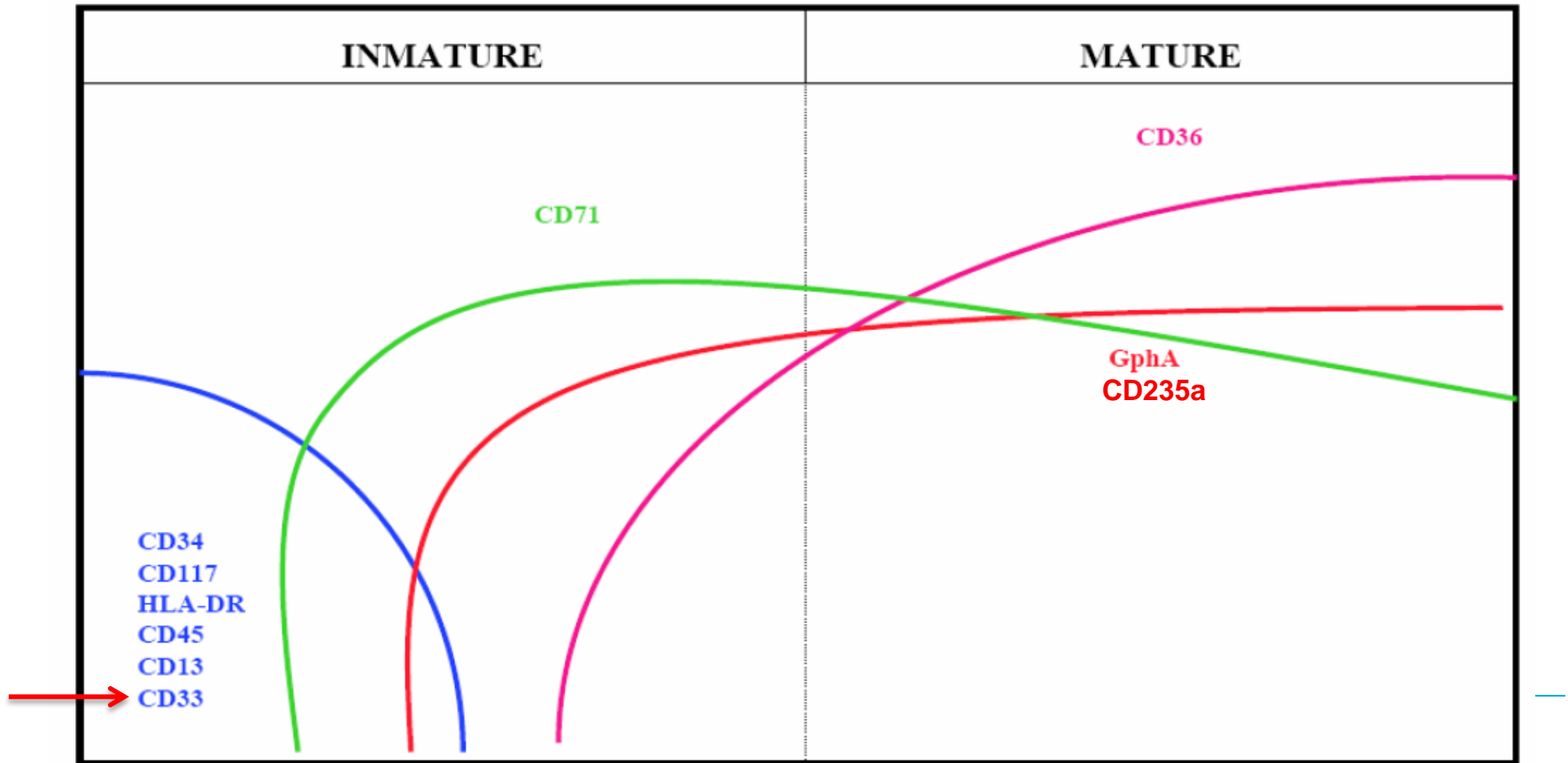
# Eliminatie van myeloïde cellen uit de erythroïde lijn

## (CD36+CD33-)



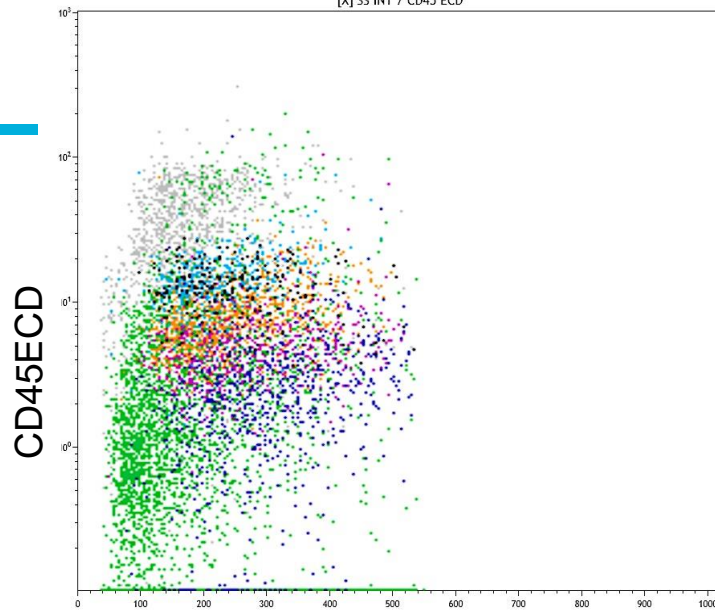


# Normale erythroïde maturatie

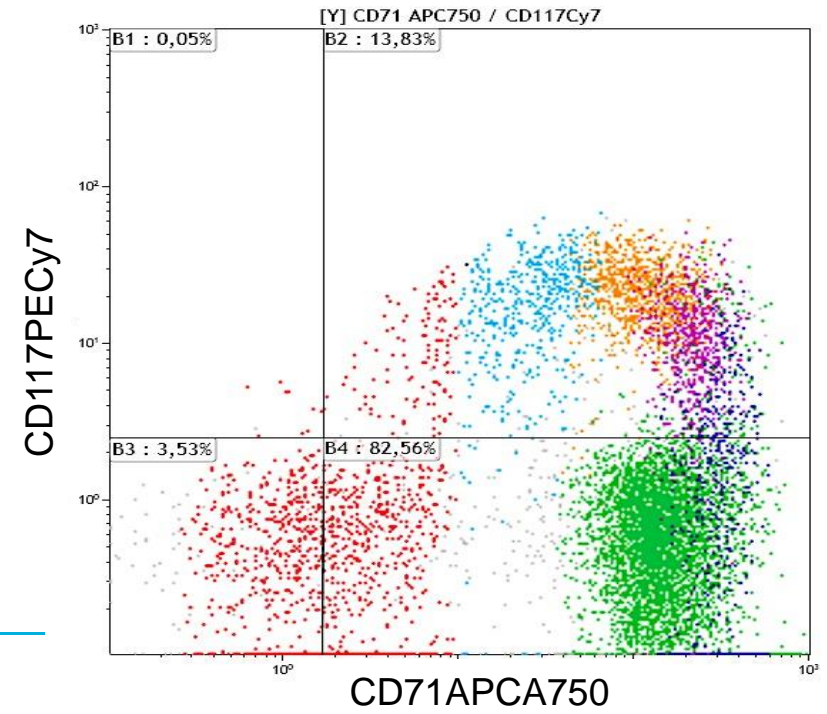
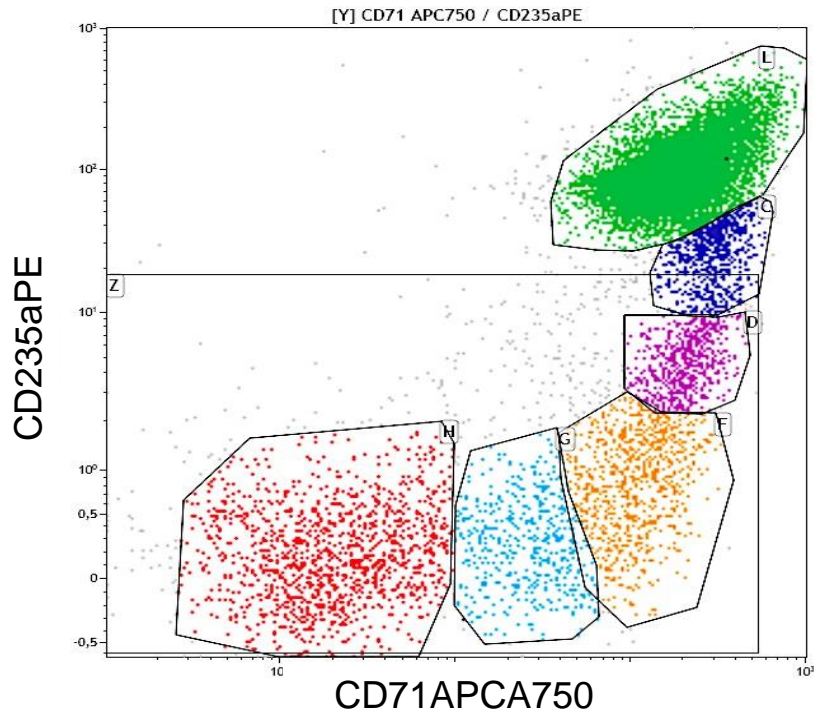


# 5.c. Erythroide pathway (focus op erythroid)

D.m.v.  
CD235a/CD117/CD71

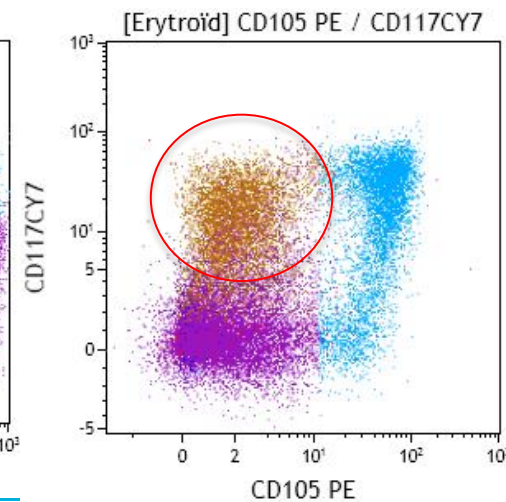
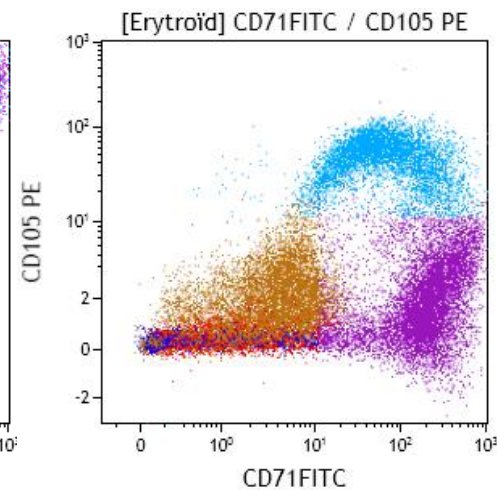
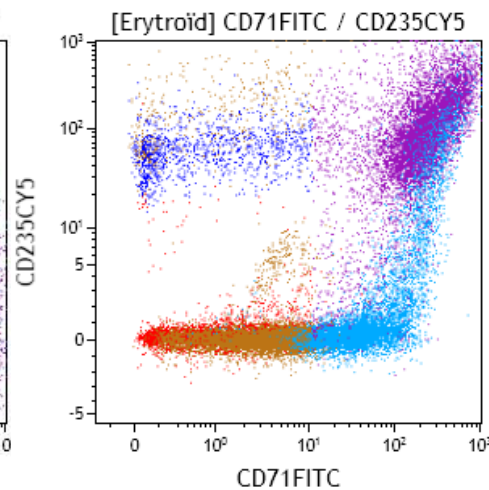
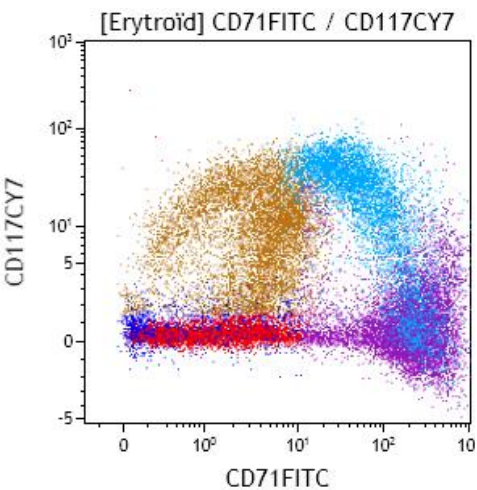
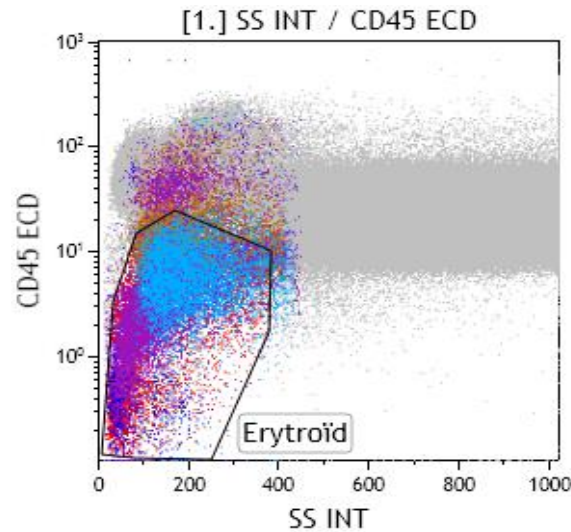


SS



# 5.d. CD33 is ook aanwezig op erythroïde precursors:

## Selectie van de erythroïde lijn door CD105



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# Samenvatting

1. Myeloïde differentiatie
2. Monocytaire differentiatie
3. Separatie van myeloïd and monocytair
4. Erythroïde differentiatie

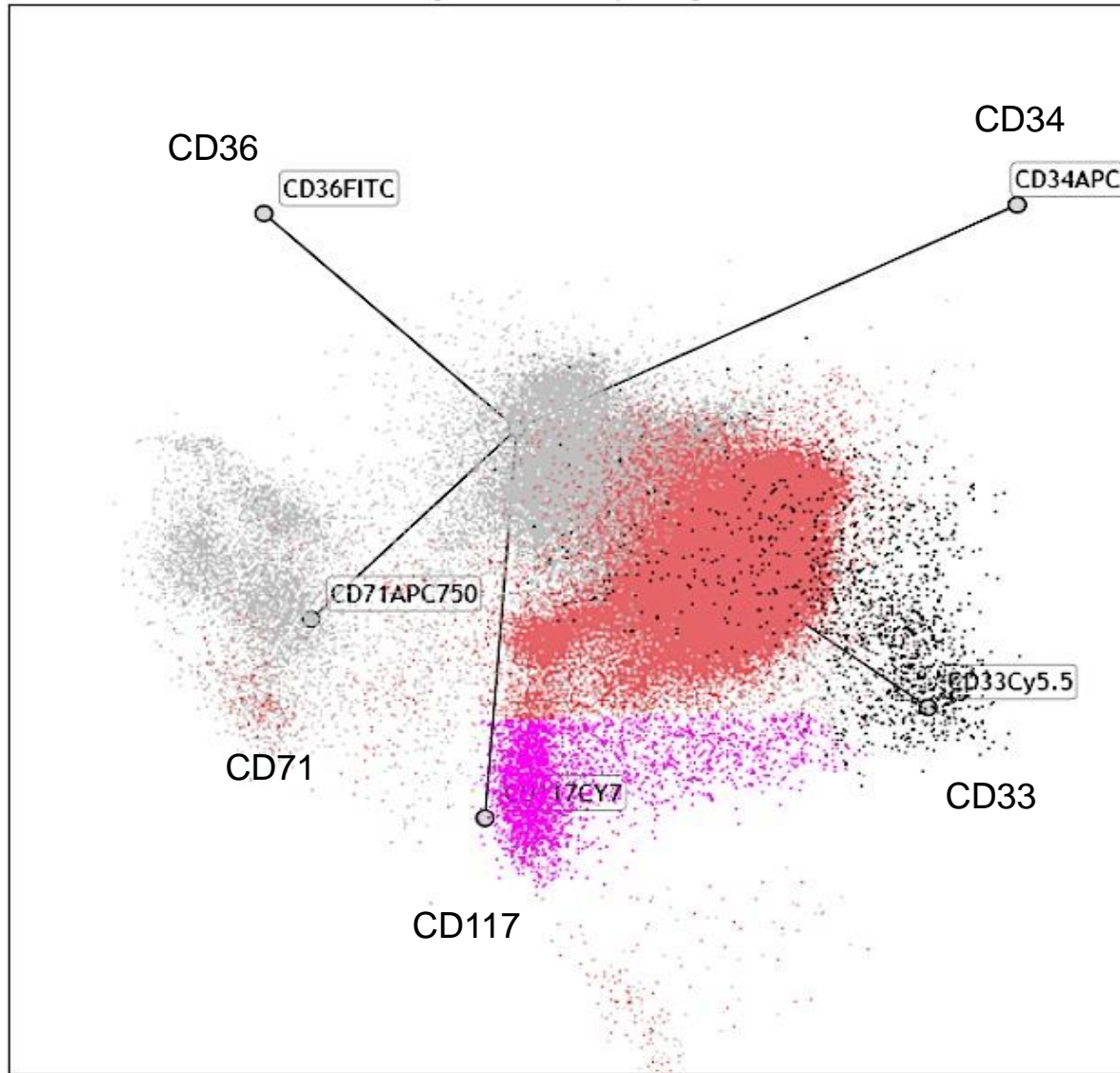
**Hoe kun je alle lijnen in één plot aantonen??**

***De Radar plots***

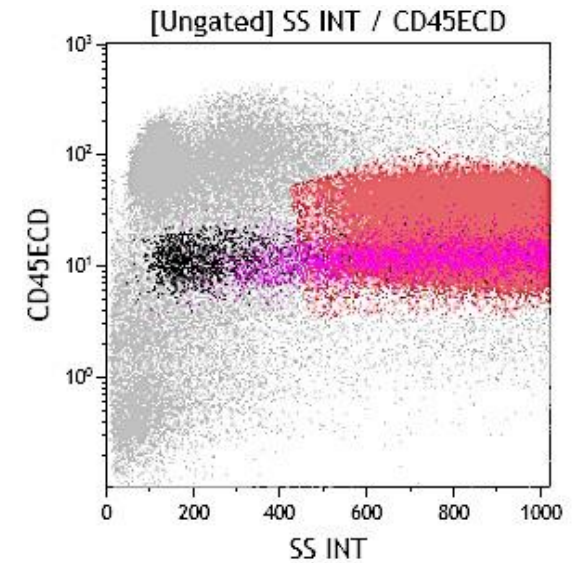
# Myeloipoiese

CD45+/-/CD34+ precursors,  
Promyelocyten + Mature myeloide cellen

[Totale hematopoiese]

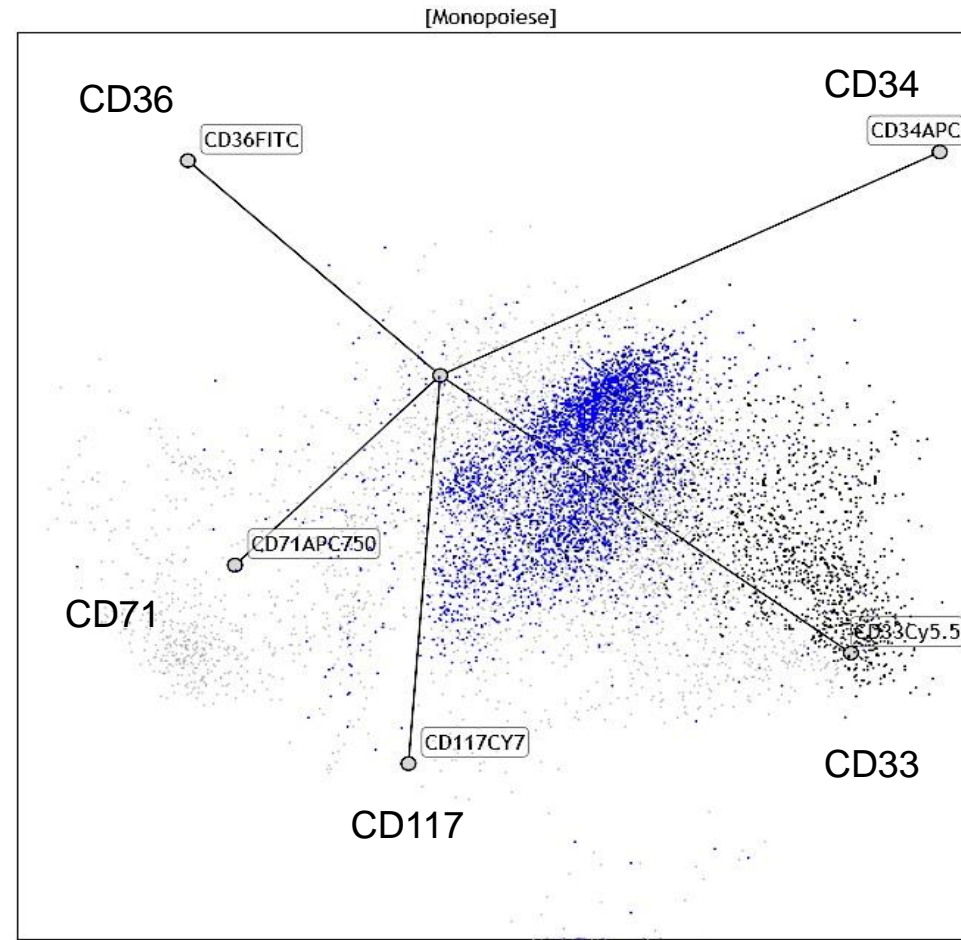
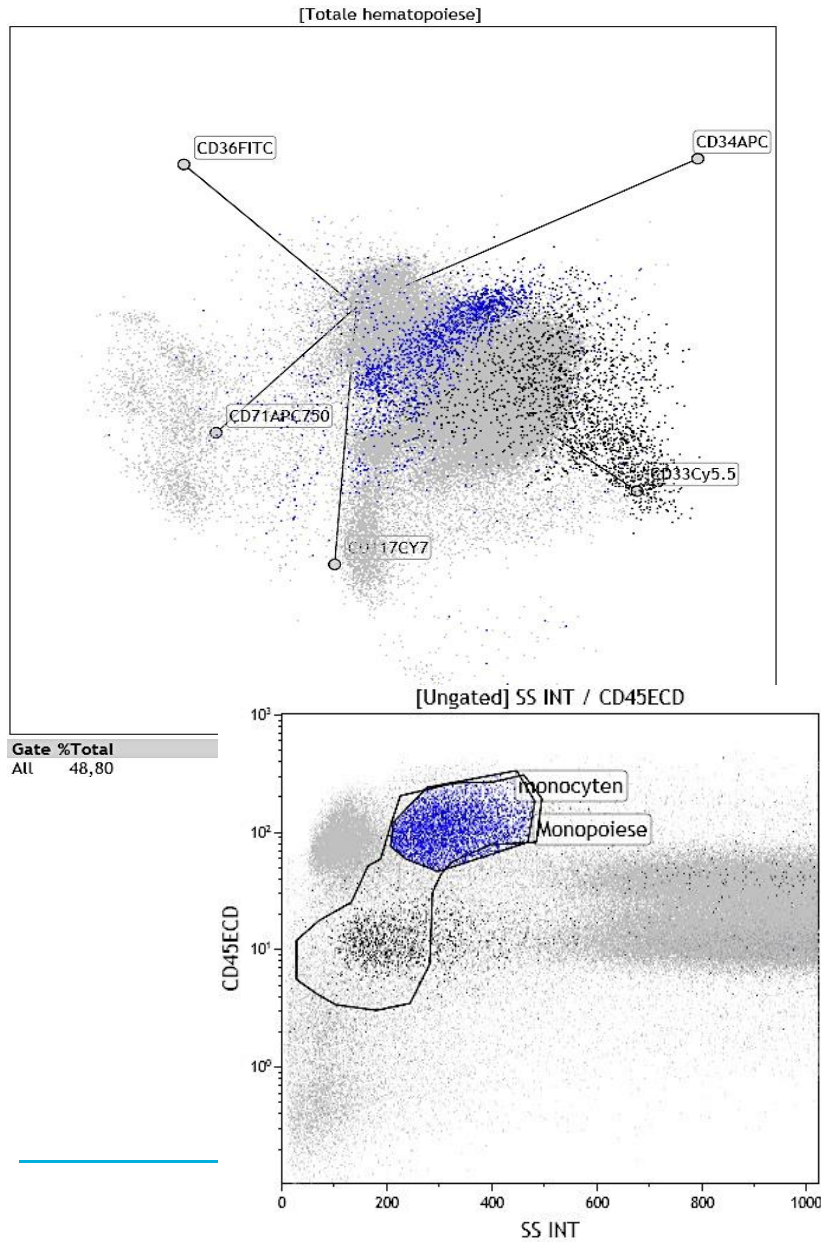


Gate %Total  
All 50,77



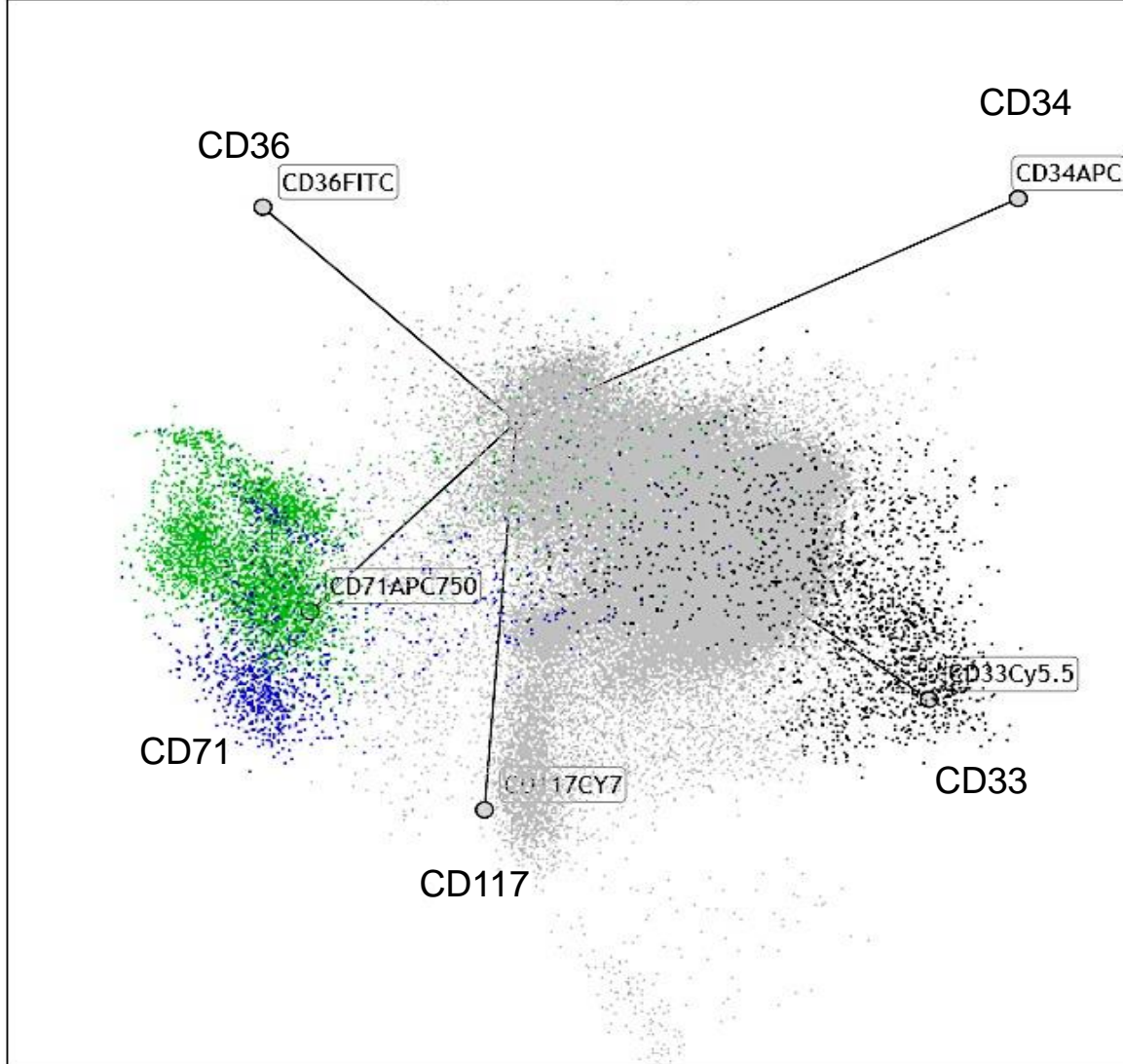
# Monopoïese

## Mature monocyten en CD45<sup>+</sup>/CD34<sup>+</sup> precursors



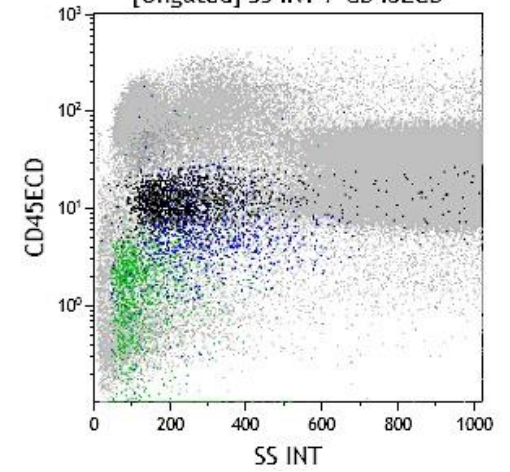
# Erythropoiese

[Totale hematopoiese]

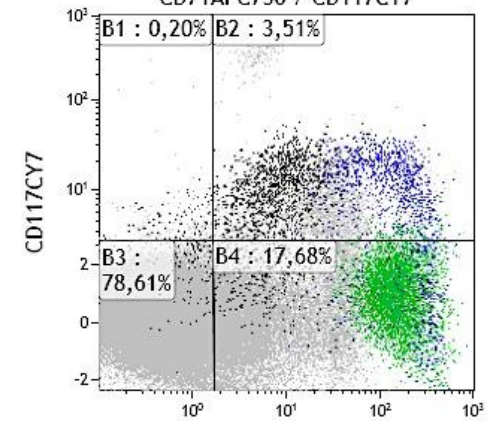


Gate %Total  
All 50,01

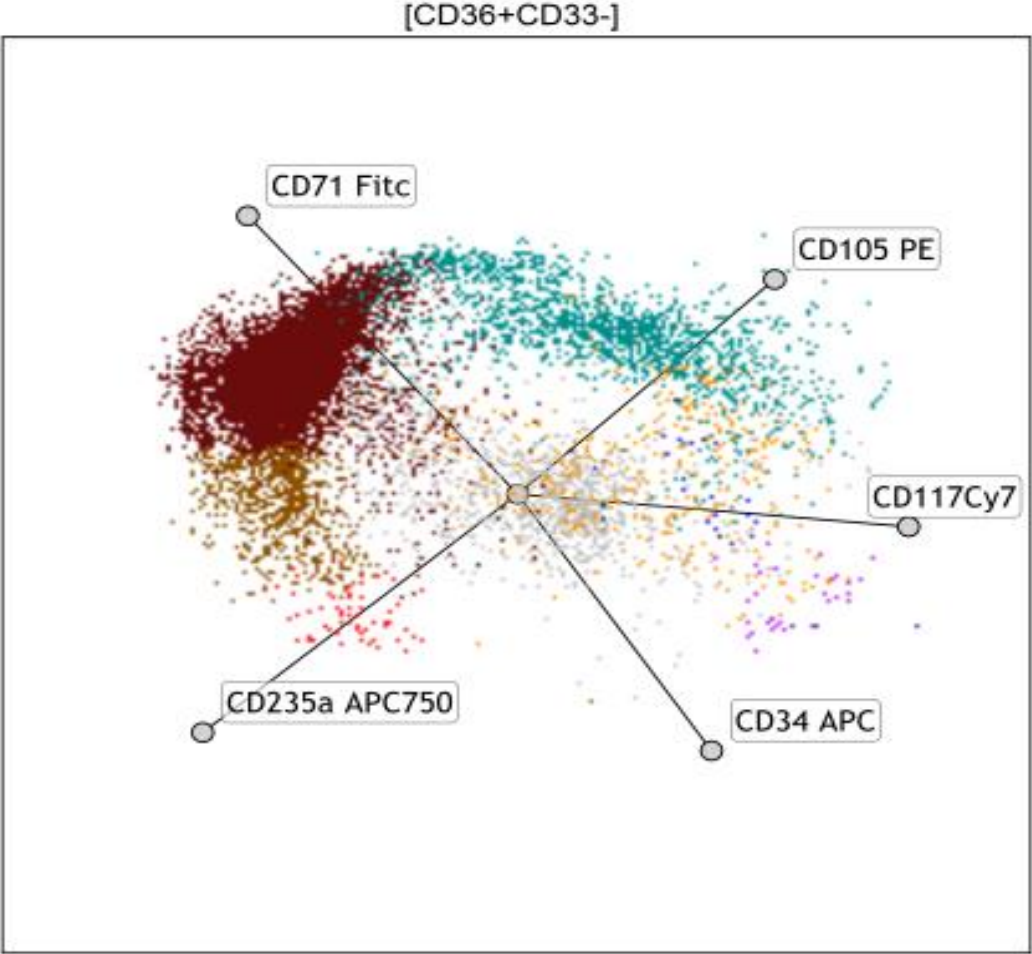
[Ungated] SS INT / CD45ECD



[Totale hematopoiese]  
CD71APC750 / CD117CY7

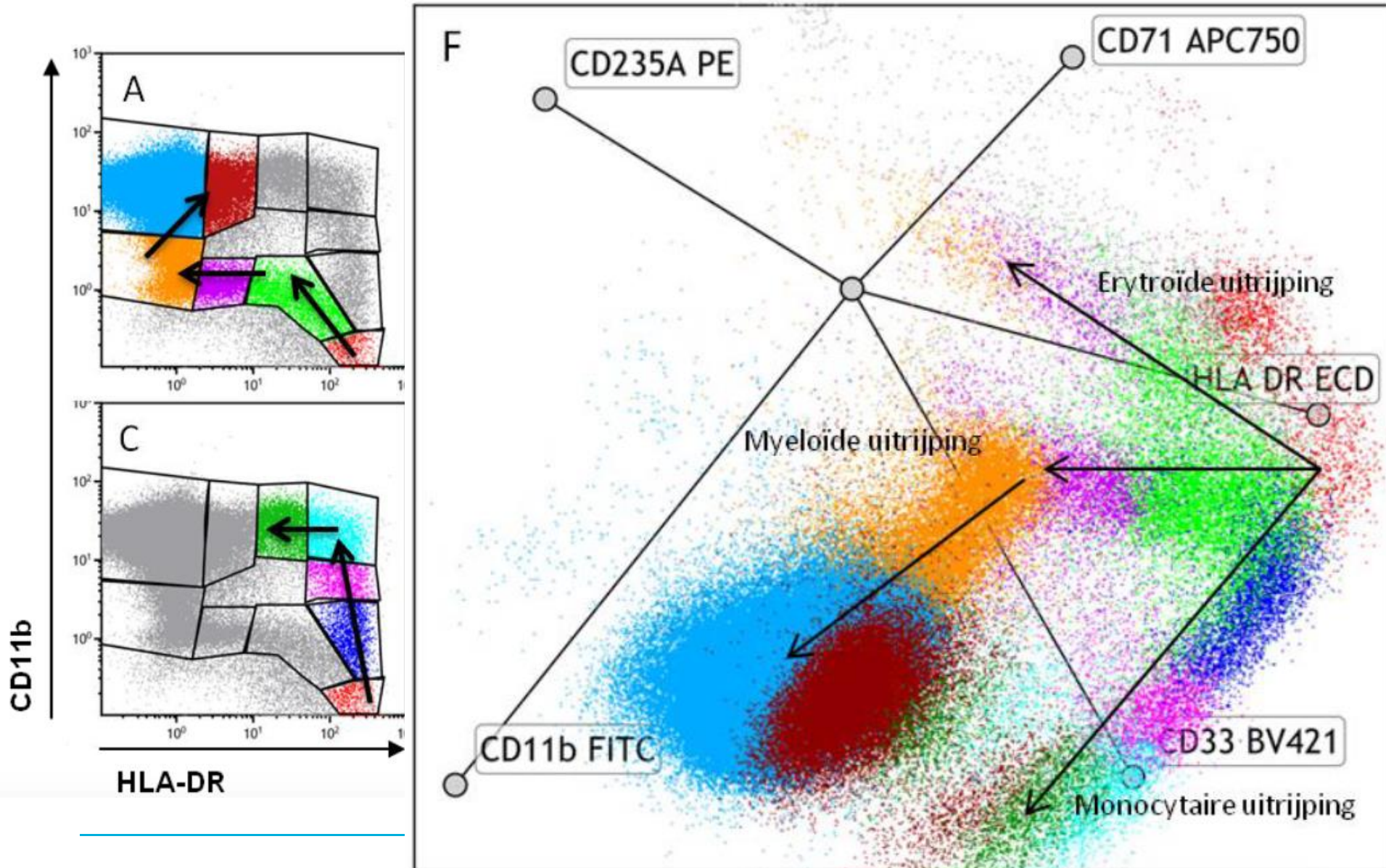


# Erythropoiese (CD36+CD33- en CD105/CD117)

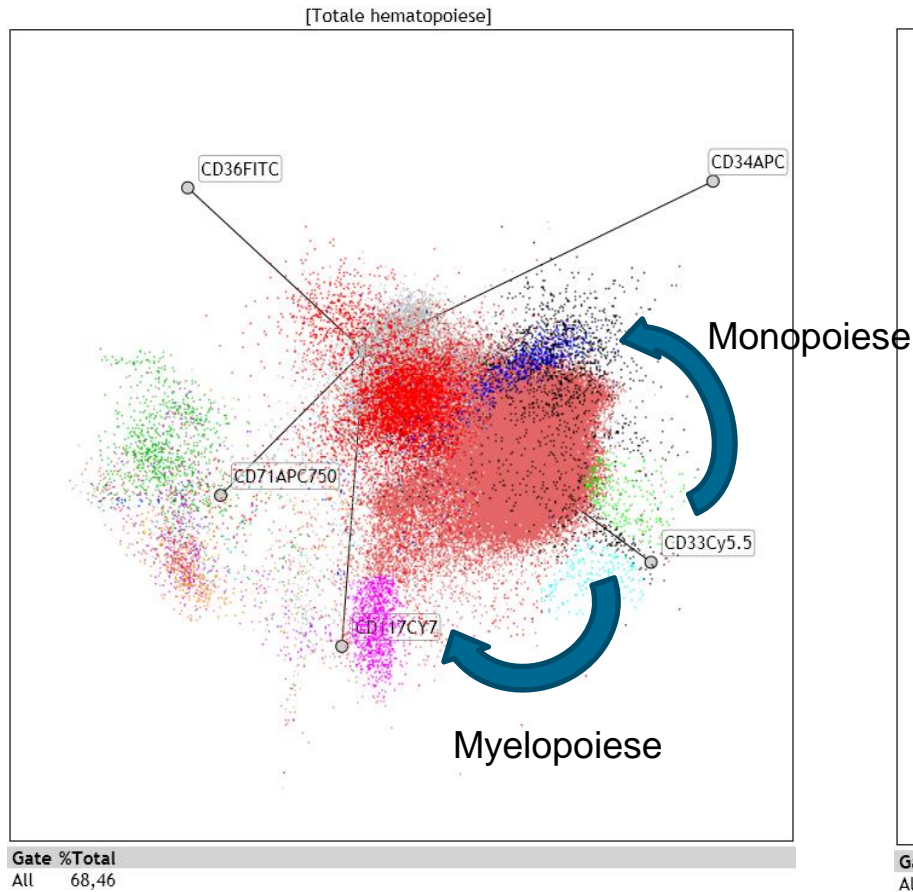




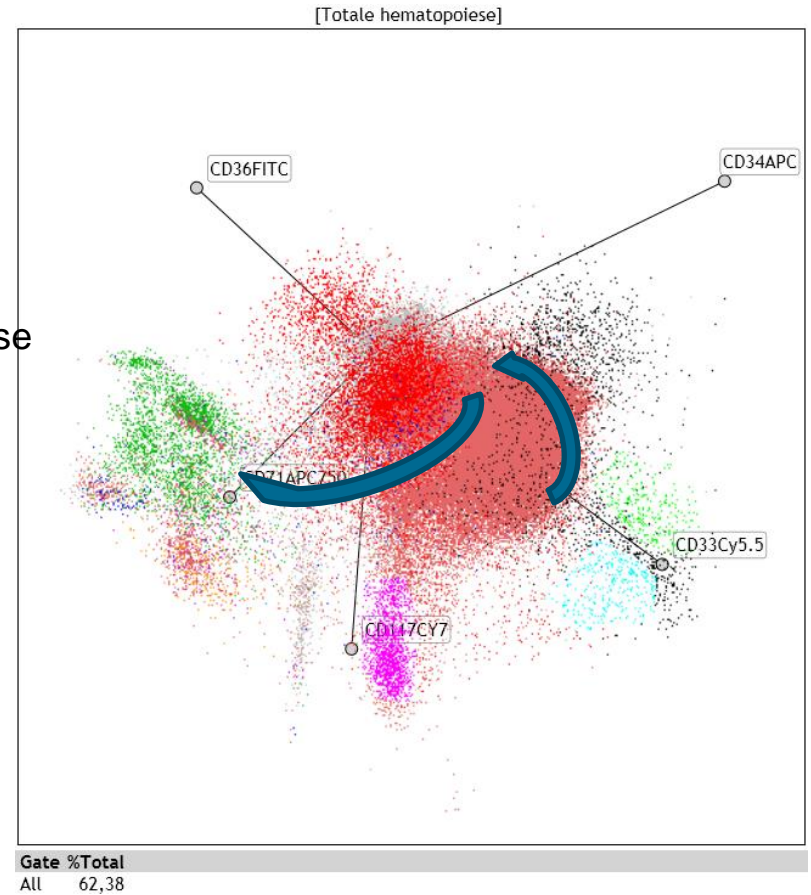
# Hematopoïese (Myeloid-Monocytair-Erythroid)



# Normale hematopoiese

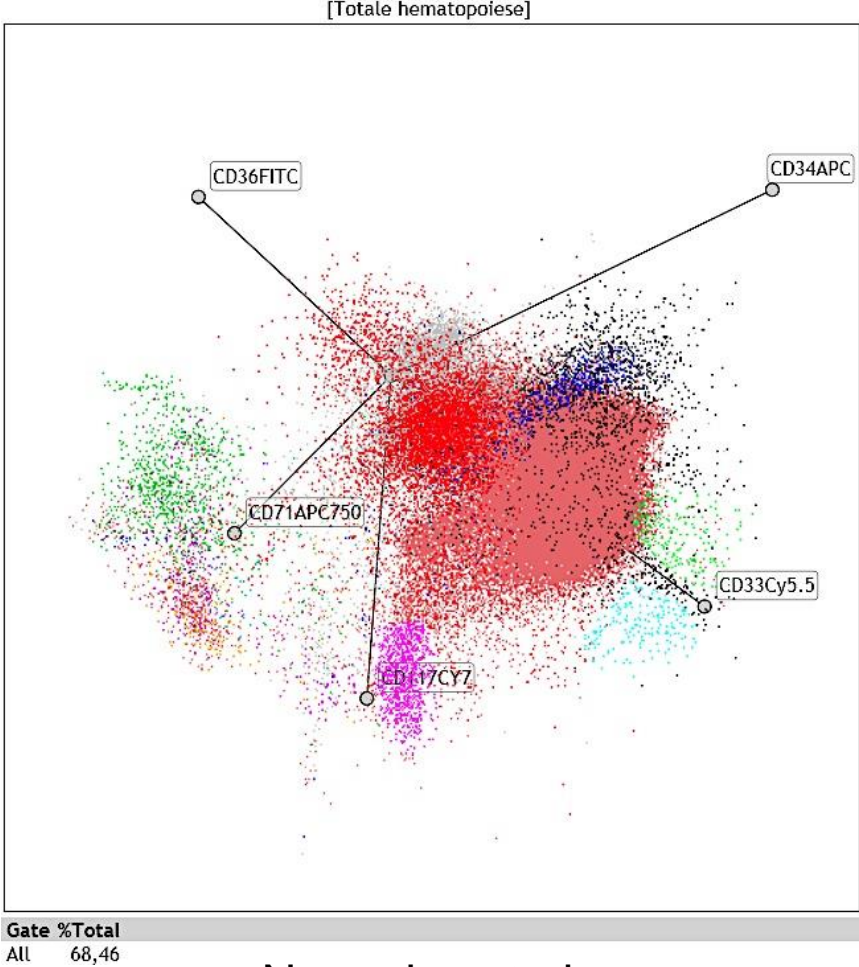
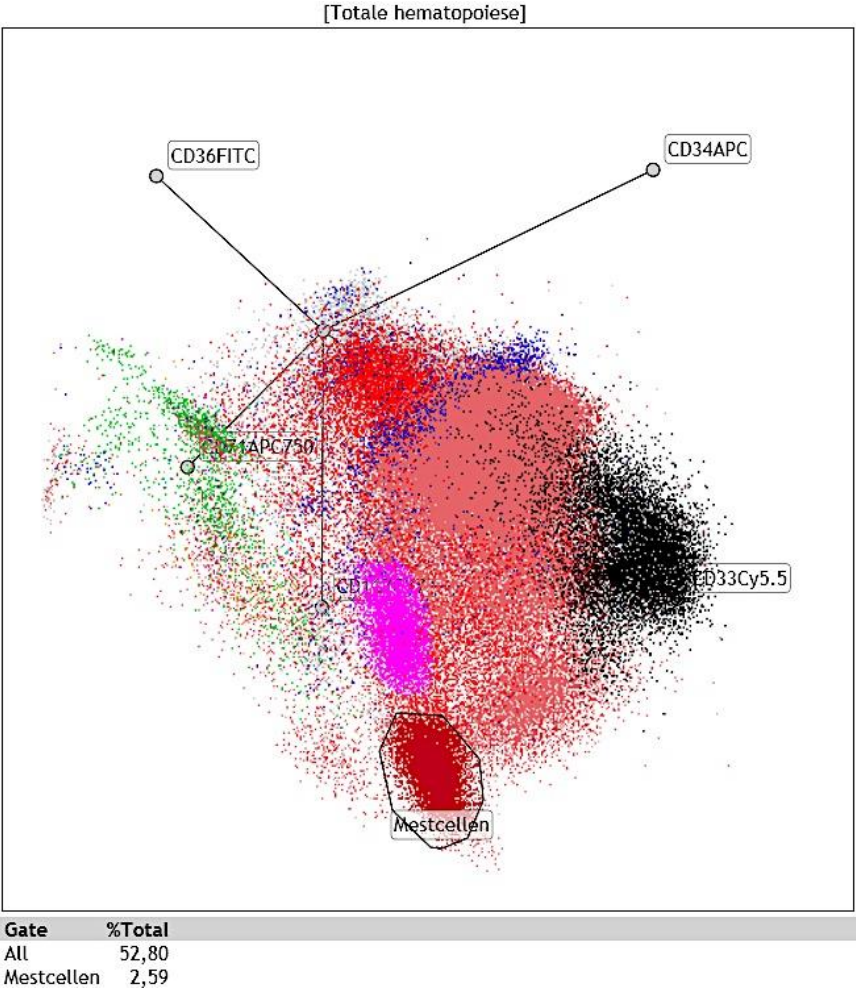


Myeloid and monocytic  
hematopoiesis



Erythroid  
hematopoiesis

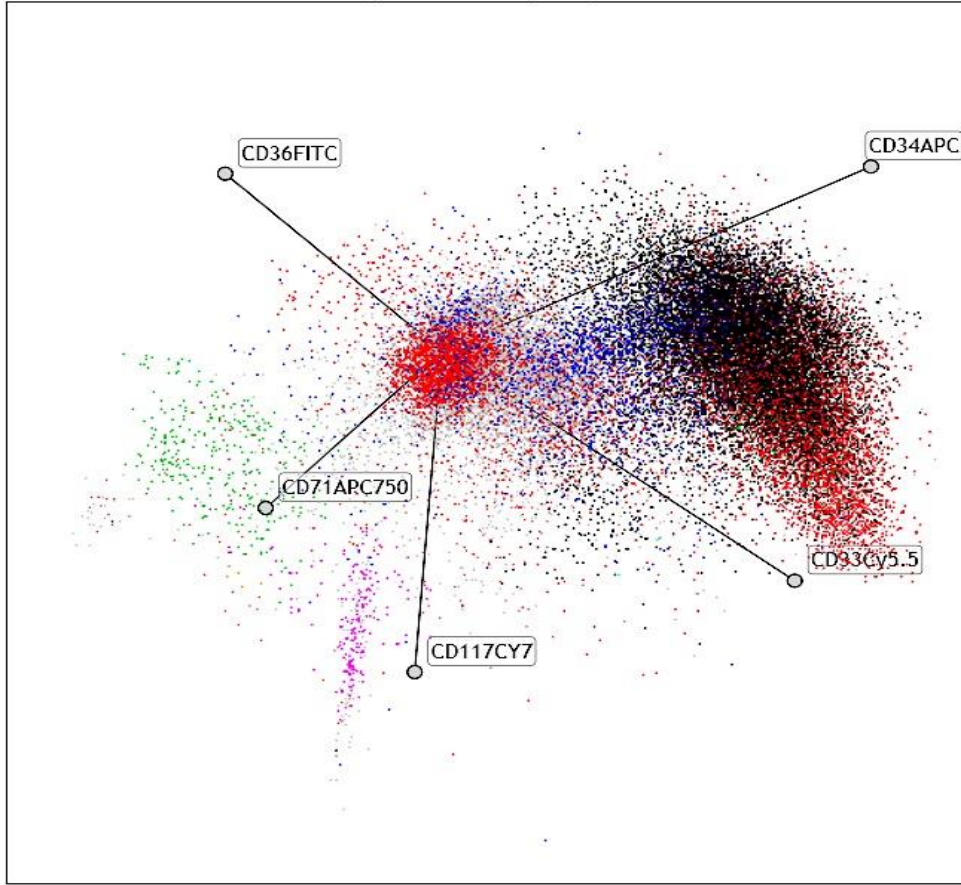
# CMML and mastocytose



Normal control

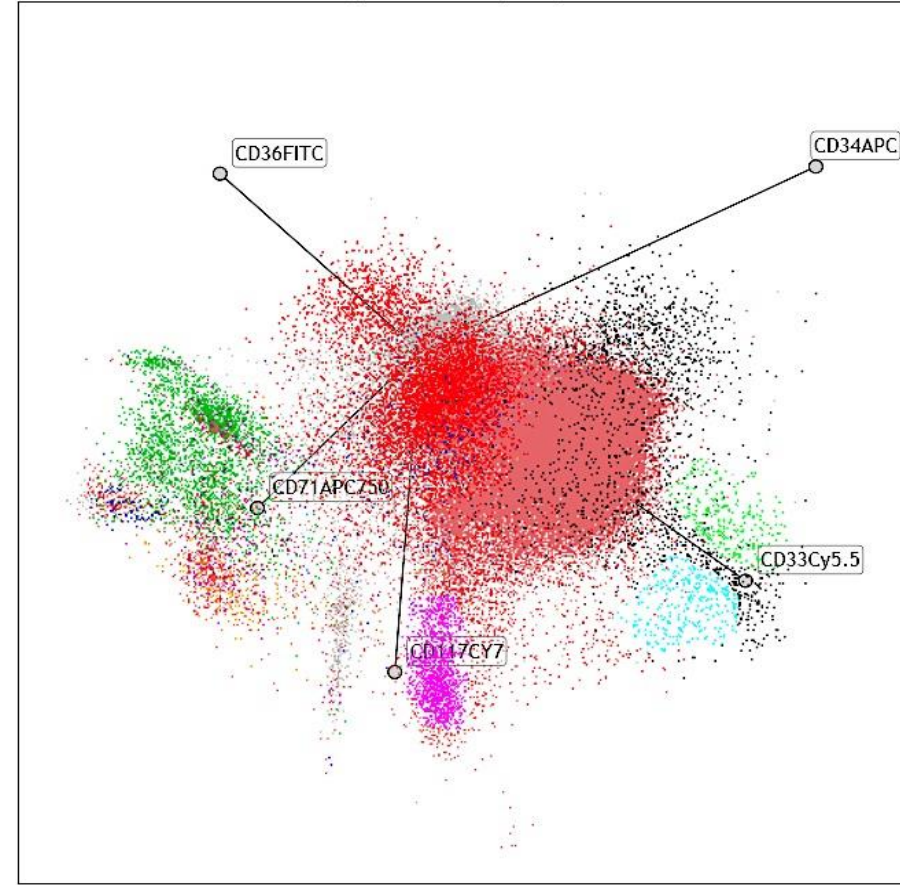
# AML zonder differentiatie en monocytair eigenschappen

[Totale hematopoïese]



Gate %Total  
All 92,83

[Totale hematopoïese]



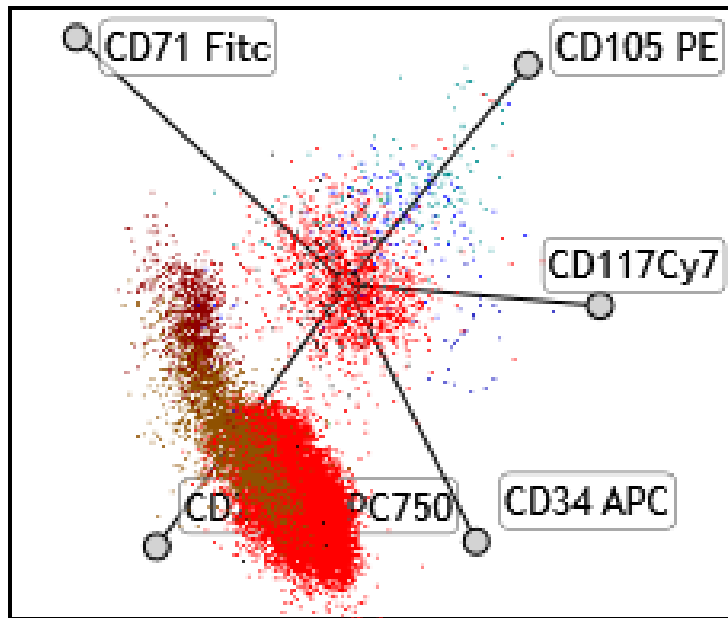
Gate %Total  
All 62,38

**Normal control**

# MDS met dyserythropoïese (toename van CD71dim/CD71CV)

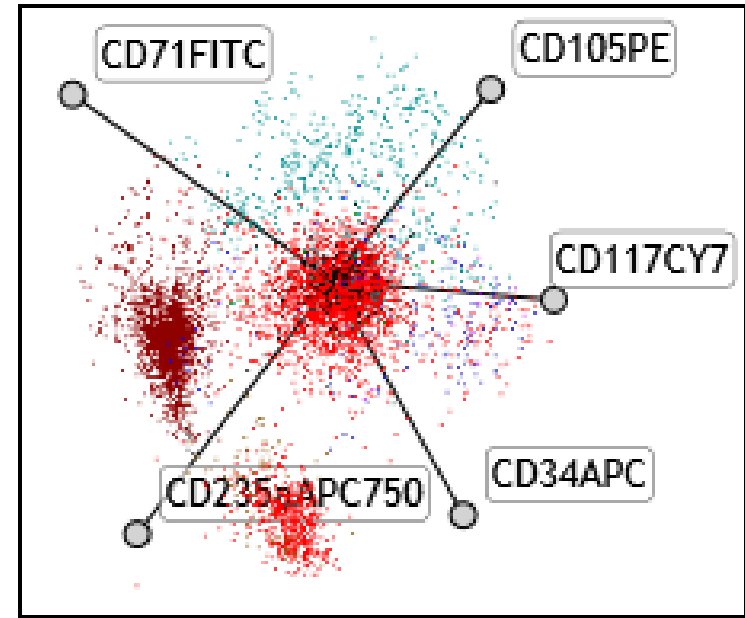
MDS

[Erythropoïese]



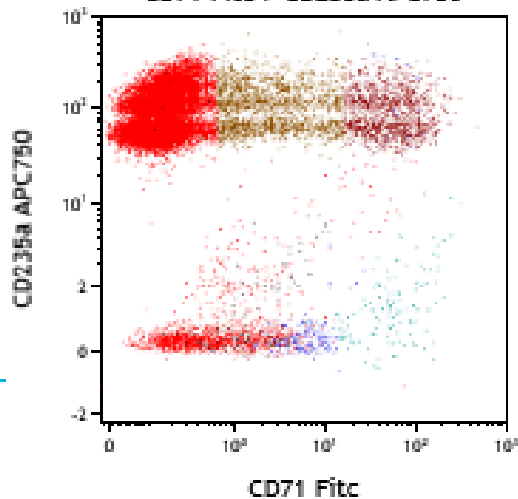
Normale controle

[Erythropoïese]



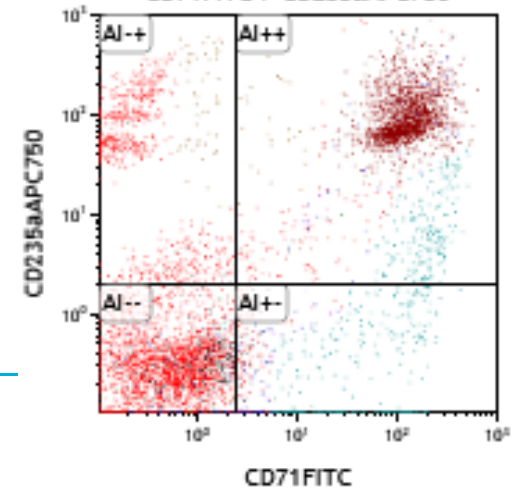
[Erythropoïese]

CD71 Fitc / CD235a APC750



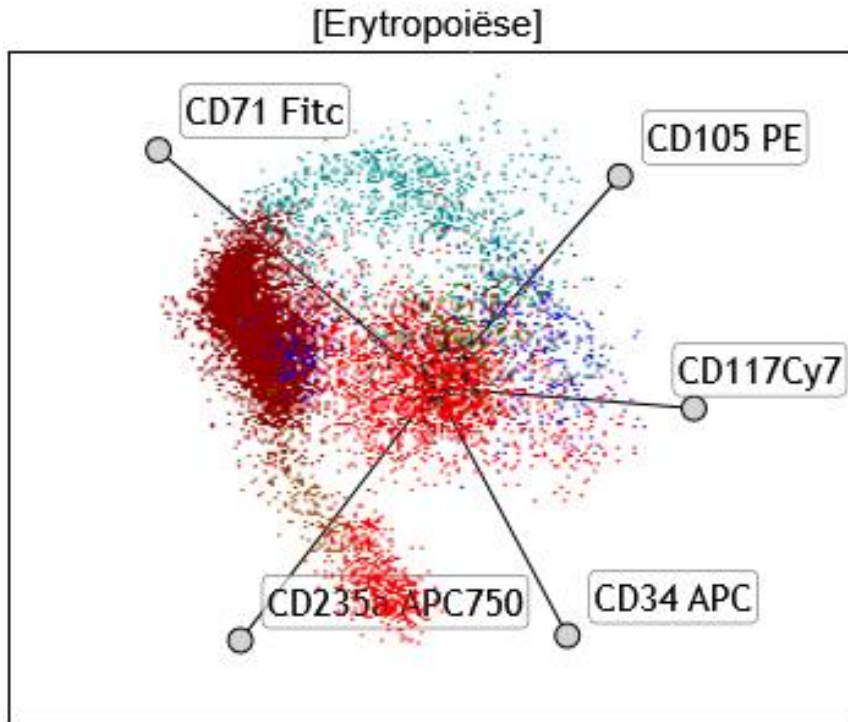
[Erythropoïese]

CD71FITC / CD235aAPC750

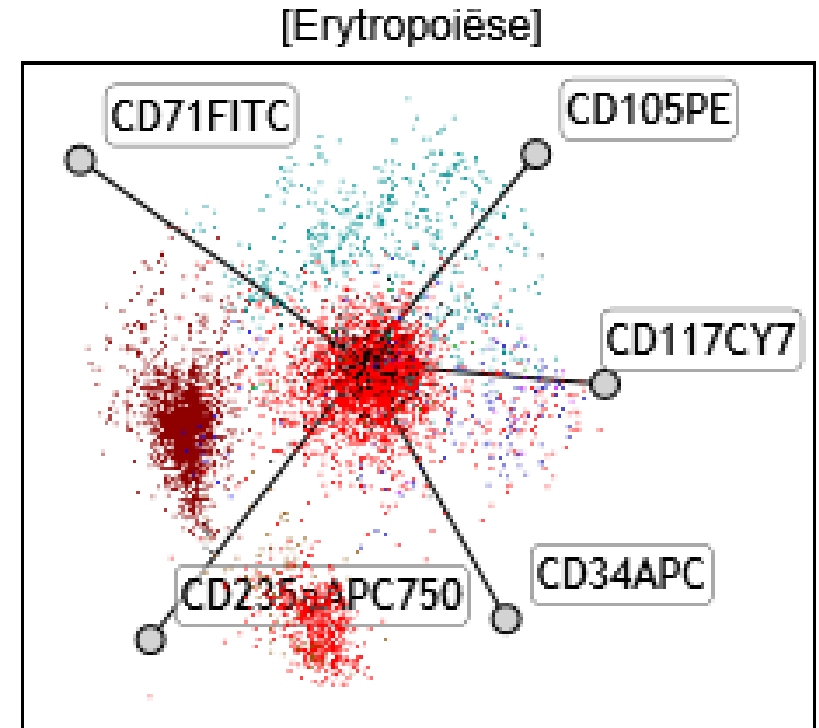


# MDS met dyserythropoïese (toename CD71 CV)

## MDS



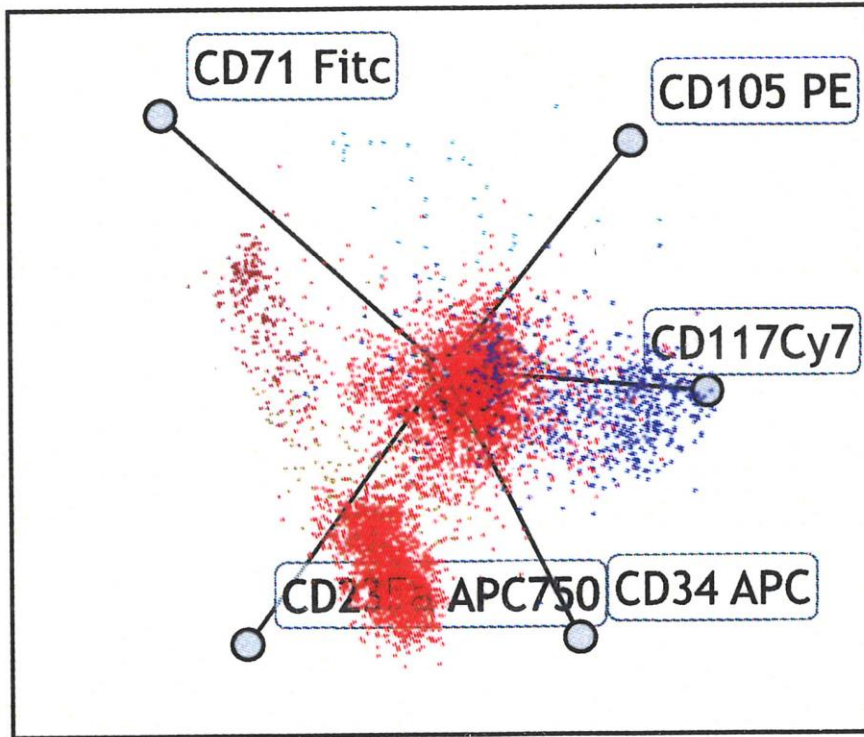
## Normale controle



# MDS met dyserythropoïese (afname onrijp)

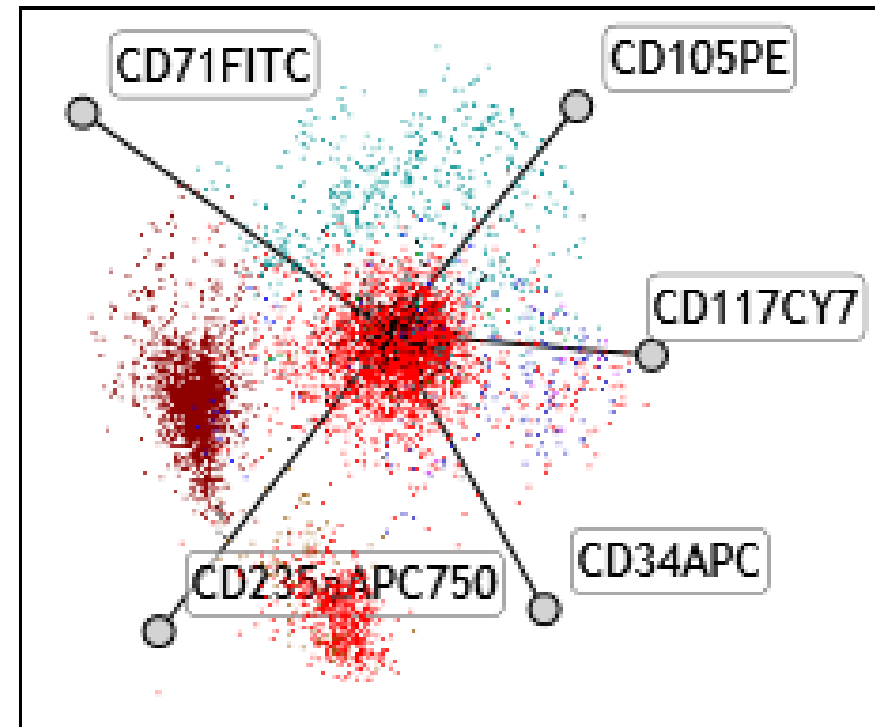
**MDS**

[Erythropoïese]



**Normal control**

[Erythropoïese]



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

**Thanks for your attention**

