



# Wie is de expert?

Immuunfenotypering van hematologische maligniteiten

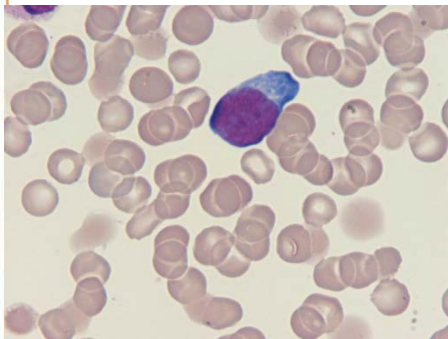
**Christa Homburg**

**Sanquin Diagnostiek, hoofd laboratorium Immunocytologie**

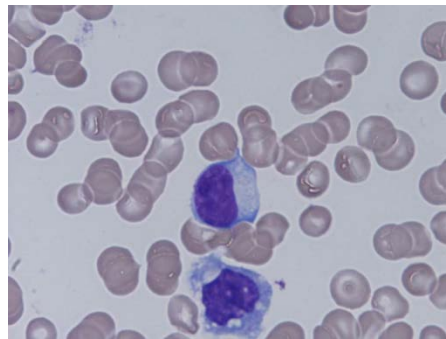
**Namens nomenclatuurcommissie, Leu/Ly typering, sectie IMCD**

Vaststellen hematologische maligniteit = vaststellen welk celtype woekert

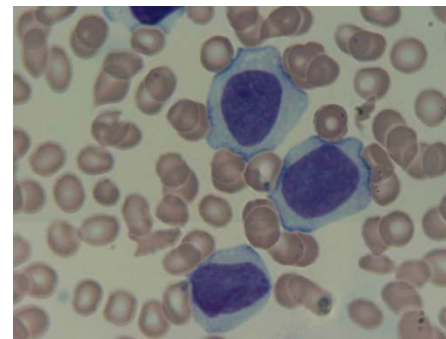
<1980 benoeming bloedcellen op basis van morfologie



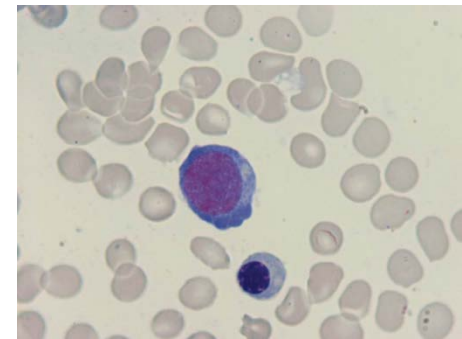
myeloblast



lymfoblast



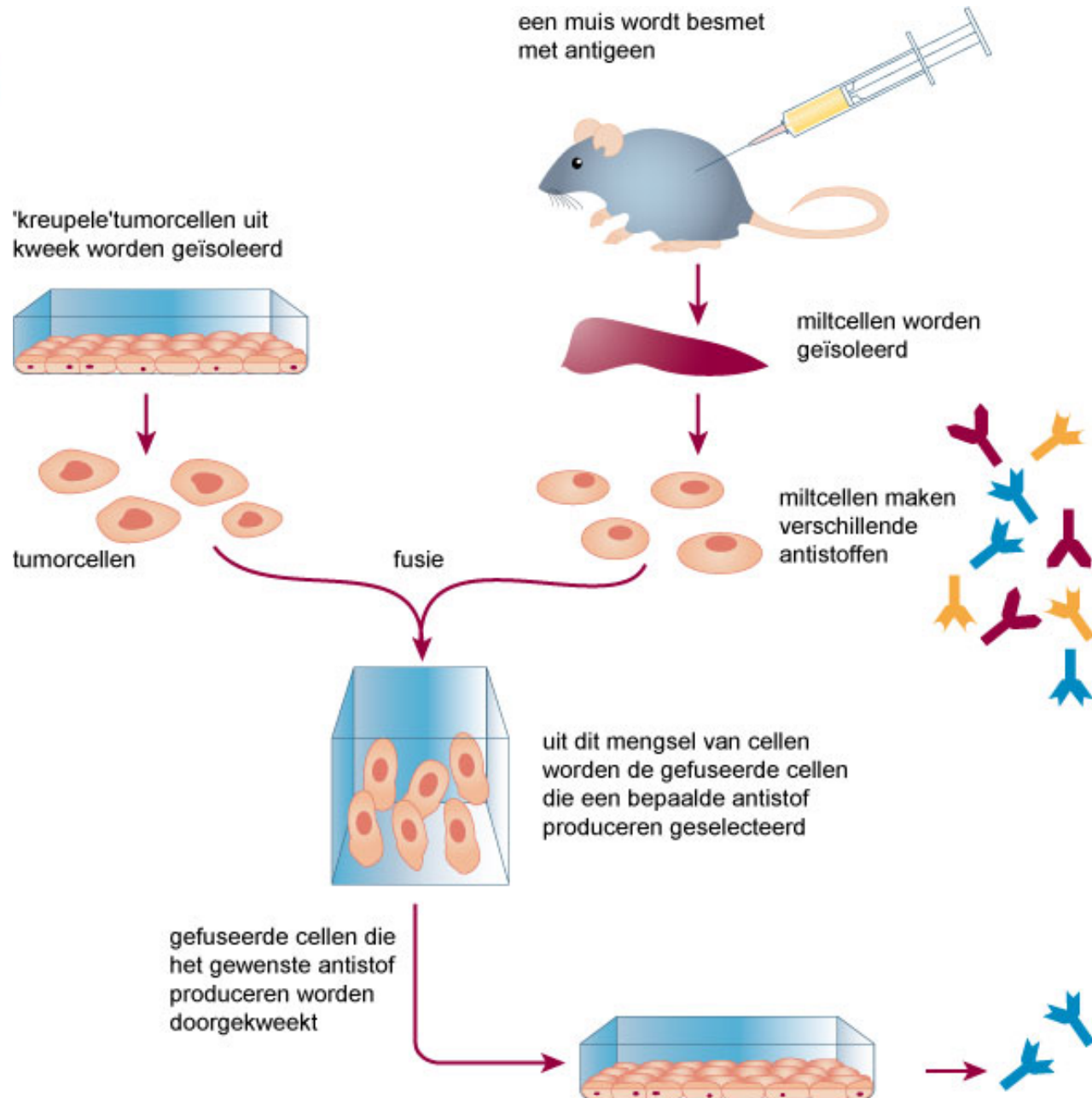
monoblast



erythroblast

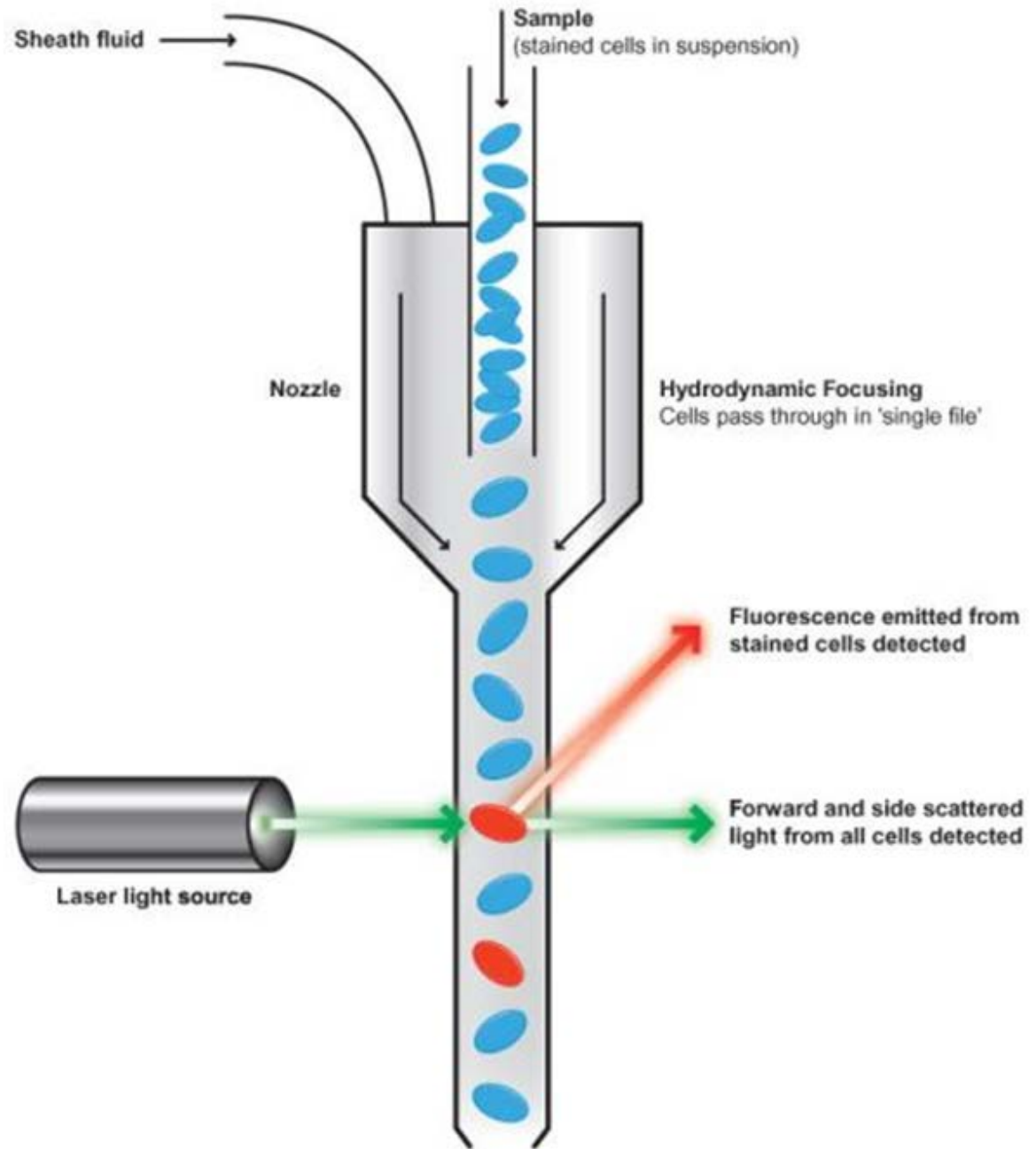
1975: Köhler en Milstein: beschrijving monoklonale antistoffen

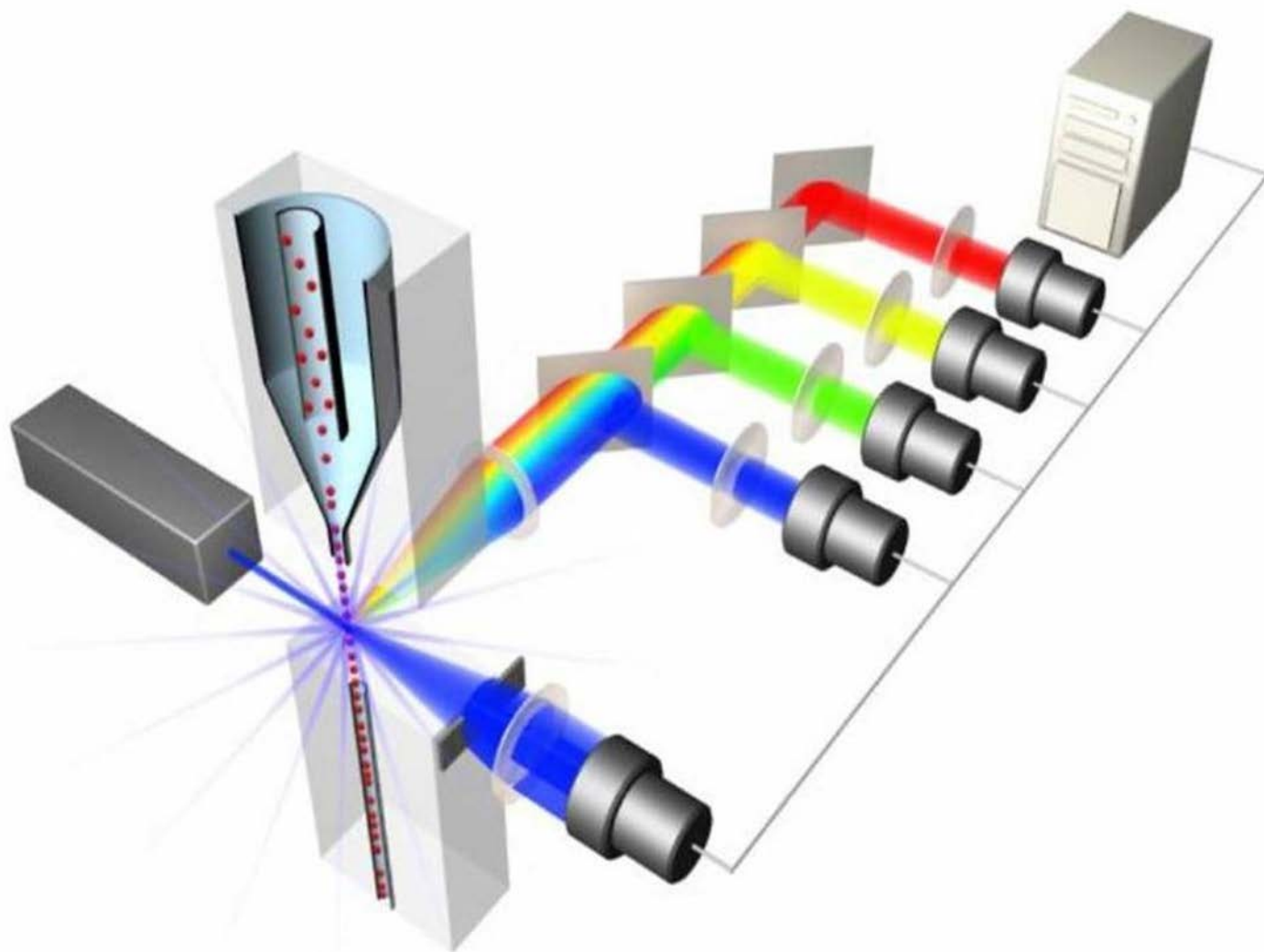
1984: nobelprijs



- indirecte aankleuring m.b.v. geit-anti-muis
- conjugaat gelabeld met fluorescent label
- beoordeling onder fluorescentie-microscop
- ontwikkeling direct gelabelde antistoffen met verschillende fluorescente labels
- ontwikkeling flowcytometers:
  - objectieve beoordeling fluorescentie
  - grotere aantallen cellen te beoordelen





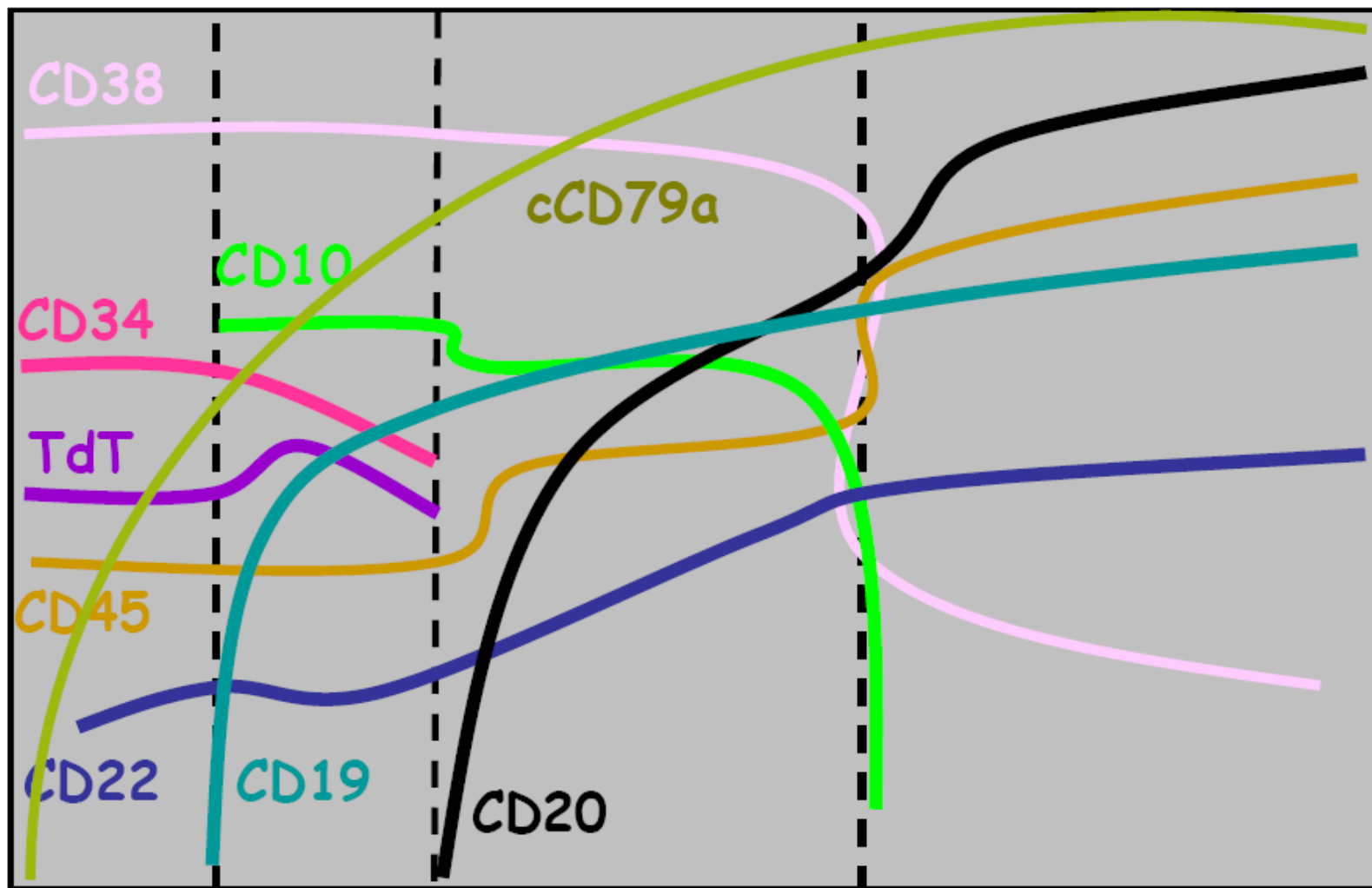


momenteel machines met 4 lasers  
en 15 parameters/13 kleuren

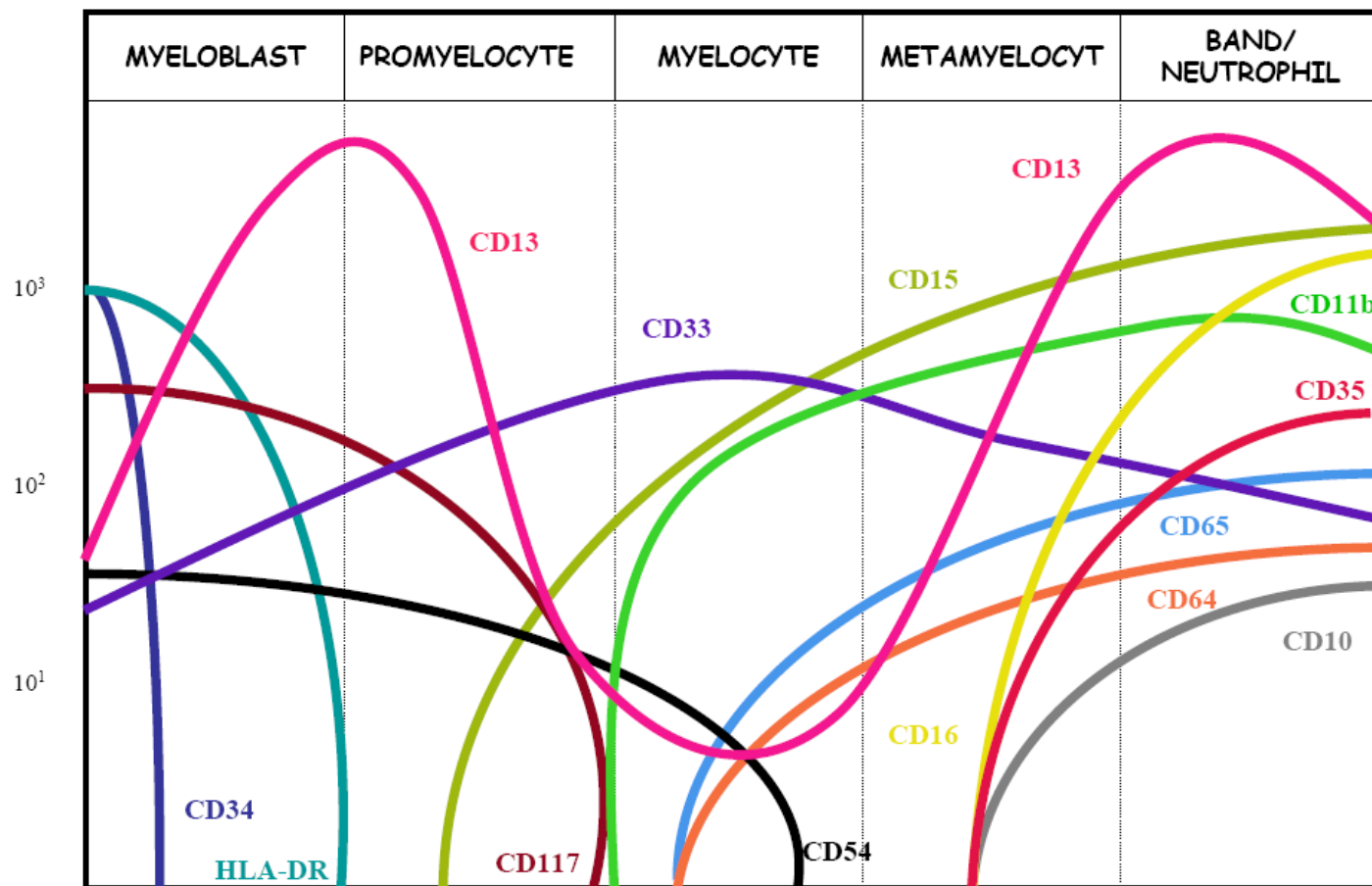
- jaren 80 gebruik monoklonale antistoffen bij typering leukemie/lymfoom ingevoerd
- Vaststellen uitrijping- en expressie patronen



## B-cel-ontwikkeling

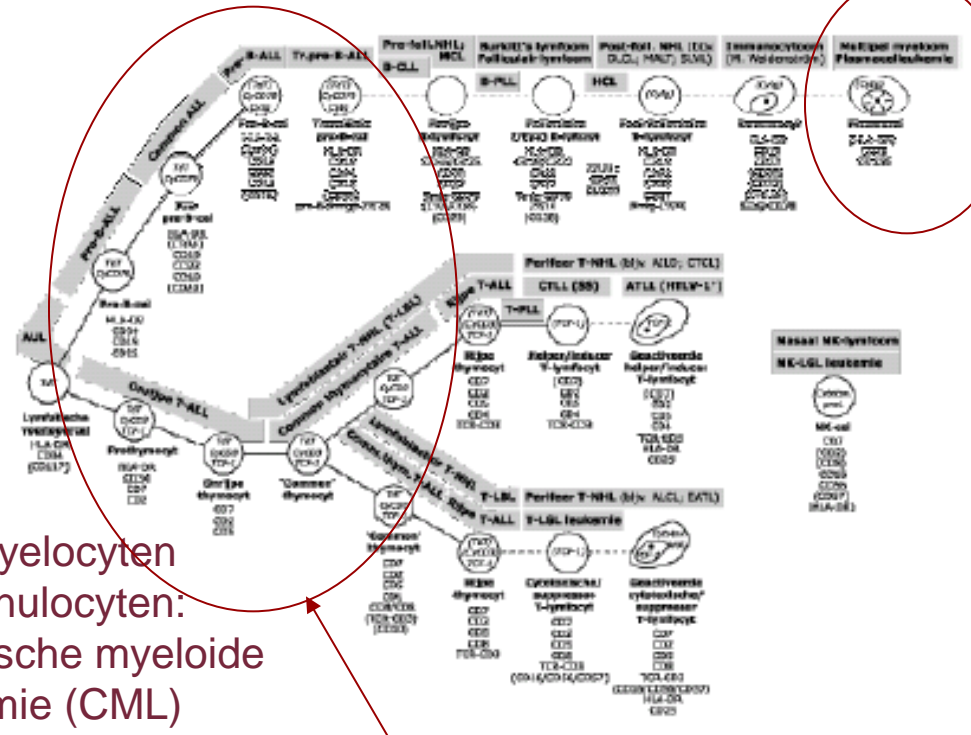
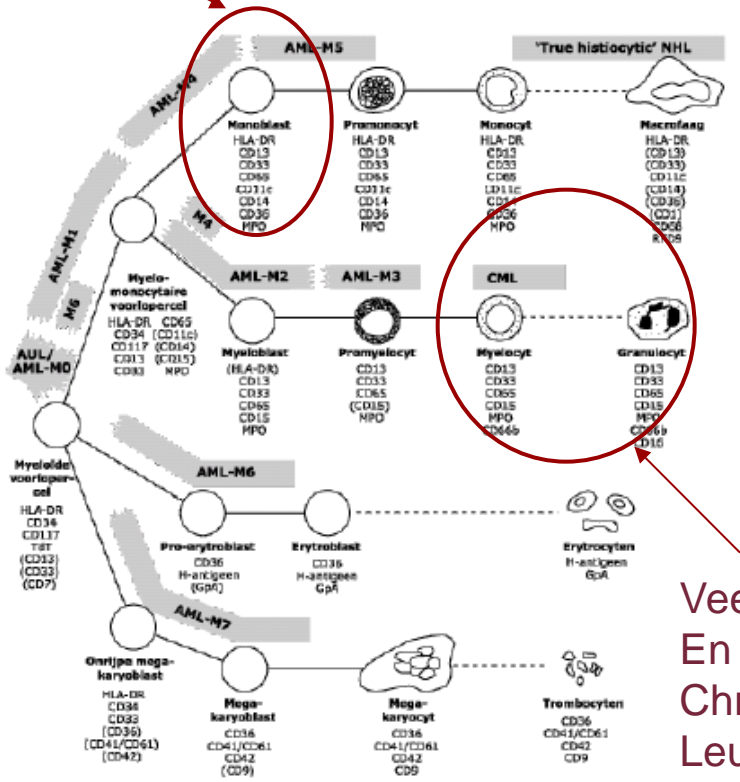


# Granulocyten ontwikkeling



Veel monoblasten:  
Acute monocytair leukemie (M4 of M5)

Veel plasmacellen:  
Multiple Myeloom



Schema myeloïde antigeen markers zoals opgesteld door laboratorium immunologie van het Erasmus MC

Veel myelocyten  
En granulocyten:  
Chronische myeloïde  
Leukemie (CML)

Veel lymfoblasten:  
Acute lymfatische leukemie

- 1985 oprichting SIHON  
Samenwerkingsverband  
Immunofenotypering  
Hematologische  
Oncologie  
Nederland
- doel: het standaardiseren en verbeteren van de immunofenotypering van hematologische maligniteiten (acute en chronische leukemieën en lymfomen).



## Middelen:

- Rondzendingen  
discussie over resultaten tijdens nabespreking
- Presentaties/gast sprekers tijdens nabespreking
- Scholing (Almelo conference/Zwolle  
symposium icm NVC/MDS cursus door WG)

- eerste formele rondzendingen

## beoordeling door nomenclatuurcommissie:

6 leden : aantoonbare deskundigheid op gebied IF

- 2 vanuit academische centra
- 1 vanuit algemeen Nederlands ziekenhuis
- 1 vanuit algemeen Belgisch ziekenhuis
- 1 medicus

- 1996 SIHON-score

pre-analytisch

analytisch

post-analytisch



- 2006 SIHON omgevormd tot wetenschappelijke vereniging: Nederlandse Vereniging voor Cytometrie



- rondzendingen naar SKML sectie ICD

## Standaardisatie

1990: richtlijnen classificatie acute leukemieën

1995: richtlijnen classificatie chronische leukemieën en lymfomen

1998: herziening richtlijnen



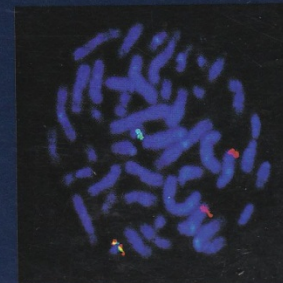
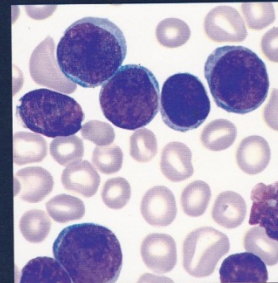
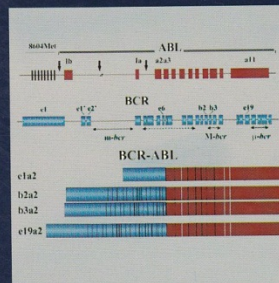
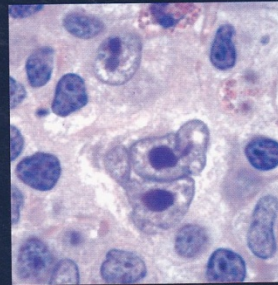
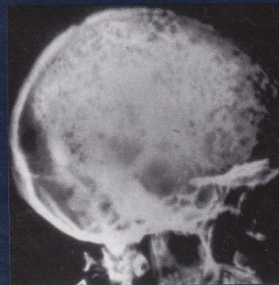


## Pathology & Genetics

# Tumours of Haematopoietic and Lymphoid Tissues

Edited by Elaine S. Jaffe, Nancy Lee Harris, Harald Stein, James W. Vardiman

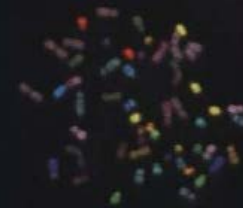
2001



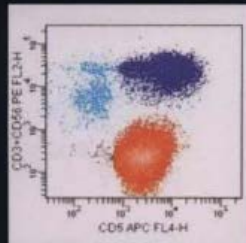
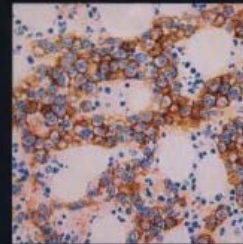
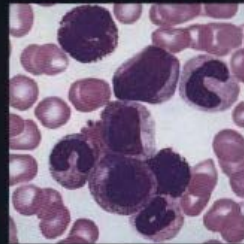
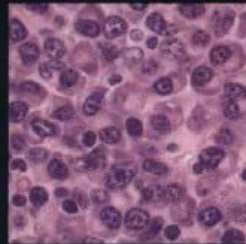
- Richtlijnen voor flowcytometrische immunofenotypering van onrijpe en rijpe maligniteiten: protocollen voor drie-voudige labelingen en richtlijnen voor interpretatie
- Monoklonale antilichaamcombinaties voor drie-kleuren labelingen ten behoeve van flowcytometrische immuunfenotypering van onrijpe- en rijpe hematologische maligniteiten
  - 1<sup>e</sup> screening
    - CD34<sup>FITC</sup>CD117<sup>PE</sup>CD45<sup>PeRCP of PE-Cy5</sup>
    - CD36<sup>FITC</sup>CD13.33<sup>PE</sup>CD14<sup>PeRCP of PE-Cy5</sup>
    - CD5<sup>FITC</sup>CD7<sup>PE</sup>CD3<sup>PeRCP of PE-Cy5</sup>
    - CD4<sup>FITC</sup>CD8<sup>PE</sup>CD3<sup>PeRCP of PE-Cy5</sup>
    - CD3<sup>FITC</sup>CD16.56<sup>PE</sup>CD38<sup>PeRCP of PE-Cy5</sup>
    - Kappa<sup>FITC</sup>Lambda<sup>PE</sup>CD19<sup>PeRCP of PE-Cy5</sup>
    - CD10<sup>FITC</sup>CD20<sup>PE</sup>CD19<sup>PeRCP of PE-Cy5</sup>
  - 2<sup>e</sup> lijnspecifieke typering
  - 3<sup>e</sup> onderzoeksvragen
- Atlas met praktische voorbeelden

# WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

Edited by Steven H. Swerdlow, Elias Campo, Nancy Lee Harris, Elaine S. Jaffe,  
Stefano A. Pileri, Harald Stein, Jürgen Thiele, James W. Vardiman



**2008**



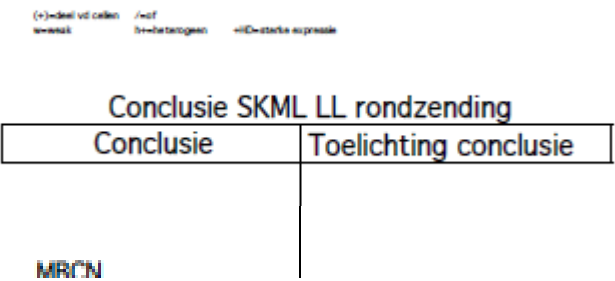


**Acute Myeloid Leukemia and Related Precursor Neoplasms**

AML with balanced translocations/inversions  
 AML with t(8;21)(q22;q22); RUNX1-RUNX1T1 (AML1-ETO)  
 AML with inv(16)(p13;q22) or t(16;16)(p13;q22); CBFB-MYH11  
 AML with t(15;17)(q22;q12); PML-RARA  
 AML with t(9;11)(p22;q23); MLLT3-MLL (in adults)  
 AML with t(9;11)(p22;q23); MLLT3-MLL (in children)  
 AML with t(6;9)(p23;q34); DEK-NUP214 (DEK-CAN)  
 AML with inv(3)(q21;q26.2) or t(3;3)(q1;q26.2); RRM1-CV11  
 AML (megakaryoblastic) with t(1;22)(p13;q13); RMI1-SMR1  
 AML with gene mutations  
 AML with mutated NRX1  
 AML with mutated CEBPA  
 AML with myelodysplasia-related changes (therapy related AML)  
 AML, not otherwise specified

**Conclude SKML LL rondzending**  
 Conclusie Toelichting conclusie

AML	Conclusie	Toelichting conclusie
AML t(8;21)	TdT+ CD13+ CD15+ CD19+ CD33+ CD34+HD CD56	several blast populations: CD3+ CD4+ CD11b+ CD11c
AML inv(16)	CD11b+w CD11b+w CD13w+ CD15+w CD18+w CD3	CD4+HD CD13+ CD14+ CD23+HD CD34+ CD56+HD
AML t(15;17)	CD4+ CD11b+ CD11c+ CD24+ CD36+ CD56+	TdT+ CD13+ CD15+ CD33+ CD34+ CD38+ CD64+ Cl
AML t(9;11) adult	CD7+ CD13+ CD33+ CD34+ CD38+ CD41+ CD11+ Cl	TdT- CD7- CD13+ CD33+ CD34+ CD36+ CD41+ CD4
AML t(9;11) child	CD11b+ CD13+ CD14+ CD33+ CD34+ CD36+ MPO+	CD7+ CD11b+ CD13+ CD14+ CD15+ CD33+ CD34+ C
AML t(6;9)		
AML inv(3)		
AML t(1;22)		
AML MPM1		
AML NCEBPA		



**Mature B-cell neoplasms**

CLL/SLL	CD5+ CD10- CD11cw CD19+ CD20+ CD22+ CD23+ CD43+ cCD79a+ CD79b-/w FMC7-/w IgDw IgMw
MBL CD5+	PB <5x10 <sup>9</sup> /L monoclonal B-cells with CLL phenotype and healthy (no cytopenie, lymphadenopathy or extramedullary tissue involvement)
MBL CD5-	PB <5x10 <sup>9</sup> /L monoclonal B-cells with non-CLL phenotype and healthy (no cytopenie, lymphadenopathy or extramedullary tissue involvement)
B-PLL	CD5+ CD19+ CD20+ CD22+ CD23+ CD38+ cCD79a+ CD79b+ FMC7+ IgD+HD IgM+HD/-
SMZL	CD5- CD10- CD20+ CD23- CD43- cCD79a+ CD103- IgD+ IgM+
HCL	CD5- CD10- CD11c+HD CD20+HD CD22+HD CD23- CD25+ CD43- CD103+ CD123+ FMC7+ monotypic slg+HD
HCLv	CD11c+ CD19+ CD20+ CD22+ CD25- CD72+ cCD79a+ CD79b+ CD103+ CD123- FMC7+ slgG+HD
LPL	abnormal B-lymphoid cells: CD5- CD10- CD19+ CD20+ CD22+ CD23- CD25+ CD38+ CD43- cCD79a+ CD103- IgD- IgM+/IgG+/IgA+ abnormal plasmacytic cells: CD138+ clgM+/clgG+/clgA+
MGUS	normal plasma cells: CD19+ CD38+HD CD56- polyclonal aberrant plasma cells: CD19- CD38+wHD CD56+ monoclonal clgG+/clgM+/IgD+/clgA+
PCM	CD10+ CD19- CD20+ CD38+HD CD52+ CD56+ CD79a+ CD117+ CD138+ slg- monotypic clgG+/clgM+/IgD+/clgA+
PCL	PB >2x10 <sup>9</sup> /L or >20% monoclonal plasma cells: CD56-

**Plasma cell**

**PCL**

**Human B-cell neoplasia**

Chronic B

Chronic B  
 B-cell lymphocytoid leukemia CD5-  
 B-cell plasmacytoid leukemia  
 Splenic marginal zone lymphoma  
 Hairy cell leukemia  
 Splenic B-cell lymphoma/leukemia, unclassifiable  
 Splenic diffuse red pulp small B-cell lymphoma  
 Hairy cell leukemia variant  
 Lymphoplasmacytic lymphoma  
 Plasma cell neoplasms  
 Monoclonal gammopathy of undetermined significance  
 Plasma cell myeloma  
 Plasma cell leukemia

MBL CD5-  
 B-PLL  
 SMZL  
 HCL  
 HCLv  
 LPL  
 MGUS  
 PCM  
 PCL

PB <5x10<sup>9</sup>/L monoclonal B-cells with non-CLL phenotype and healthy (no cytopenie, lymphadenopathy or extramedullary tissue involvement)  
 CD5+ CD19+ CD20+ CD22+ CD23+ CD38+ cCD79a+ CD79b+ FMC7+ IgD+HD IgM+HD/-  
 CD5- CD10- CD20+ CD23- CD43- cCD79a+ CD103- IgD+ IgM+  
 CD5- CD10- CD11c+HD CD20+HD CD22+HD CD23- CD25+ CD43- CD103+ CD123+ FMC7+ monotypic slg+HD  
 CD11c+ CD19+ CD20+ CD22+ CD25- CD72+ cCD79a+ CD79b+ CD103+ CD123- FMC7+ slgG+HD  
 abnormal B-lymphoid cells: CD5- CD10- CD19+ CD20+ CD22+ CD23- CD25+ CD38+ CD43- cCD79a+ CD103- IgD- IgM+/IgG+/IgA+  
 abnormal plasmacytic cells: CD138+ clgM+/clgG+/clgA+  
 normal plasma cells: CD19+ CD38+HD CD56- polyclonal  
 aberrant plasma cells: CD19- CD38+wHD CD56+ monoclonal clgG+/clgM+/IgD+/clgA+  
 CD10+ CD19- CD20+ CD38+HD CD52+ CD56+ CD79a+ CD117+ CD138+ slg- monotypic clgG+/clgM+/IgD+/clgA+  
 PB >2x10<sup>9</sup>/L or >20% monoclonal plasma cells: CD56-

Immunologische  
markers die minimaal  
vereist zijn  
bij de SKML-  
Leukemie/Lymfoom  
rondzendingen

rijp B-cel	
CD45	(lymfocyten)
CD5	(T-cel)
CD10	
CD19	(B-cel)
CD20	(B-cel)
CD23	
CD25	
CD38	
CD103	
Smlgkappa	(B-cel)
Smlglambda	(B-cel)
<i>additioneel:</i>	
CD11c	
FMC7	(B-cel)
CD22	(B-cel)
CD79b	
CD43	

# Rapportage rondzendingsresultaten

## diverse merkers

immunol. diff.

PRE	ANA	POST	Concluse	T-lymfocyten	%	B-lymfocyten	%	NK-lymfocyten	%	Myeloid	%	Monocytair	%	Afwijkende populatie	%	CD 1	CD 2	CD 3	CD 4	CD 5	CD 7	CD 8	CD 10	CD 11b	CD 11c	CD 13	Expr.	CD 14	CD 15	CD 16	CD 19	
B	B	A	AML	CD3/SSC	4	CD19/SSC	<1	CD3-CD16.56	3	CD13/CD33/SSC	82	CD14/SSC	3	CD45/SSC	4				-	+			-		+	Zwak	-	-	-	-		
B	A	A	AML	CD3/SSC	4	CD19/SSC	<1	CD3-CD16.56/SSC	3	CD13/SSC	83	CD13/CD45/SSC	4	CD34/HLA-DR/CD	5				-	+			-		+	Zwak	-	-	-	-		
C	B	A		CD3/SSC	4	CD19/SSC	<1	CD3-CD16.56/SSC	3	CD15/SSC	89	CD14/SSC	3	CD34/SSC	2																	
A	B	A		CD3/SSC	4	CD19/SSC	<1	CD3-CD16.56/SSC	3	CD13/SSC	87,8	CD14	2,6	CD34/SSC	3,8																	
C	B	A		CD3/SSC	4	CD19/SSC	<1	CD3-CD16.56/SSC	3	CD13/SSC	80	CD14/SSC	3	CD34/SSC	4																	
A	C	A	AML	CD3/SSC	5	CD19/SSC	1	CD3-CD16.56	3	CD15/SSC	79	CD14/SSC	5	CD34/SSC	6												Zwak	-	-	-	-	
B	A	A		CD3/SSC	3	CD19/SSC	1	CD3-CD16.56	2	CD13/SSC	82	CD14/SSC	2	CD45/SSC	6												Zwak	-	-	-	-	
B	B	B	AUL	CD3/SSC	5	CD19/SSC	1	CD3-CD16.56	3	CD13/SSC	82	CD14/SSC	4	CD45/SSC	5												Zwak	-	-	+	-	
B	A	A	AML	CD3/SSC	4	CD19/SSC	1	CD3-CD16.56/SSC	2	CD45/CD15	80	CD14/SSC	3	CD45(zwak)/CD7	4								NV			Norme	-	-	-	-		
A	A	A	AML	CD3/SSC	6	CD19/SSC	1	CD3-CD16.56/SSC	3	CD13/SSC	75	CD14/SSC	3	CD34/SSC	7												Zwak	-	-	-	-	
A	B	A	AML	CD3/SSC	5	CD19/SSC	1	CD3-CD16.56/SSC	4	CD66abce/SSC	79	CD14/SSC	4	CD45/SSC	7				+	+							Zwak	-	-	-	-	
A	C	A	AML	CD3/SSC	4	CD19/SSC	<<1	CD3-CD16.56/SSC	2	CD13/CD33/SSC	80	CD14/SSC	3	CD45/SSC	6									+			Zwak	-	-	-	-	
C	B	A		CD3/SSC	4	CD19/SSC	0,4	CD3-CD16.56	4	CD13/CD33/SSC	90	CD14/SSC	2	CD34 CD45zwak	1				+	+					+		Zwak	-	-	-	-	
A	B	B	AUL	CD3	4	CD19	1	CD3-CD16.56	3	CD13/SSC	82	CD14	3	CD34	5																	
C	B	A	AML	CD3/SSC	4	CD19/SSC	0,5	CD3-CD16.56/SSC	3,5	CD13/CD33/SSC	88,5	CD14/SSC	2	Anders	1,5				+	+				NV			Norme	-	NV	-	-	
A	B	A	AML	CD3/SSC	4	CD19/SSC	1	CD3-CD16.56/SSC	4	CD13/CD33/SSC	82	CD14/SSC	3	CD34/CD71/CD45	5												Zwak	-	-	-	-	
A	B	A	AML	CD3	4	CD19	<1	CD3-CD16.56	4	CD13/CD33/SSC	86	CD14/SSC	3	CD34/SSC	4												Zwak	-	-	-	-	
B	B	B		CD3	4	CD19	1	CD3-CD16.56	3	CD13/CD33/SSC	77	CD14	4	CD34	3				+	+							Zwak	-	-	+	-	
A	A	A		CD3/SSC	3	CD19/SSC	<1	CD3-CD16.56/SSC	2	CD13/SSC	88	CD14/SSC	2	34+/SSC	3												Zwak	-	-	-	-	
C	C	A		CD3/SSC	4	CD19/SSC	0,4	CD3-CD16.56	4	CD15/SSC	73	CD14/SSC	4	CD45/SSC	6				+	-							Norme	-	+	-	-	
A	B	B	AUL	CD3/SSC	8	CD19/SSC	1	CD3-CD16.56/SSC	4	CD13/SSC	72	CD14/SSC	5	CD45/SSC	9												Zwak	-	-	-	-	
B	B	A		CD3/SSC	4	CD19/SSC	1	CD3-CD16.56/SSC	3	CD13/CD33/SSC	83	CD14/SSC	2	CD45/SSC	7												Norme	-	-	-	-	
A	B	A		CD3/SSC	8	CD19/SSC	1	CD3-CD16.56/SSC	4	CD15/SSC	75	CD14/SSC	4	CD45/SSC	8													-	-	-	-	
A	B	A		CD3/SSC	3,5	CD19/SSC	0,4	CD3-CD16.56/SSC	2,2	CD13/CD33/SSC	90,3	CD14/SSC	0,3	SSC/CD34	3,3												Norme	-	-	-	-	
A	B	A		CD3/SSC	3	CD19/SSC	<1	CD3-CD16.56	2	CD13/SSC	88	CD36, CD14	3	CD34 CD117	3				+	+				NV			Zwak	-	-	-	-	
B	A	B		CD3/SSC	4	CD19/SSC	1	CD3-CD16.56/SSC	4	CD13/CD33/SSC	83	CD14/SSC	4	CD45/SSC	4												Norme	-	-	+	-	
C	A	A	AML		7		1		4		75		5	CD45/SSC	8													-	-	-	-	
C	B	A		CD3/SSC	4,6	CD19/SSC	0,4	CD3-CD16.56/SSC	3,8	CD45/SSC	57,2	CD14/SSC	2	CD34/SSC	8				+								Norme	-	-	-	-	
		D																														
A	A	A		CD3/SSC	5	CD19/SSC	1	CD3-CD16.56/SSC	3	CD13/CD33/SSC	81	CD14/SSC	4	CD34/CD117/CD	5													Zwak	-	-	-	-
B	A	A		CD3/CD45/SSC	4,1	CD19/CD45/SSC	0,5	CD56/CD16/SSC	3,4	CD15/CD45/SSC	84	CD33/CD45/SSC	2,7	CD34/CD45/SSC	1,9													Norme	-	-	-	-
A	A	B	AUL	CD3/SSC	3,6	CD19/SSC	0,6	CD3-CD16.56/SSC	3,2	CD33/SSC	87	CD14/SSC	3,6	CD45/SSC	3,5													Zwak	-	-	-	-

# MUSE

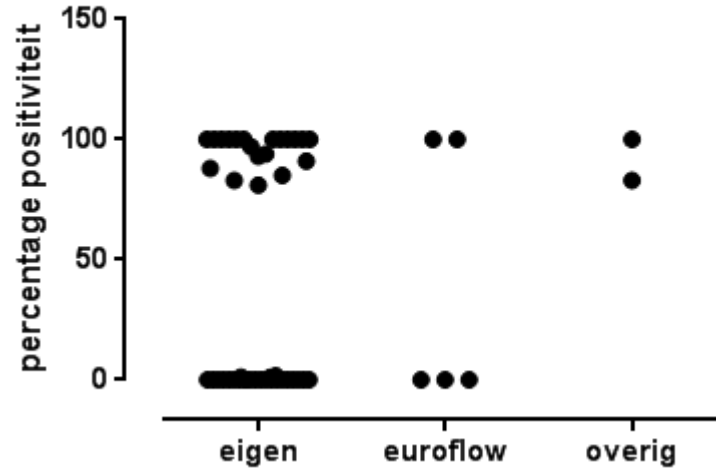
								mean referentielabs	mean groep
<b>T-lymfocyten</b>	<b>49</b>	<b>50</b>	<b>46</b>	<b>49</b>	<b>50</b>	<b>58</b>		<b>50</b>	<b>49</b>
<b>B-lymfocyten</b>	<b>2</b>	<b>2</b>	<b>3</b>	<b>2</b>	<b>2</b>	<b>2</b>		<b>2</b>	<b>2</b>
<b>NK-lymfocyten</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>&lt;1</b>		<b>1</b>	<b>1</b>
<b>Myeloid</b>	<b>39</b>	<b>41</b>	<b>36</b>	<b>36</b>	<b>37</b>	<b>29</b>		<b>36</b>	<b>36</b>
<b>Monocytair</b>	<b>8</b>	<b>5</b>	<b>8</b>	<b>8</b>	<b>7</b>	<b>8</b>		<b>7</b>	<b>7</b>
<b>Afwijkende populatie</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>2</b>	<b>2</b>		<b>2</b>	<b>2</b>
<b>Totaal</b>	<b>100</b>	<b>100</b>	<b>96</b>	<b>98</b>	<b>99</b>	<b>99</b>			

# Heterogeniteit in expressie

							Mean ref.labs	Mean groep
CD5	0	0	0	0	0	0	0	5
CD10	0	0	0	0	0	0	0	0
CD19	100	100	100	100	100	100	100	93
CD20	100	100	100	100	100	100	100	97
CD23	0	0	0	0	0	0	0	5
CD25	0	0	100	0	97	-	0	55
CD38	0	0	0	0	12	0	0	13
CD45	100	100	100	100	100	100	100	100
CD103	0	0	0	0	0	0	0	0
SmlgKAPPA	100	100	100	100	100	100	100	93
SmlgLAMBDA	0	0	0	0	0	0	0	2
CD11c		0		0	0	0	0	
CD22	100	100	100	100	100	100	100	
CD43	0	-	0	36	0	-	0	
CD79b	100	-	-	100	100	100	100	
FMC7	100	100	0	-	78	-	100	

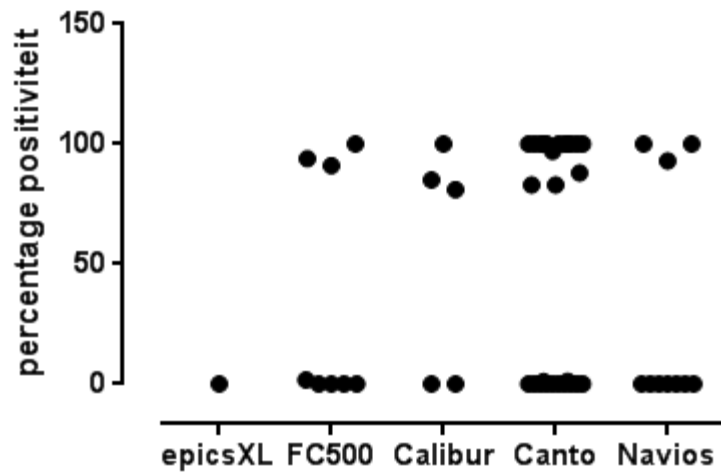


### antistofpanel

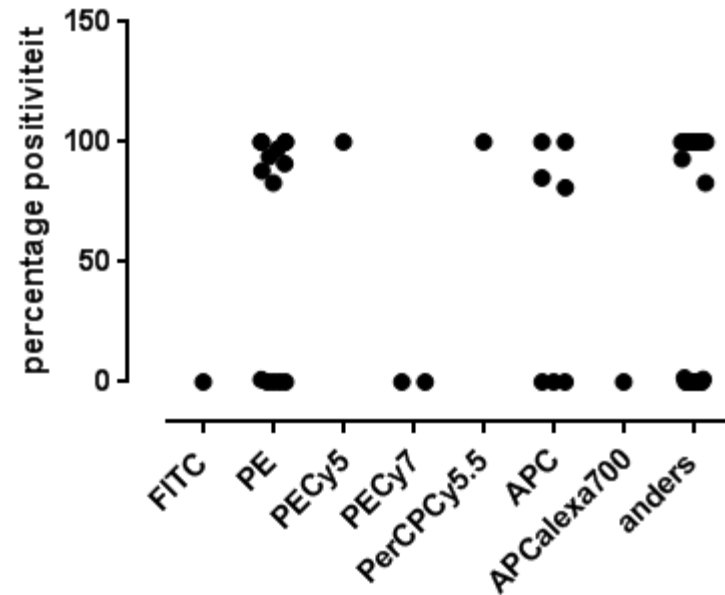


- pre-analyse?

### Flowcytometer

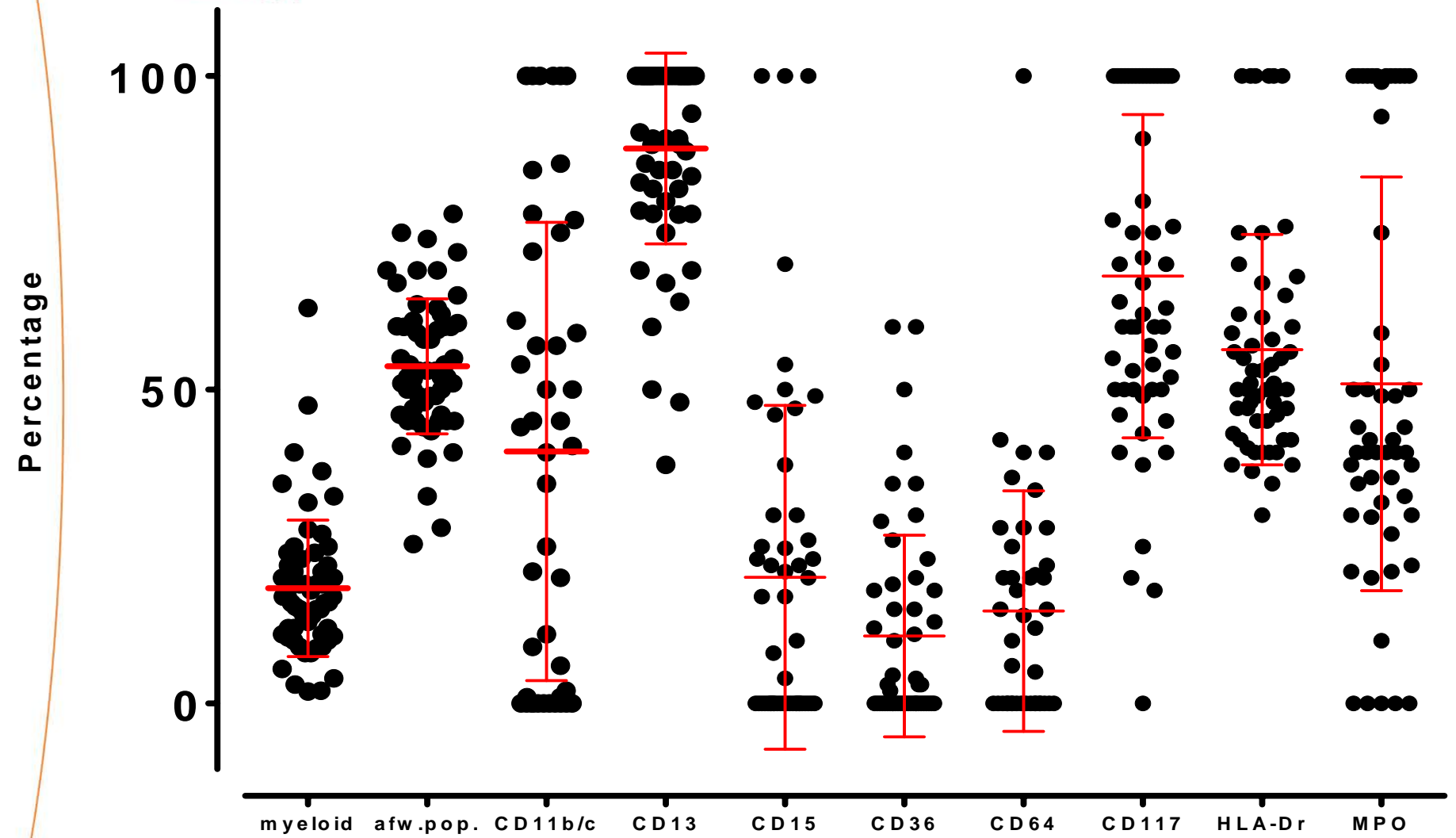


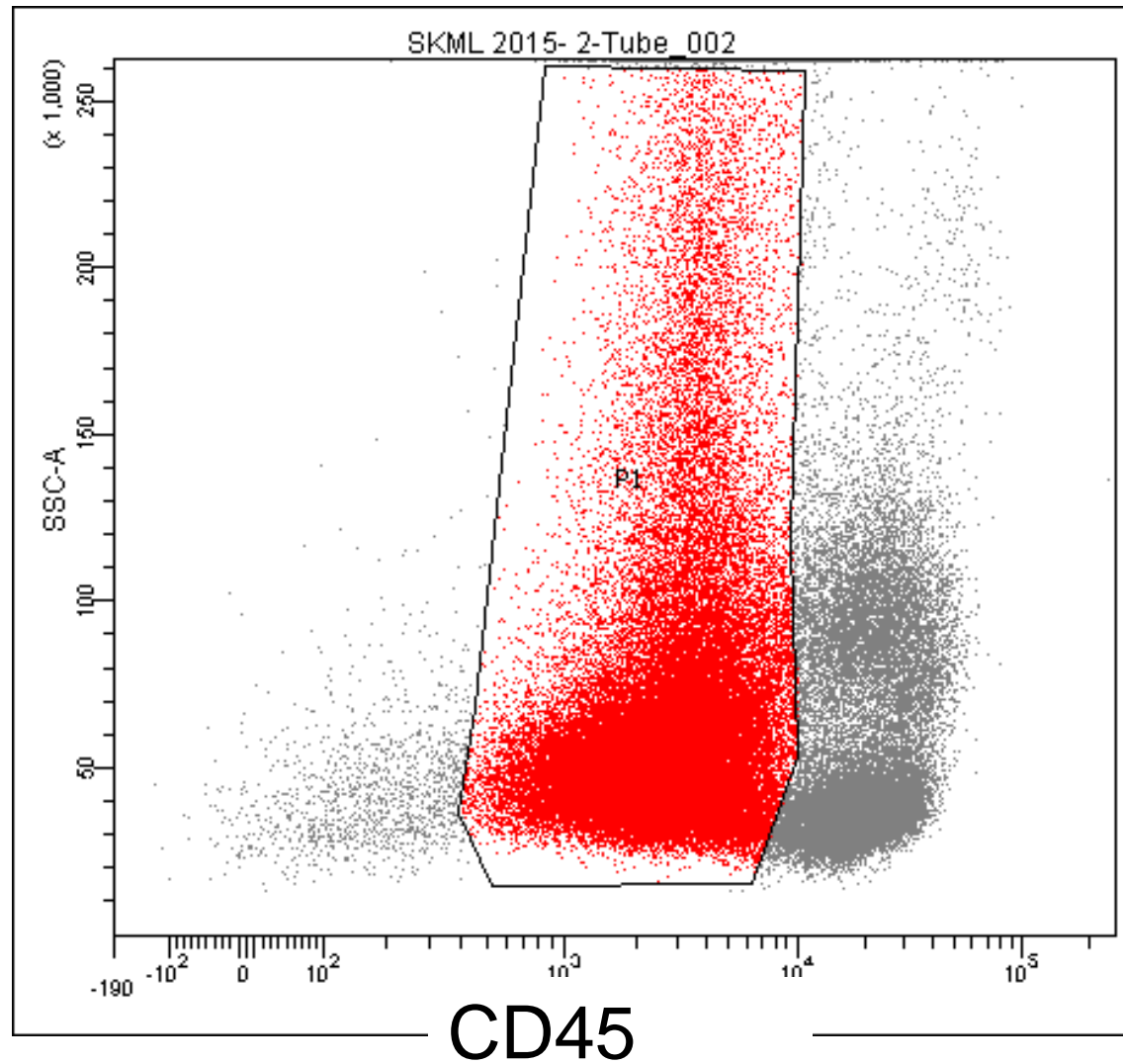
### fluorochroom



# Heterogeniteit in gatesetting

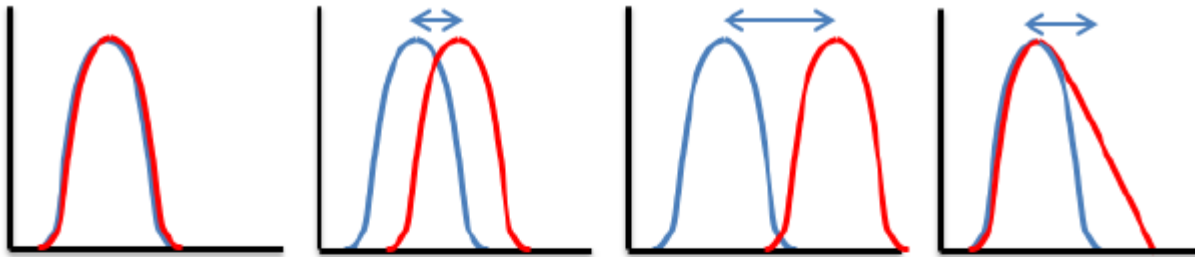
								mean referentielabs	mean groep
T-lymfocyten	10	6	8	10	10	10		9	10
B-lymfocyten	4	1	3	5	5	5		4	5
NK-lymfocyten	1	1	1	4	2	1		2	2
Myeloid	37	14	22	11	24	<1		22	18
Monocytair	8	4	9	11	12	8		9	9
Afwijkende populatie	40	74	53	60	45	75		58	54
Totaal									



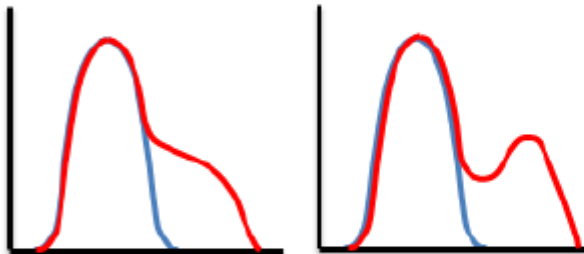


# Verdere standaardisatie - richtlijnen positiviteit

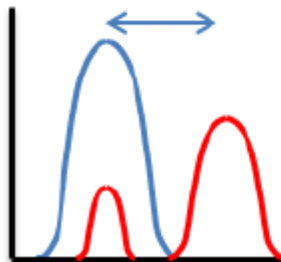
Ad 1.



Ad 2.



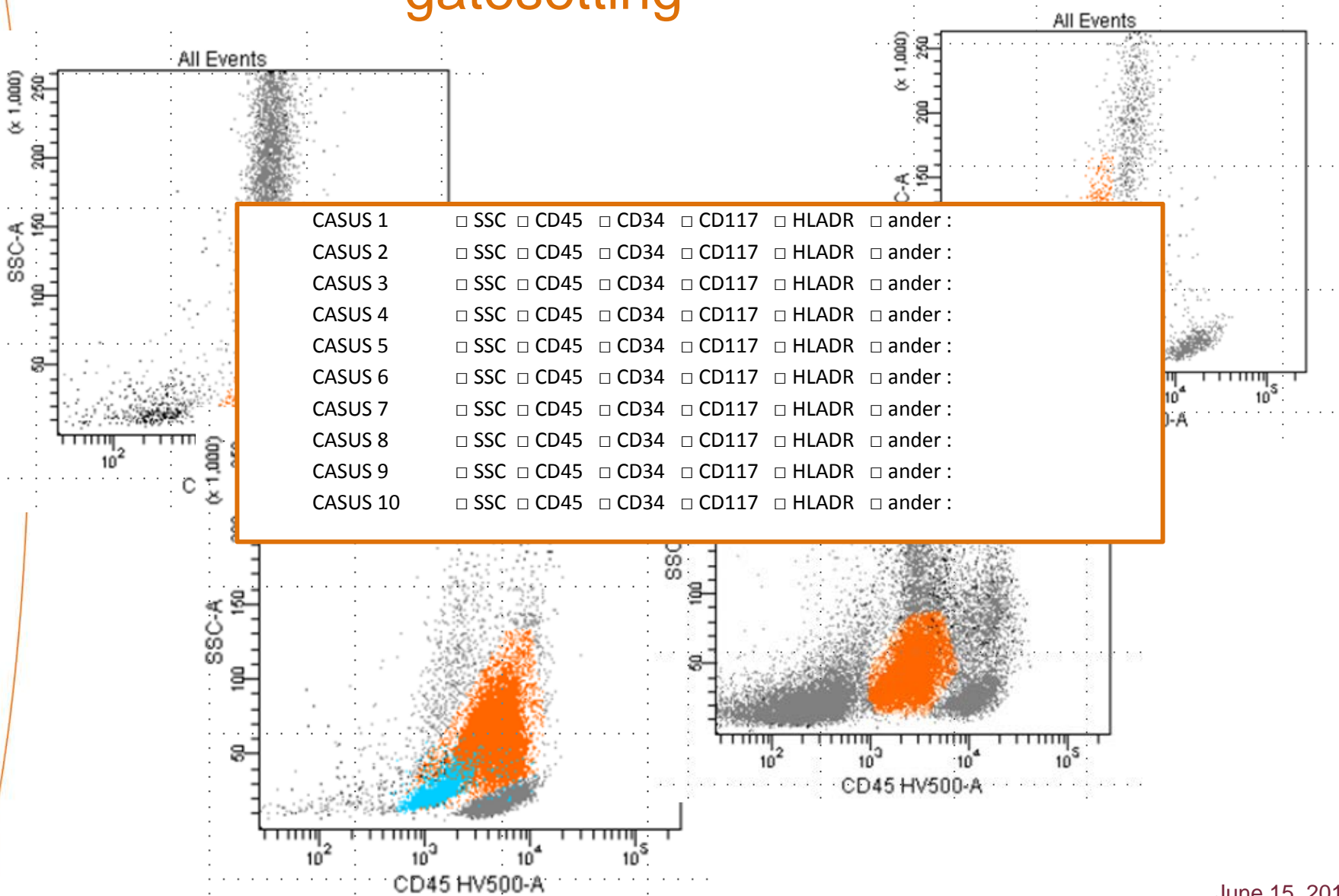
Ad 3.



$$\frac{\text{MFI}_{\text{rood}}}{\text{MFI}_{\text{blauw}}}$$

3.3-10 = zwak pos  
>10 = pos

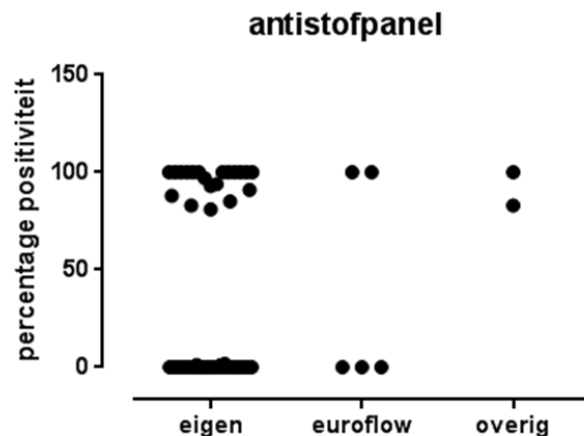
# Verdere standaardisatie - gatesetting



## EuroFlow consortium

### 20 diagnostische/research groepen

- opzetten en standaardiseren van snelle, accurate en gevoelige flow cytometrische bepalingen voor de diagnose en vaststellen van prognostische factoren van hematologische maligniteiten
- Protocollen voor instellen apparatuur, pre-analyse (8-kleuren panels met vastgestelde fluorochromen, klonen en verdunningen) en analyse (zelf-ontwikkelde software)



Ook niet altijd gelijke resultaten/interpretatie

## Conclusie

- Binnen leukemie en lymfoom typering wordt gewerkt met consensus waarden: geen expertwaardes
- Referentielabs bereiken ook niet altijd consensus
- Gemiddelde dan meestal wel vergelijkbaar met groep
- Oorzaak van variatie vaak niet vast te stellen
- Niet alle variaties in bewerking bekend (bewerking materiaal, antistofklonen, instellingen apparatuur etc)



# DEELNEMERSGROEP IS EXPERT



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