

Basic workshop MB&C24: Blood and bone marrow differentiation by flowcytometry

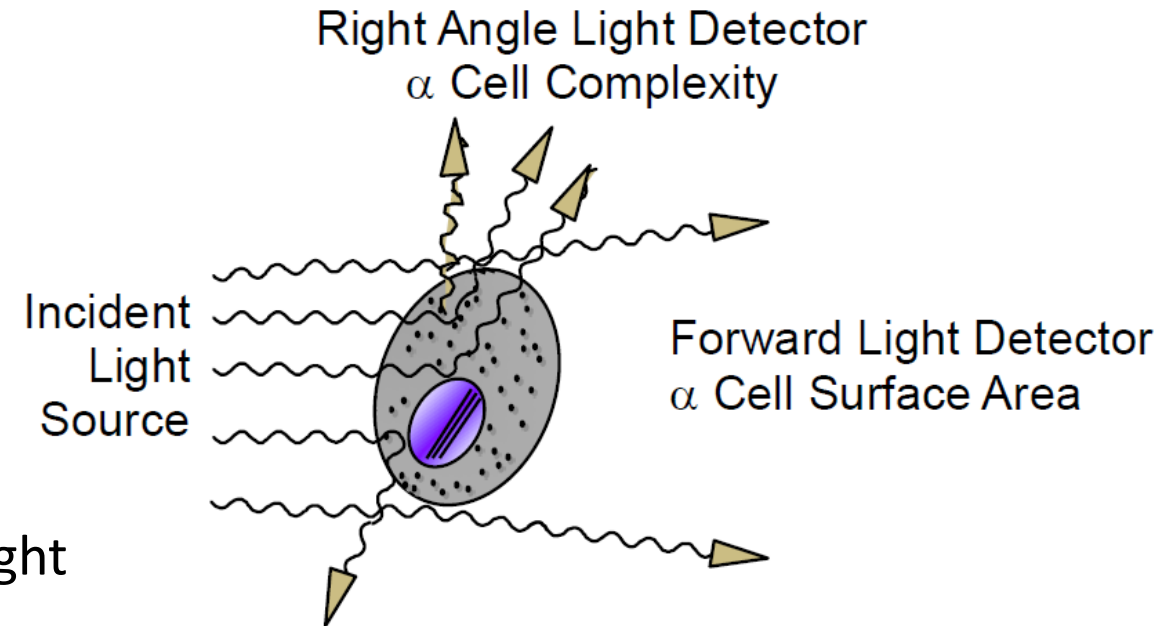
Prof. Apr. Biol. Barbara Depreter
AZ Delta

General

- Unique properties of flow cytometry
 - Multiparametric
 - Rapid analysis of large number of cells
 - Information at single cell level
- What Can a Flow Cytometer Tell Us About a Cell?

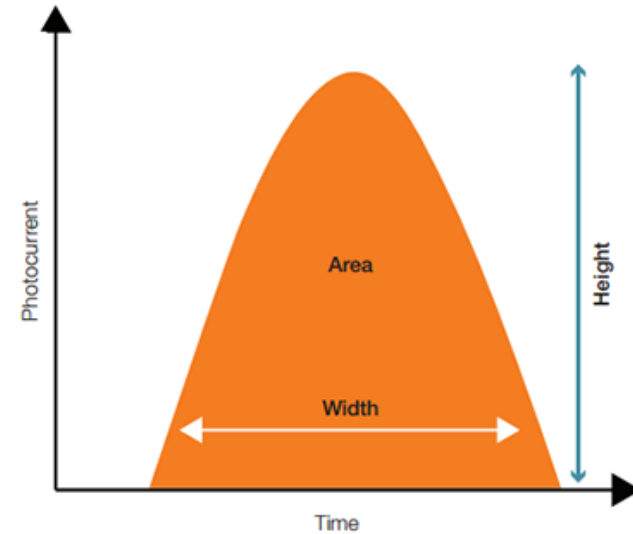
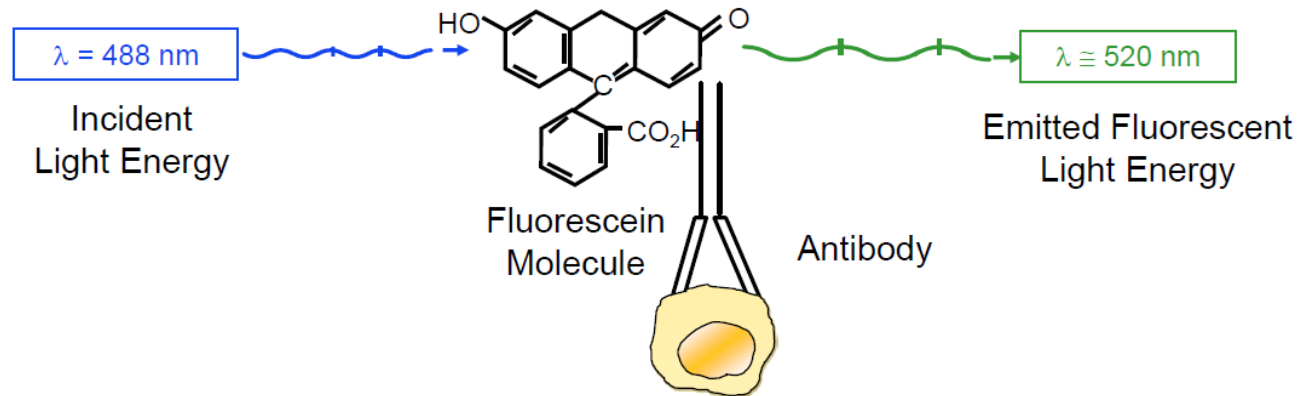
General

- Scatter parameters
 - Forward Scatter (FSC) = diffracted light
 - Detected along axis of incident light
 - \sim relative size (cell surface area)
 - Side Scatter (SSC)=reflected and refracted light
 - Detected at 90° to the laser beam
 - \sim relative granularity or internal complexity



General

- Fluorescence parameters



Signal intensity: area (A) or height (H)

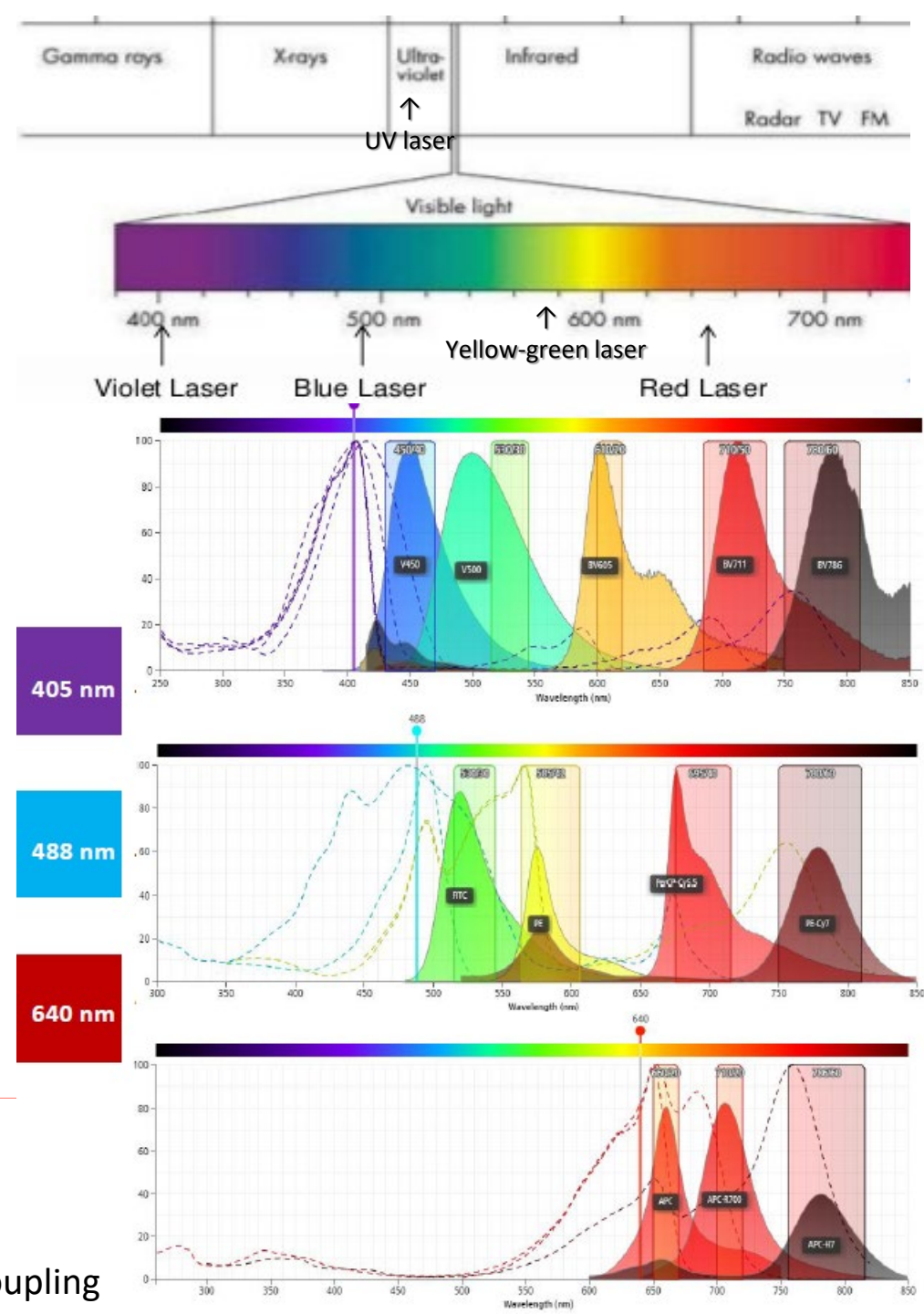
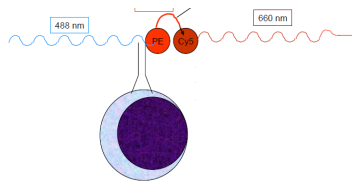
Time that the cell spends in the laser: width (W)

- mAb-fluorochrome binds cluster of differentiation (CD) antigen on the cell → fluorochrome absorbs energy from the laser and releases the absorbed energy (decreased)

General

• Fluorochromes

1. Violet laser: 405 nm
 - Horizon V450, V500
 - Brilliant violet BV605, BV771, BV786...
2. Blue laser: 488 nm
 - FITC
 - PE
 - PerCP(-Cy5.5), PE-Cy7
3. Red laser: 635-640 nm
 - APC
 - APC-Cy7, APC-H7, APC-R700
4. Yellowgreen laser: 561nm
5. Ultraviolet laser: 355nm



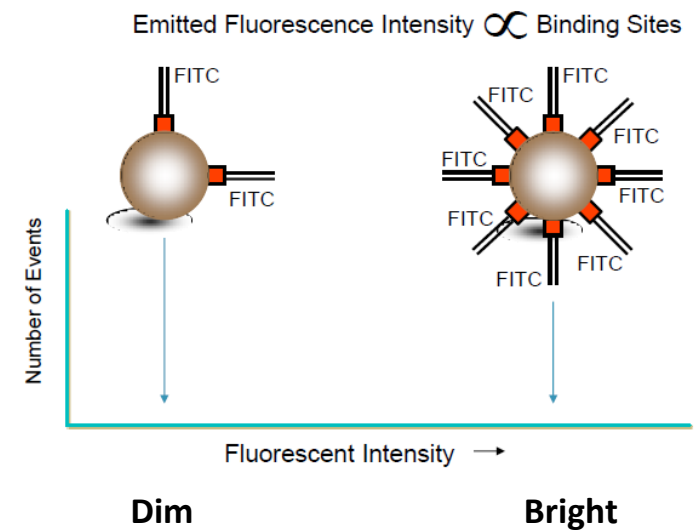
! Tandem fluorochromes: bleedthrough emission and decoupling

General

- Fluorochromes
 - Shotgun approach
 - Screening approach
 - Cocktails: use of a mix of markers because a lack of specificity

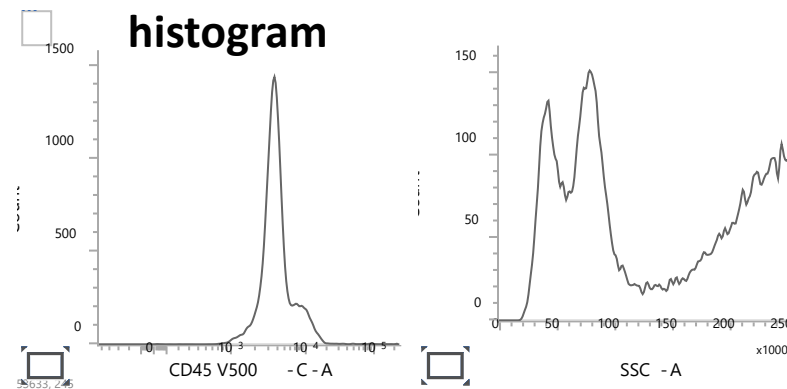
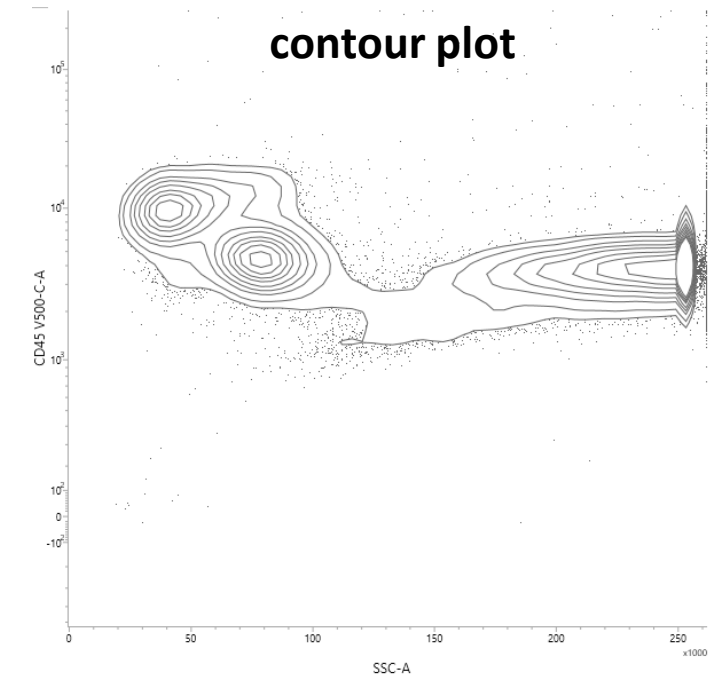
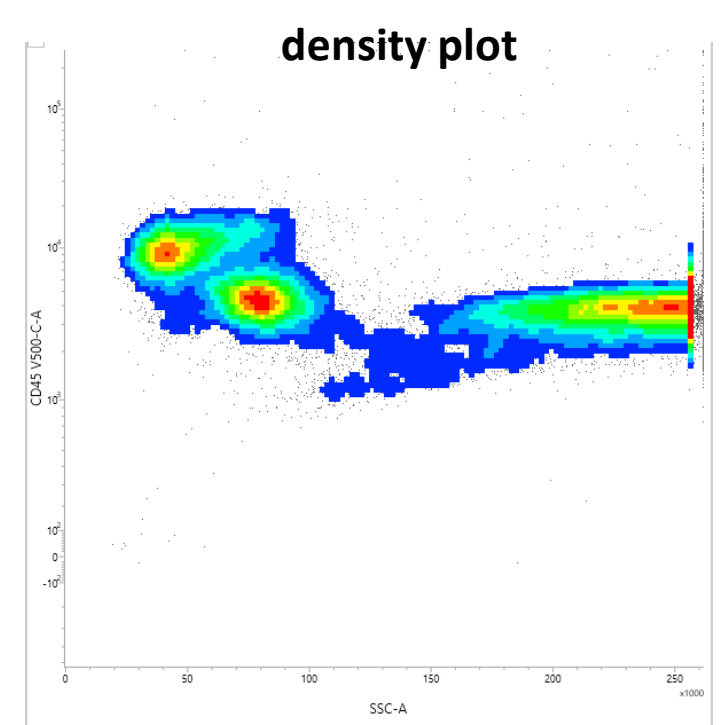
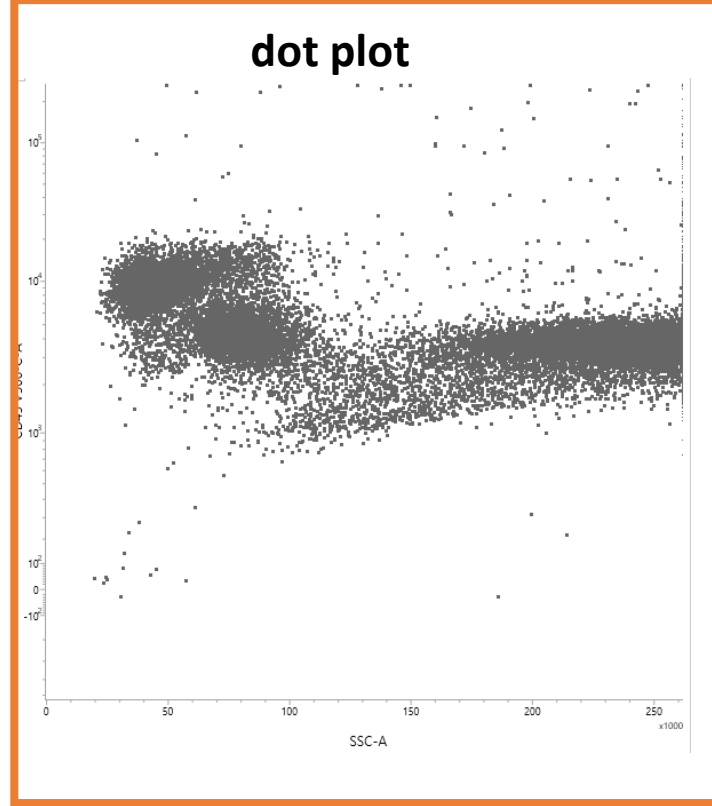
! Panel design = other workshop but some key rules:

- 1) Watch out for high spillover fluorochromes
- 2) Match fluorochrome brightness with expression level
- 3) Avoid data spread



General

- Plot types



General

- Cleaning data: removal of debris (air, clogs, small particles) and aggregated cells
- Gating: setting upper and lower limits on the type and amount of throughpassing cells using electric windows (gates): judge fluorescence intensity based on a cut-off for positivity
 - Solid cut-offs i.e. 1E3
 - Negative reference populations (for example lysed red-cells fraction)
 - Fluorescence-minus one (FMO) controls
- Isotype controls

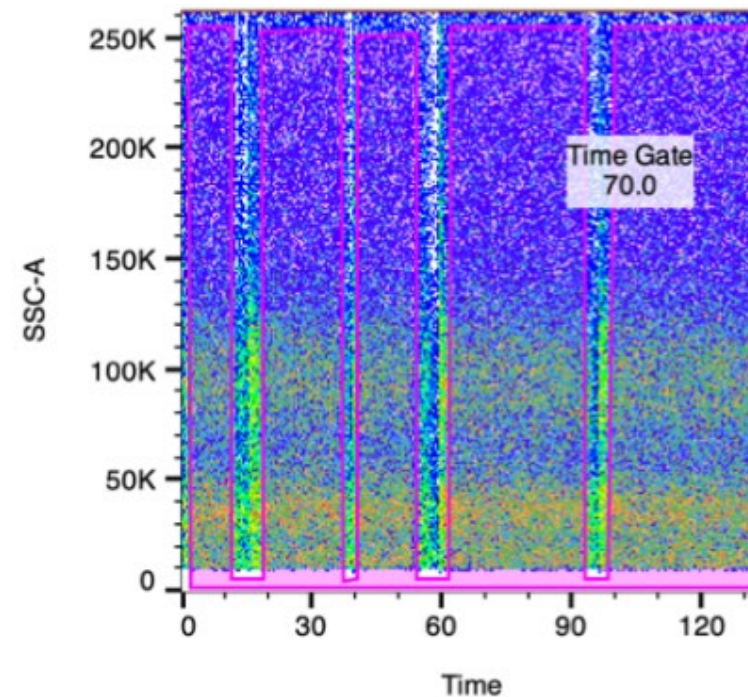
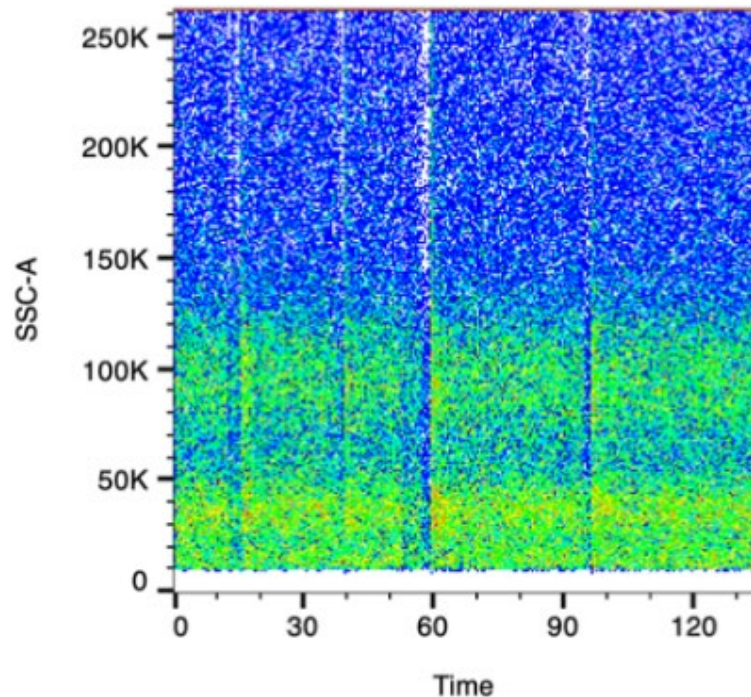
→ Select only a certain population for analysis

Flow cytometry analysis

- Standard analysis starts with

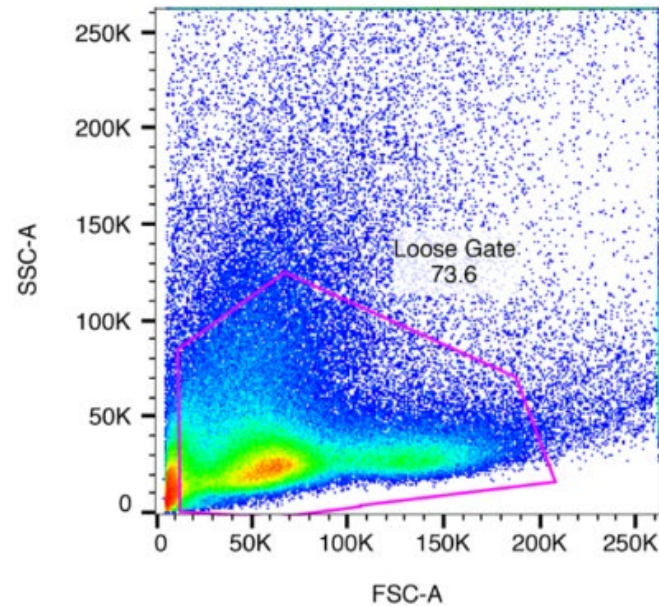
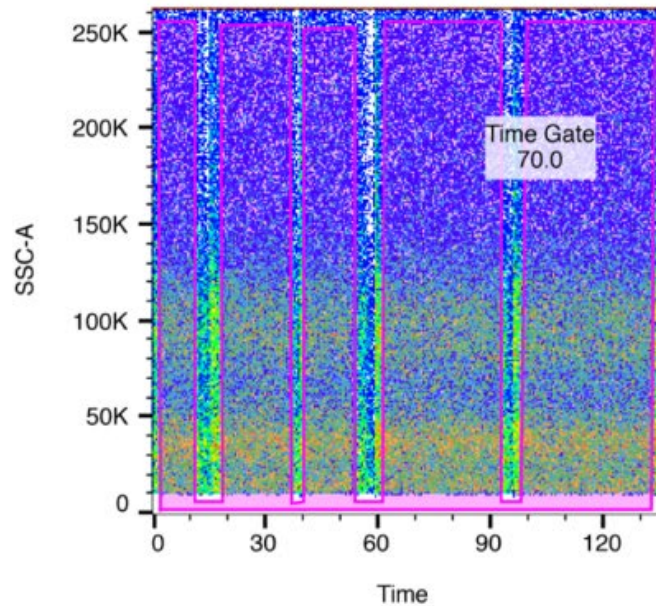
1. Time plot

- Microbubbles, clogs and air
- Acquisition issues → eliminate spikes



Flow cytometry analysis

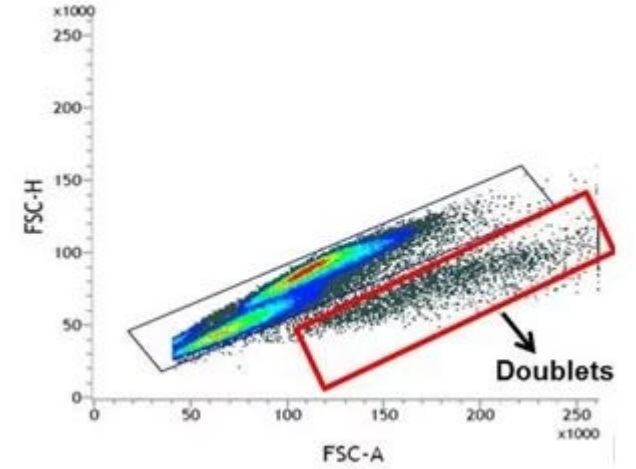
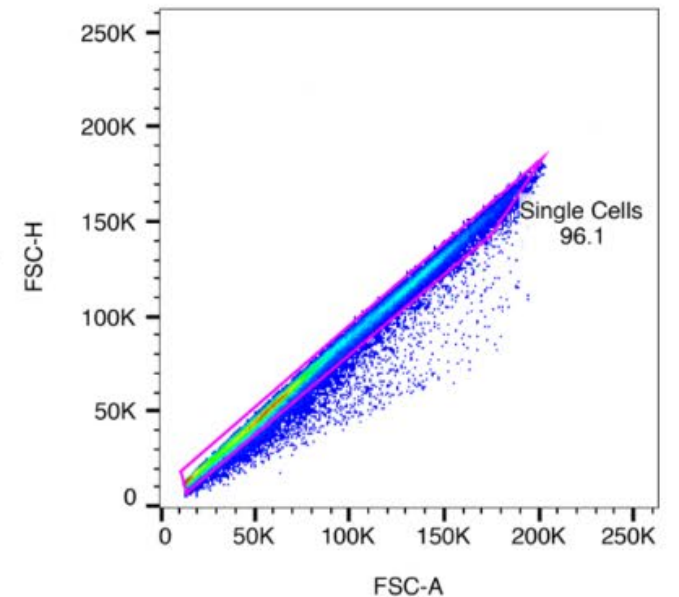
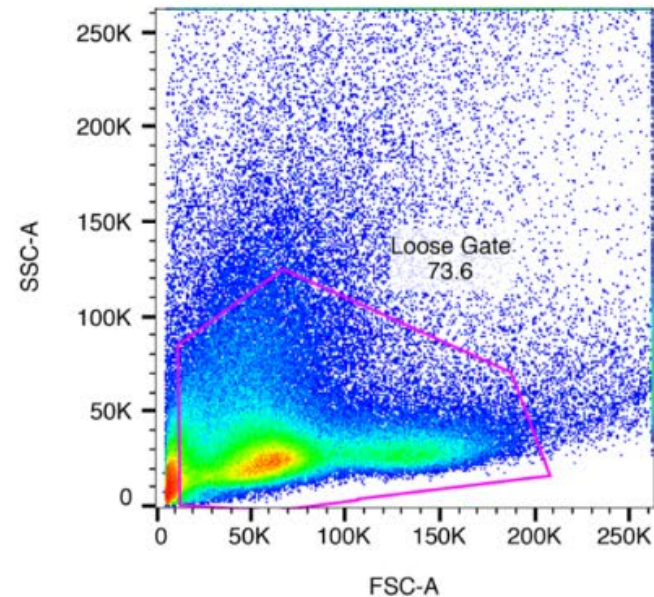
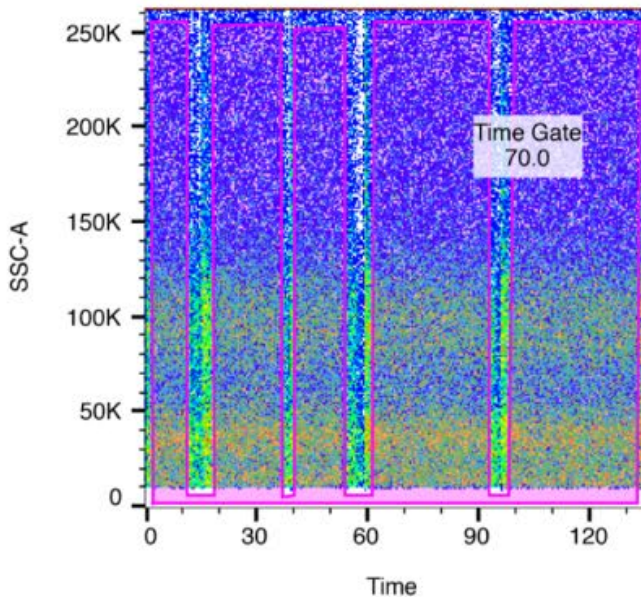
- Standard analysis starts with
 1. Time plot
 2. Removal of debris and platelets



Flow cytometry analysis

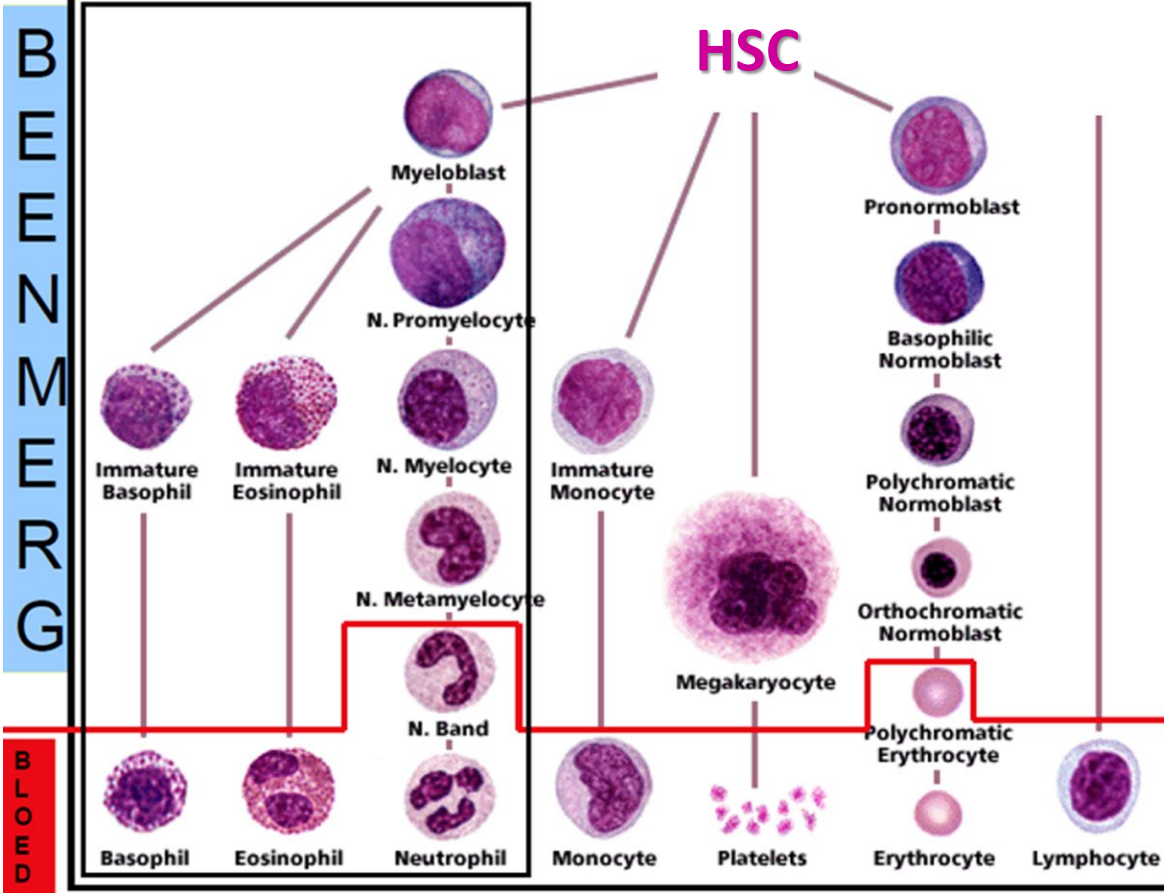
- Standard analysis starts with
 1. Time plot
 2. Removal of debris and platelets
 3. Removal of doublets

- FSC-A x FSC-H
- SSC-H x SSC-W
- FSC-H x FSC-W



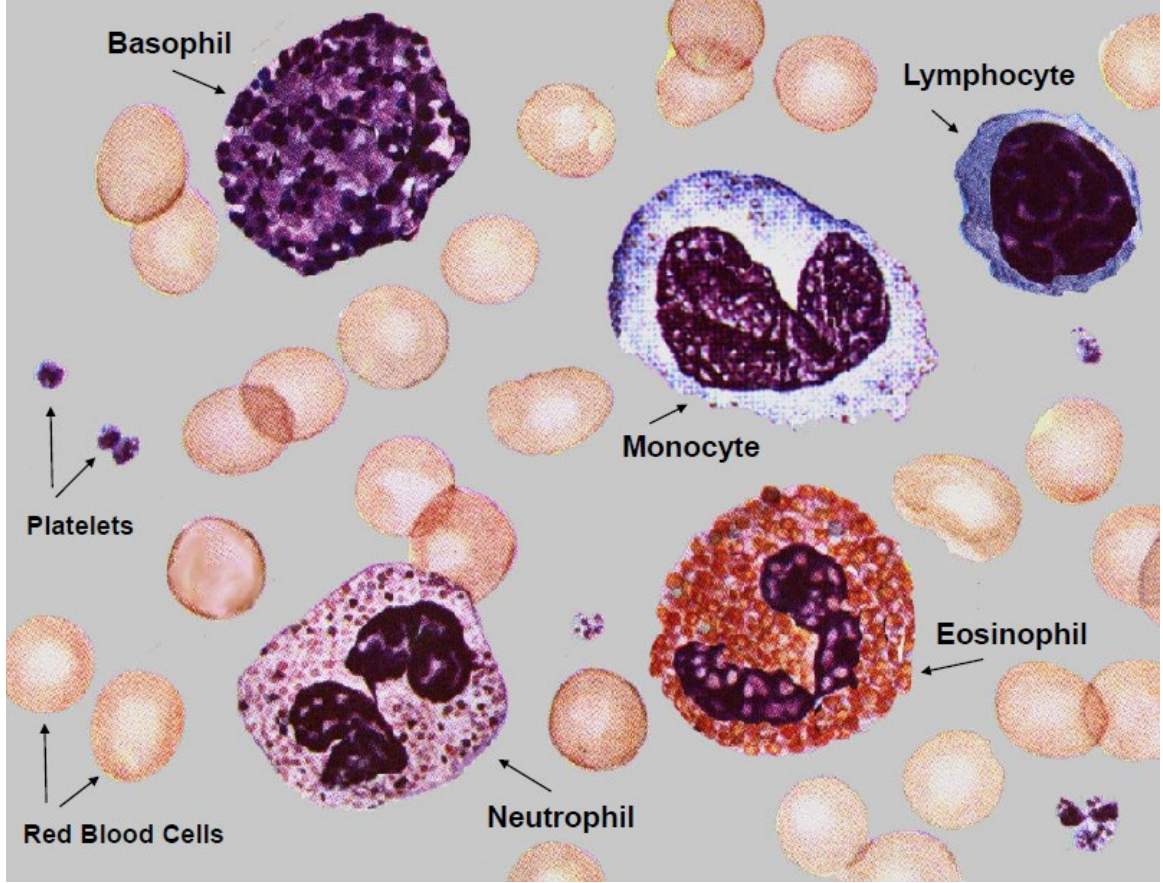
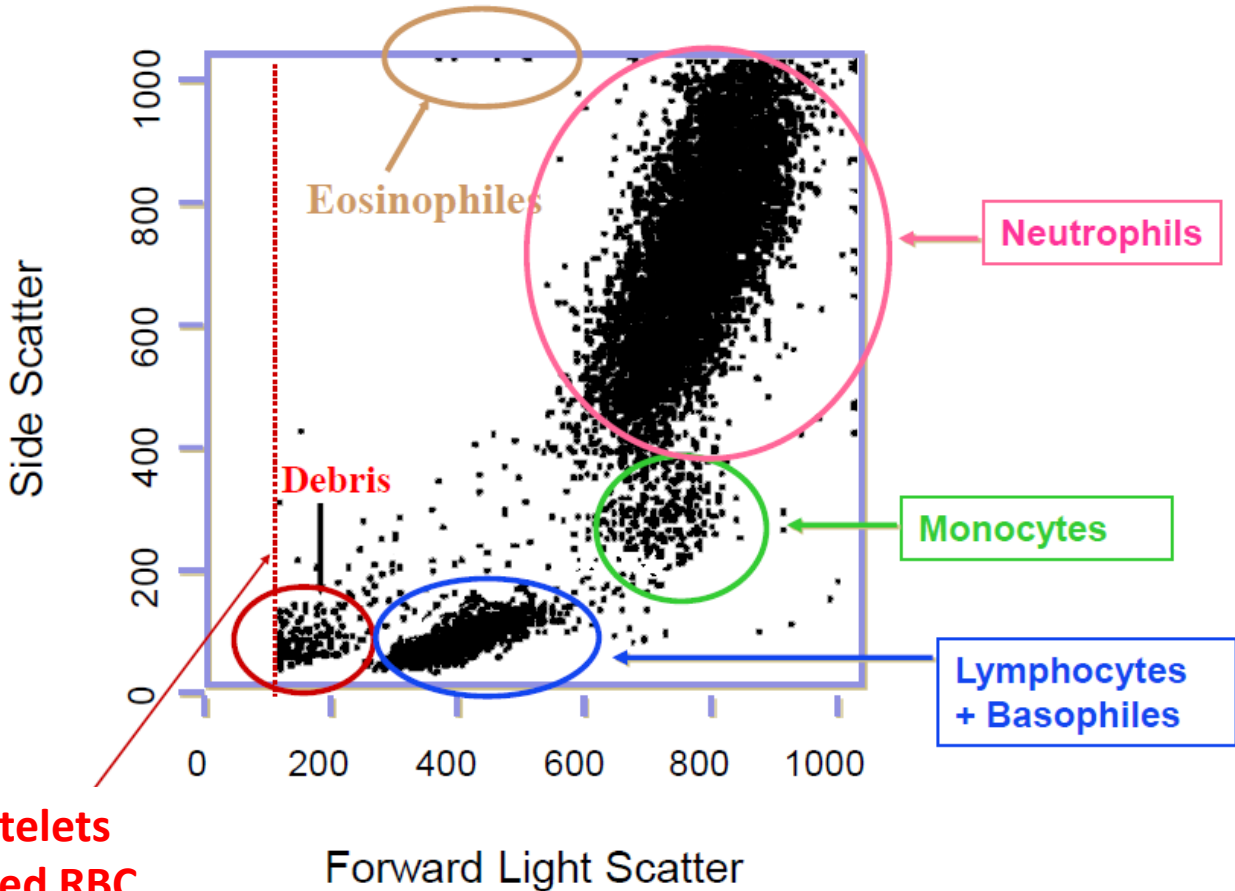
Matrix

- WBC differentiation by flow cytometry as reliable as by standard cytology, already with 5-color FCM (Faucher et al 2007, Bjornsson et al 2008)



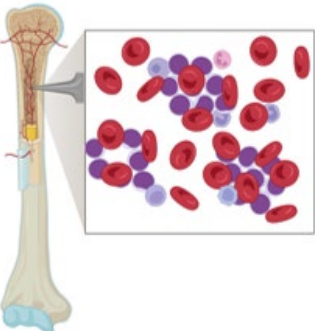
Matrix

- Whole blood



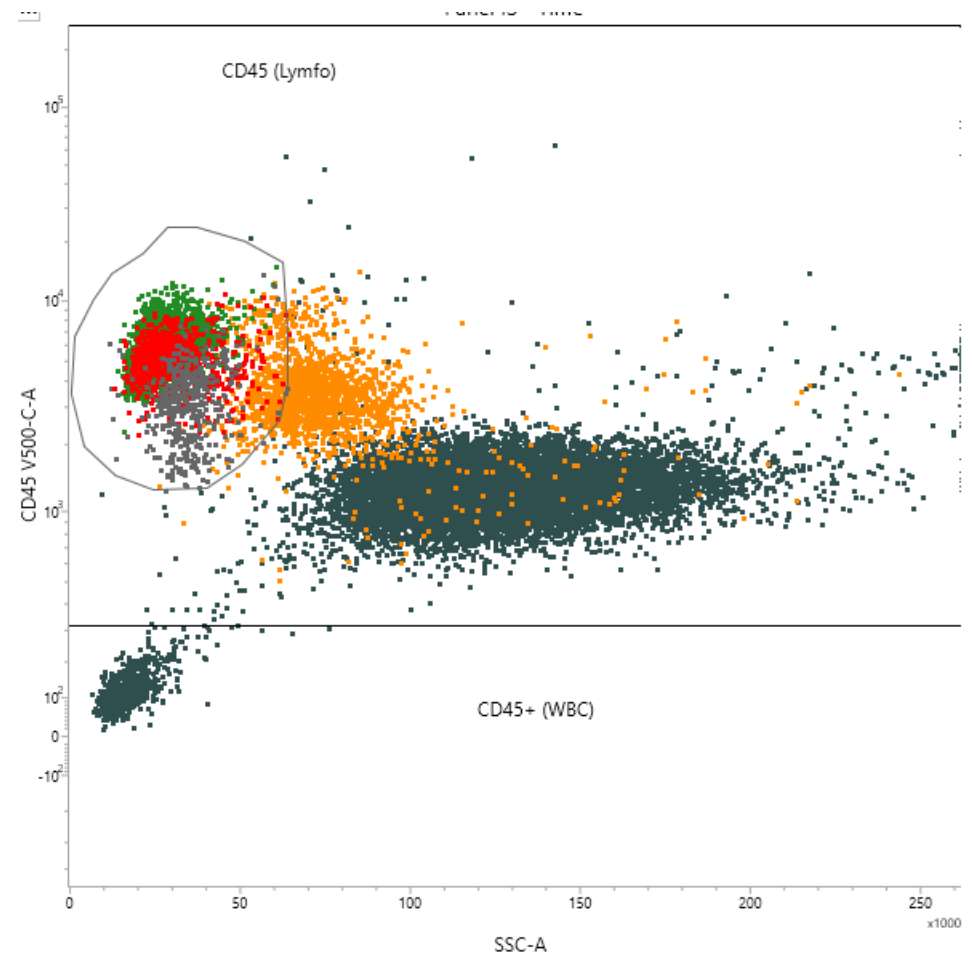
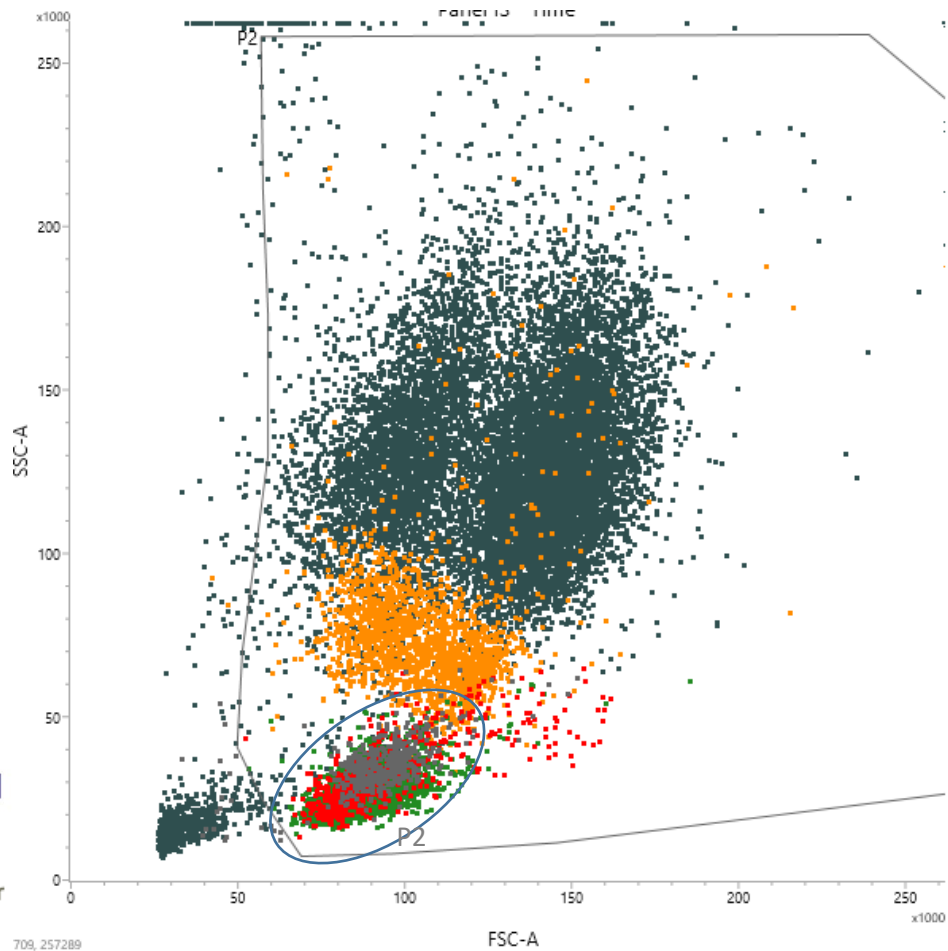
Matrix

- Bone marrow: assess hemodilution
 - First pull aspirate (2 mL)
 - Importance: follow-up and staging, detection of measurable residual disease (MRD), MDS (maturation-associated abnormalities)
 - (Predicted) BM purity
 - Quantification of BM-restricted nucleated cells: mast cells, plasma cells, pDCs and/or NRBCs → Contamination tube: CD13/CD38/CD34/CD117/CD138/CD10/CD16/CD45

BM pull 1	Formula for detecting hemodilution	Disease measured	Additional requirements
3 mL Bone Marrow aspiration	Bone marrow purity = $[1 - (\text{erythrocytes BM} / \text{erythrocytes PB}) \times (\text{leukocytes PB} / \text{leukocytes BM})] \times 100\%$	MDS	Matched PB
	PB contamination index = $-3.052 + 0.065 \times (\%CD10+ \text{ neutrophils of granulocytes}) - 0.609 \times (\%CD34+) - 2.008 \times (\%plasma \text{ cells})$	MM (not used post induction)	CD10, CD38, CD138, CD34 positive cells and plasma cells
	Normalized blast count = $(80\% / \% \text{ dim CD16}) \times \text{blast count}$	MDS/ AML	CD16 (maturing neutrophils)
	Predicted bone marrow purity = $[1 - (\text{Lymphocytes FCM} / \text{Lymphocytes PB}) \times (\text{Leukocytes PB} / \text{Leukocytes FCM})] \times 100\%$	AML/ MDS	Matched PB
	Suggested blood contamination if mast cell population $(CD117^+) \leq 0.002\%$	MM	CD117 positive mast cells

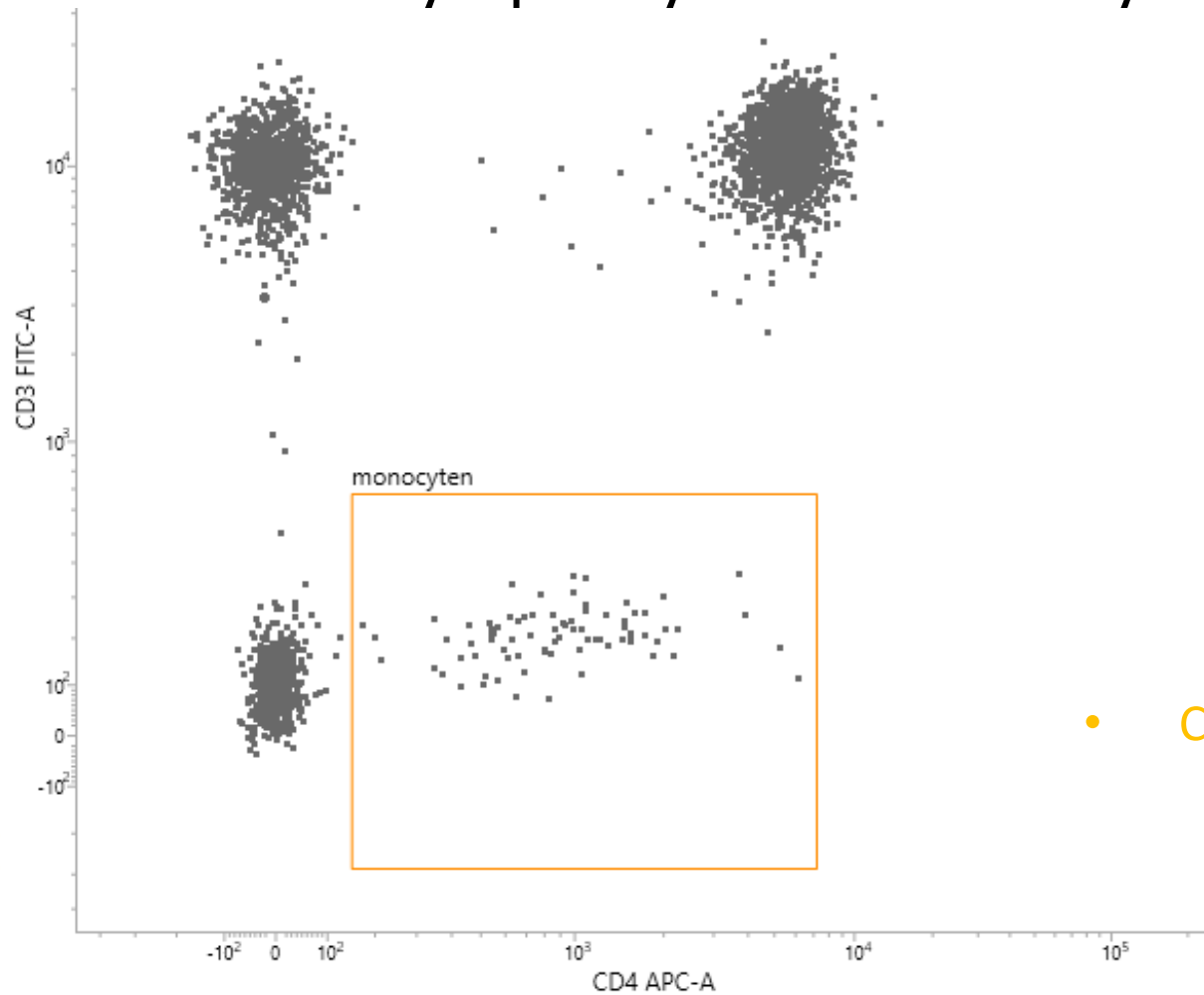
Normal cell populations

- Whole blood: lymphocyte subset analysis



Normal cell populations

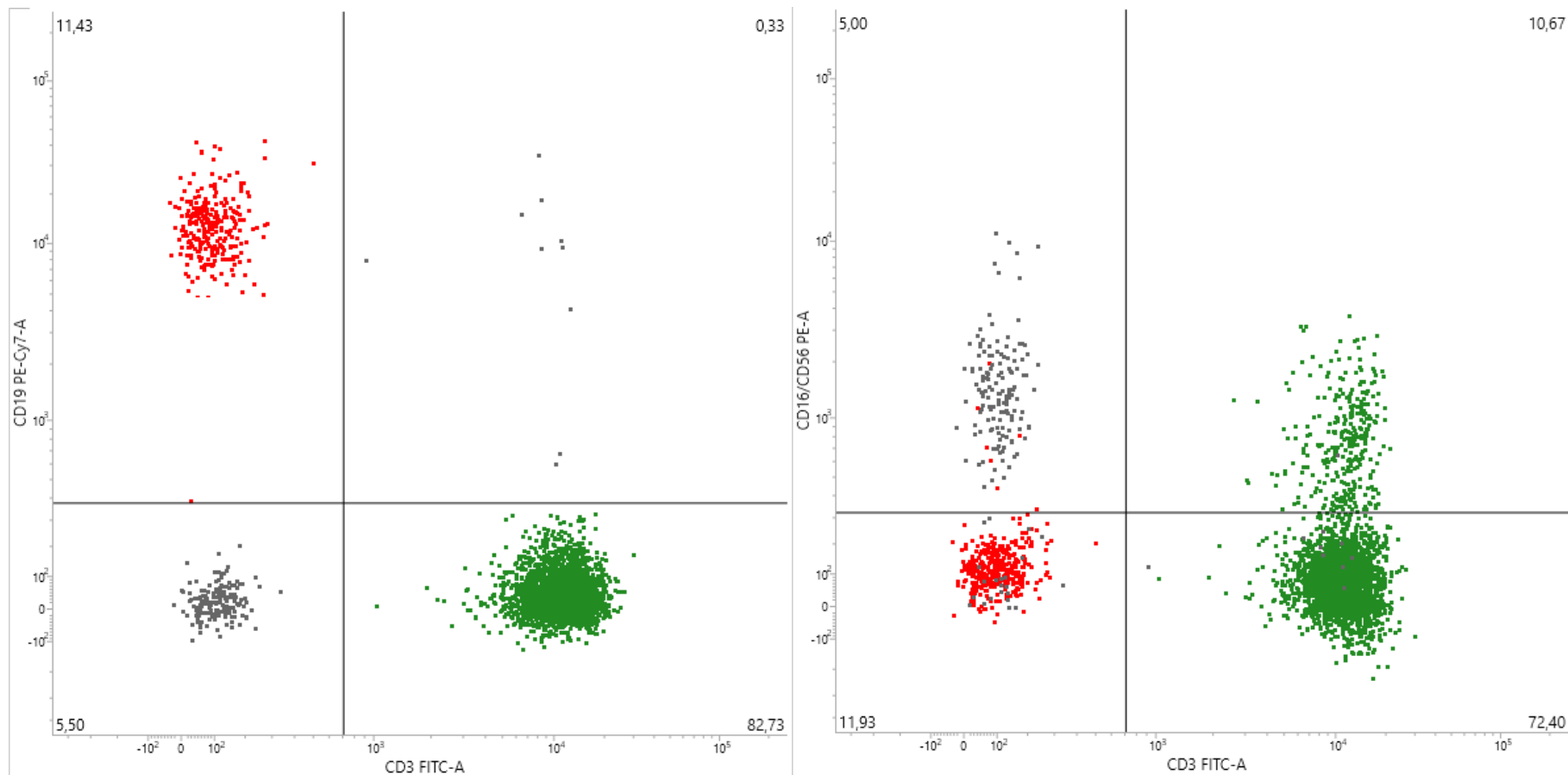
- Whole blood: lymphocyte subset analysis



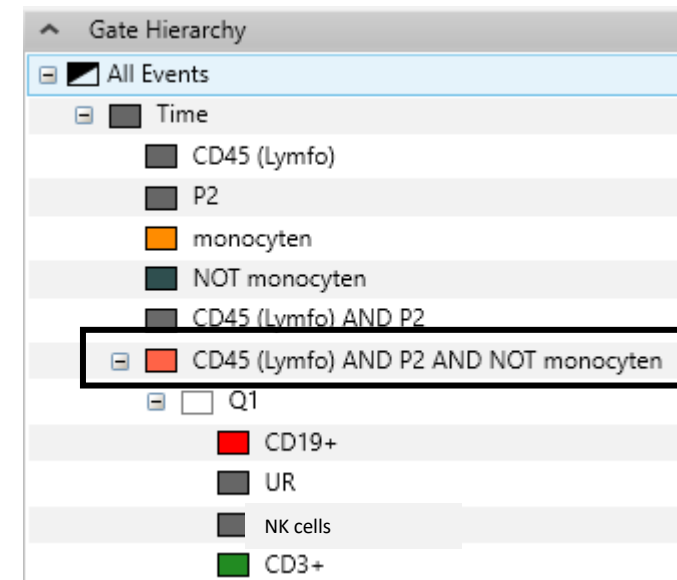
- CD4 = exclude monocytic interference

Normal cell populations

- Whole blood: lymphocyte subset analysis

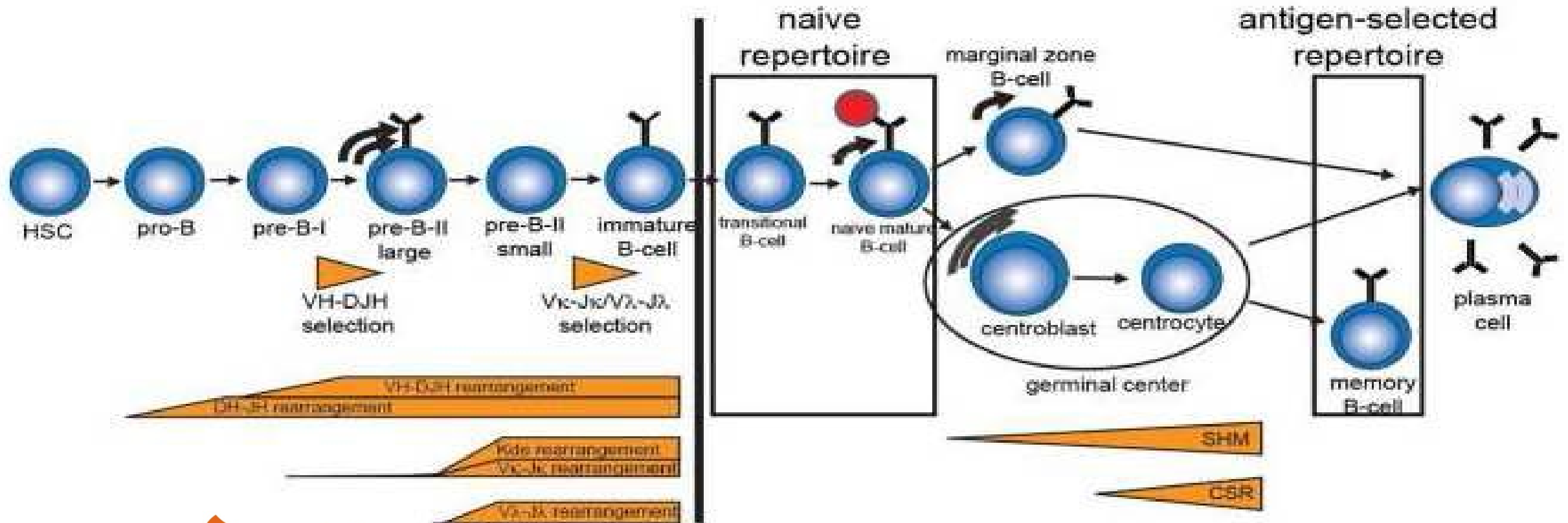


- CD19 = B cell lineage
- CD3 = T cell lineage
- CD16+CD56 = NK marker



Normal cell populations

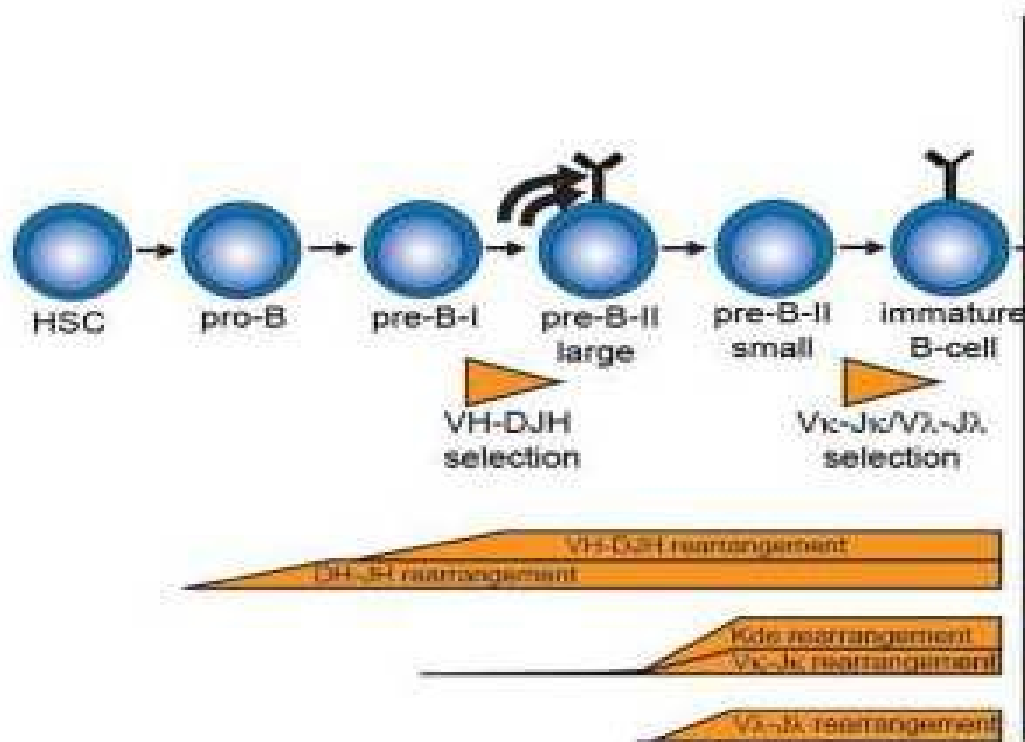
- B-cells



Normal cell populations

- B-cells

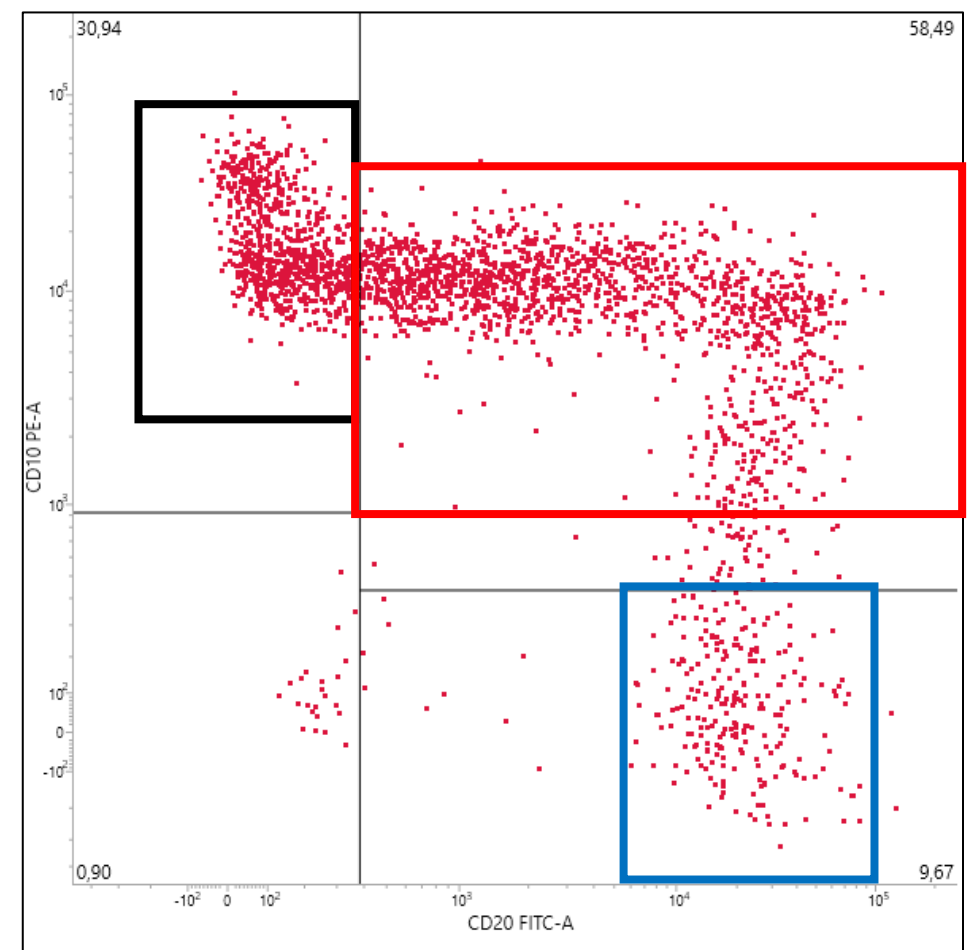
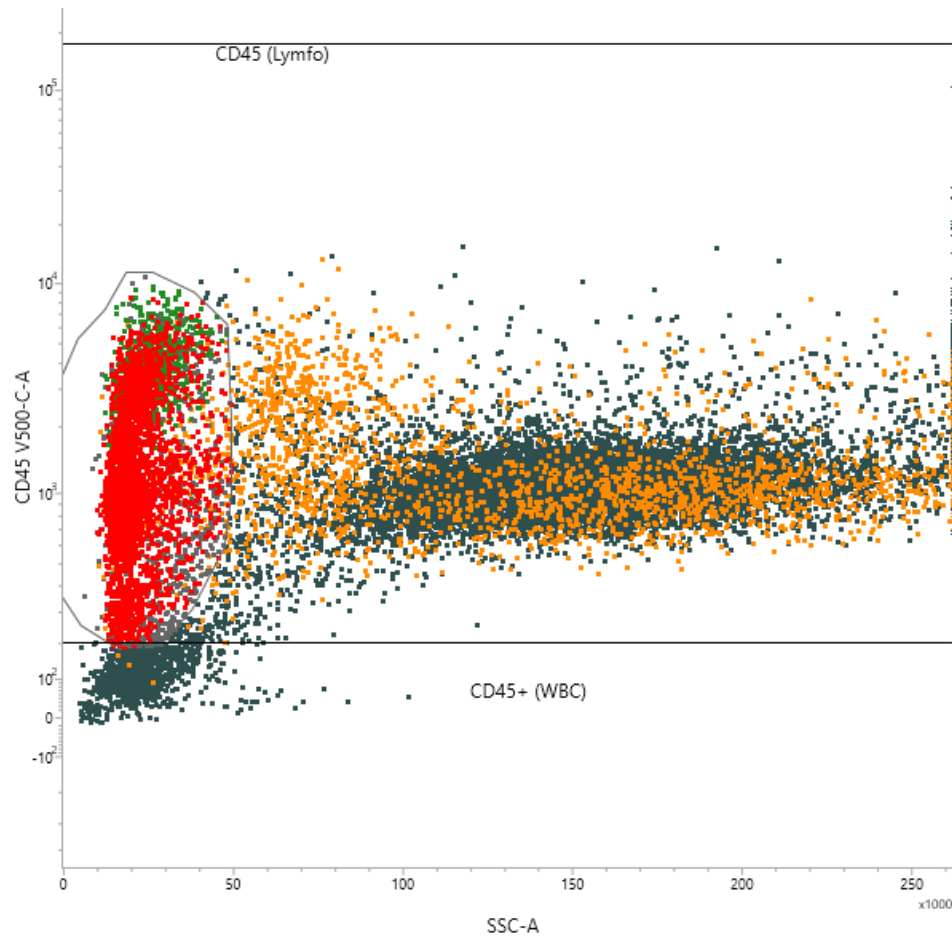
BEENMERG



- proB: **CD19-/CD34+/CD10-/CD20-**
- pre B I: **CD19+/CD34+/CD10+/CD20-/(CD38++)**
- Pre B II: **CD19+/CD34-/CD10+/CD20-/(CD38++)**
- Immature B: **CD19+/CD34-/CD10±/CD20+/(CD38++)**

Normal cell populations

- Immature B-cells (BM): CD10 vs CD20

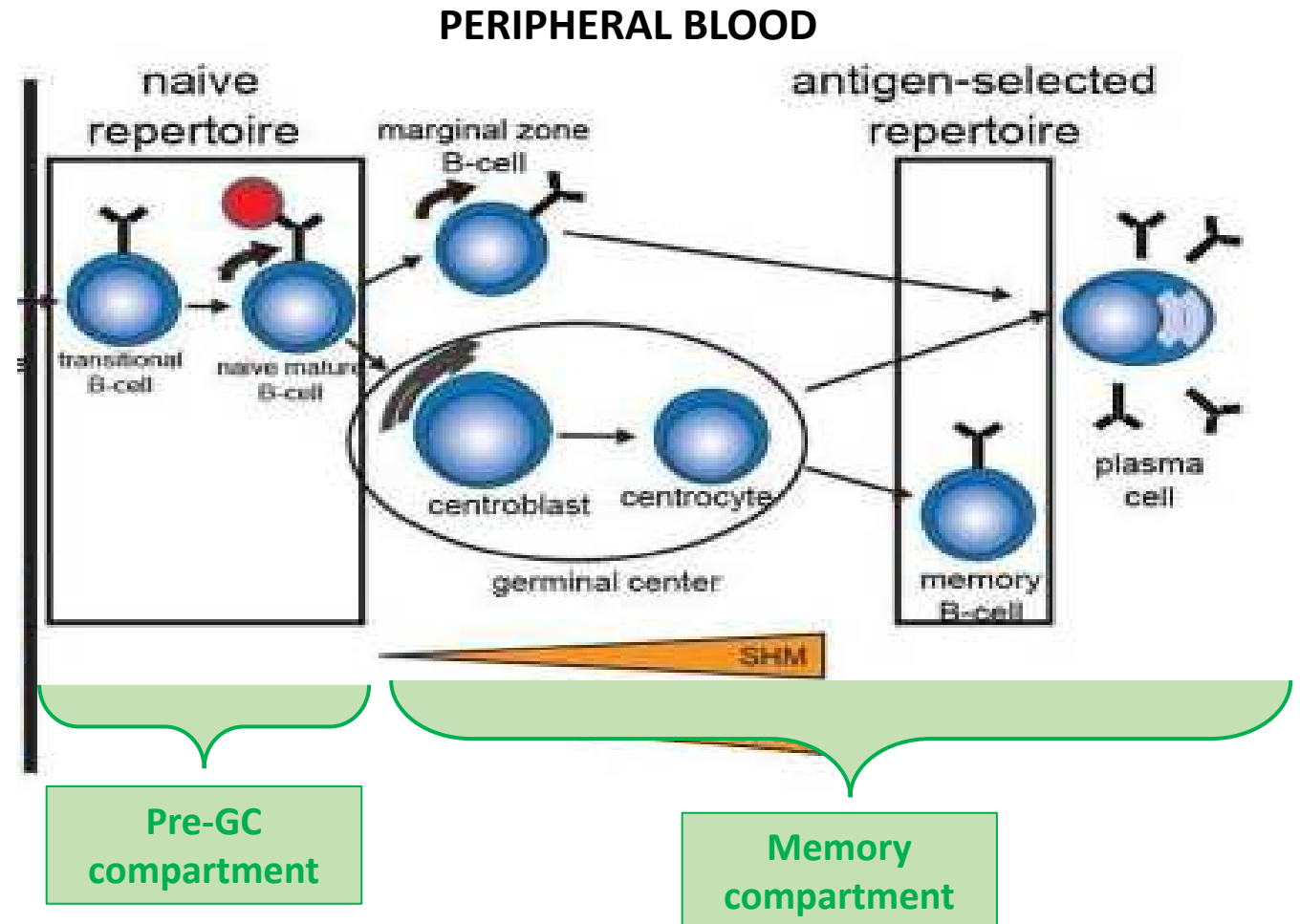


- Immature B-cells (hematogones)
 - **Early**: CD45^{low}/CD34⁺/CD10⁺⁺/CD20⁻
 - **Late**: CD45^{int}/CD34⁻/CD10⁺/CD20[±]
- **Mature** B-cells in PB (10-30%):
CD45^{high}/CD20⁺/CD22⁺/CD34⁻/CD10⁻

Normal cell populations

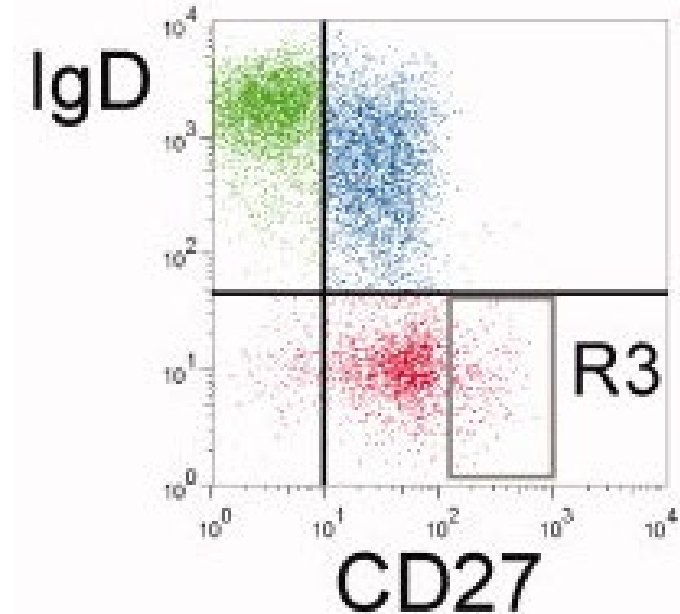
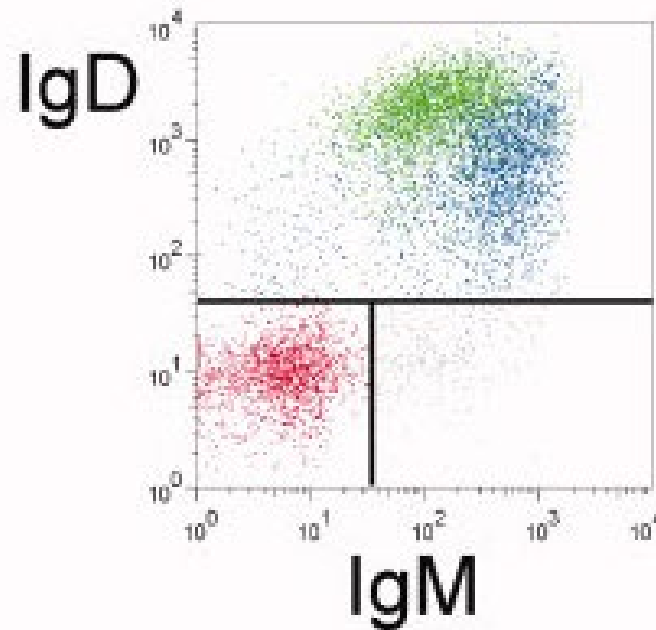
- Mature B-cells: IgD/IgM/CD27/CD38

- Pre-GC (CD19+/CD27-/IgD+/IgM+)
 1. Transitional CD24++/CD38++
 2. Naïve CD24-/CD38-
- Post-GC = memory (CD19+/CD27+)
 3. marginal zone/unswitched IgD+/IgM+
 4. Switched memory IgD-/IgM-
 5. Plasmacells CD38++



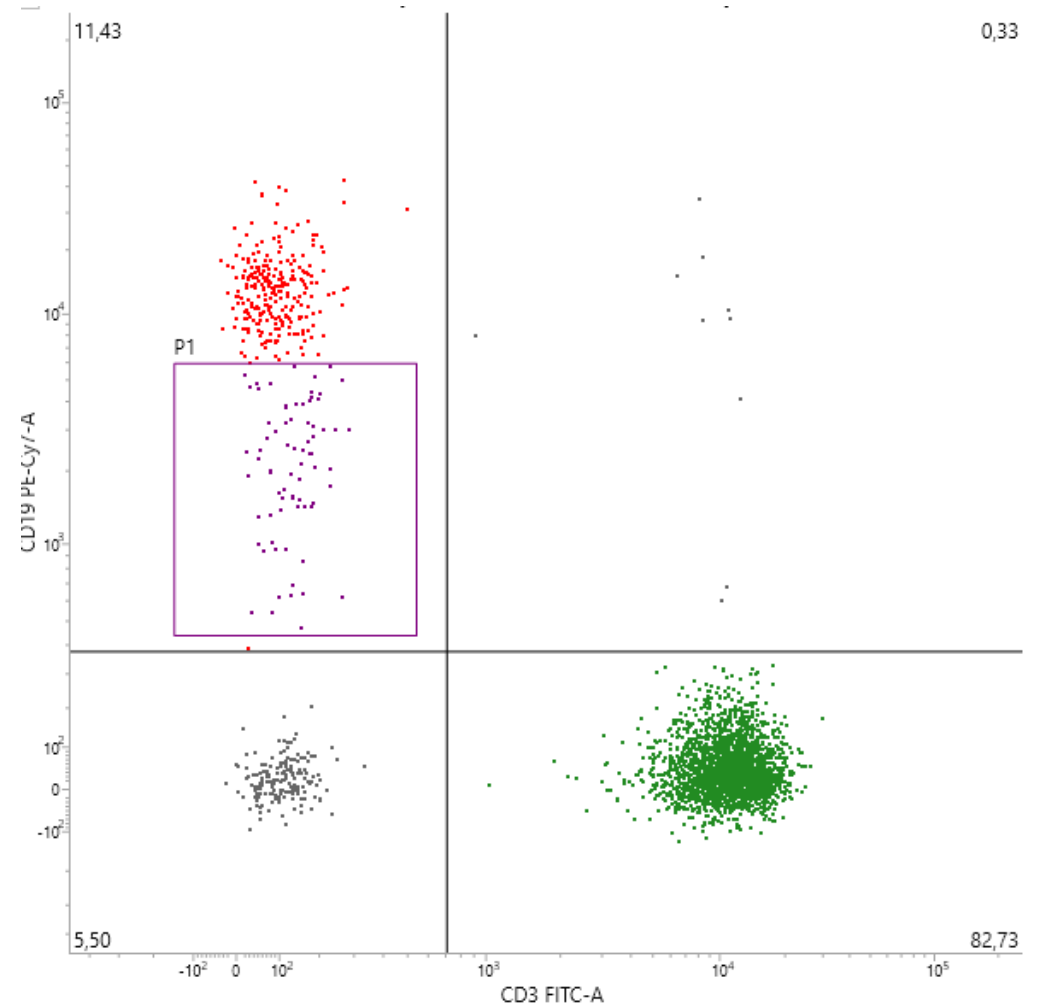
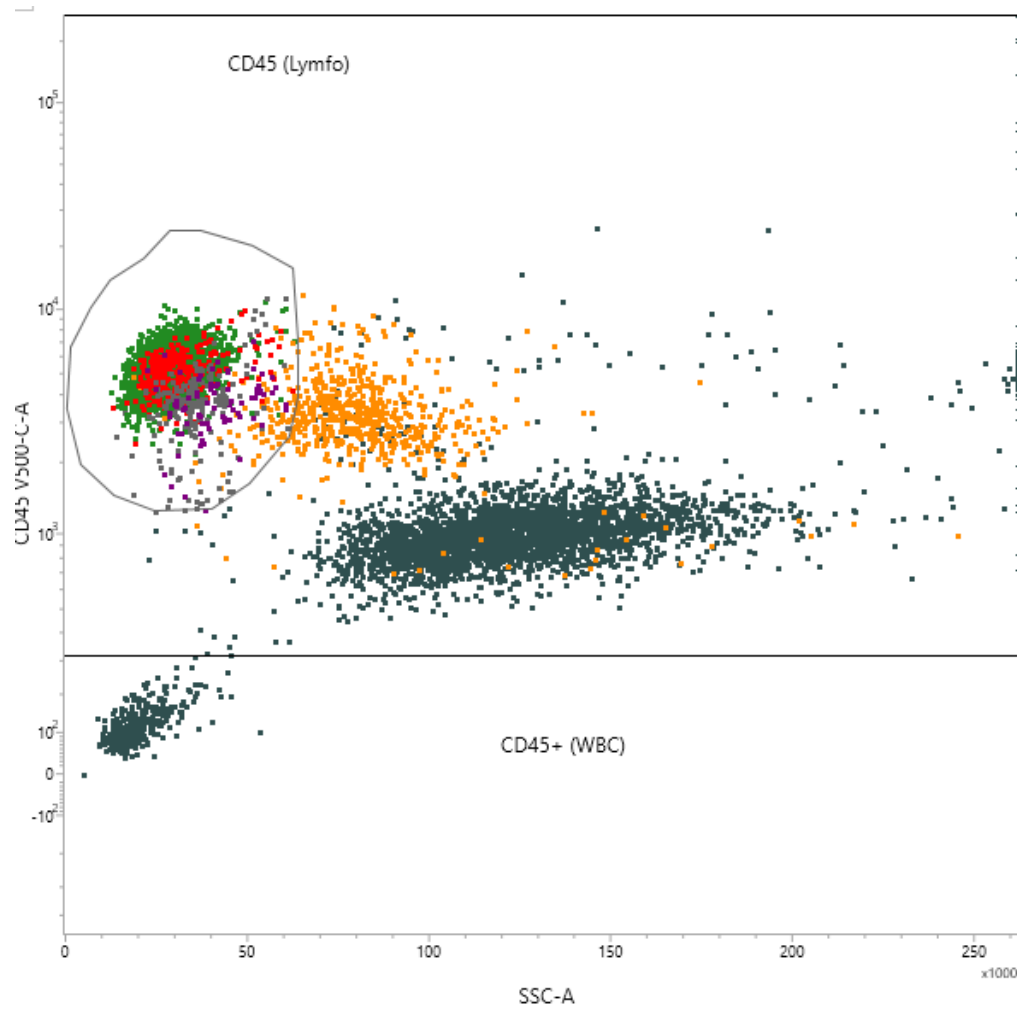
Normal cell populations

- Mature B-cells: IgD/IgM/CD27/CD38
- Pre-GC (CD19+/CD27-/IgD+/IgM+)
 1. Transitional (R4) CD24++/CD38++
 2. Naïve CD24-/CD38-
- Post-GC = memory (CD19+/CD27+)
 3. marginal zone/unswitched IgD+/IgM+
 4. Switched memory IgD-/IgM-
 5. Plasmacells (R5) CD38++



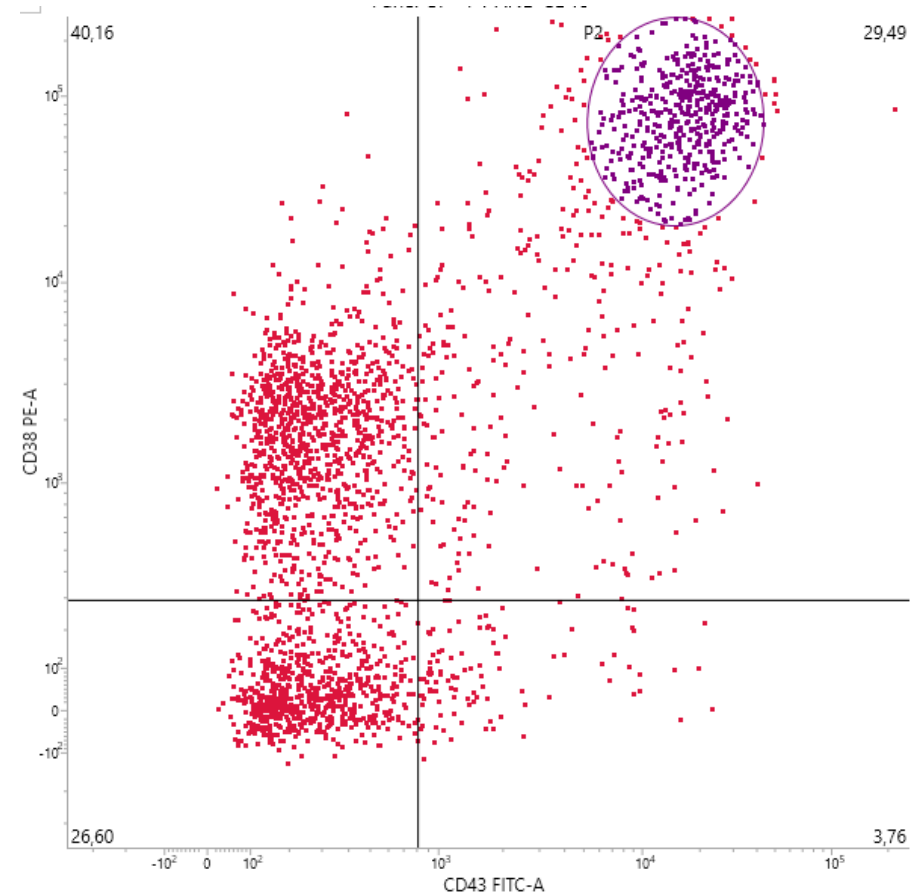
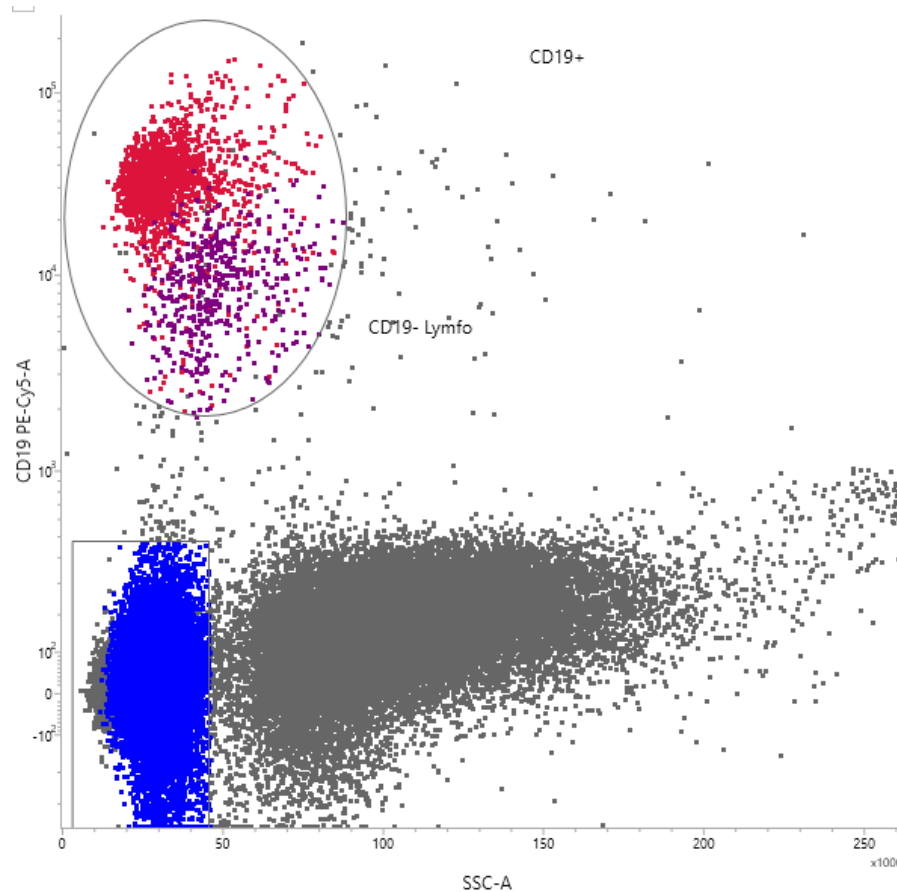
Normal cell populations

- Plasmacells



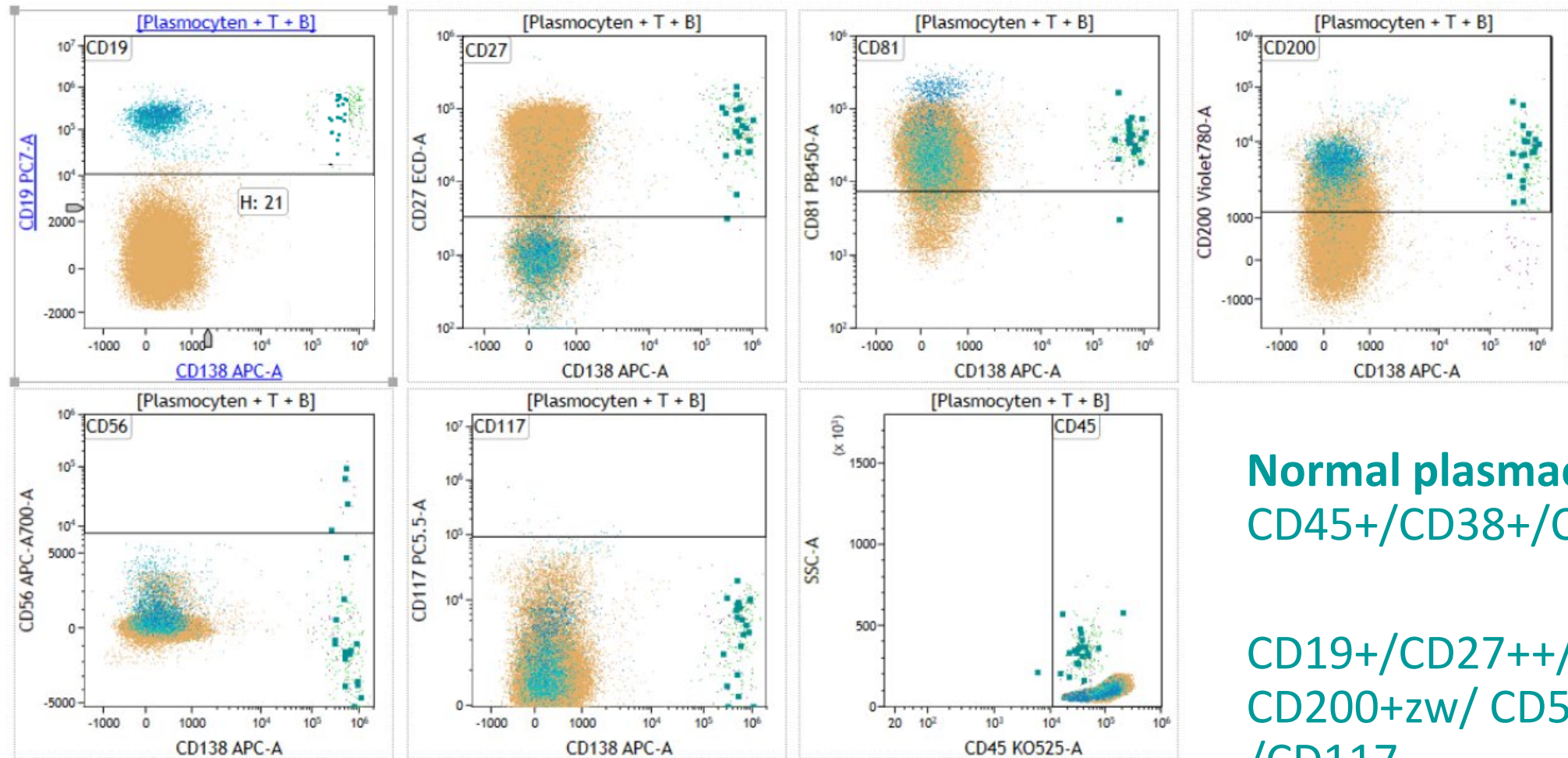
Normal cell populations

- Plasmacells



! CD138 (*SDC1*) decreases rapidly after sampling

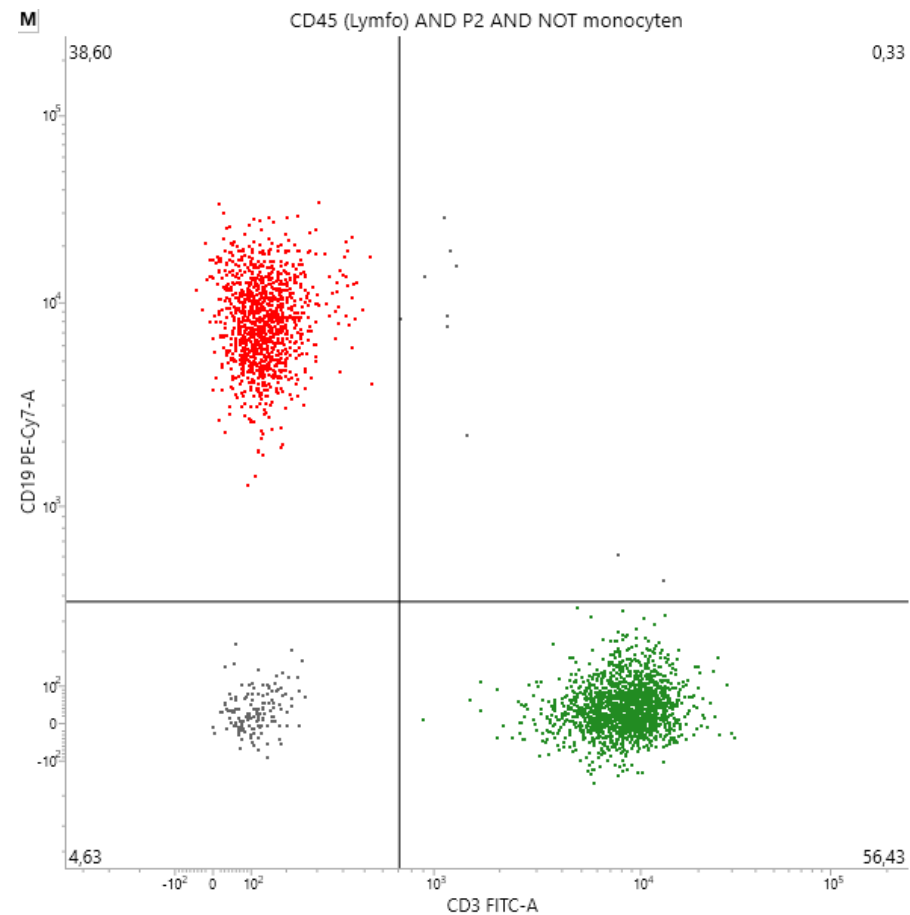
Normal cell populations



Normal plasmacells:
CD45+/CD38+/CD138+
CD19+/CD27++/CD81+/
CD200+zw/ CD56-
/CD117-

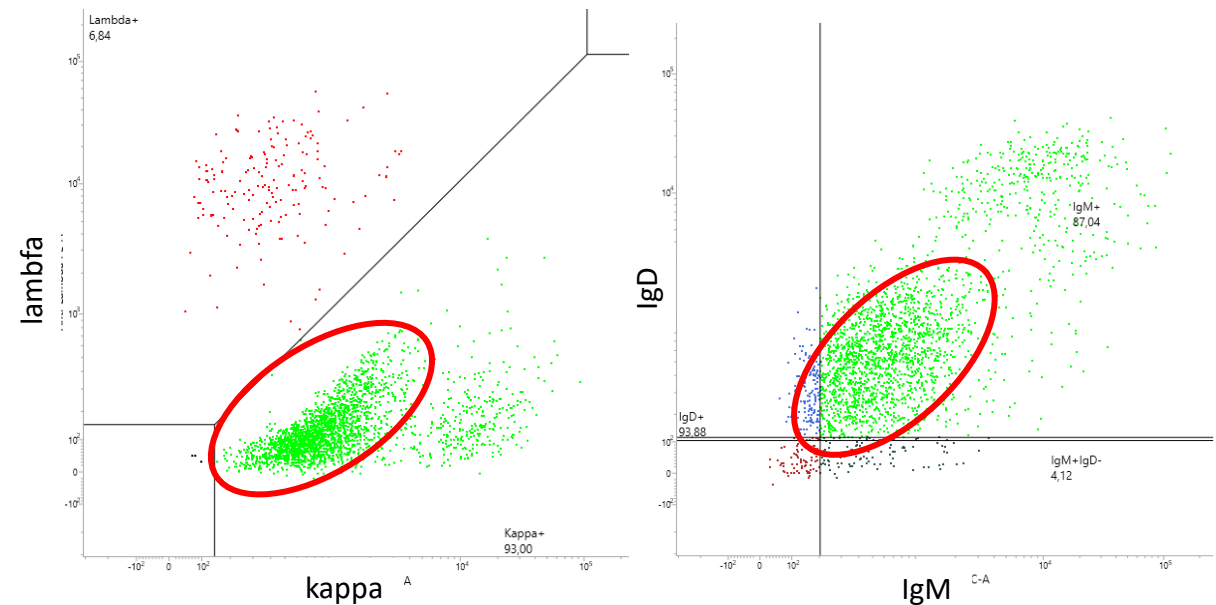
Malignant cell populations

- How to differentiate between normal and neoplastic B cells
 - Relative increased percentage



Malignant cell populations

- How to differentiate between normal and malignant B cells
 - Relative increased percentage
 - Monoclonality
 - Ig light chain class restriction
 - 60-75% kappa
 - 25-40% lambda
 - Cut off k/λ ratio \pm [0.8 2.1]
 - Ig heavy chain class restriction
 - 80-85% IgM and IgD
 - 12% IgG
 - 3-8% IgA



Malignant cell populations

- How to differentiate between normal and malignant B cells
 - Relative increased percentage
 - Monoclonality
 - Aberrant marker expression
 - CD5
 - CD23
 - FMC7
 - CD79b
 - CD43
 - CD81
 - CD200
 - CD38
 - CD138
 - CD25
 - CD11c
 - CD103
 - CD180
 - CD305
 - ROR1
 - CD10
 - CD22
 - CD45

Malignant cell populations

- How to differentiate between normal and malignant B cells
 - Relative increased percentage
 - Monoclonality
 - Aberrant marker expression: normal B-cells
 - CD5-
 - CD23-/weak+
 - FMC7+
 - CD79b+
 - CD43-
 - CD81+
 - CD200+weak
 - CD38-/weak+
 - CD138-
 - CD25-/weak+
 - CD11c-
 - CD103-
 - CD180+
 - CD305
 - (LAIR1)+(weak)
 - ROR1-
 - CD10-
 - CD22+
 - CD45++

	CLL /SLL		B-PLL		HCL		SLVL		FL		MCL		LPL		PLASMA-CYTOOM		DLBCL		BURKITT		MALT		NMZL	
CD5	+	pos 80-100%	±	neg/pos 30%	-	neg	-	neg	±	80%neg	+	pos	-	neg	-	neg	-	neg (110% pos)	-	neg	-	neg	-	neg
CD10	-	neg	-	mstl neg	-	neg	-	neg	+	pos	-	neg	-	neg	±	vaak pos	-	neg 40-70%	+	pos	-	neg	-	neg
CD11c	-	neg (meestal)	+	zwak pos	+	pos	+	pos 50%	-	neg of zz pos	-	neg	-	neg (meestal)	-	neg	-	neg, zz pos	-	neg	±	pos soms	±	soms pos
CD19	+	pos matig	+	pos 80-100%	+	pos zwak	+	pos	+	pos	+	pos	+	pos	-	neg (aberrant)	+	pos	+	pos	+	pos	++	pos sterk
CD20	+	pos zwak	++	pos sterk (60%)	++	pos sterk	+	pos	+	pos	+	pos	+	pos	±	pos (20%)	+	pos	+	pos	+	pos	++	pos sterk
CD22	±	neg/zwak pos (10-40%)	+	pos	++	pos sterk	+	pos	+	pos	++	pos sterk (70%)	±	soms zwak pos	-	neg	++	pos sterk	+	pos	+	pos	++	pos sterk
CD23	+	pos (80-100%)	±	neg/pos 10-20%	±	80% neg	-	neg	+	pos (10-40%)	-	neg meestal	-	neg (meestal)	-	neg	-	neg, zz pos	-	neg	-	neg	±	neg/zwak
CD25	-	neg (meestal)	-	neg	+ -	pos variant neg	±	pos 30%	-	neg	-	neg	+	vaak pos	-	neg	+	pos meestal						
CD38	±	neg/ zwak pos	+	pos 46%	-	neg	-	neg	±	pos (10-40%)	±	pos (52%)	+	vaak pos	+	zwak pos	-	meestal neg	+	pos	-	neg	+	pos
CD43	+	pos	+	meestal pos	-	neg	-	neg	-	neg	±	meestal pos	±	soms zwak pos	±	pos/neg	-	neg	+	pos			-	neg
CD79b	±	neg/zwak pos (10-40%)	+	pos (80-100%)	+ -	pos variant meestal neg	+	pos	+	pos	+	pos	++	pos sterk			+	pos	+	pos	+	pos	++	pos sterk
CD81	-	neg			+	pos			+	pos	+	pos	+	pos	±	pos (aberrant)	+	pos	++	pos sterk			+	pos
CD103	-	neg (meestal)	-	neg	+	pos 2/3 pos bij variant	-	neg	-	neg	-	neg	-	neg (meestal)	-	neg	-	neg	-	neg	-	neg	-	neg
CD138	-	neg	-	neg	-	neg	-	neg	-	neg	-	neg	-	neg	+	pos	-	neg	-	neg	-	neg	-	neg
CD200	++	sterk pos			+	pos	+	pos	-	neg	-	neg	+	pos			±	pos/neg	-	neg			+	pos
LAIR-1	-	neg	-	neg	++	pos sterk	+	pos	-	neg	±	kan pos	+	pos			-	neg	-	neg			-	neg
FMC7	-	neg	+	vaak pos	+	pos	+	pos	+	pos	+	pos	+	pos (meestal)									-	neg
IgM	+	pos zwak	++	sterk pos	++	pos sterk	+	pos	+	pos (samen)	+	matig-sterk	+	meestal (M>G/A)	±	meestal neg	+	50-75% (M>G)	+	matig-sterk	+	zwak pos	+	zwak pos
IgD	+	pos zwak	++	ev. sterk pos	++	pos sterk	+	pos (meestal)	+	pos (samen)	-	mstl neg	-	neg	±	meestal neg	-	meestal neg	-	neg	-	meestal neg	+	zwak pos
HLADR	+	pos (80-100%)	+	pos (80-100%)	+	pos			+	pos	+	pos	+	pos			++	pos sterk	+	pos			+	pos

Malignant cell populations

- How to differentiate between normal and malignant B cells
 - Relative increased percentage
 - Monoclonality
 - Aberrant marker expression
 - CD5
 - CD23
 - FMC7
 - CD79b
 - CD43
 - CD81
 - CD200
 - CD38
 - CD138
 - CD25
 - CD11c
 - CD103
 - CD180
 - CD305
 - ROR1
 - CD10
 - CD22
 - CD45

Example: CLL → ERIC & ESCCA harmonization project Reproducible diagnosis of CLL by flow cytometry

Malignant cell populations

- How to differentiate between normal and malignant B cells
 - Relative increased percentage
 - Monoclonality
 - Aberrant marker expression in CLL
 - CD5+
 - CD23+
 - FMC7
 - CD79b
 - CD43
 - CD81
 - CD200
 - CD38
 - CD138
 - CD25
 - CD11c
 - CD103
 - CD180
 - CD305
 - ROR1
 - CD10
 - CD22
 - CD45

Example: CLL → ERIC & ESCCA harmonization project Reproducible diagnosis of CLL by flow cytometry:
- 4 **required** markers: weak CD20, weak light chain restriction, CD5/CD23

Malignant cell populations

- How to differentiate between normal and malignant B cells
 - Relative increased percentage
 - Monoclonality
 - Aberrant marker expression in CLL
 - CD5+
 - CD23+
 - FMC7
 - CD79b weak+/-
 - CD43+
 - CD81 weak+/-
 - CD200weak+
 - CD38
 - CD138
 - CD25
 - CD11c
 - CD103
 - CD180
 - CD305
 - ROR1+
 - CD10-
 - CD22
 - CD45

Example: CLL → ERIC & ESCCA harmonization project Reproducible diagnosis of CLL by flow cytometry:

- 4 **required** markers: weak CD20, weak light chain restriction, CD5/CD23

-6 **recommended** markers: CD79b/CD43/CD81/CD200/CD10/ROR1

= score /8 or /10

Malignant cell populations

- How to differentiate between normal and malignant B cells
 - Relative increased percentage
 - Monoclonality
 - Aberrant marker expression in CLL
 - CD5+
 - CD23+
 - FMC7-
 - CD79b weak+/-
 - CD43+
 - CD81 weak+/-
 - CD200weak+
 - CD38
 - CD138
 - CD25
 - CD11c
 - CD103
 - CD180
 - CD305
 - ROR1+
 - CD10-
 - CD22
 - CD45

Example: CLL → ERIC & ESCCA harmonization project Reproducible diagnosis of CLL by flow cytometry:

- 4 **required** markers: weak CD20, weak light chain restriction, CD5/CD23

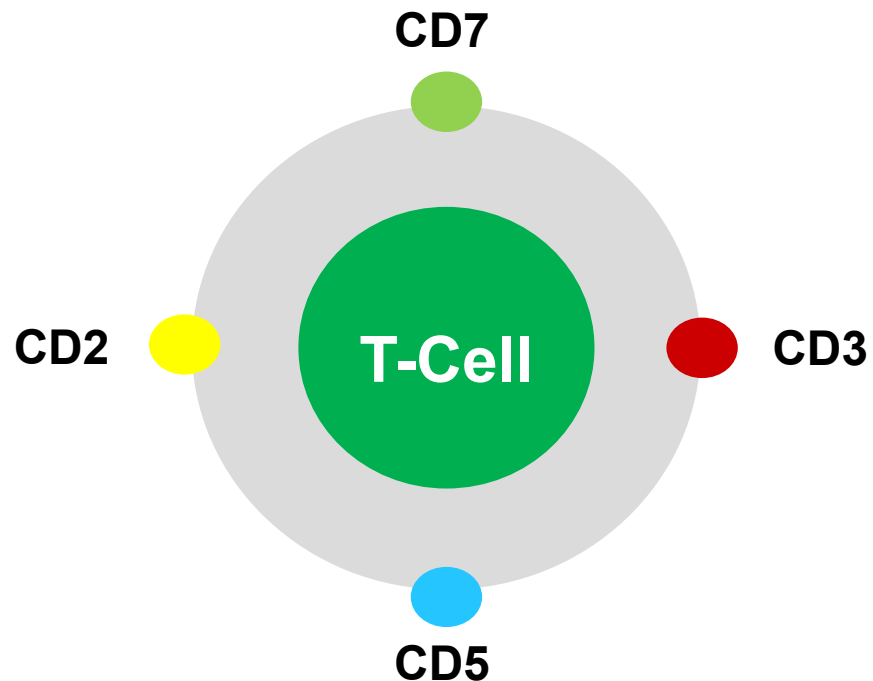
-6 **recommended** markers: CD79b/CD43/CD81/CD200/CD10/ROR1

= score /8 or /10

- Individual lab preference: CD22/FMC7/CD45/CD38

Normal cell populations

- T-cells and NK-cells

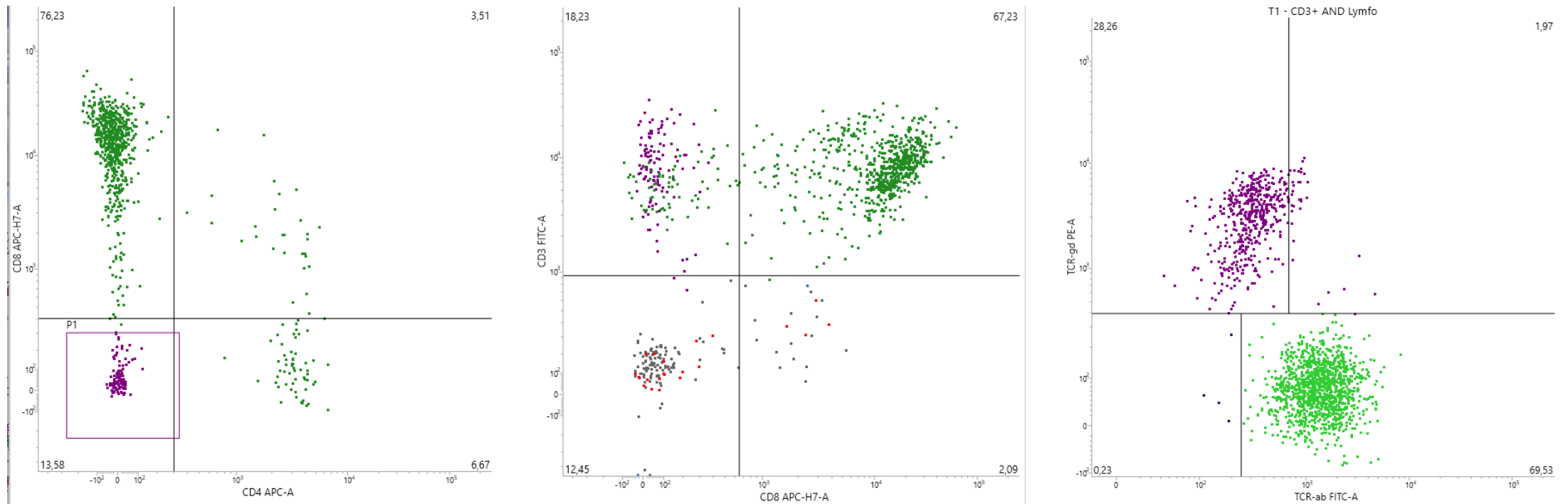


CD3+ T cells (70-90%)

- TCR $\gamma\delta$ + T-cells (5%)
 - CD4-/CD8-
 - Decreased expression of CD5 and CD7
 - Ethnic/genetic and environmental factors
 - Extramedullary tissue

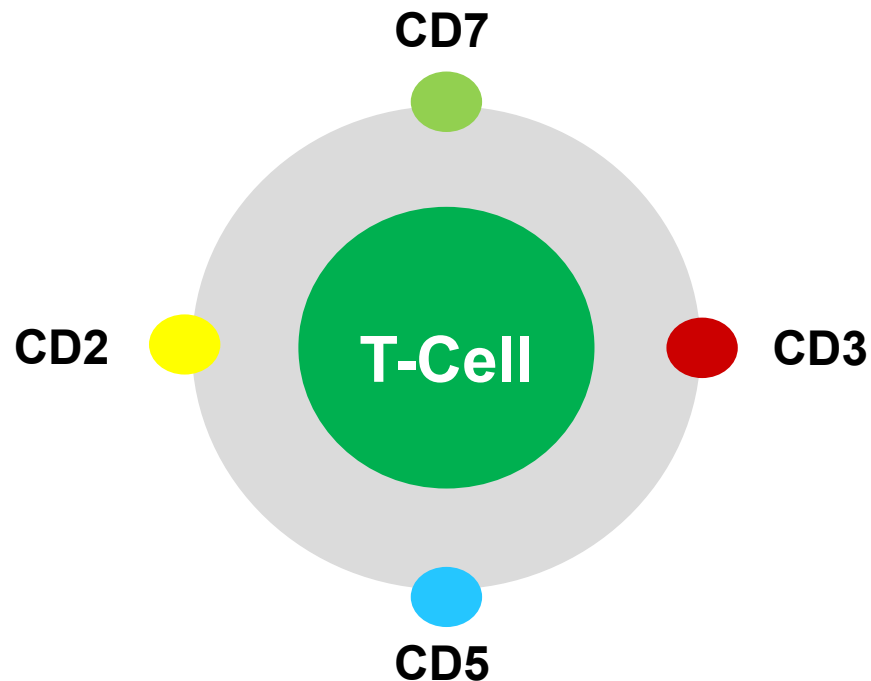
Normal cell populations

- TCR $\gamma\delta$ + T-cells (CD4- and CD8-)



Normal cell populations

- T-cells and NK-cells

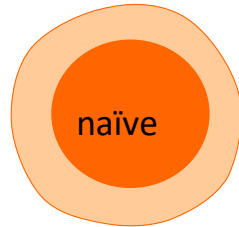


CD3+ T cells (70-90%)

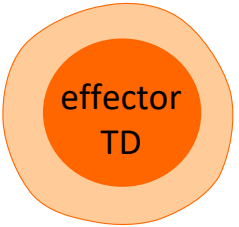
- TCR $\alpha\beta$ + (95%)
 - Helper T-cells: CD4+/CD8-
 - Cytotoxic T-cells: CD4-/CD8+
→ CD4/CD8 ratio (PB): 1.3 – 3.6
 - CD4+/CD8+ double positive: normal thymus, thymic hyperplasia and thymoma (CD3/CD1a) dd (cortical) T-ALL and mature T-cell neoplasms

Normal cell populations

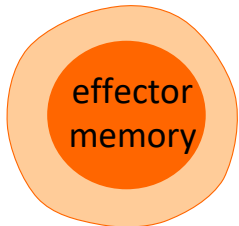
- Naïve and memory T-cells (CD4+ and CD8+)



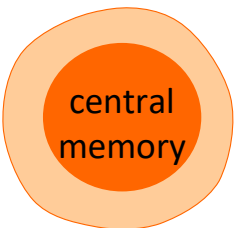
CD45RO-
CD45RA+
CD27+
CD28+



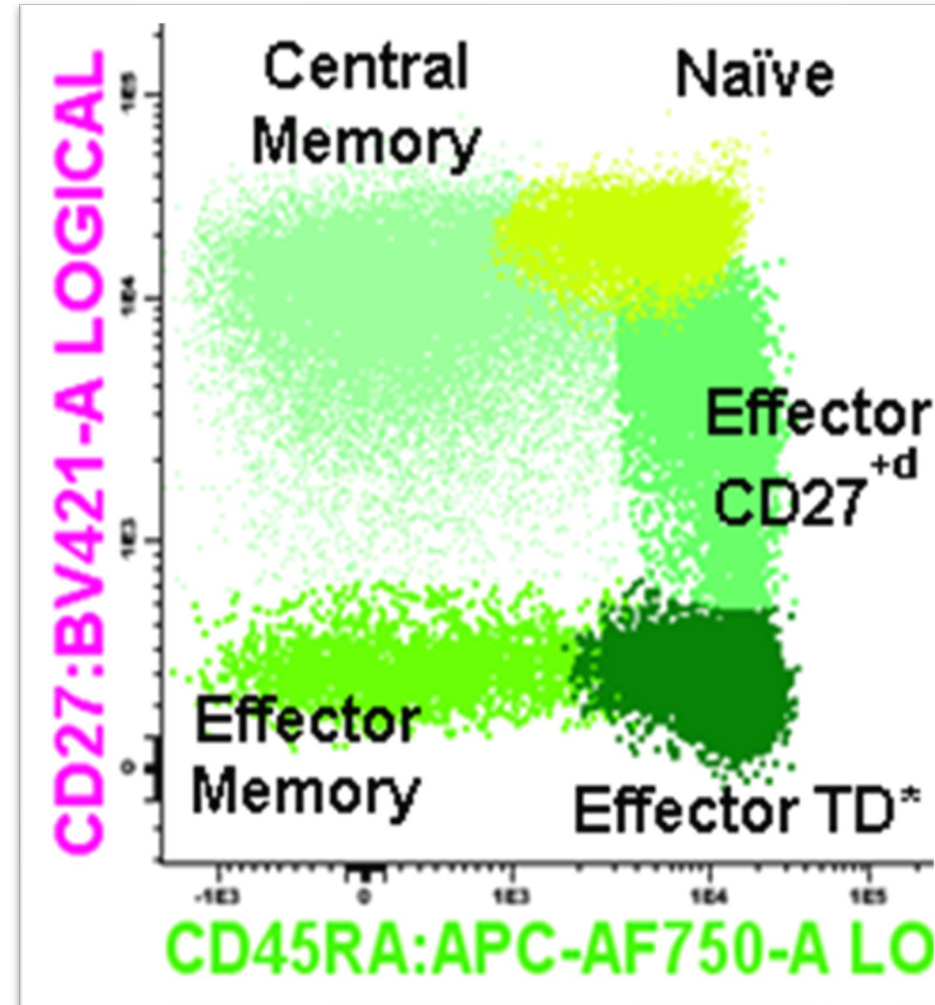
CD45RO-
CD45RA+
CD27-



CD45RO+
CD45RA-
CD27-



CD45RO+
CD45RA-
CD27+
CD28+



Normal cell populations

- T-cells and NK-cells

CD3- NK-cells:

- Most cases: CD5-/CD7+/CD2+/CD94+/CD38+
 - Immature: CD56(bright+)/CD16/CD57(dim+)/NKp46(bright+)
 - Mature: CD56(dim+)/CD16/CD57(bright+)/NKp46(dim+)

CD3+ NK-like T-cellen

- mCD3+/CD5±/CD7+/CD2+
CD4+ >> CD4-CD8- >> CD8+
CD16+/CD56+/CD57+

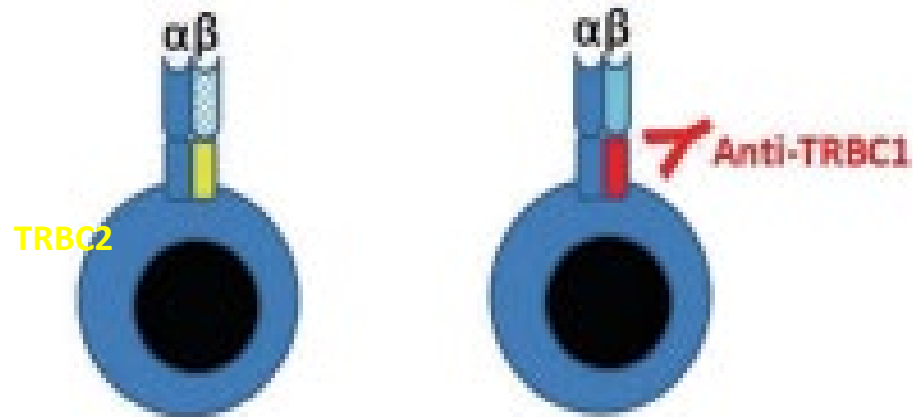
Malignant cell populations

- How to differentiate between normal and neoplastic T- cells
 - CD4/CD8 ratio
 - Shifts
 - Increased double positive or double negative populations
 - ! DD reactive conditions

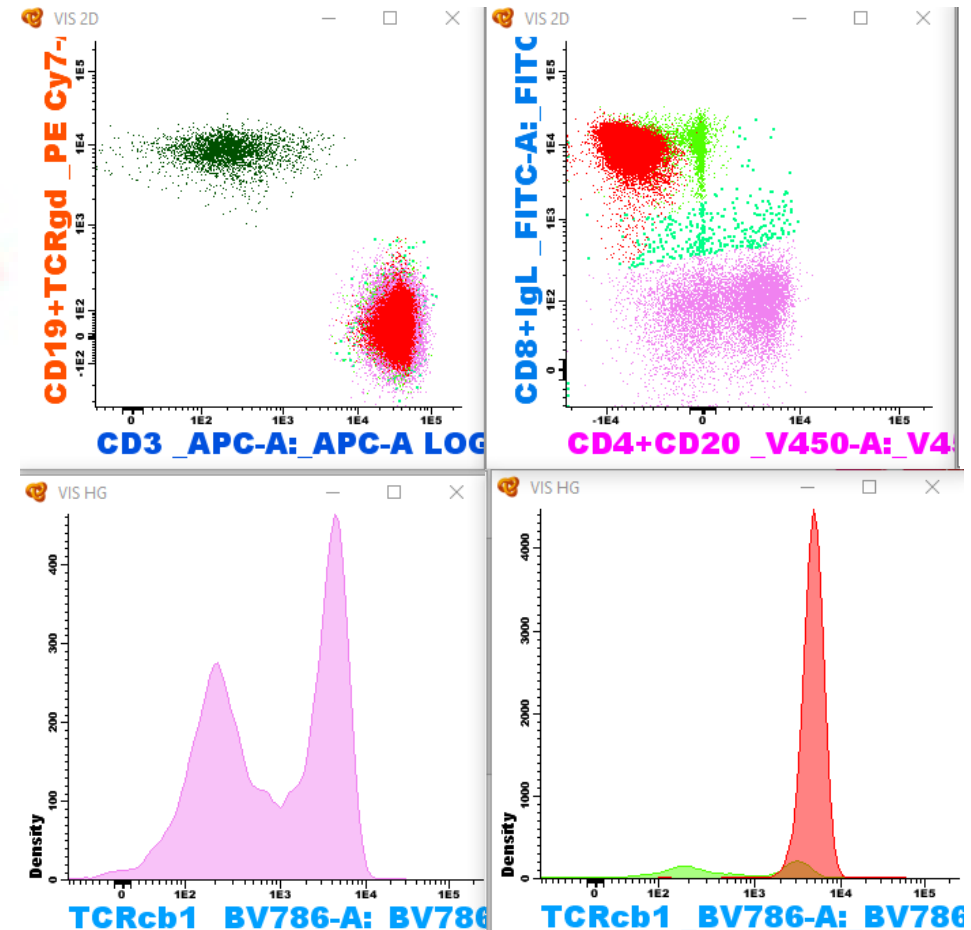
Malignant cell populations

- How to differentiate between normal and malignant T- cells
 - CD4/CD8 ratio
 - JOVI-1 (TRBC1)

Mature TCR $\alpha\beta$ ⁺
T-cells



Flow
cytometry

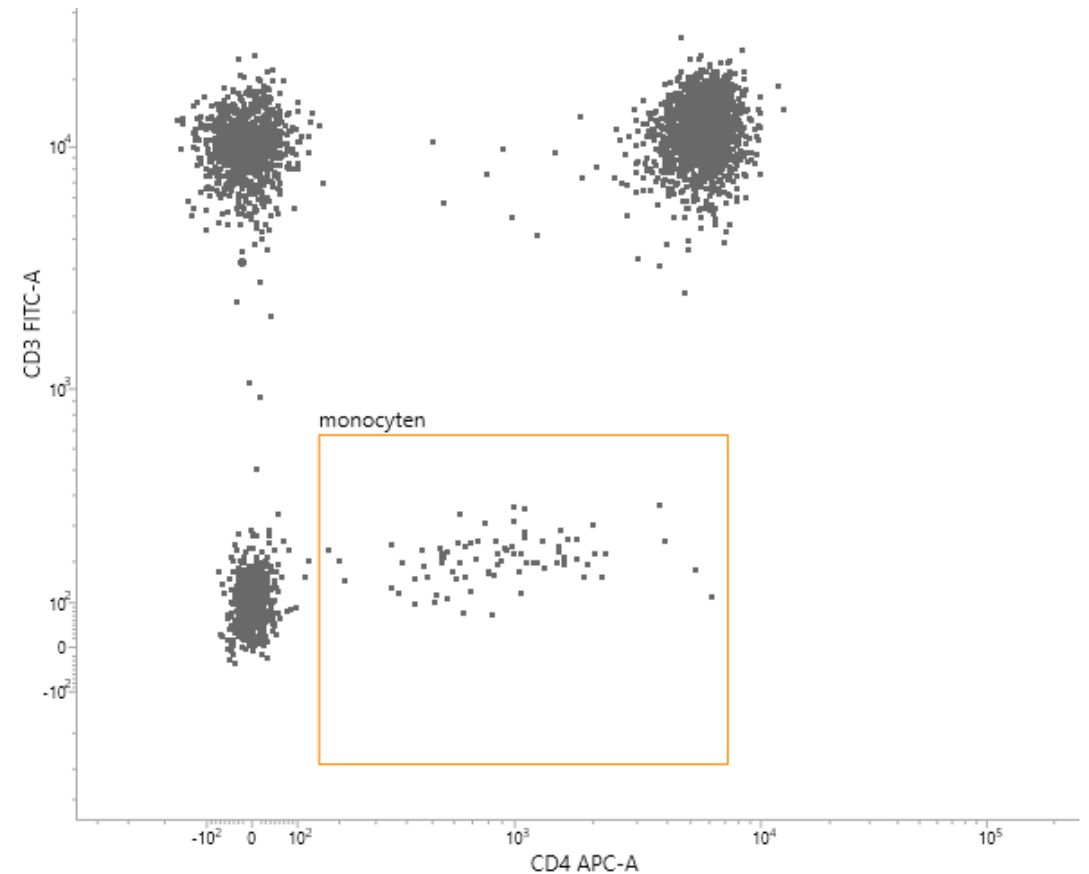
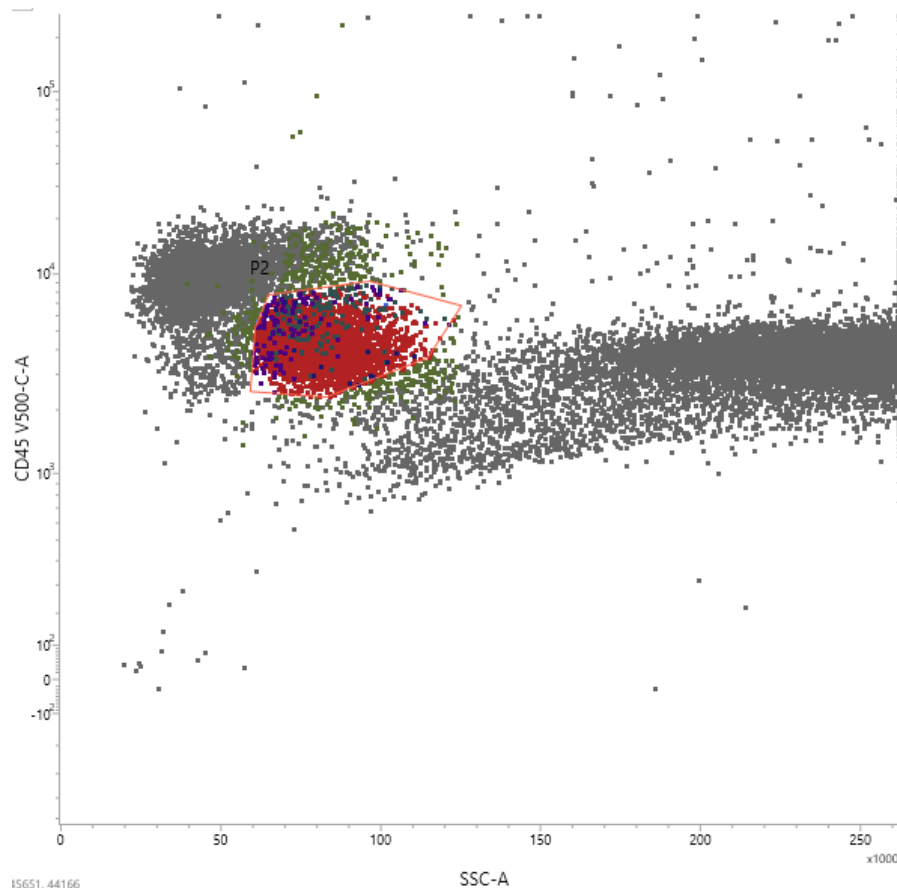


Malignant cell populations

- How to differentiate between normal and malignant T- cells
 - CD4/CD8 ratio
 - JOVI-1
 - Aberrant antigen expression
 - CD7
 - CD2
 - CD5
 - HLA-DR
 - CD26
 - CD16
 - CD56
 - CD57
 - CD52
 - CD10
 - CD1a
 - CD99

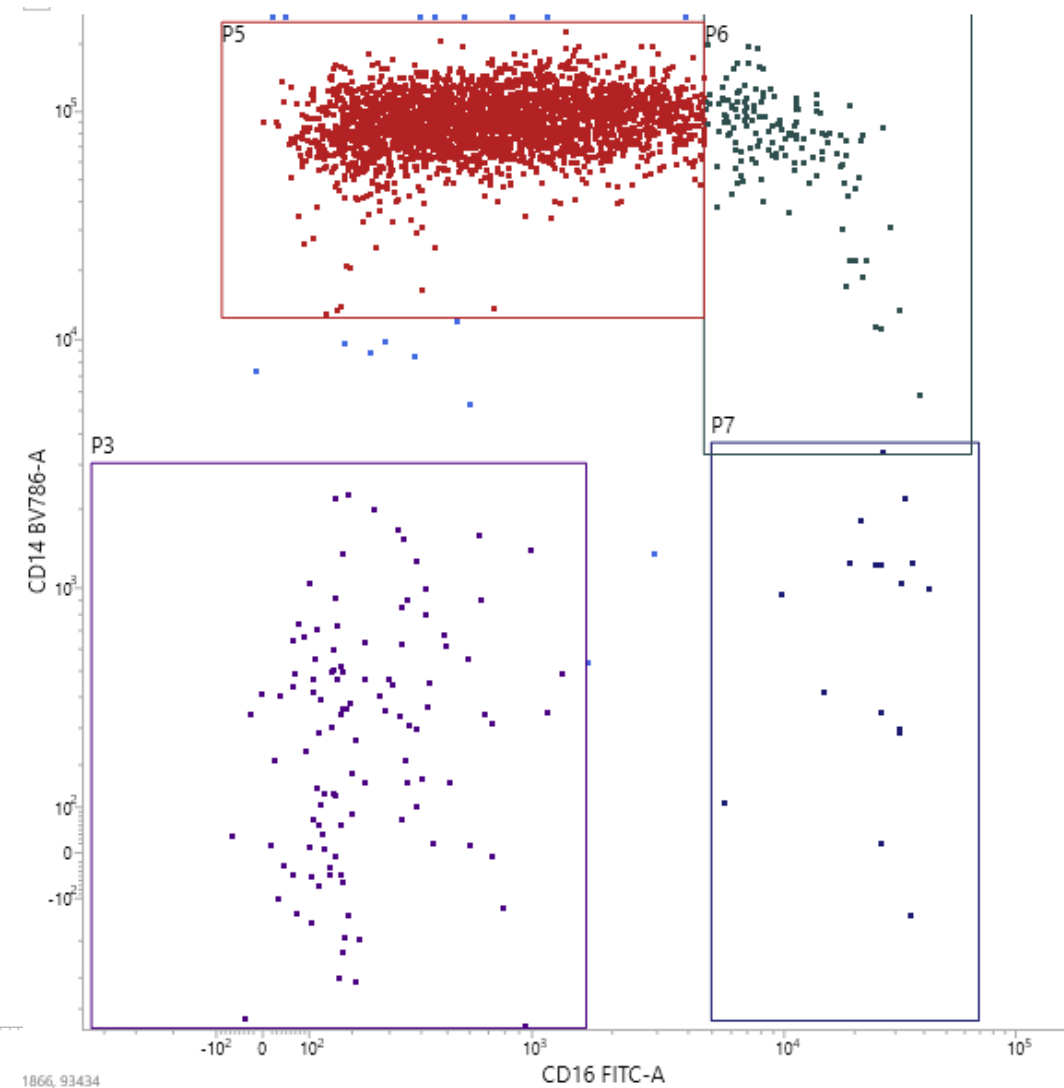
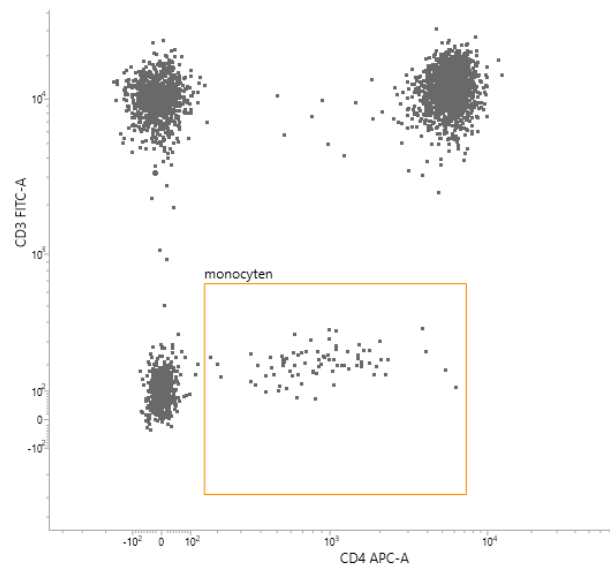
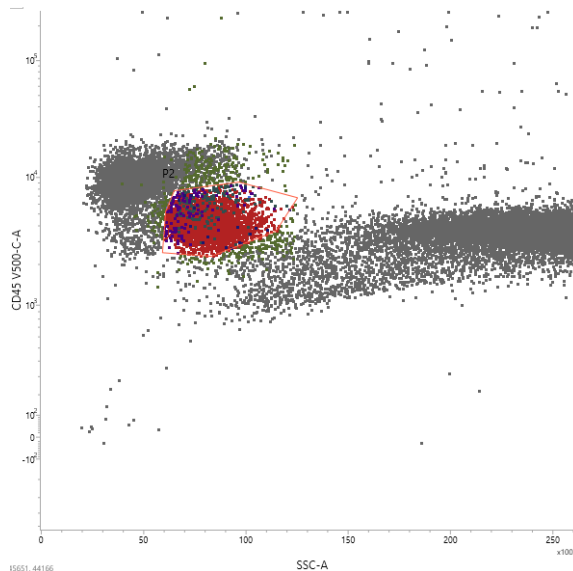
Normal cell populations

- Whole blood: monocyte subset analysis
 - Selection based on CD45/CD4+/CD3-



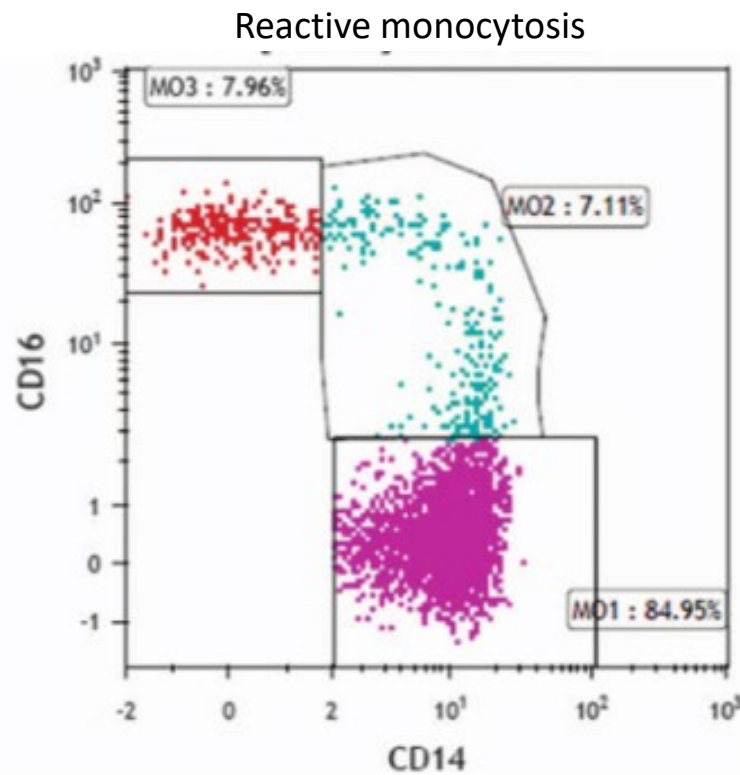
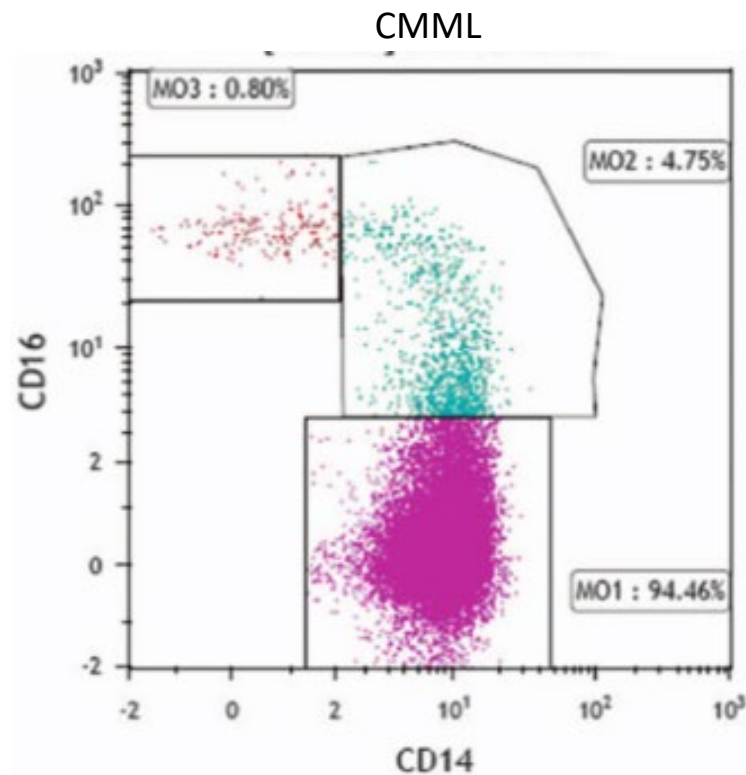
Normal cell populations

- Whole blood: monocyte subset analysis
 - Selection based on CD45/CD4+/CD3-
 - **3 groups** circulating mature monocytic cells (Mukerjee et al 2015)
 1. CD14+/CD16- (classical, 80-85%): cMo
 2. CD14+/CD16+ (intermediate, <15%): iMo
 3. CD14lo/CD16+ (non-classical, <15%): ncMo



Malignant cell populations

- FCM helpful to distinguish **neoplastic** from **reactive** monocytosis
 - **cMo** (CD14+/CD16-) are **increased** in CMML
 - **iMo** (CD14+/CD16+) and **ncMo** (CD14-/CD16+) are **decreased** in CMML



cMO \geq 94%: CMML
(96% (14/15) and 92% (98/107) of patients)

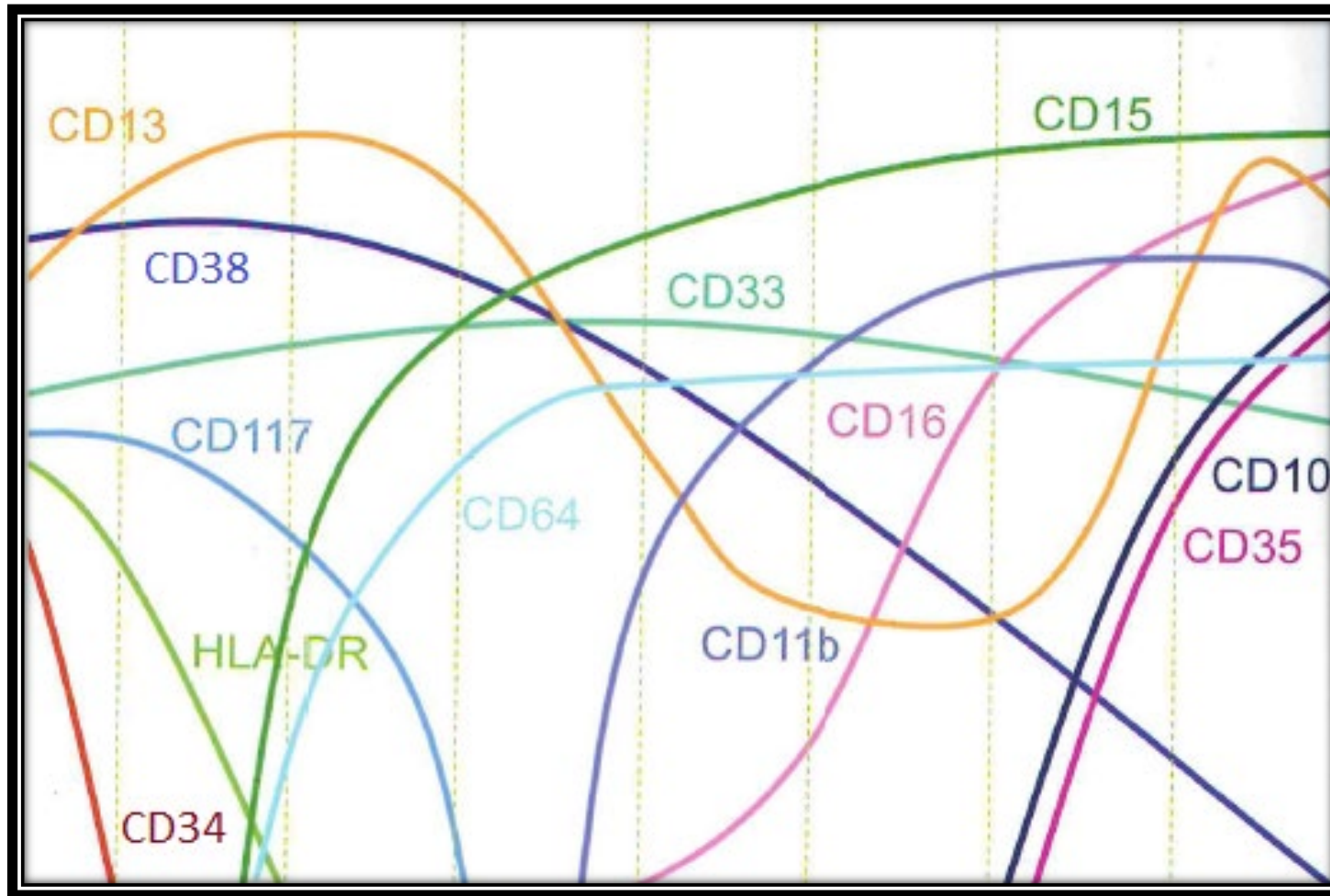
cMO < 92%: other monocytosis

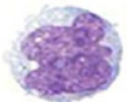




Co-occurrence inflammatory disease (increased iMO)

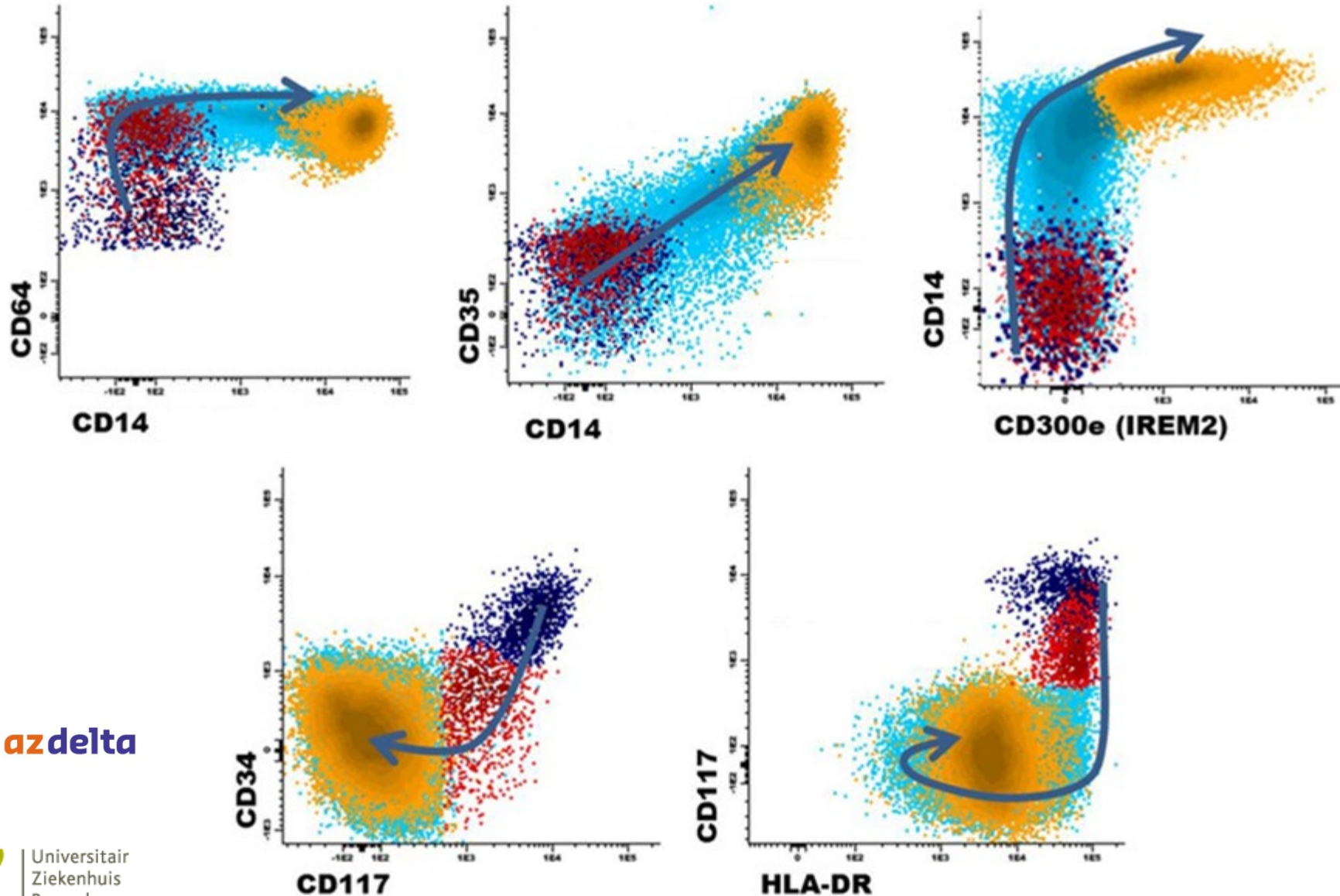
Normal cell populations

- Bone marrow: monocytic compartment



- Monoblasts 
 - Promonocytes 
 - Monocytes 
- Macrophages
→ Dendritic cells

Monocytic development



■ Monocytic precursors
incl. monoblasts

CD34±/CD117+/CD64zw+/ CD14-
/DR+/CD33+

■ Promonocytes
CD34-/CD117-/CD64++/
CD14±/CD300e-/DR+/CD33++

■ Mature monocytes
CD34-/CD117-/CD64++/CD14++/
CD300e+/DR+/CD33++

Monocytic development

CD marker	Monoblast	Promonocyte	Monocyte
CD4	++	++	++
CD11b	-	++	+++
CD11c			
CD13	+	+ / ++	++ / +++
CD14	-	+ / -	+++
CD15	-	++	+ / ++
CD16	-	-	- / +
CD33	+++	+++	+++
CD34	+ / -	-	-
CD35	-	+ / -	+
CD36	-	++	+++
CD45	+	++	+++
CD64	+++	+++	+++
CD117	+	+ / -	-
CD300e	-	-	+
HLA-DR	+++	+++	++ / +++

Immunohenotypic changes

~ maturation

~ activation

Monocytic development

CD marker	Monoblast	Promonocyte	Monocyte
CD4	++	++	++
CD11b	-	++	+++
CD11c			
CD13	+	+ / ++	++ / +++
CD14	-	+ / -	+++
CD15	-	++	+ / ++
CD16	-	-	- / +
CD33	+++	+++	+++
CD34	+ / -	-	-
CD35	-	+ / -	+
CD36	-	++	+++
CD45	+	++	+++
CD64	+++	+++	+++
CD117	+	+ / -	-
CD300e	-	-	+
HLA-DR	+++	+++	++ / +++

Some anti-CD14 clones only recognize mature monocytic cells, while others recognize promonocytes and mature monocytes.

*Neoplastic monoblasts or promonocytes may not always be CD14 negative
!correlation with morphology*

ncMo have decreased expression of CD14 should not be confused with immature monocytic cells.

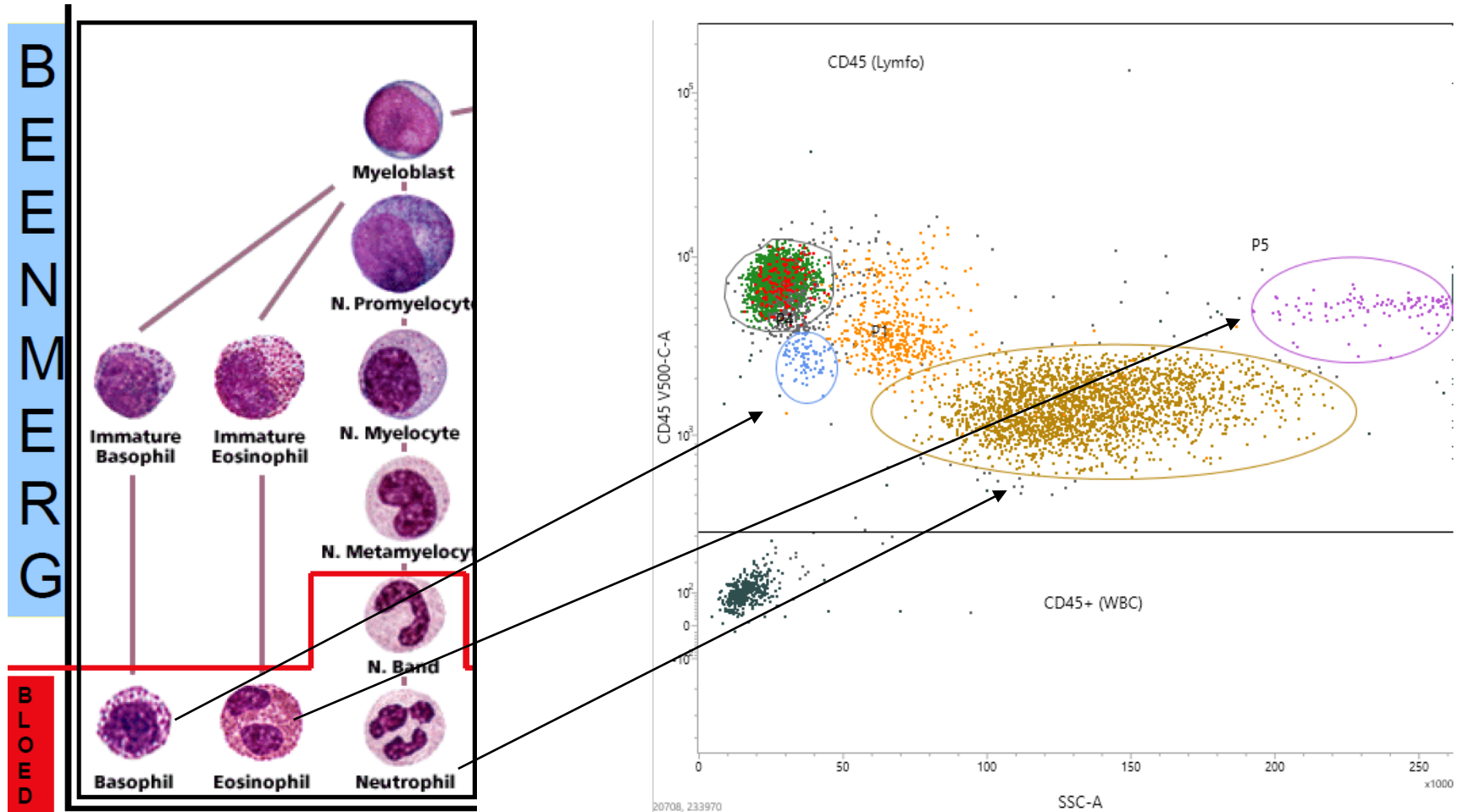
Monocytic development

CD marker	Monoblast	Promonocyte	Monocyte
CD4	++	++	++
CD11b	-	++	+++
CD11c			
CD13	+	+ / ++	++ / +++
CD14	-	+ / -	+++
CD15	-	++	+ / ++
CD16	-	-	- / +
CD33	+++	+++	+++
CD34	+ / -	-	-
CD35	-	+ / -	+
CD36	-	++	+++
CD45	+	++	+++
CD64	+++	+++	+++
CD117	+	+ / -	-
CD300e	-	-	+
HLA-DR	+++	+++	++ / +++

Activation influences the level of CD15 and HLA-DR expression

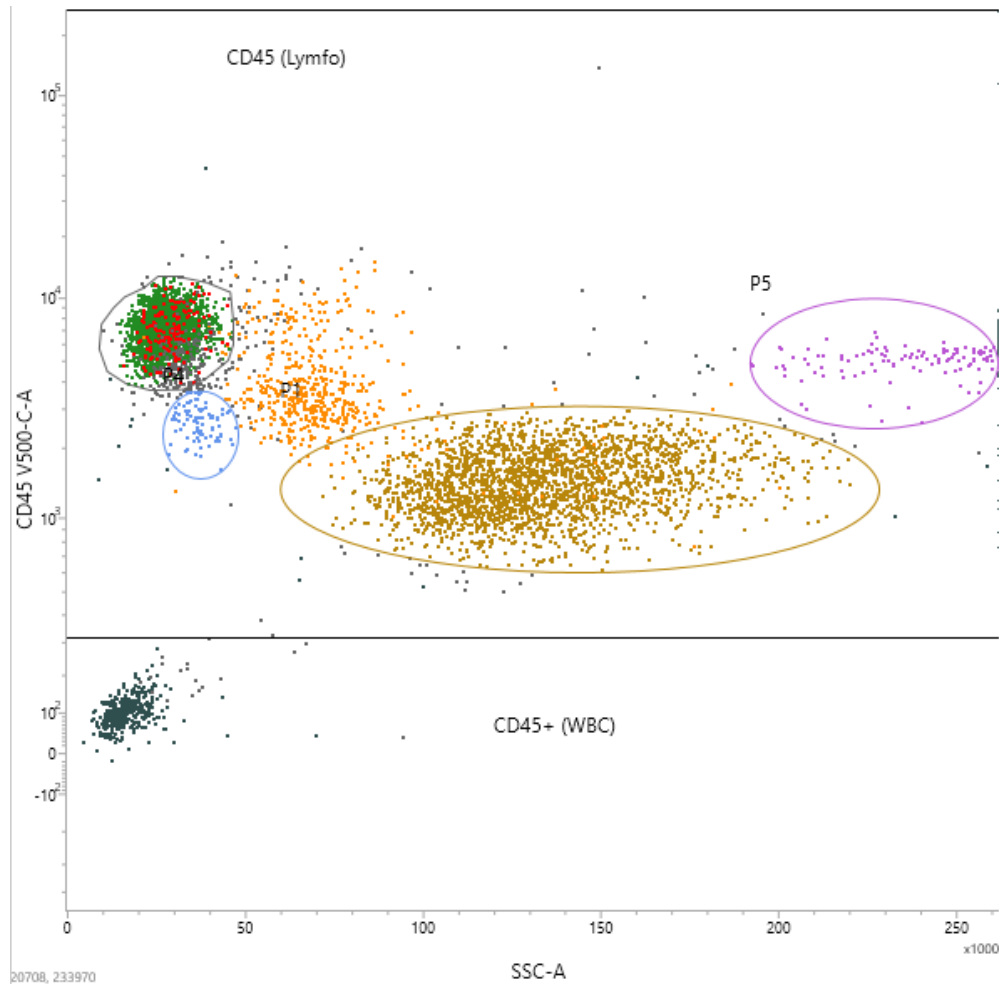
Normal cell populations

- Peripheral blood: granulocytic/myeloid compartment



Normal cell populations

- Peripheral blood: granulocytic/myeloid compartment



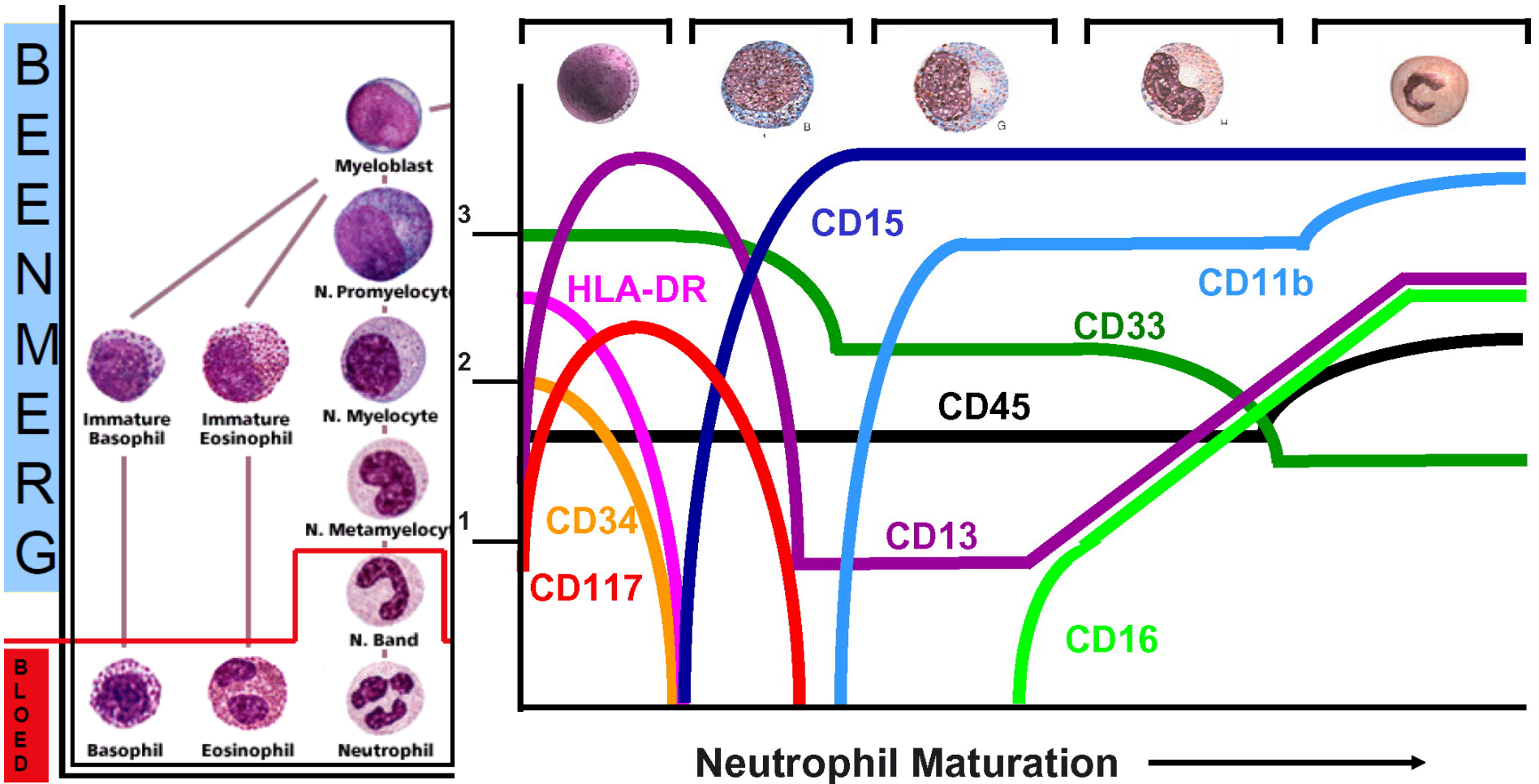
Basophils: CD45^{int}/**CD123++**/HLA-DR-
/CD9+/CD22+/CD25+/CD13+/CD33+/CD36+/CD38+

Eosinophils: **SSC^{high}**/FSC^{low}/CD45++/CD14-/**CD16-**/HLA-DR-
/CD11b+/CD11c+/CD13+/**CD15+**/CD33+

Granulocytes:
CD11b+/CD11c+/CD13++/CD15+/CD33+/**CD16+**/**CD10+**

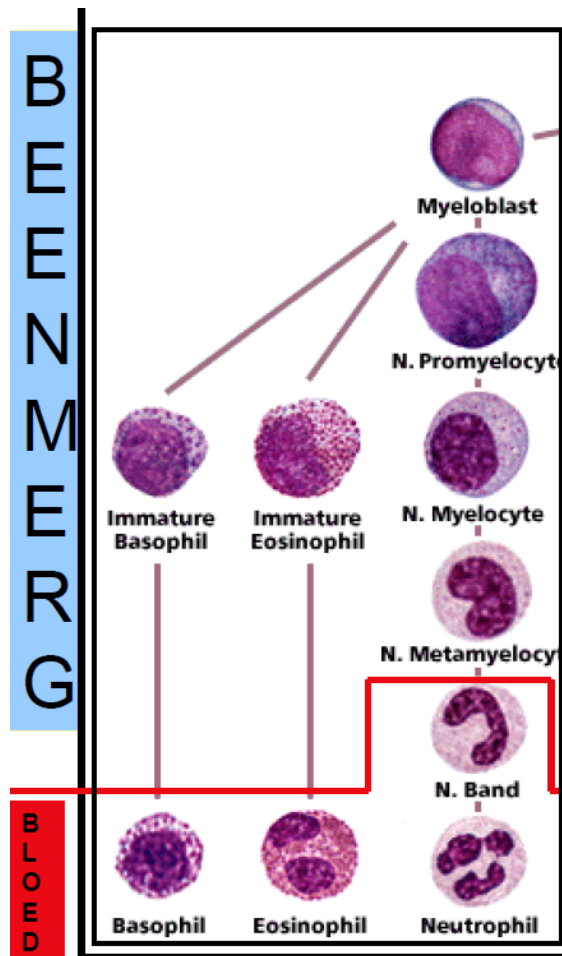
Normal cell populations

- Bone marrow: granulocytic/myeloid compartment



Normal cell populations

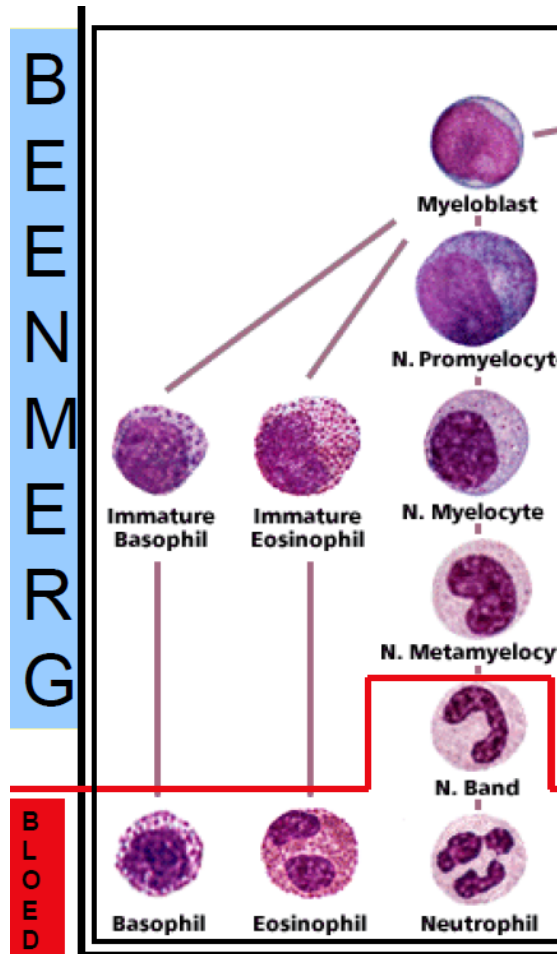
- Bone marrow : granulocytic/myeloid compartment



- HSC/LSC:
SSC^{low}/CD45moderate/CD34+/CD38-
- **Blast window = intermediate CD45 and low SSC**
 - CD34+ (myeloblasts)
 - CD34- (monoblasts and megakaryoblasts)
- Myeloblasts:
SSC^{low}/CD45moderate/CD34+/CD38+/CD117+/CD13+/HLA-DR+/CD33+/TdT±
 - Monocytoid: CD36/CD64/CD14/CD33
 - Erytroid: CD36/CD71
 - Megakaryocytic: CD36/CD9/CD41/CD64

Normal cell populations

- Bone marrow : granulocytic/myeloid compartment

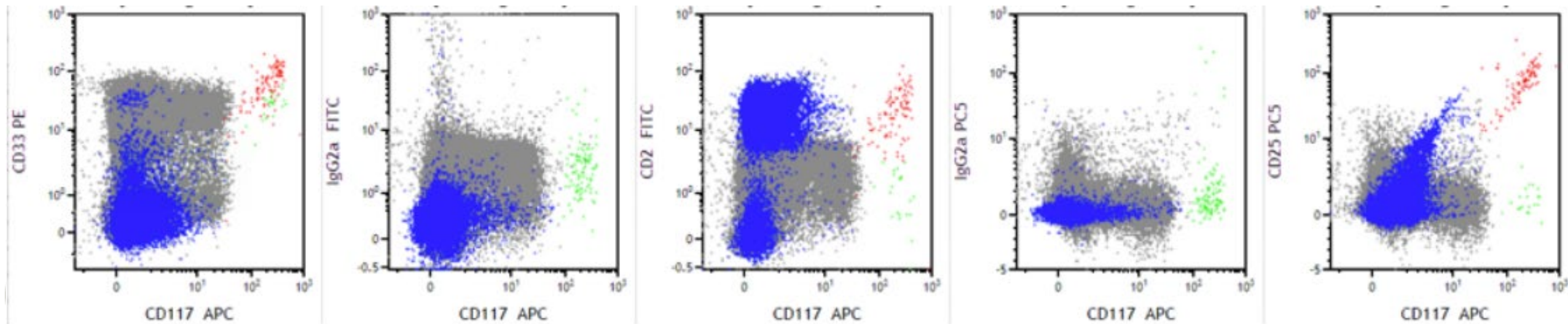


- Promyelocytes:
SSC^{high}/CD34-/CD117+/CD13++/CD33+/HLA-DR-/CD15-/CD16-/CD11b-/CD11c-
- Myelocytes:
SSC^{high}/CD34-/CD117-/CD13+/CD33+/HLA-DR-/CD16-/CD15+/CD11b±/CD11c±
- Metamyelocytes:
SSC^{high}/CD34-/CD117-/CD13+/CD33+/HLA-DR-/CD16+/CD15+/CD11b±/CD11c±

Normal cell populations

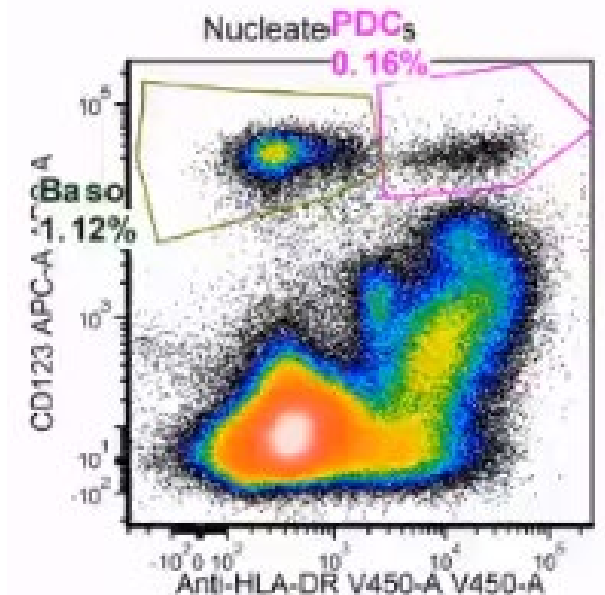
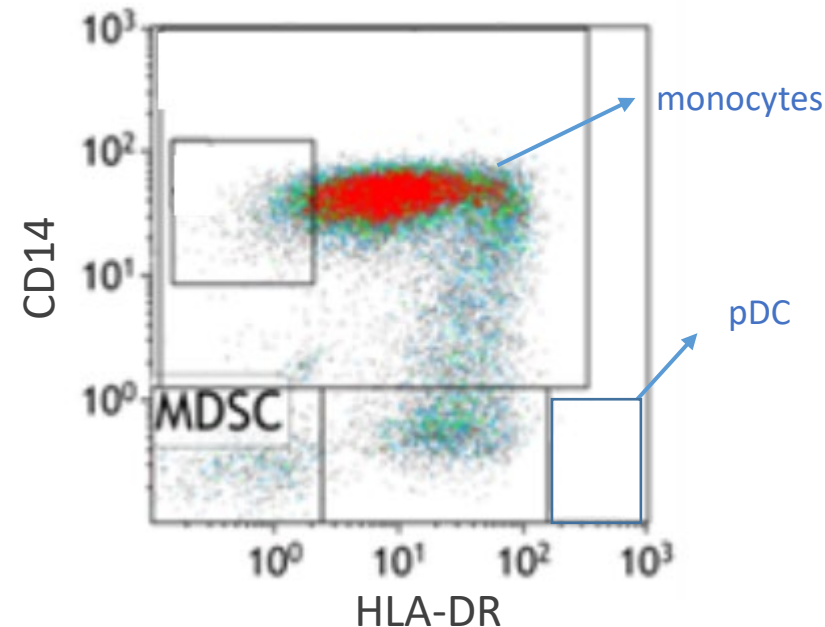
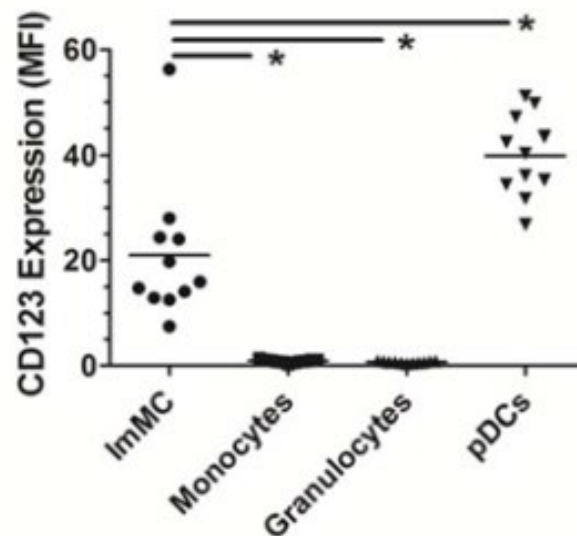
- Bone marrow : granulocytic/myeloid compartment
- **Mast cells**
 - <1% BM
 - SSC^{high}/FSC^{low}/CD45⁺⁺ (~eosinophils)
 - **CD117⁺⁺/CD33⁺/CD9⁺/CD71⁺/CD11b^{low}/HLA-DR^{low}**
 - Systemic mastocytosis: aberrant expression of **CD25**, with or without **CD2**

■ Normal mast cells
■ Abnormal mast cells



Normal cell populations

- Bone marrow : granulocytic/myeloid compartment
 - **Plasmacytoid dendritic cells (pDC):** CD45+/CD14-/CD13+/CD33+/CD11c+/HLA-DR++/CD4+/CD45RA+/CD36+/CD16-/CD123++
 - BM: 0.082% ± 0.025%
 - PB ± 0,65%
 - Malignant: CD56 expression



Zhang et al (2019), Matarraz, S et al. (2017)

Thank you for your attention!

