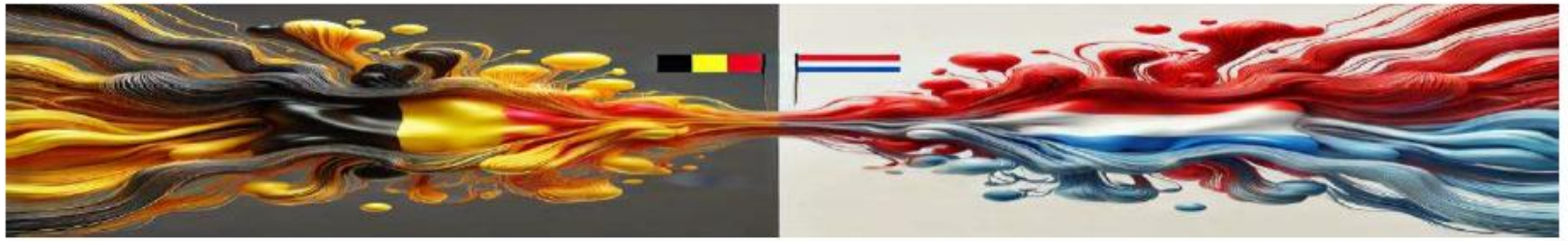
BSAC
Belgian Society for Advancement of Cytometry

NVC
CYTOMETRIE.NL
NEDERLANDSE VERENIGING VOOR CYTOMETRIE



"Flow across borders"

CytHem LMMC

Flow cytometry analysis of CMML: monocyte partitioning & slan marker

Orianne WAGNER-BALLON

AP-HP. Hôpitaux universitaires Henri-Mondor

UPEC UNIVERSITÉ PARIS-EST CRÉTEIL | FACULTÉ DE SANTÉ

IMRB INSTITUT MONDOR DE RECHERCHE BIOMÉDICALE

March, 11th 2026

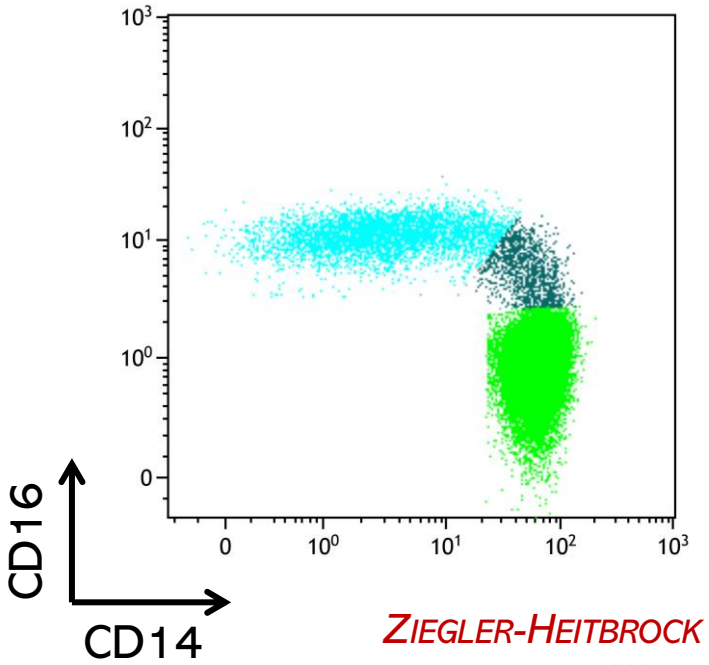
CONFLICT OF INTEREST

	No, nothing to disclose
✓	Yes, as specified below:

Company Name	Specification
Alexion Pharmaceuticals France	Board, Conferences, Research funding
Alexion Pharmaceuticals Spain	Conference
Novartis	Board, Conferences, Research funding
Sobi	Research funding

Monocyte partitioning: the Monocyte assay

Relative quantification of the monocyte subsets in the peripheral blood



Ziegler-Heitbrock et al, Blood 2010
Wong et al, Blood 2011

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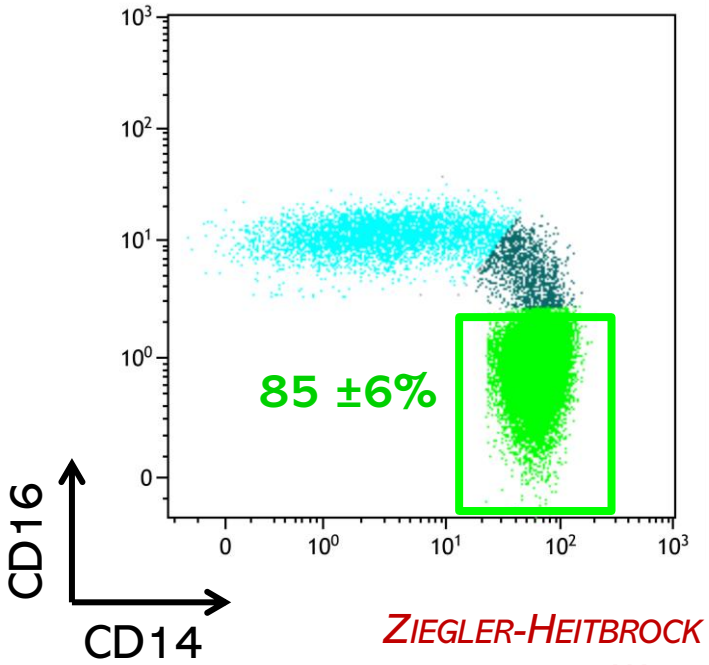

cMo
Classical monocytes
$CD14^{++}CD16^{-}$

Ziegler-Heitbrock et al, Blood 2010
Wong et al, Blood 2011

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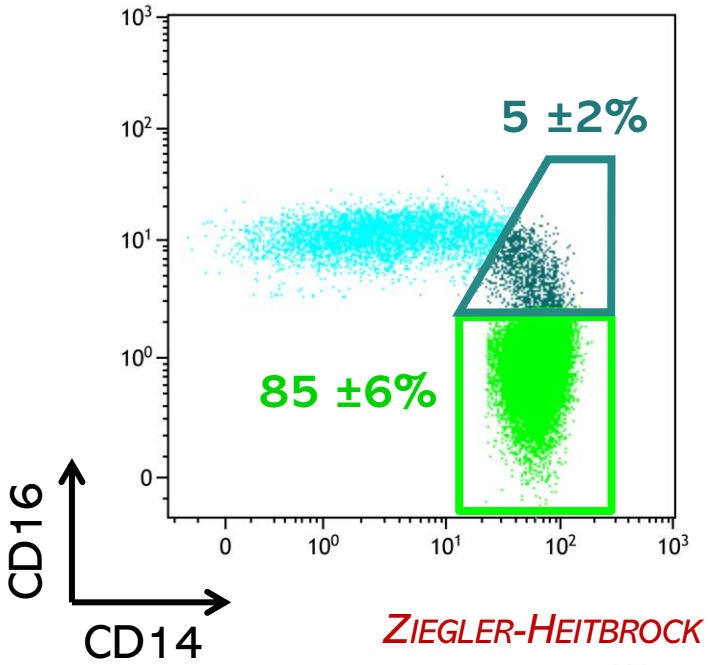
Relative quantification of the monocyte subsets in the peripheral blood



iMo
Intermediate monocytes
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Monocyte partitioning: the Monocyte assay

Relative quantification of the monocyte subsets in the peripheral blood

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Non classical monocytes
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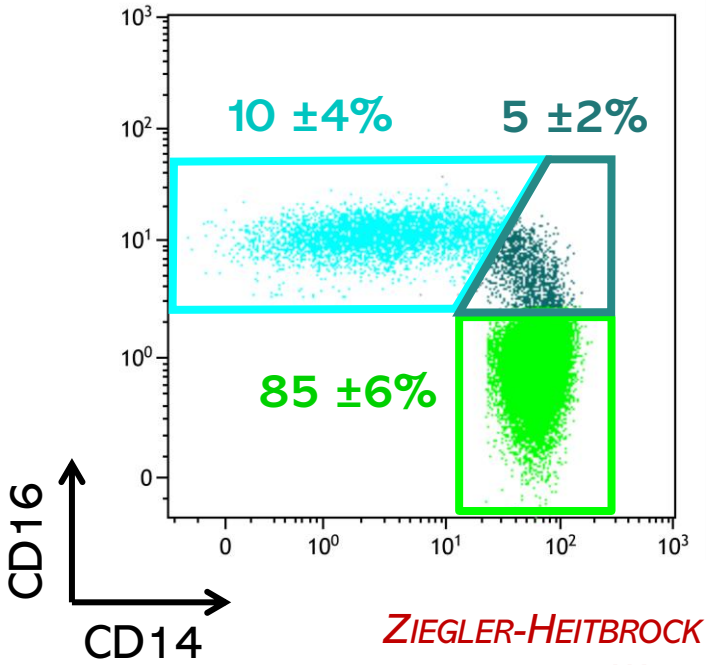

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Ziegler-Heitbrock et al, Blood 2010
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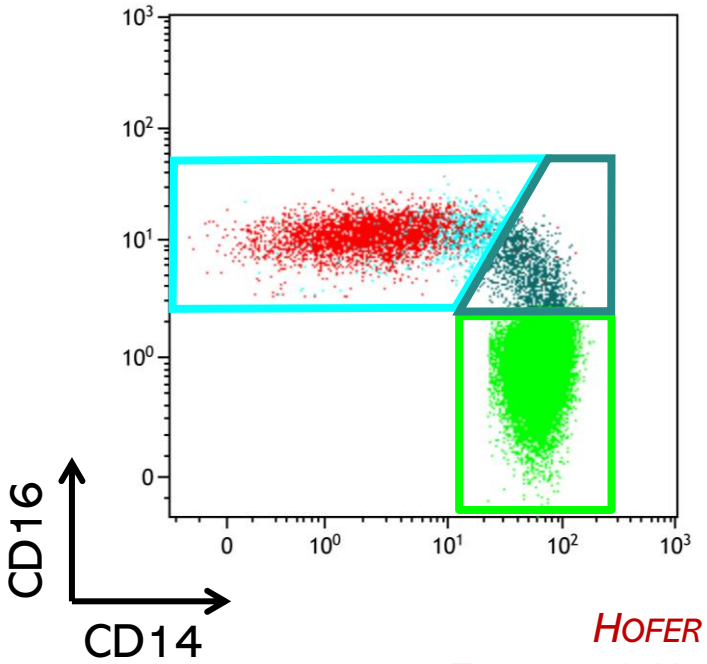
Monocyte partitioning: the Monocyte assay

Relative quantification of the monocyte subsets in the peripheral blood

slan+ ncMO
slan^{+} CD14$^{-/low}$CD16^{+}
(6.5 ±3.2%)



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Hofer et al, Blood 2015
Tarfi et al, Haematologica 2019

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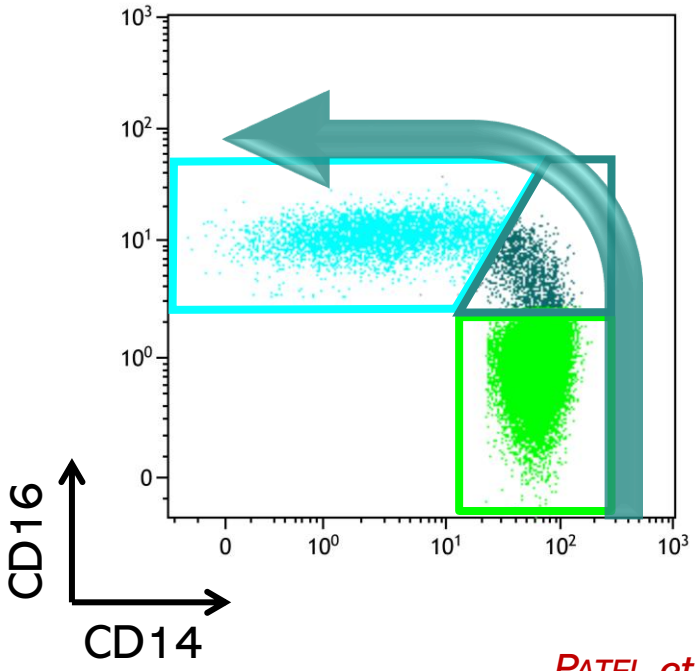

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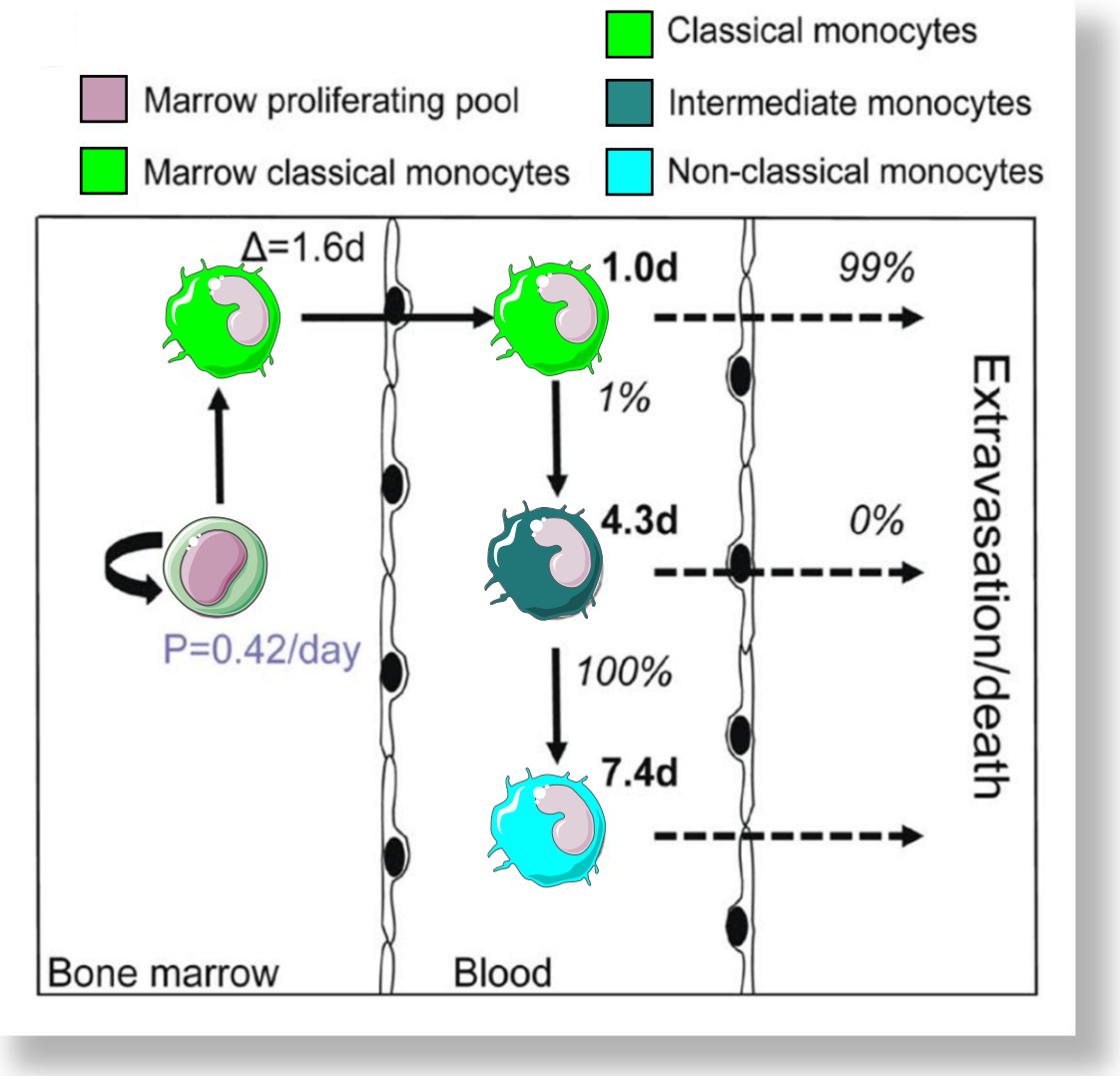

Patel et al, J Ex Med 2017

Monocyte partitioning: the Monocyte assay

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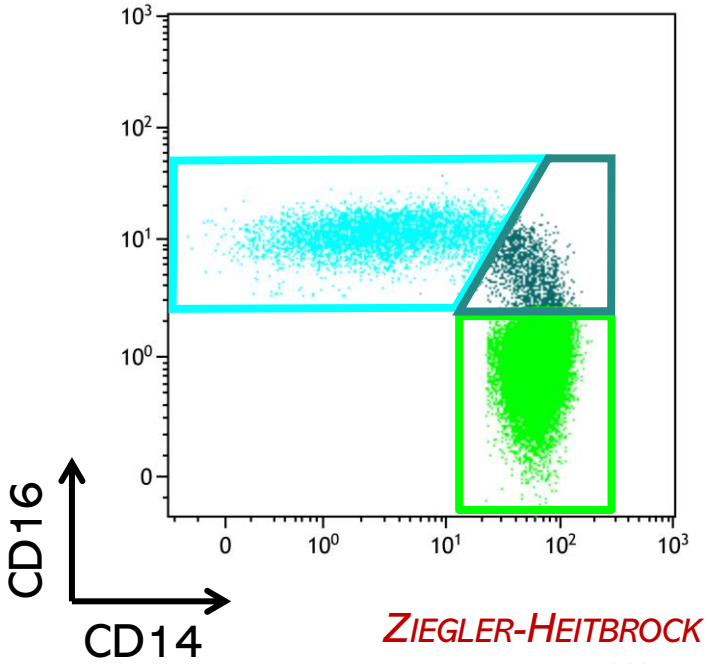

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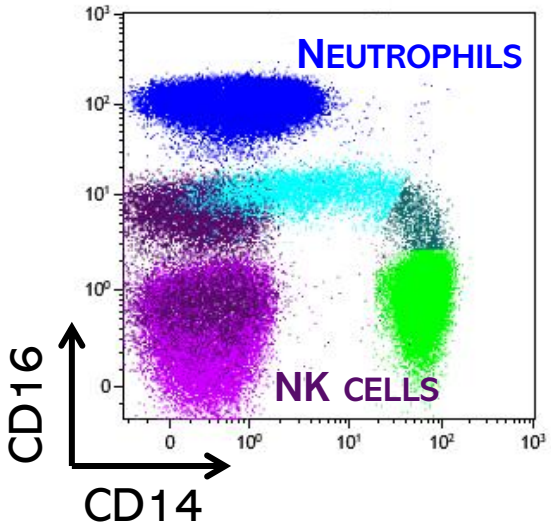
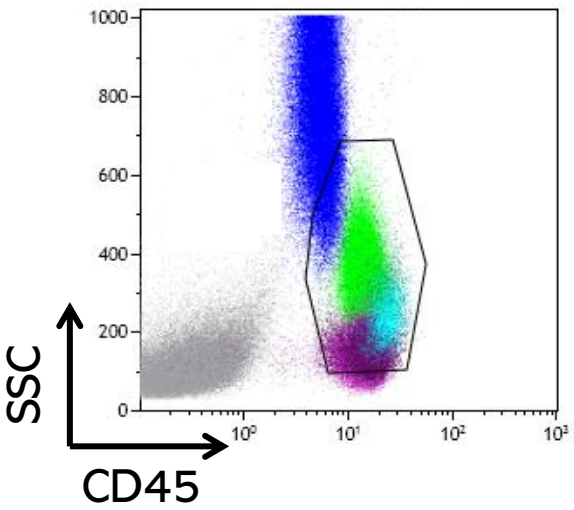
Ziegler-Heitbrock et al, Blood 2010
Wong et al, Blood 2011

Exclusion gating strategy mandatory (NK cells & neutrophils +++)





Selimoglu-Buet, Wagner-Ballon et al, Blood 2015
European patent

Monocyte partitioning: the Monocyte assay

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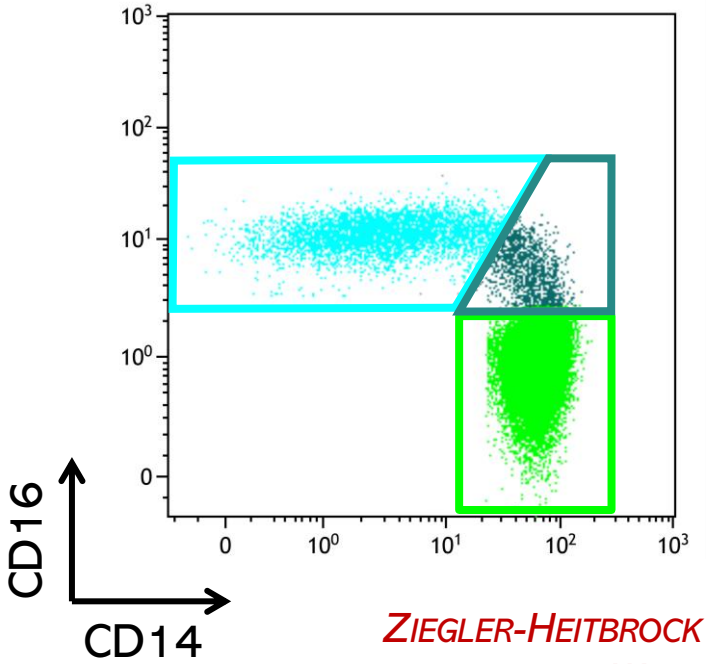

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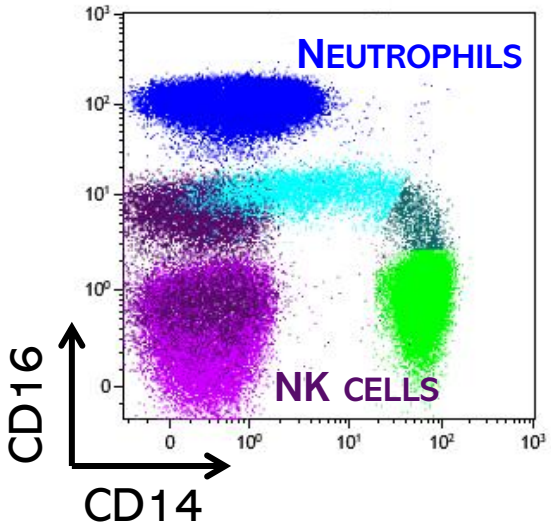
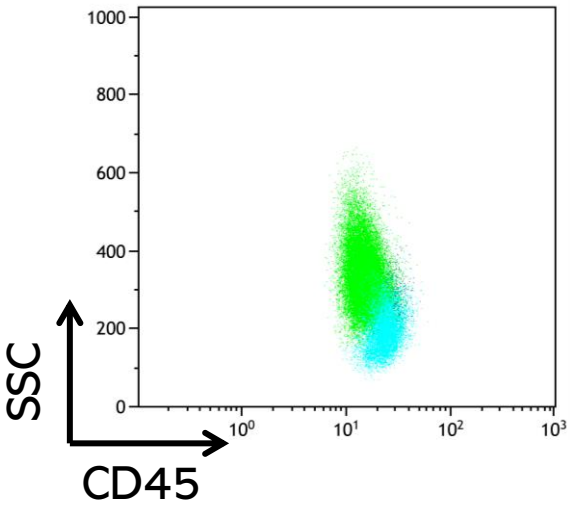
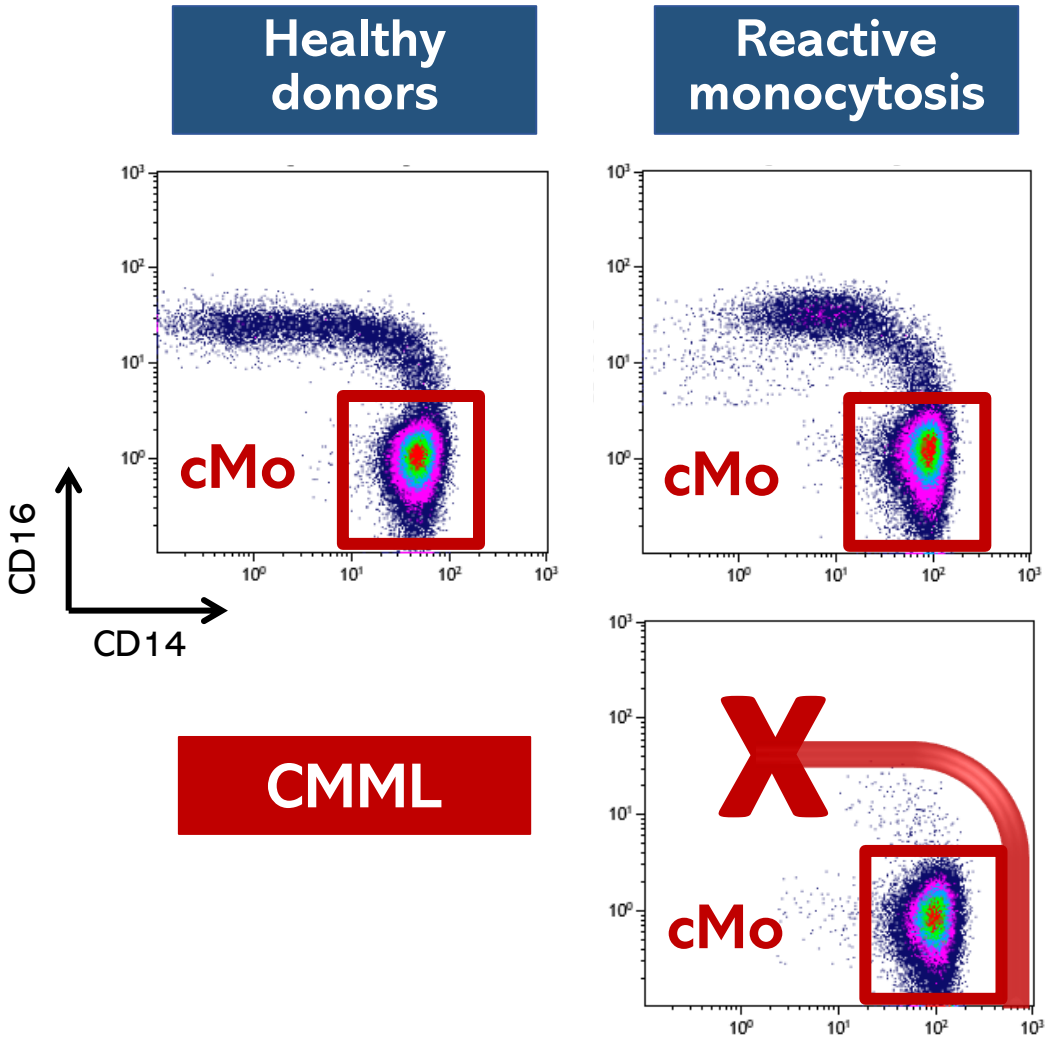




Selimoglu-Buet, Wagner-Ballon et al, Blood 2015
European patent

Relative accumulation of cMO in CMML



Selimoglu-Buet, Wagner-Ballon et al, Blood 2015

European patent

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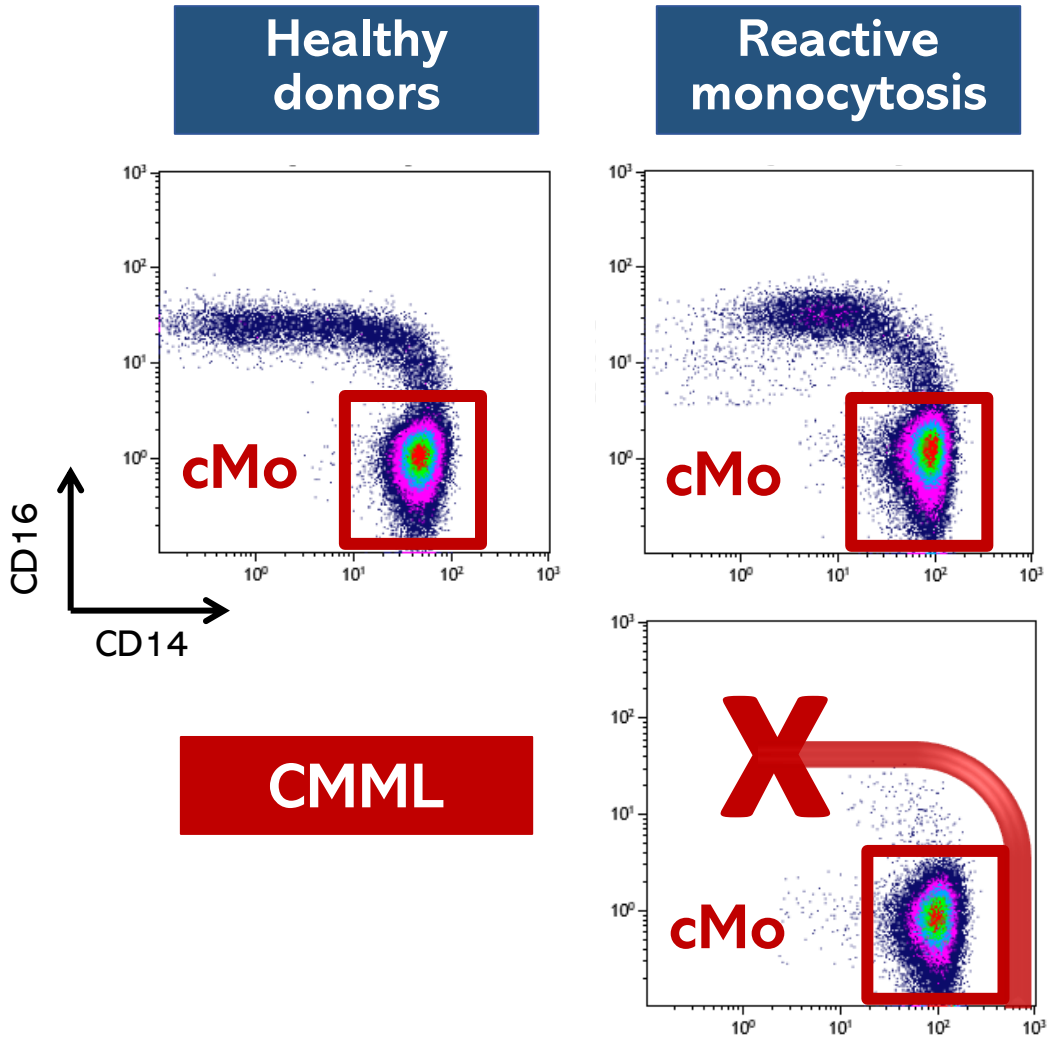

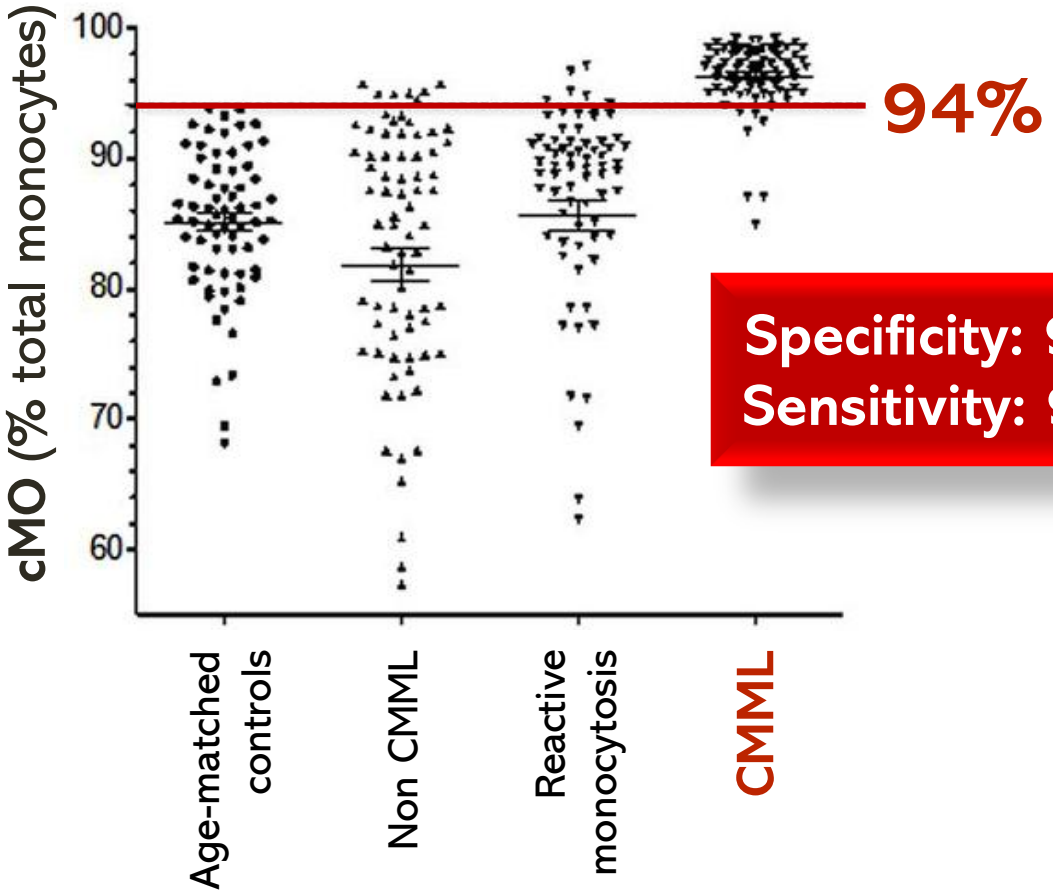

Selimoglu-Buet, Wagner-Ballon et al, Blood 2015

European patent

Relative accumulation of cMO in CMML

Is independent of

- the **absolute count** of circulating monocytes
- the **cytogenetic or mutational** background
- the **proliferative vs dysplastic** status
- the **CMML subtype** (CMML-1 *vs* CMML-2)

Selimoglu-Buet, Wagner-Ballon et al, Blood 2015
European patent

Relative accumulation of cMO in CMML

Is independent of

- the **absolute count** of circulating monocytes
- the **cytogenetic or mutational** background
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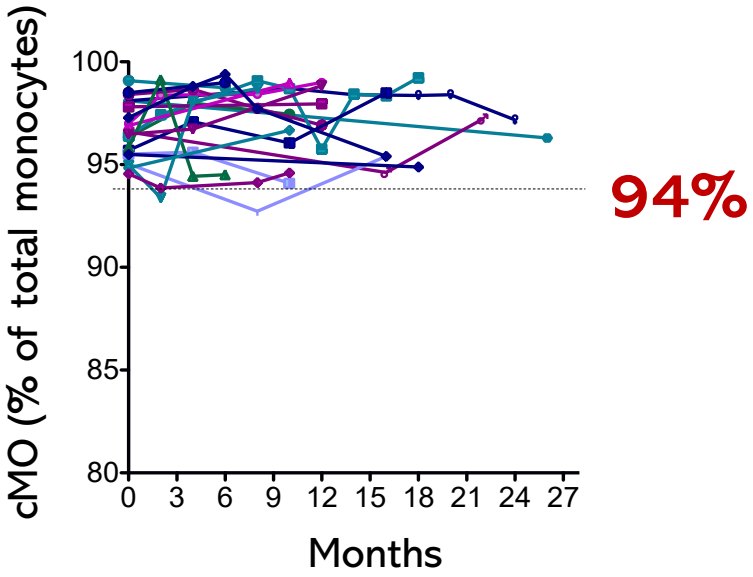
- the **CMML subtype** (CMML-1 *vs* CMML-2)

Is stable over time



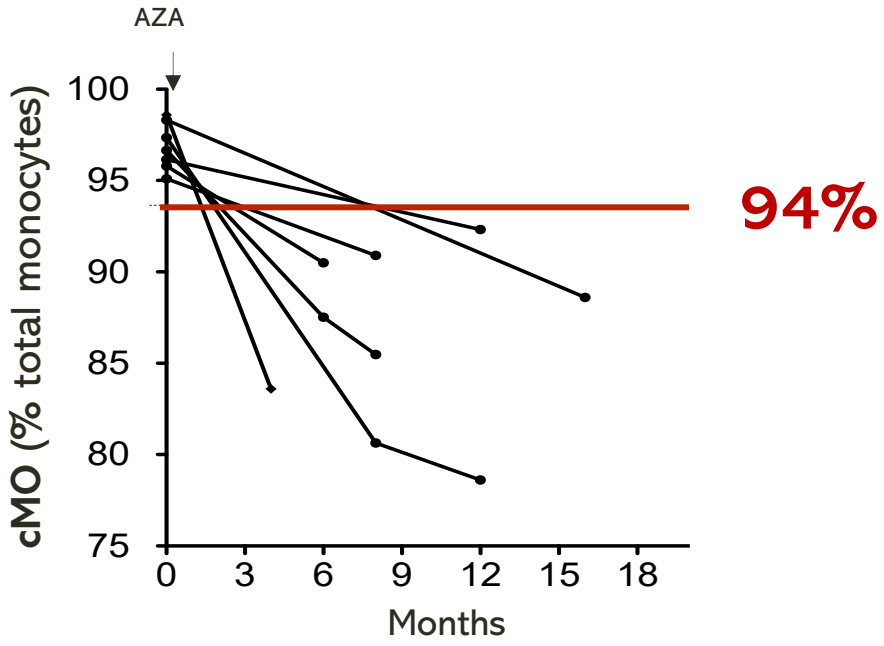
21 untreated CMML patients

Selimoglu-Buet, Wagner-Ballon et al, Blood 2015

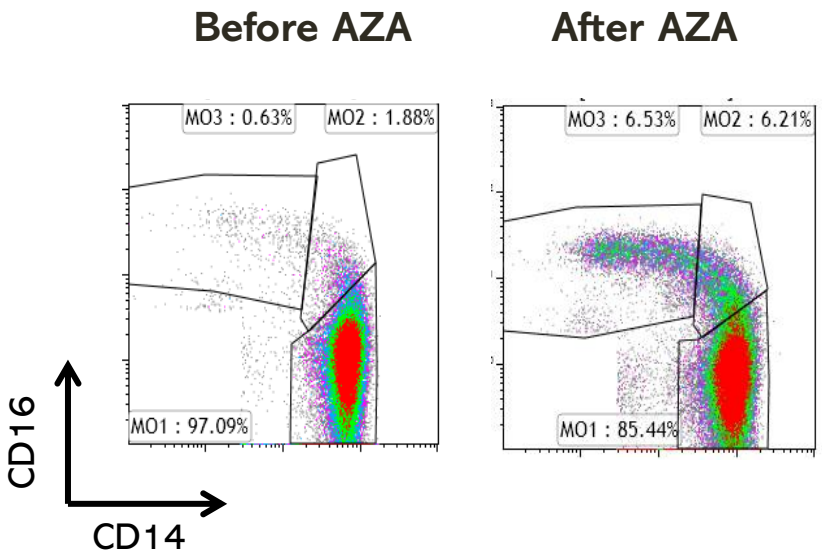
European patent

Relative accumulation of cMO in CMML

Can be reverted by Hypomethylating agents



94%



7 Patients responding to AZA therapy

Selimoglu-Buet, Wagner-Ballon et al, Blood 2015

European patent

MULTICENTER VALIDATION OF THE MONOCYTE ASSAY

MOFFITT CANCER CENTER

Sp: 86%
Se: 100%

TALATI et al, Blood 2017

UR MEDICINE | WILMOT CANCER INSTITUTE

Sp: 86%
Se: 88%

HUDSON et al, AJCP 2018

MAYO CLINIC

Sp: 95%
Se: 75%

POPHALI et al, BCJ 2019

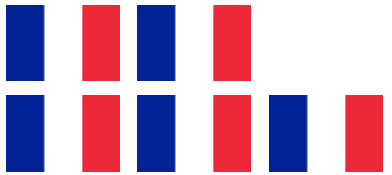
Sp: 95%
Se: -

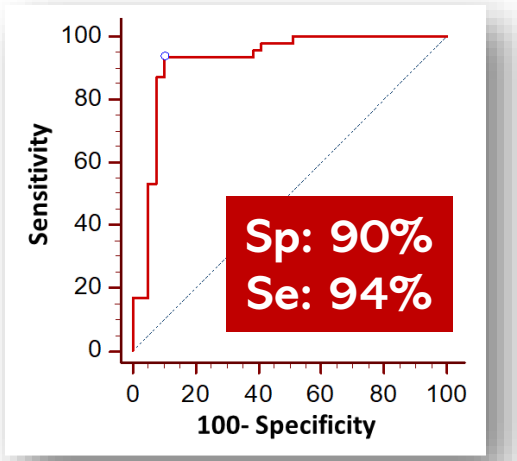
JURADO et al, Cyt PartB 2023

Sp: 89%
Se: 73%

BARGE et al, Pathology 2023

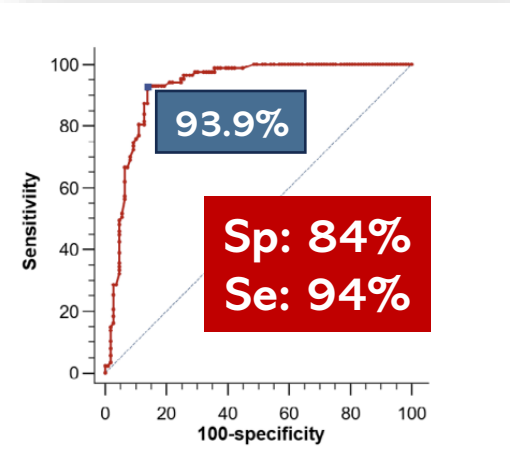
gfm





TARFI et al, BCJ 2018

ELN European iMDS Flow



WAGNER-BALLON et al, Cyt Part B 2022

NEW CLASSIFICATIONS

WHO 2022

Essential criteria
1. Persistent absolute (≥0.5 × 10^9/L) and relative (≥10%) peripheral blood monocytosis.
2. Blasts constitute <20% of the cells in the peripheral blood and bone marrow.[a]
3. Not meeting diagnostic criteria of chronic myeloid leukaemia or other myeloproliferative neoplasms.[b]
4. Not meeting diagnostic criteria of myeloid/lymphoid neoplasms with tyrosine kinase gene fusions (*PDGFRA*, *PDGRB*, *FGFR1*, or *JAK2*).[c]
Desirable criteria
1. Dysplasia involving ≥1 myeloid lineages.[d]
2. Acquired clonal cytogenetic or molecular abnormality.[e]
3. Abnormal partitioning of peripheral blood monocyte subsets.[f]

ICC 2022

Monocytosis defined as monocytes ≥ 0.5 × 10^9/L and ≥ 10% of the WBC
Cytopenia (thresholds same as MDS)*
Blasts (including promonocytes) < 20% of the cells in blood and bone marrow
Presence of clonality: abnormal cytogenetics and/or presence of at least one myeloid neoplasm associated mutation of at least 10% allele frequency†
In cases without evidence of clonality, monocytes ≥ 1.0 × 10^9/L and > 10% of the WBC, and increased blasts (including promonocytes),‡ or morphologic dysplasia, or an abnormal immunophenotype consistent with CMML would be required for its diagnosis.
Bone marrow examination with morphologic findings consistent with CMML (hypercellularity due to a myeloid proliferation often with increased monocytes), and lacking diagnostic features of acute myeloid leukemia, MPN or other conditions associated with monocytosis§
No *BCR::ABL1* or genetic abnormalities of myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions

NEED FOR HARMONIZATION

CytHem LMMC

www.cythem.fr

NEED FOR HARMONIZATION

CytHem LMMC

www.cythem.fr

BEST PRACTICE

CLINICAL CYTOMETRY WILEY

Technical, gating and interpretation recommendations for the partitioning of circulating monocyte subsets assessed by flow cytometry

Sihem Tarfi[1] | Wolfgang Kern[2] | Elodie Goulas[1] | Dorothée Selimoglu-Buet[3] | Orianne Wagner-Ballon[1,4] | the CytHem-LMMC

TECHNICAL PROTOCOL

- **EDTA whole blood samples**
- **Antibodies**


NAVIOS & DxFLEX
(Beckman Coulter)


BD FACSCanto II & FACSLyric
(BD Biosciences)

	CD45	CD7 and/or CD2	CD56	CD24	CD16	CD14	± slan
Role	Rough gating of monocytes	Exclusion of T cells and most of NK cells	Exclusion of NK cells	Exclusion of granulocytes and B cells	Fine-tuning of neutrophil exclusion	-	-

Partitioning of monocyte subsets

TECHNICAL PROTOCOL

CytHem LMMC

BECKMAN COULTER Life Sciences

Samples

200 µl of whole blood EDTA (sample dilution may be needed to adjust WBC to 10 x10^9/l)

Antibodies

Fluorochrome	FITC	PE	PC5.5	PC7	AA700	PB	KRO
Antibody	slan	CD24	CD56	CD14	CD7	CD16	CD45
Clone	M-DC8	ALB9	N901	RMO52	8H8.1	3G8	J33
Volume	2 µl	20 µl	5 µl	5 µl	5 µl	10 µl	5 µl

Lysis

1ml of VersaLyse with 25 µl of undiluted IOTest 3 10X* Fixative Solution

BD

Samples

200 µl of whole blood EDTA (sample dilution may be needed to adjust WBC to 10 x10^9/l)

Antibodies

Fluorochrome	FITC	PE	PerCP5.5	PC7	APC	BV421	V500
Antibody	slan	CD24	CD7	CD14	CD56	CD16	CD45
Clone	M-DC8	ML5	M-T701	MOP9562698	NCAM16.2	3G8	HI30
Volume	2 µl	20 µl	5 µl	5 µl	5 µl	5 µl	5 µl

Lysis

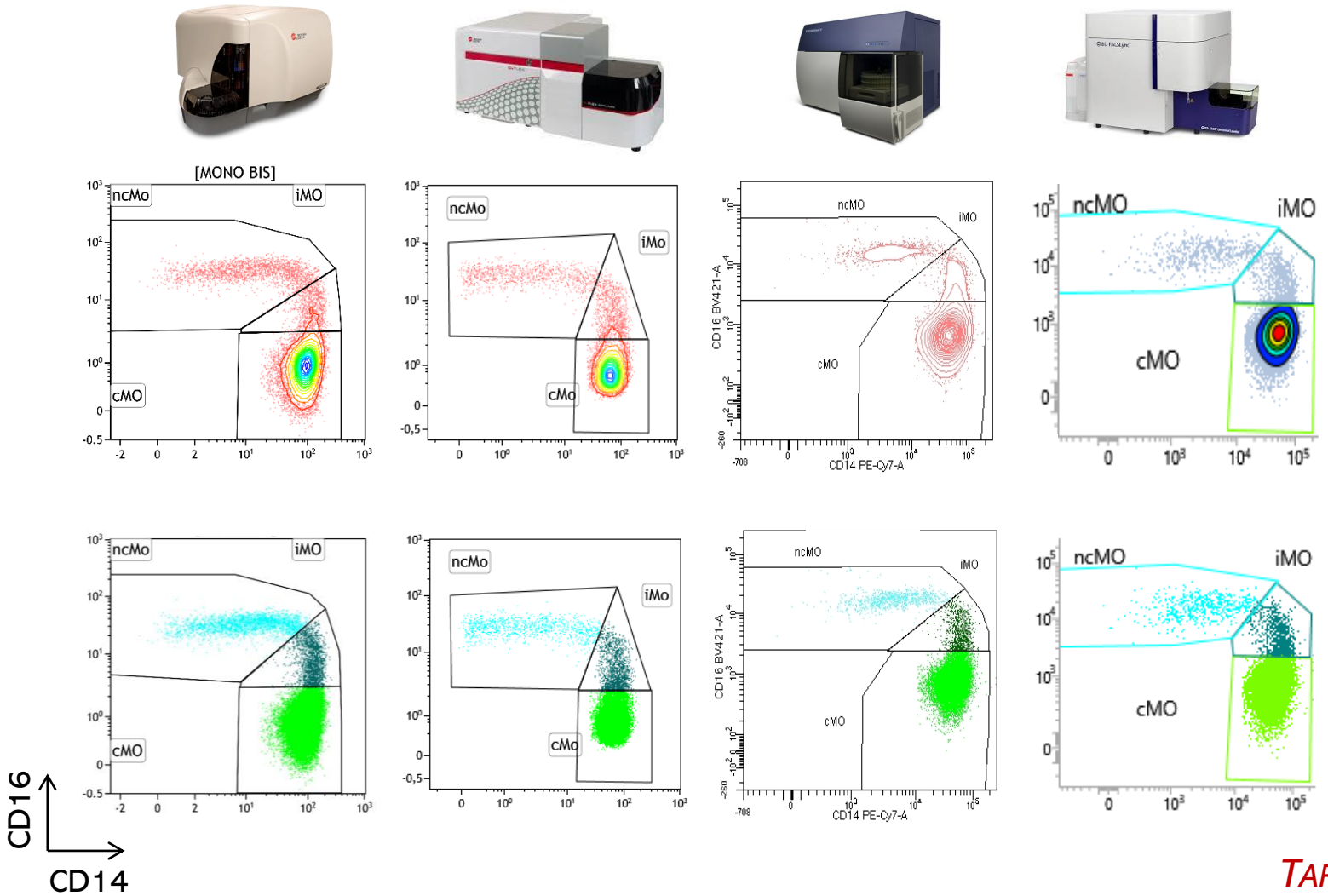
1 ml of Pharmlyse

20 minutes incubation at room temperature, with protection from light

Acquisition on cytometer at once **without washing procedure**

TARFI et al, Cyt PartB 2024

EXCLUSION GATING STRATEGY



TARFI et al, Cyt PartB 2024

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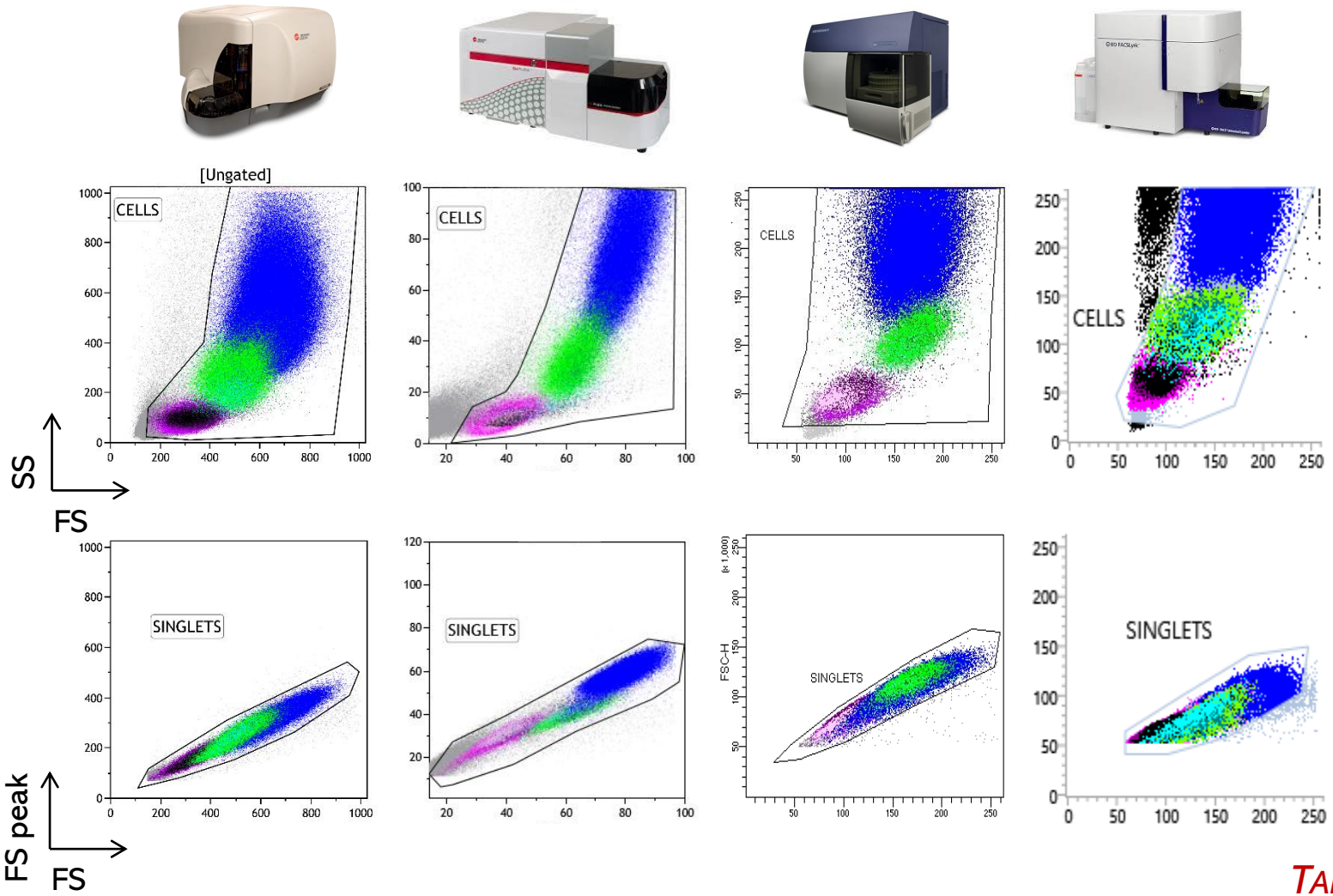

TARFI et al, Cyt PartB 2024

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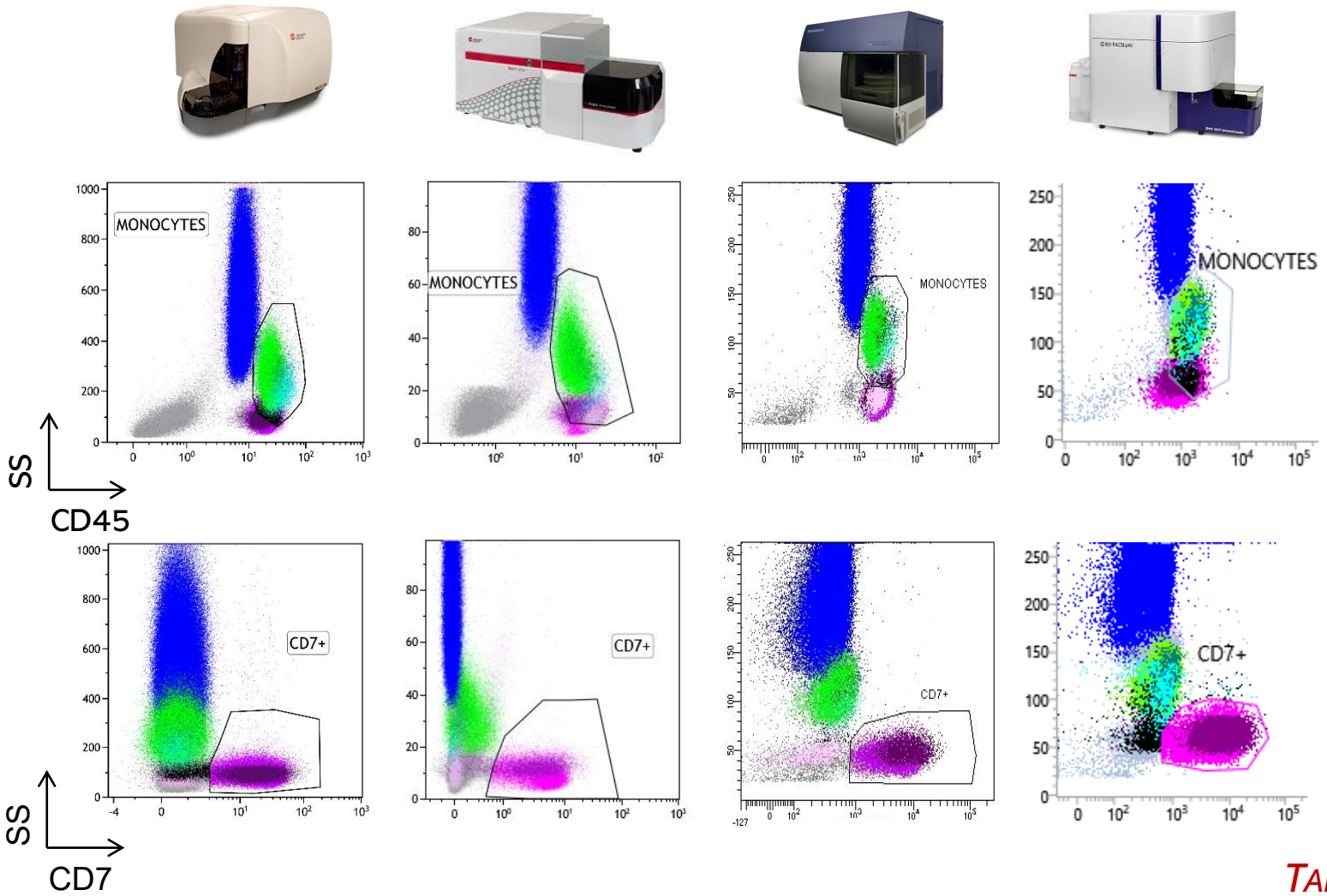

TARFI et al, Cyt PartB 2024

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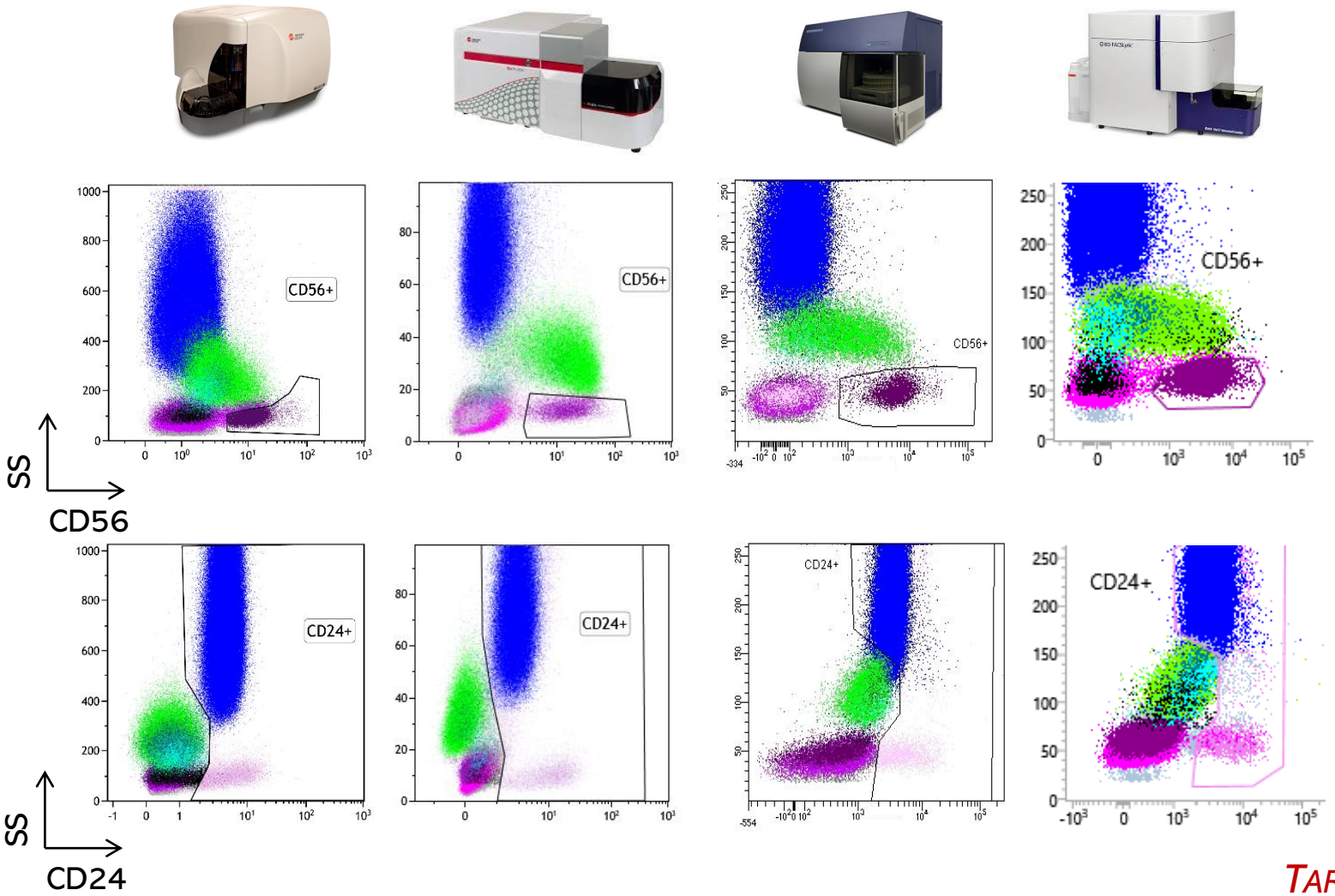

TARFI et al, Cyt PartB 2024

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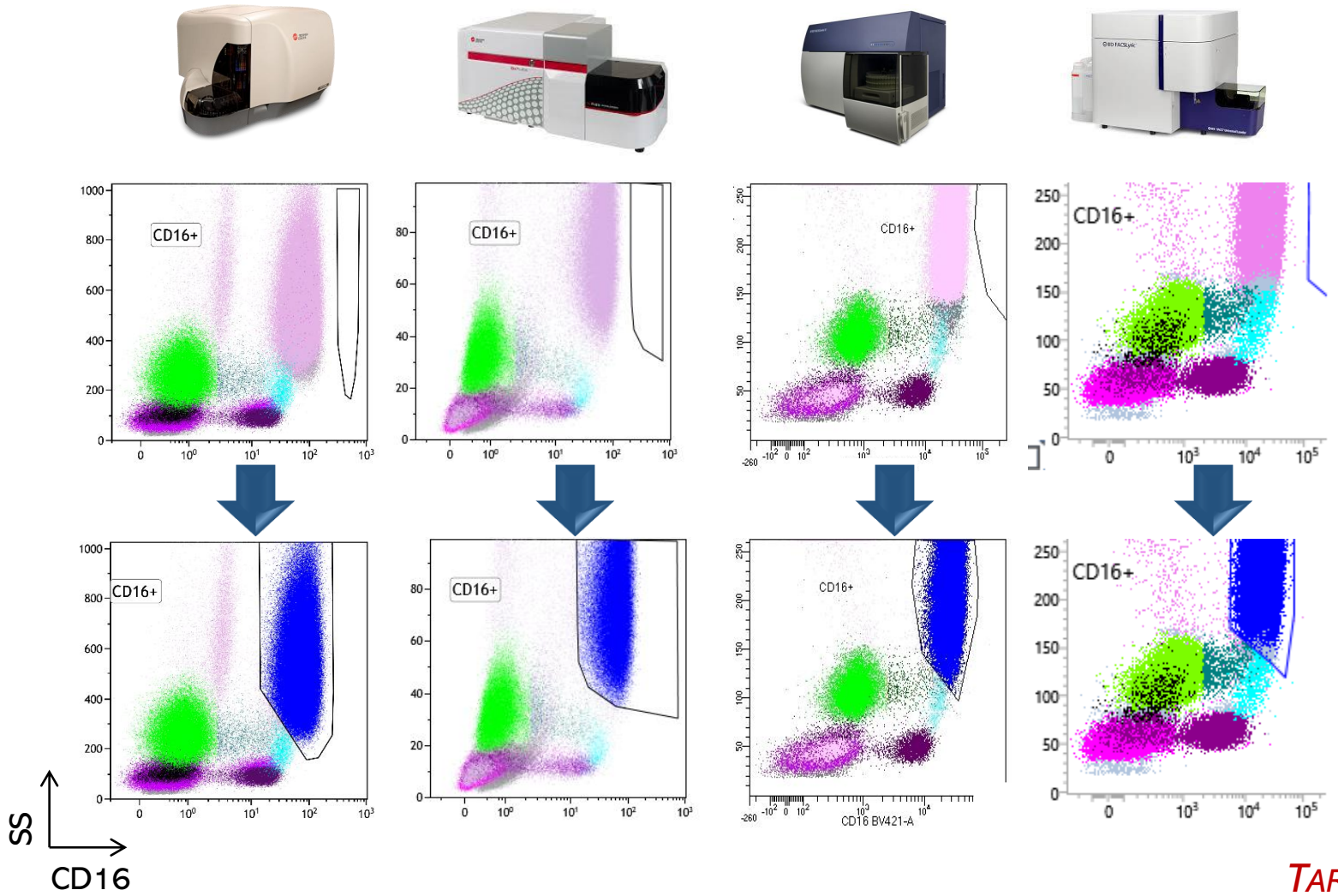

TARFI et al, Cyt PartB 2024

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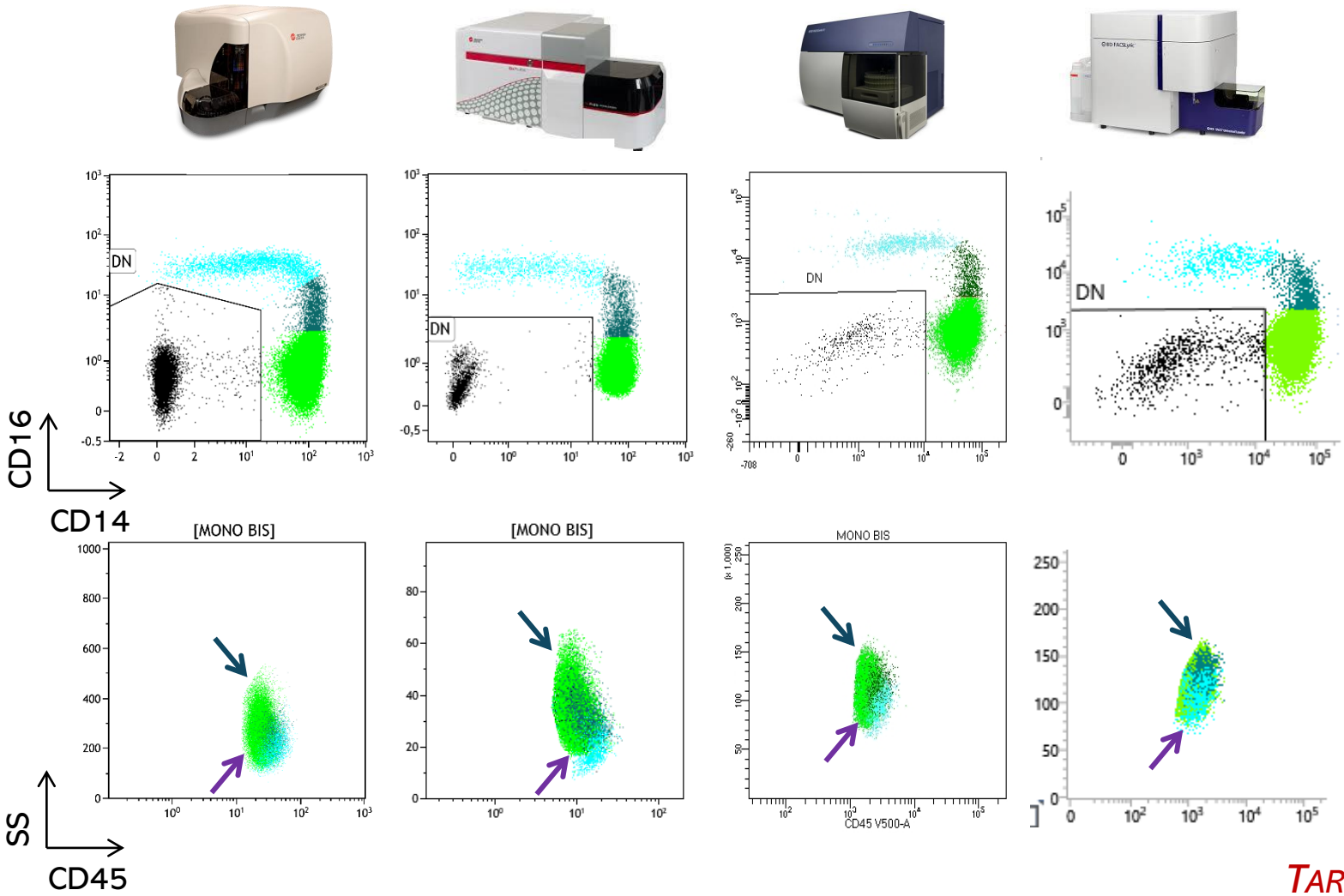

TARFI et al, Cyt PartB 2024

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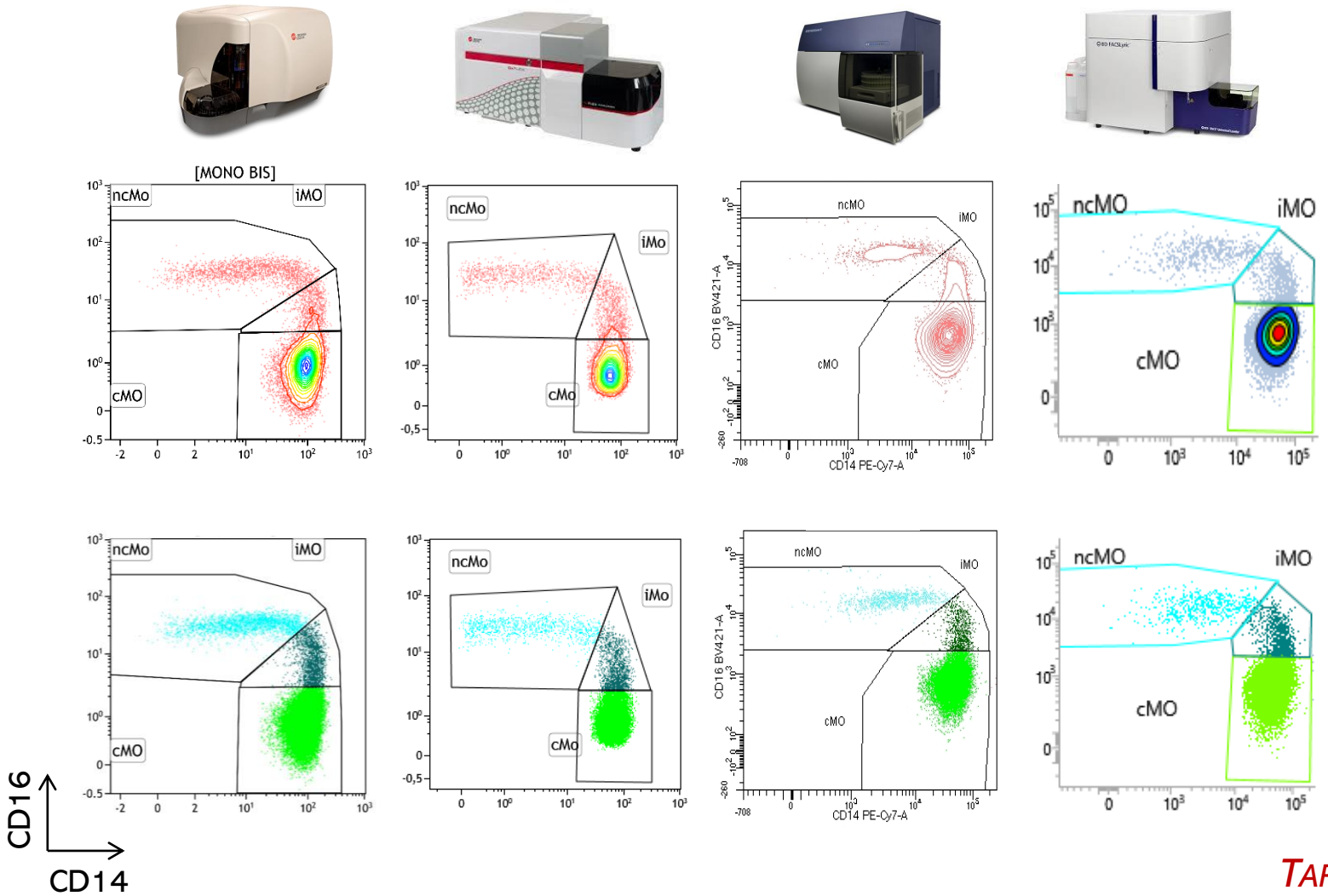


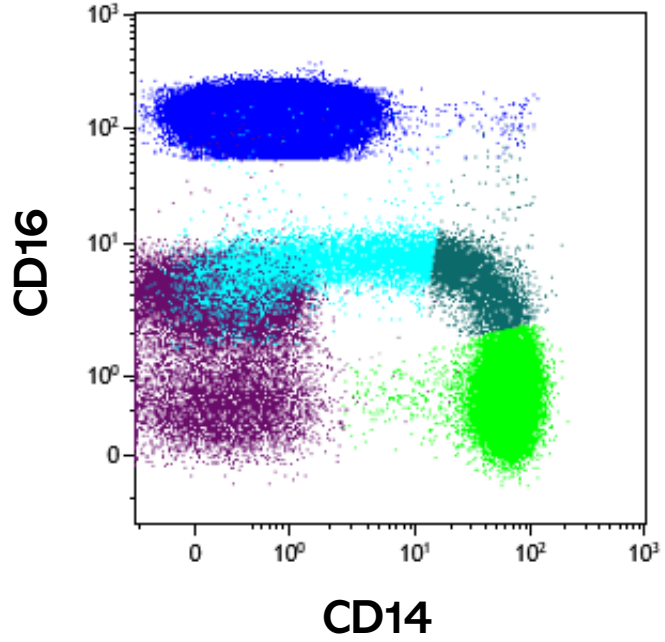
TARFI et al, Cyt PartB 2024

TECHNICAL PITFALLS (1)

A few recommendations

- Use **200 µl** of whole peripheral blood
- Be cautious of **high leukocytosis** → **CD16 +++**
- Choose your **exclusion antibody panel**

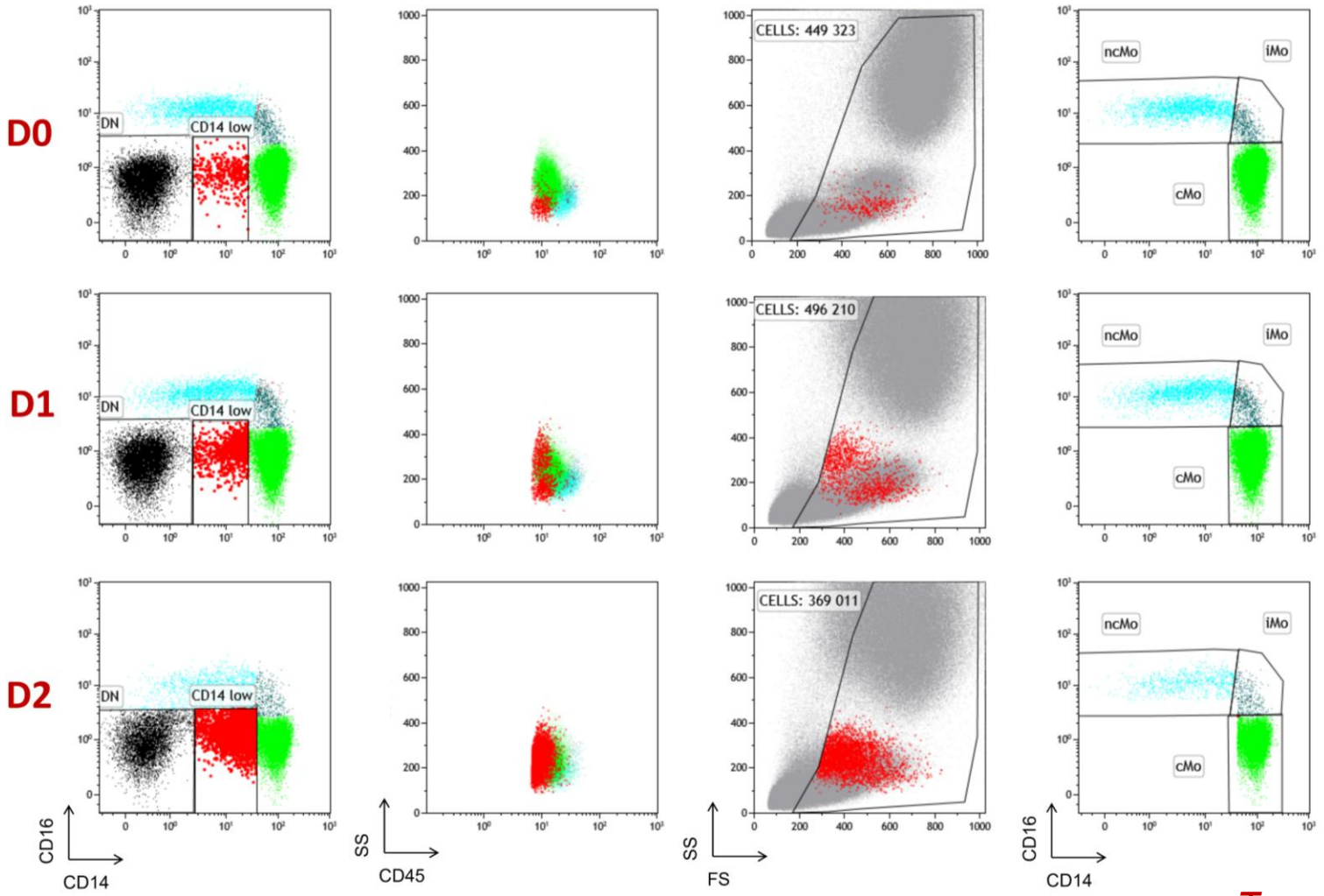
(**exclusion of NK cells and immature granulocytes +++**)



Avoid any positive gating based on monocyte marker expression!

TECHNICAL PITFALLS (2)

Process samples within 24h

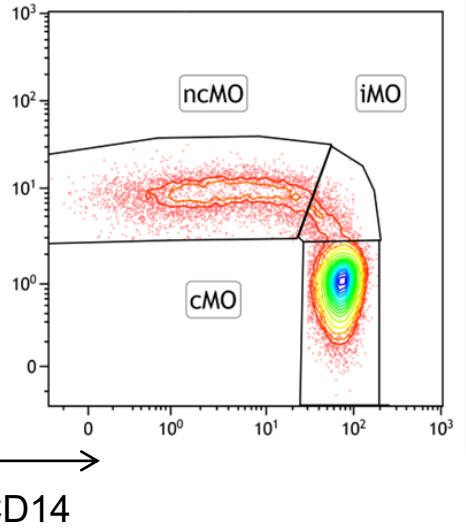


TARFI et al, Cyt PartB 2024

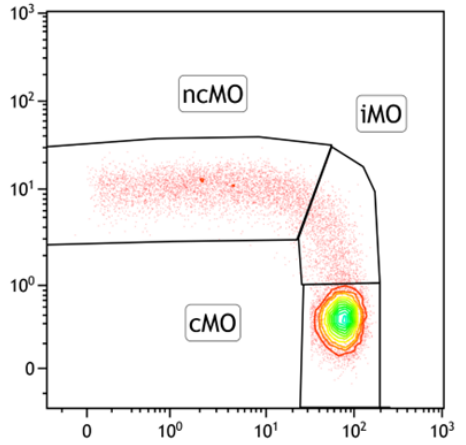
TECHNICAL PITFALLS (3)

Avoid washing procedure

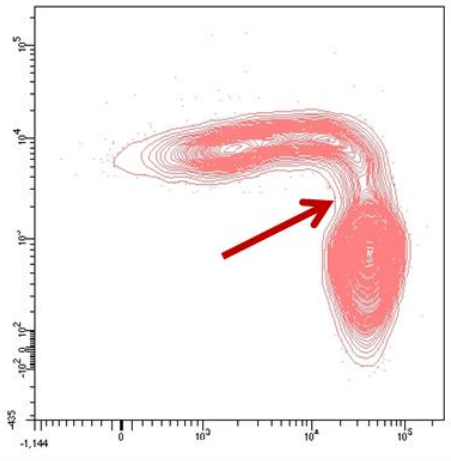
Without washing



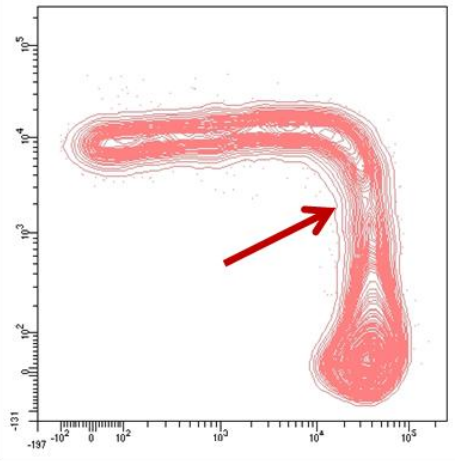
After washing

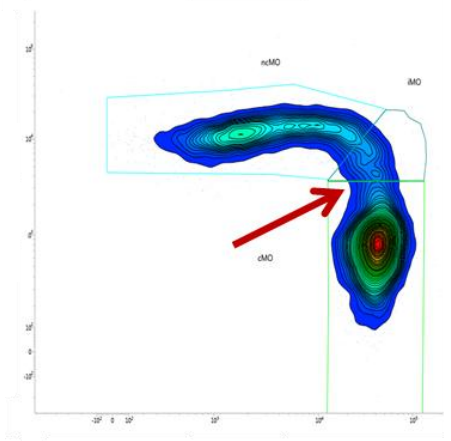


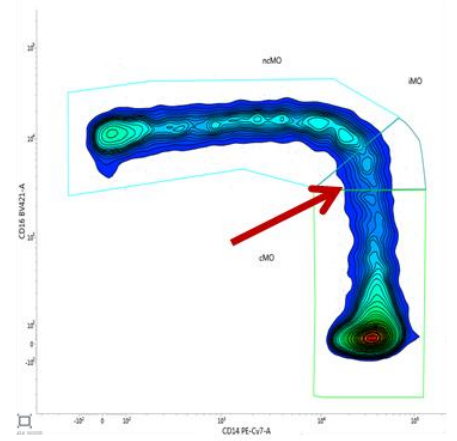
Without washing



After washing







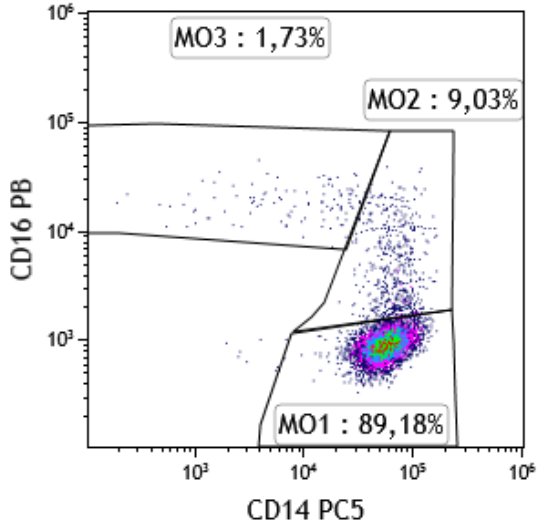
TARFI et al, Cyt PartB 2024

TECHNICAL PITFALLS (4)

Acquire ≥10,000 cMo events

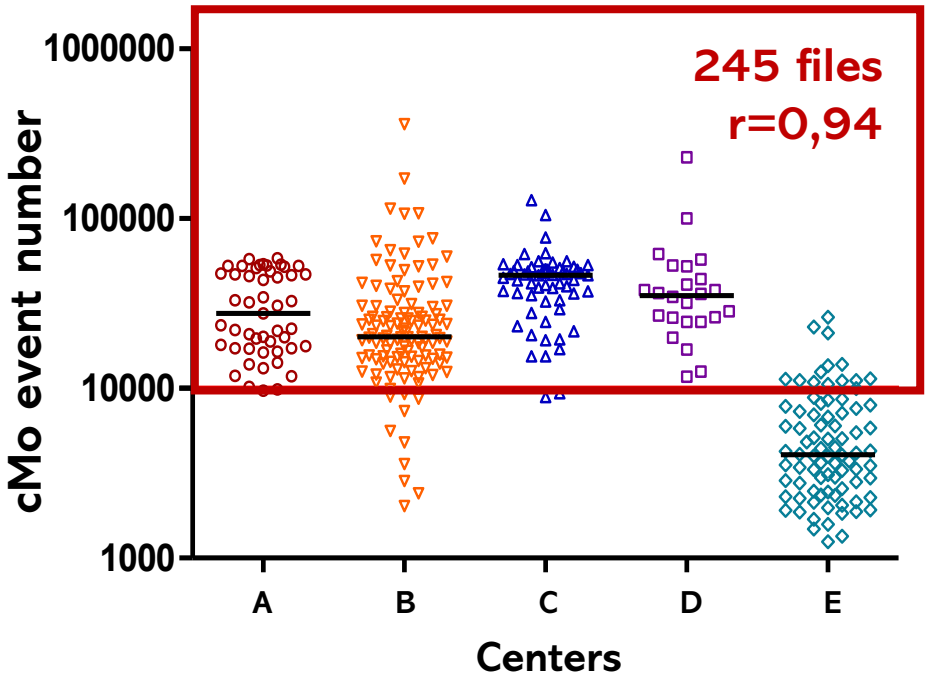
BLIND CENTRALIZED RE-ANALYSIS OF 319 FILES (FIVE CENTERS)

NAVIOS™ (Beckman-Coulter)



5817 événements cMo

Example of a divergent case

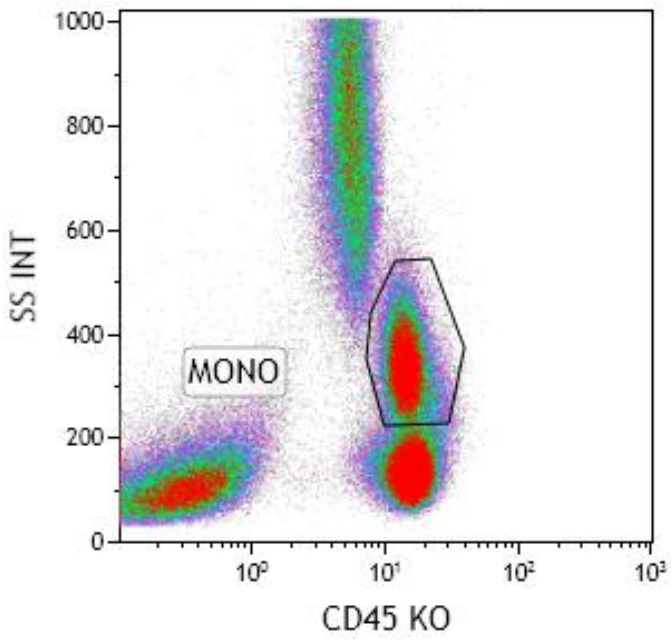


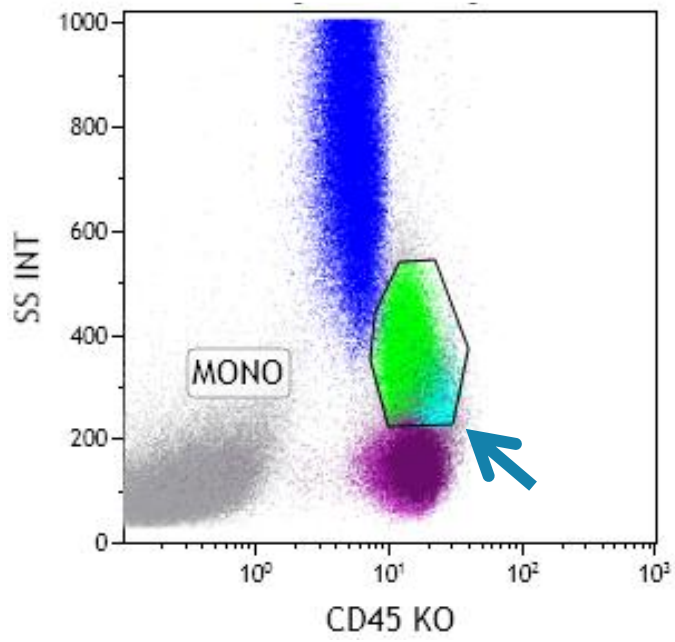
Excellent agreement

TARFI, HARRIVEL et al, BCJ, 2018

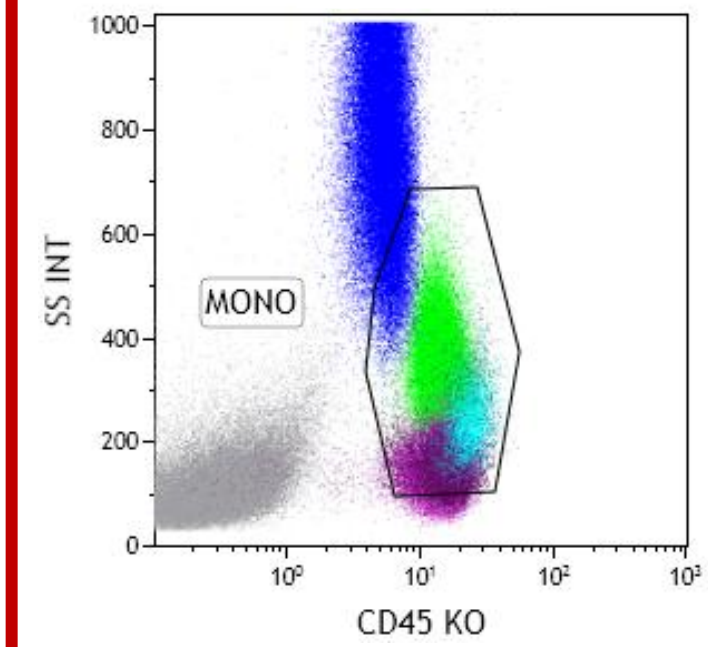
GATING PITFALLS (1)

Gate roughly the monocytes



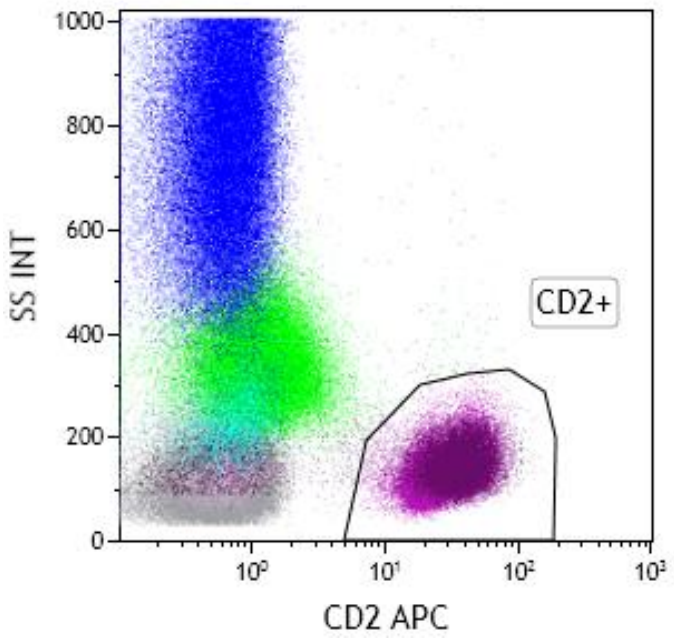


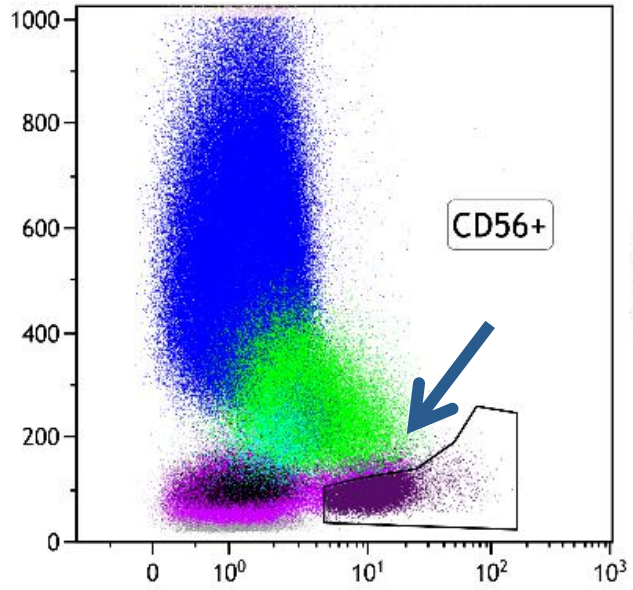
Large « MONO » gate

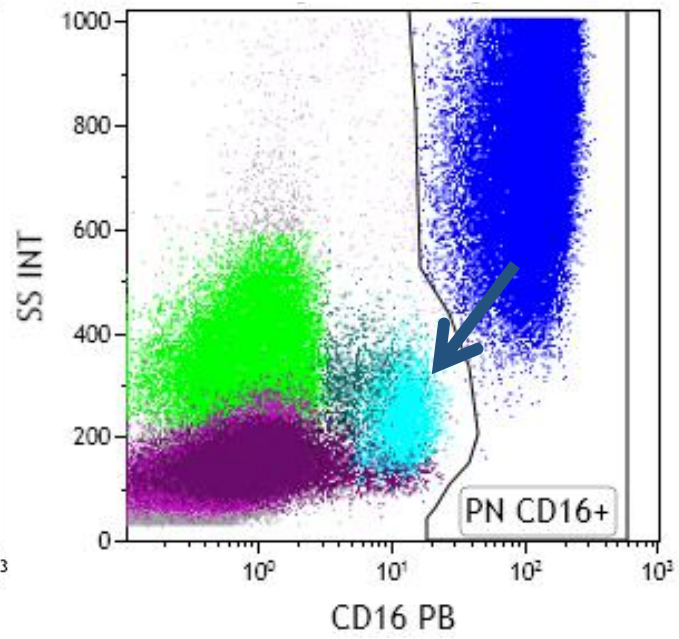


GATING PITFALLS (2)

Be careful not to exclude CD56+ monocytes or CD16+ monocytes

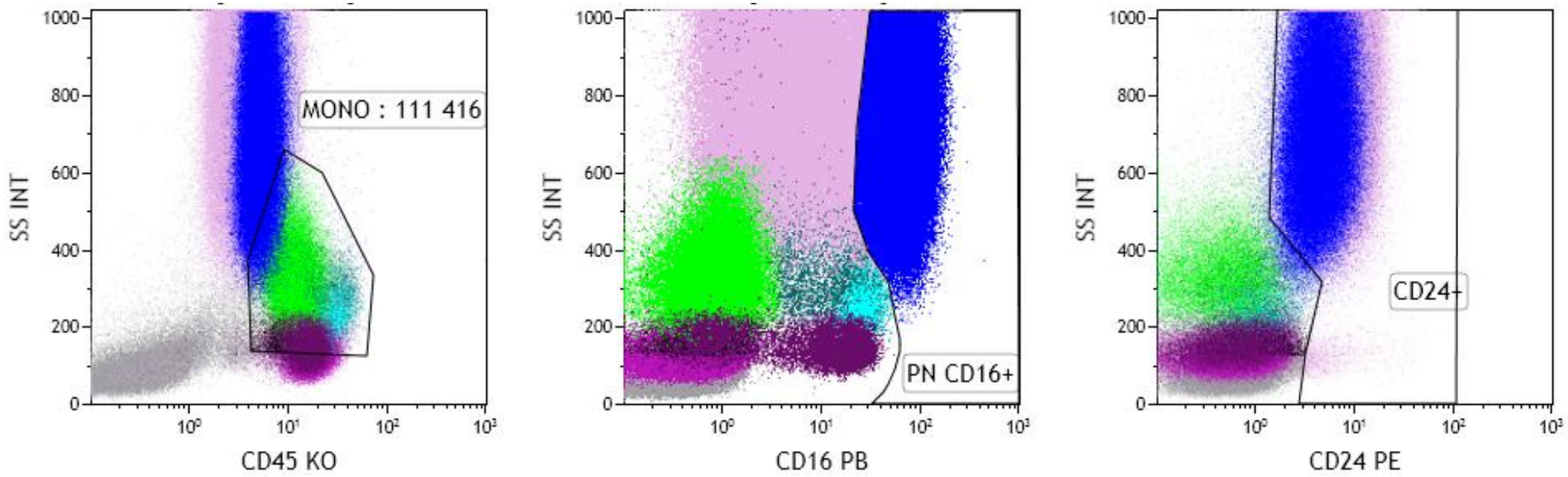






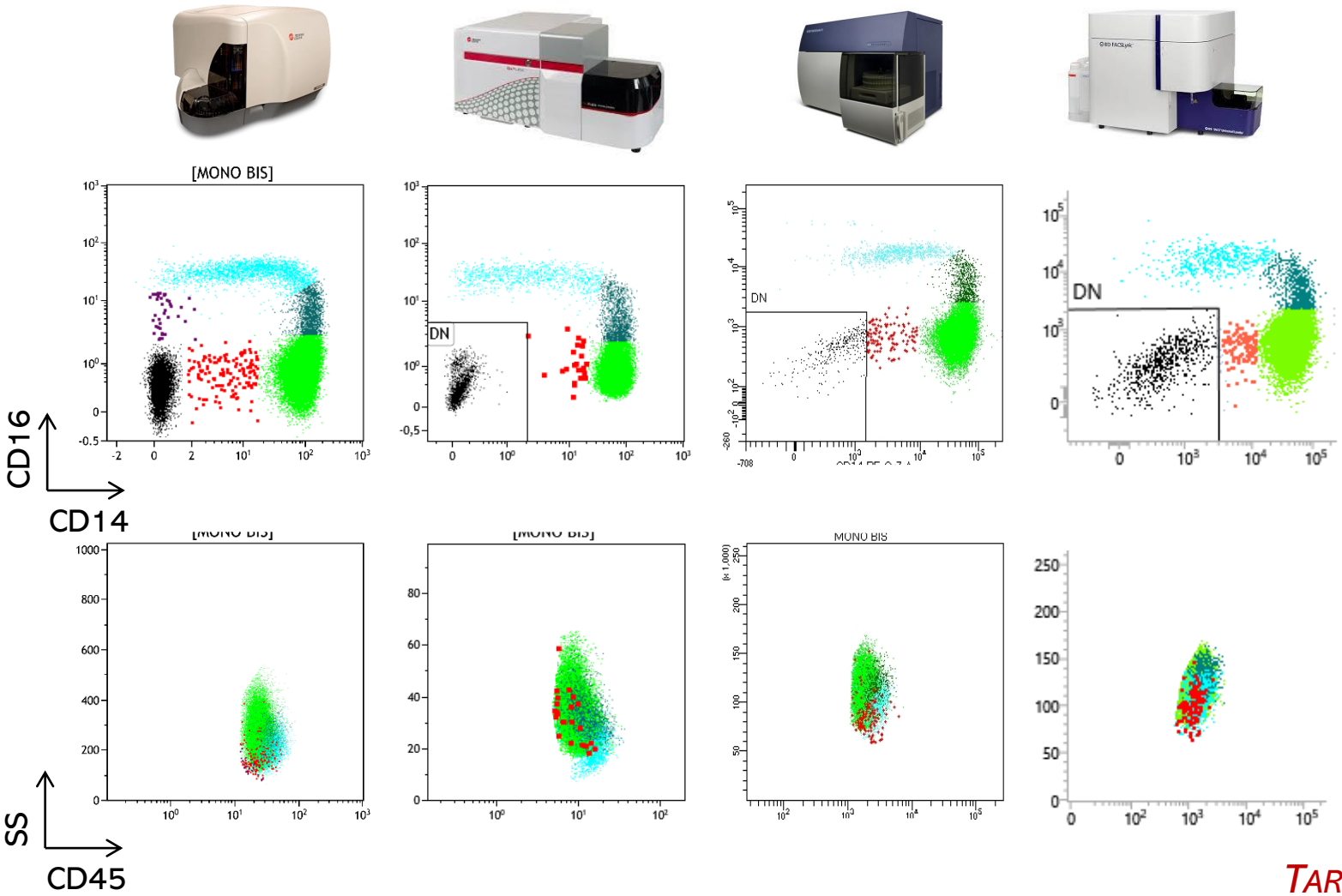
GATING PITFALLS (3)

Be careful to exclude correctly immature granulocytes



GATING PITFALLS (4)

Be careful with CD14^{low} cells

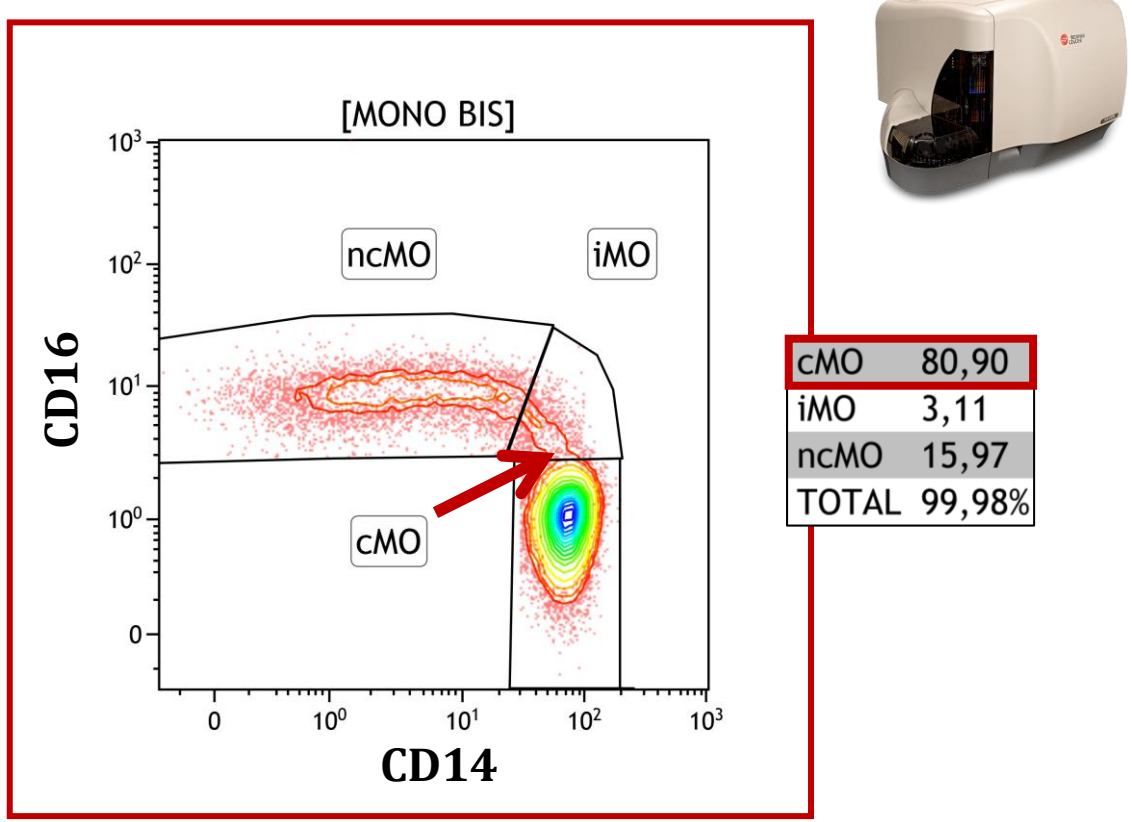


TARFI et al, Cyt PartB 2024

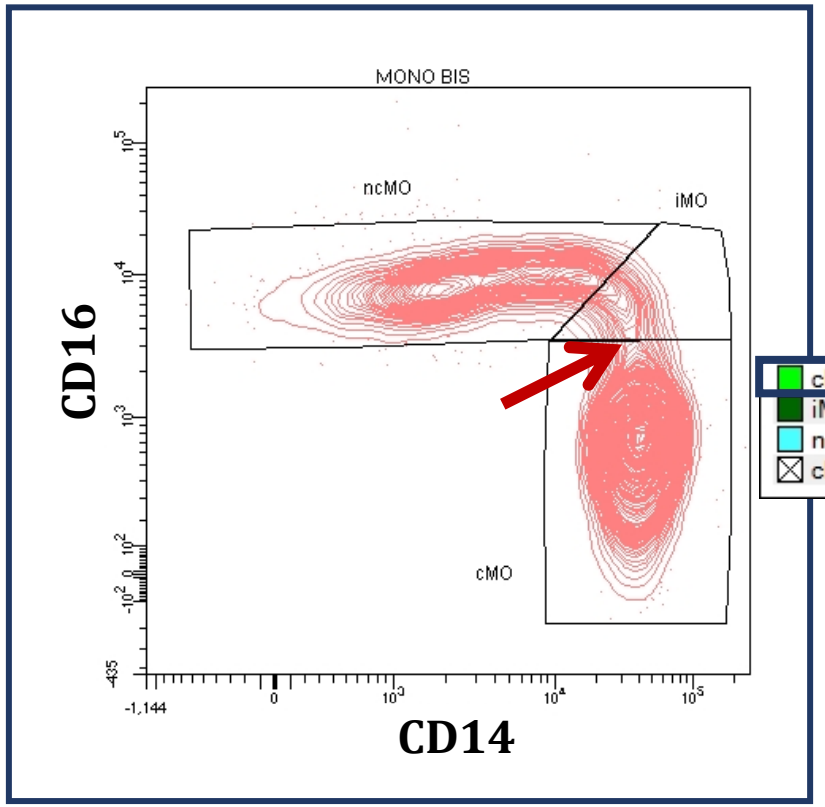
GATING PITFALLS (5)

Use contour plot representation

Check the sum of the 3 subsets
→ should be ≥ 99.5%!



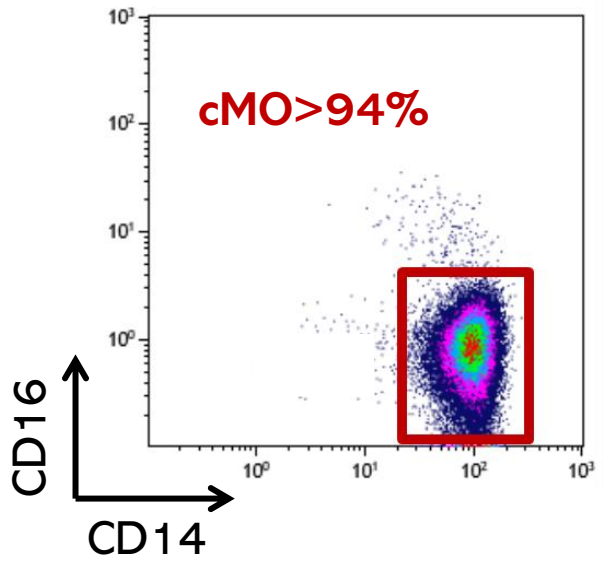
cMO	80,90
iMO	3,11
ncMO	15,97
TOTAL	99,98%



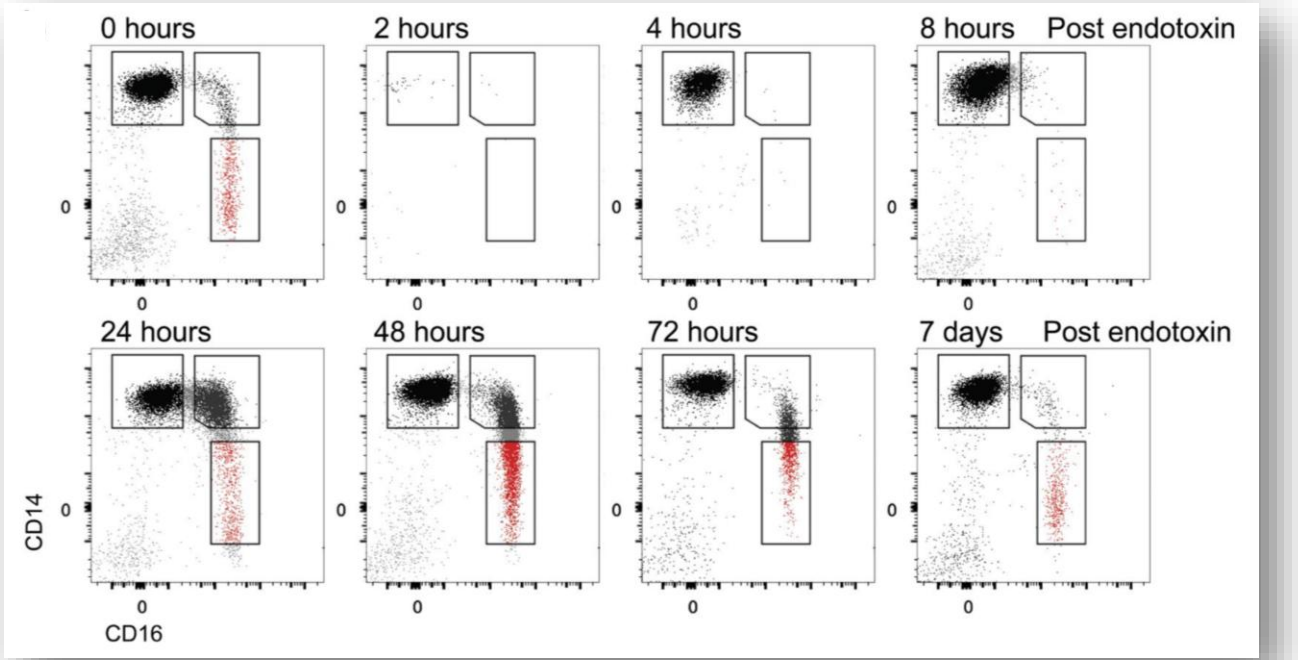
	%Parent
cMO	79.5
iMO	3.5
ncMO	16.8
cMO OR iMO OR ncMO	99.8

INTERPRETATION PITFALLS (1)

False



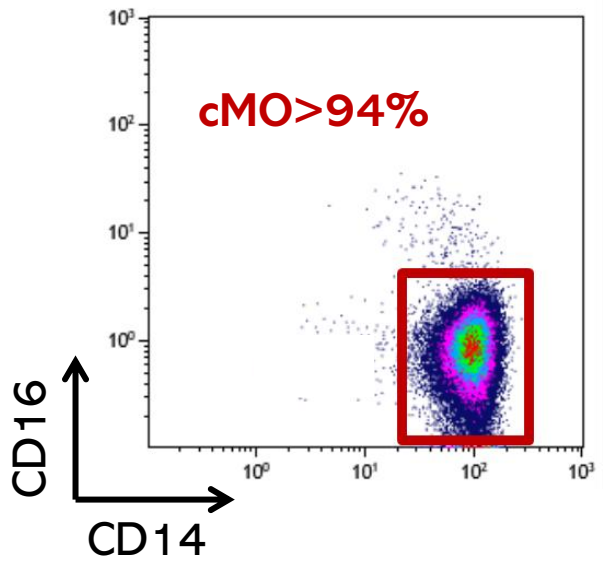
- **Recovery from bone marrow aplasia**



PATEL, ZHANG et al, J Exp Med 2017

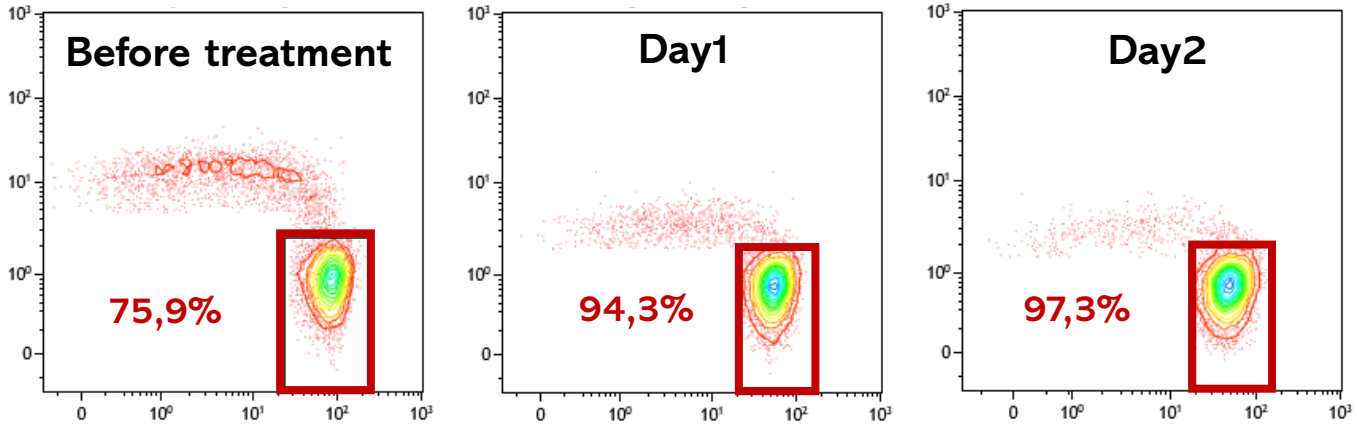
INTERPRETATION PITFALLS (1)

False



- Recovery from bone marrow aplasia
- **Treatment by corticosteroids and IV immunoglobulins**

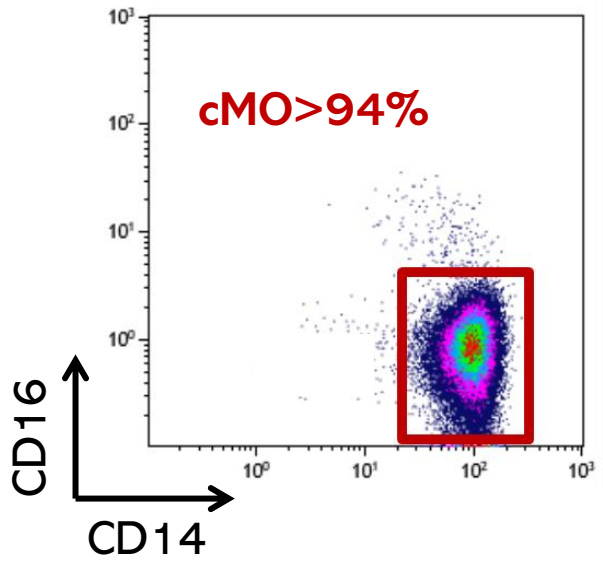
FINGERLE-ROWSON et al, Clin Exp Imm 1998
SIEDLAR et al, Clin Immunol 2011
TJON et al, Clin Immunol 2012



VOLLE, TARFI*, Unpublished data, ASH 2023, Poster*

INTERPRETATION PITFALLS (1)

False



- Recovery from bone marrow aplasia
- Treatment by corticosteroids and IV immunoglobulins
- **Severe cases of COVID-19**

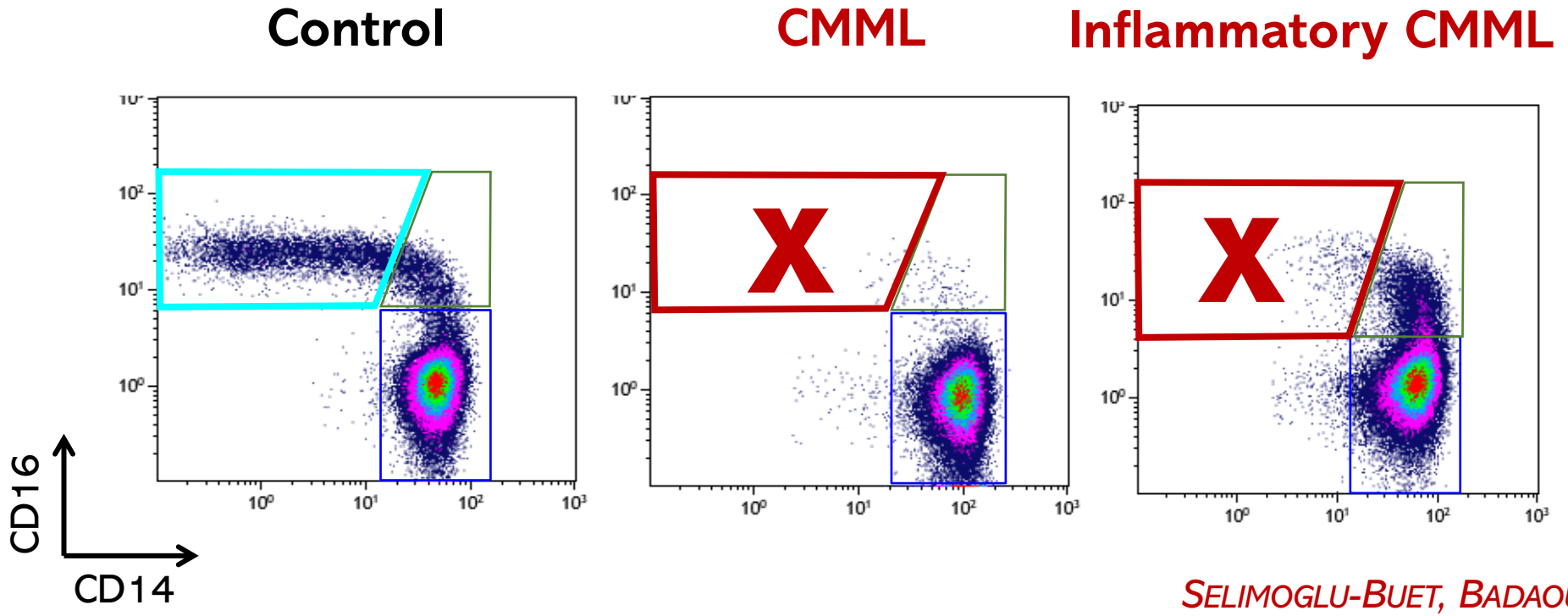
SILVIN et al, Cell 2020

INTERPRETATION PITFALLS (2)

False

cMO<94%

- **CMML with inflammatory state**



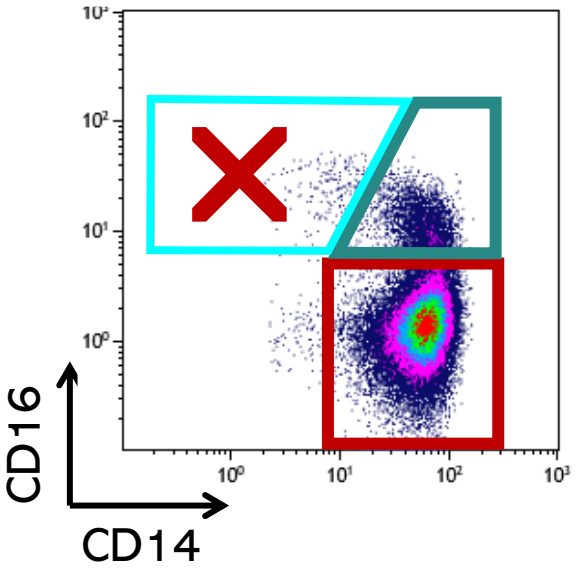
SELIMOGLU-BUET, BADAOUI et al, Blood 2017

INTERPRETATION PITFALLS (2)

False

- CMML with inflammatory state

"Bulbous" aspect profile



↗ % iMo

↘ ↘ ↘ ncMO

↘ cMo <94%

Associated inflammatory state in 16 to 20% of CMML

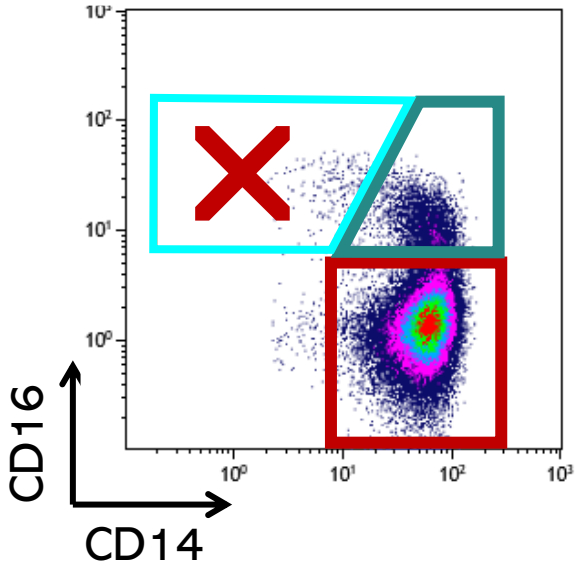
SELIMOGLU-BUET, BADAOUI et al, Blood 2017

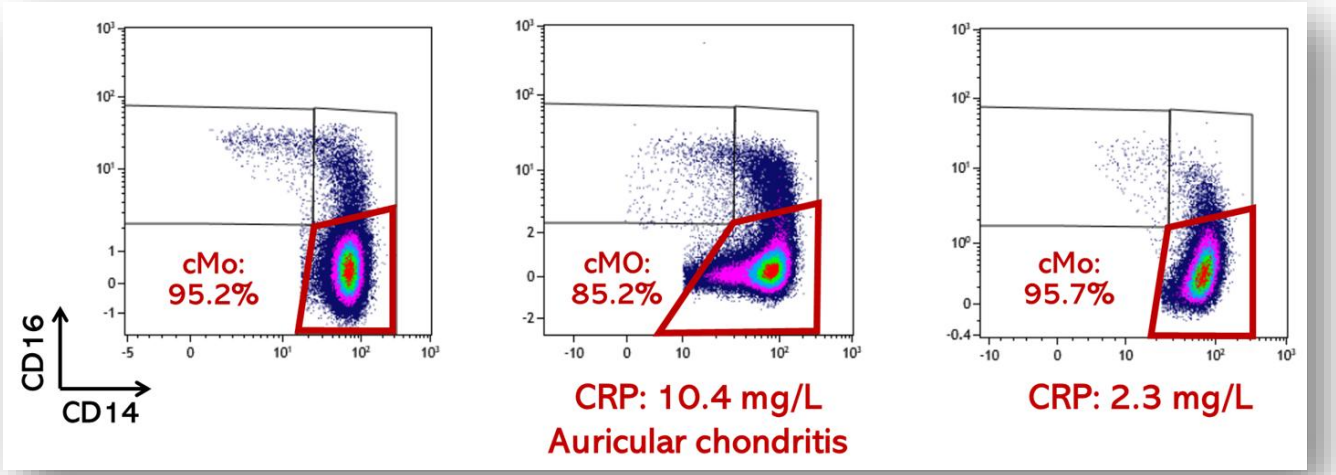
INTERPRETATION PITFALLS (2)

False

- **CMML with inflammatory state**

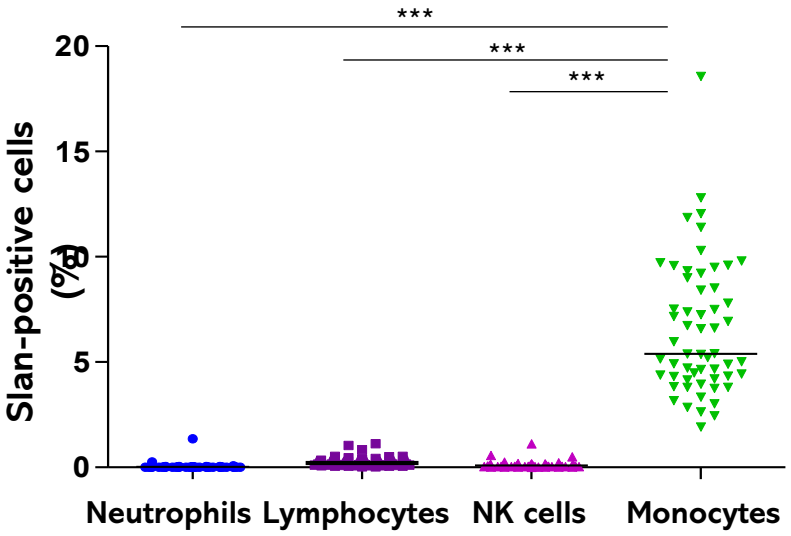
"Bulbous" aspect profile

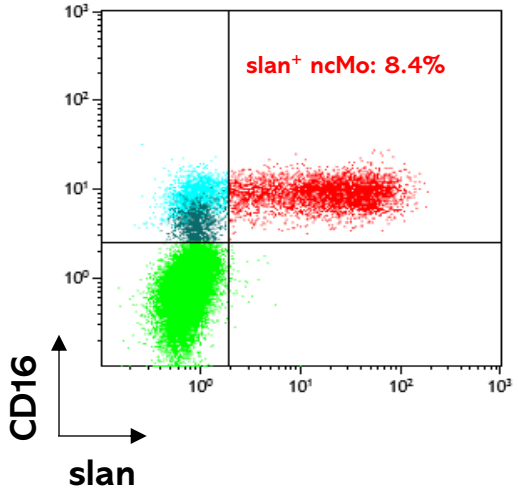


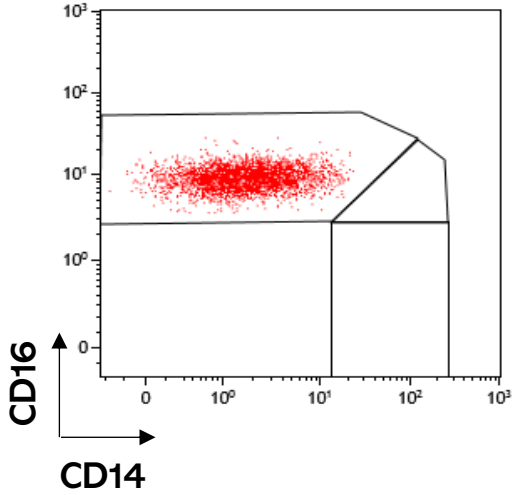


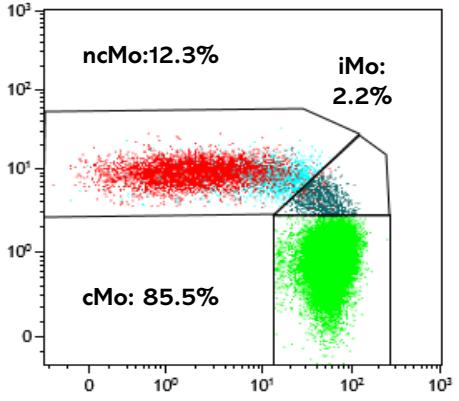
SELIMOGLU-BUET, BADAOUI et al, Blood 2017

SLAN IS A MARKER OF A FRACTION OF NCMO

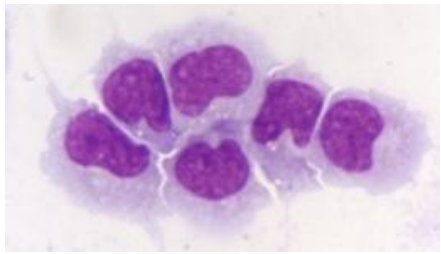




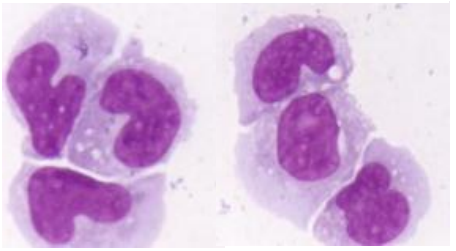




MO3 slan -



MO3 slan +

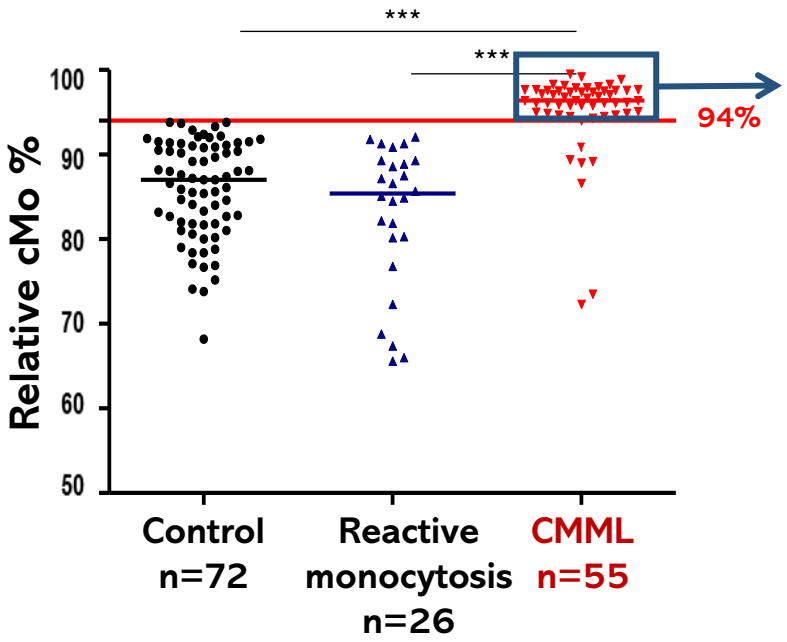


SLAN MARKER LABELS ABOUT 50% OF NCMO

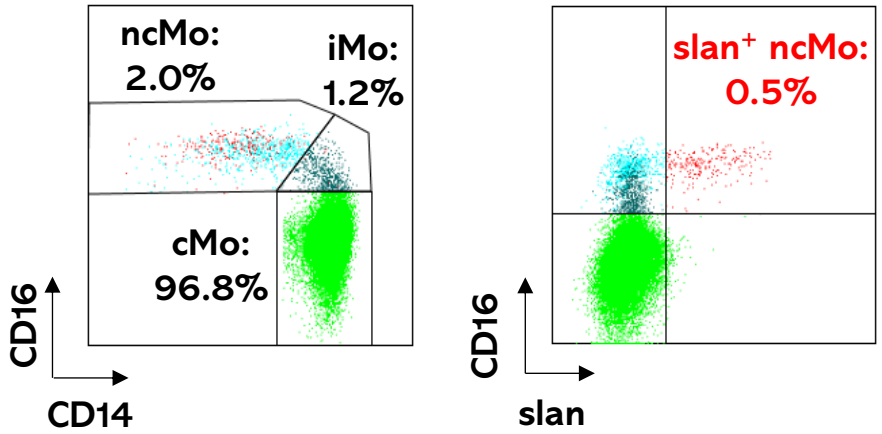
TARFI, BADAOUI et al, Haematologica 2019

Relative % of slan$^+$ ncMo in CMML patients

Relative accumulation of cMo



Example of typical CMML

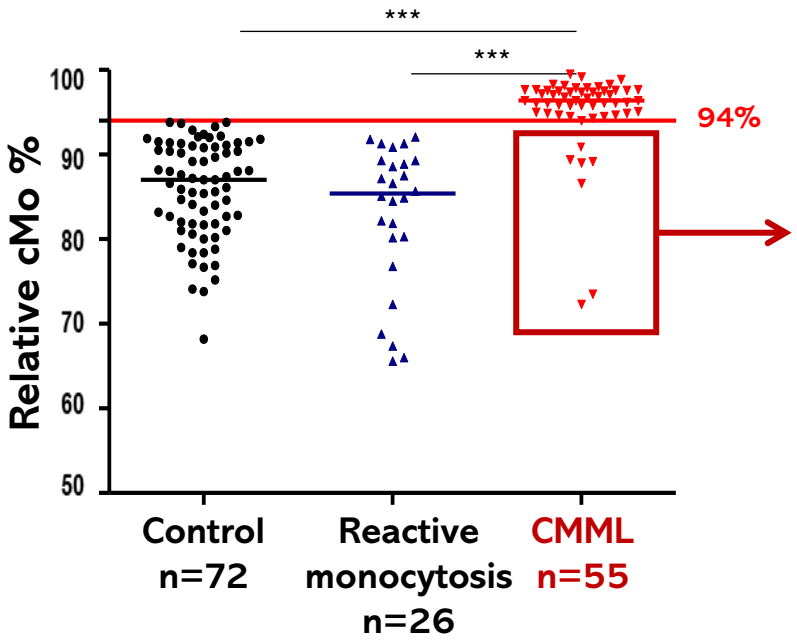


CRP : 68,3 ± 80,5 mg/l

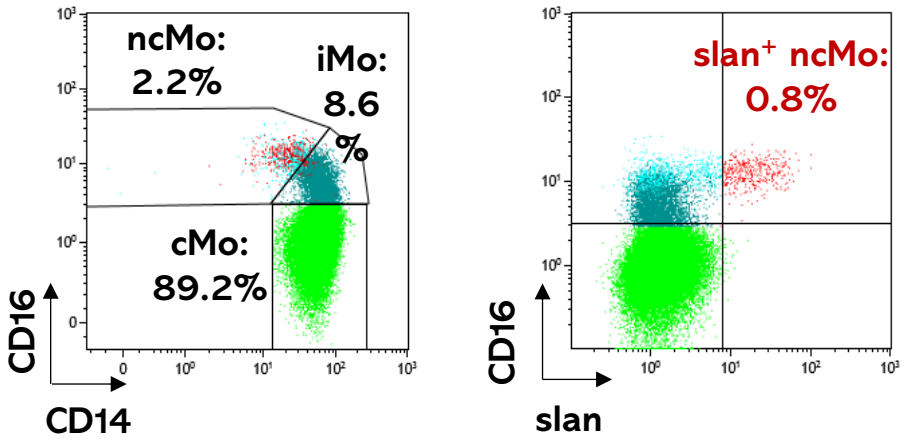
Tarfi, Badaoui et al, Haematologica 2019

Relative % of slan$^+$ ncMo in CMML patients

Relative accumulation of cMo



Example of inflammatory CMML

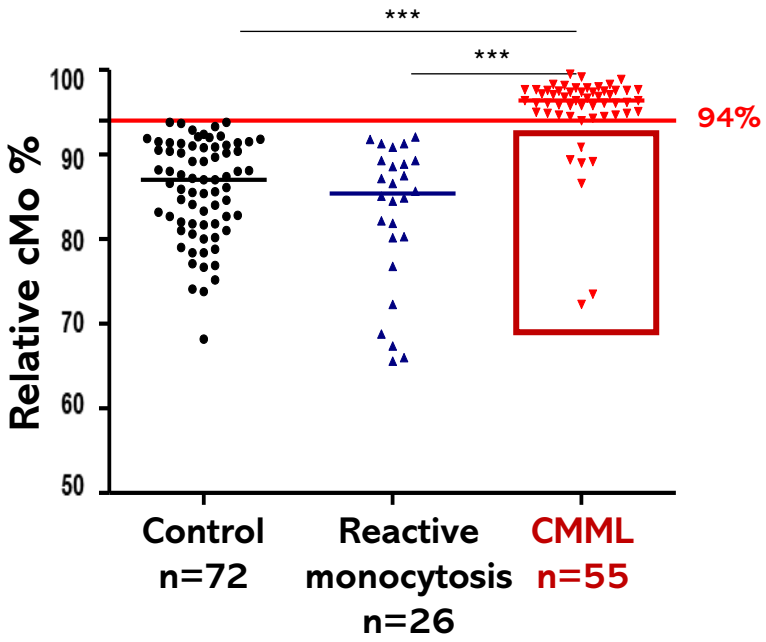


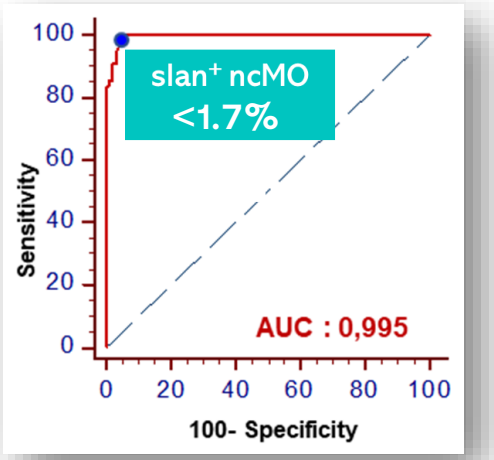
CRP : 68,3 ± 80,5 mg/l

Tarfi, Badaoui et al, Haematologica 2019

Relative % of slan$^+$ ncMo in CMML patients

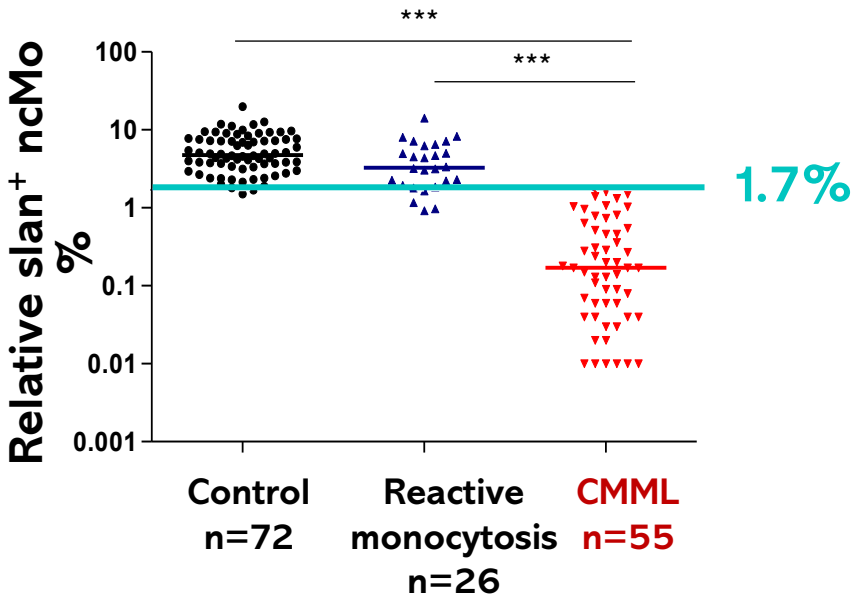
Relative accumulation of cMo





Sensitivity:100%
Specificity: 94%

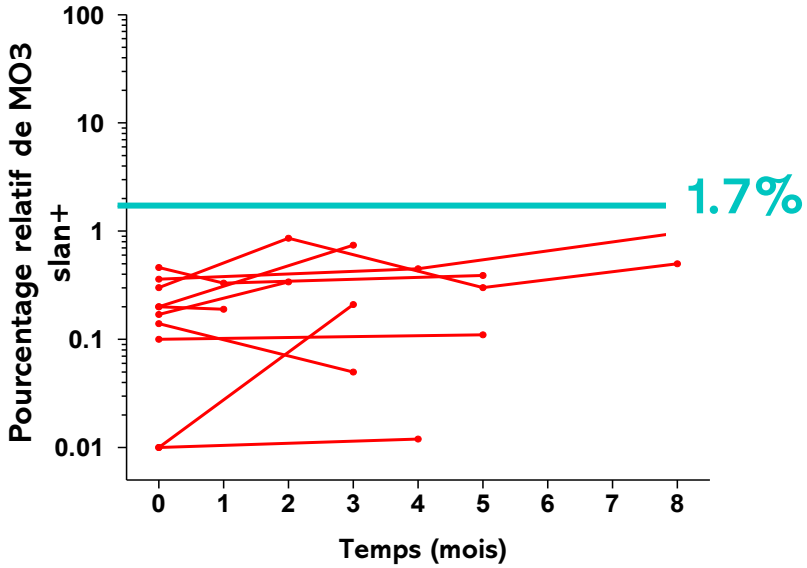
Relative diminution of slan$^+$ ncMo



Tarfi, Badaoui et al, Haematologica 2019

THE RELATIVE DECREASE IN SLAN$^+$ NCMO ≤1.7%

IS STABLE OVER TIME



Untreated CMML patients

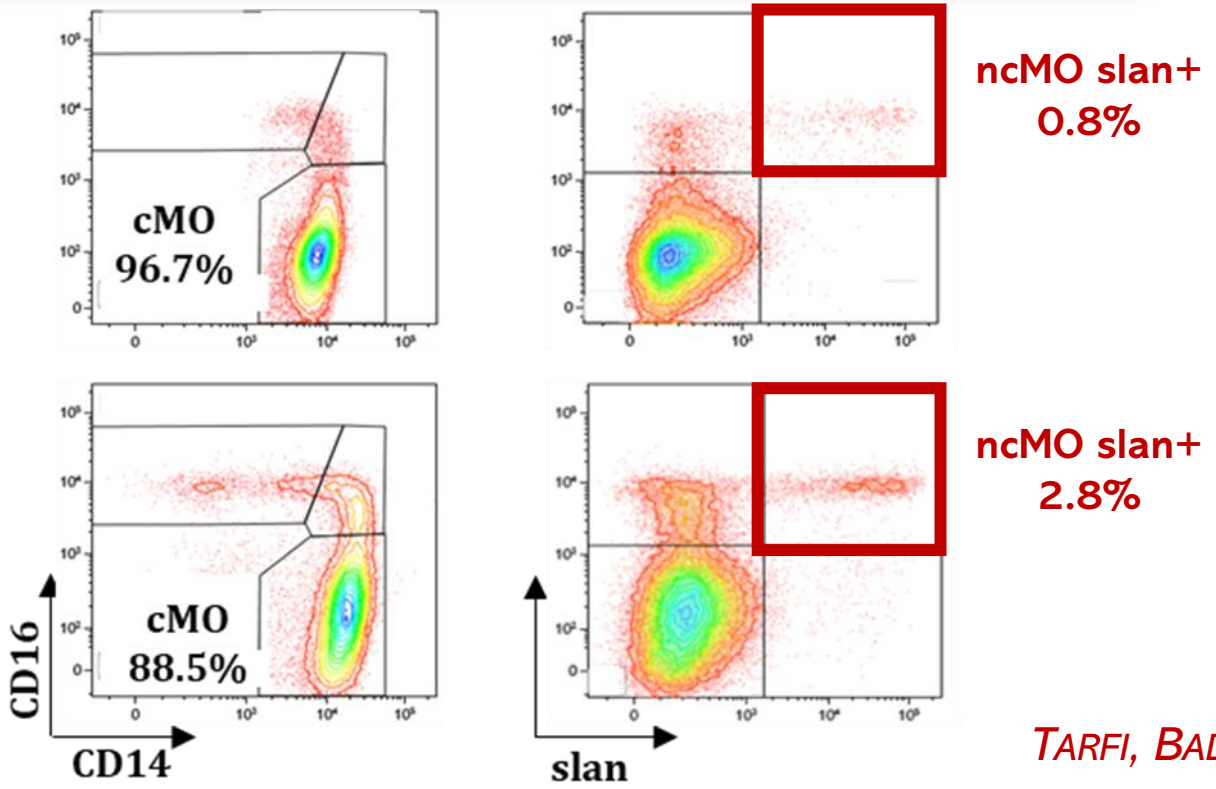
TARFI, BADAOUI et al, Haematologica 2019

The relative decrease in slan^{+} ncMo ≤1.7%

Can be reverted by Hypomethylating agents

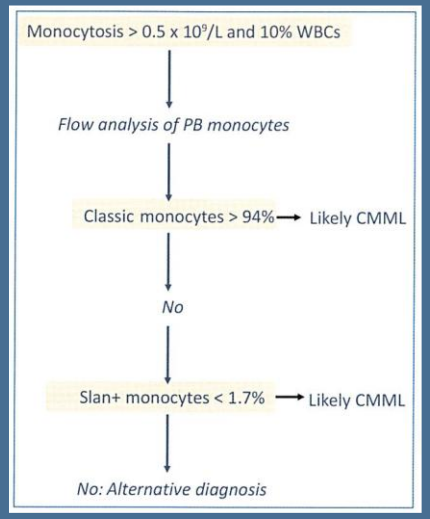
Diagnosis

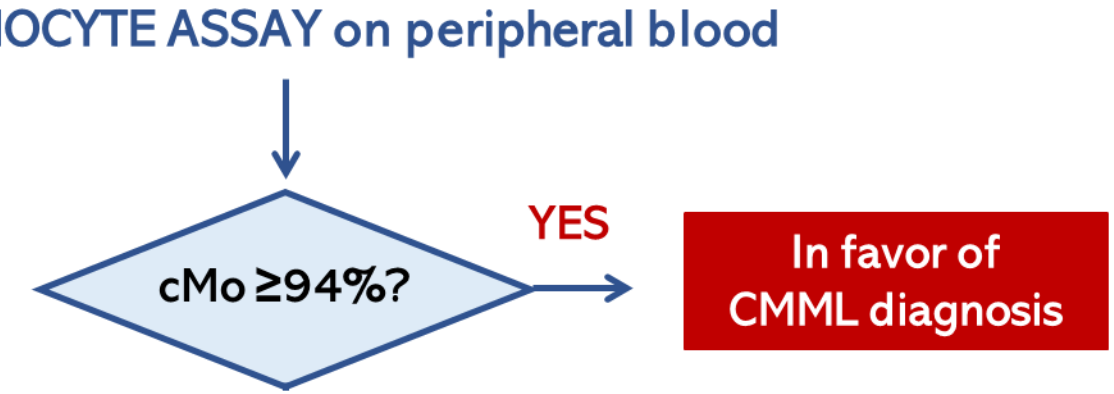
HMA



Tarfi, Badaoui et al, Haematologica 2019

Algorithm for CMML diagnosis

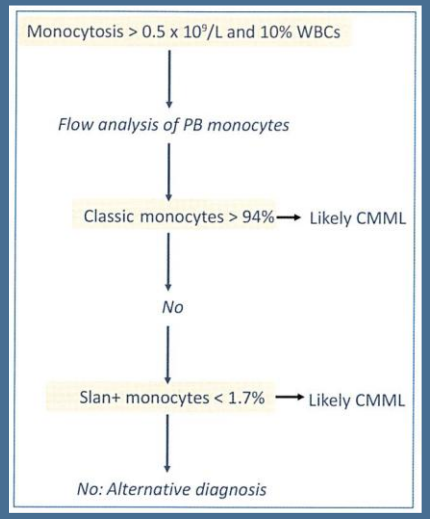


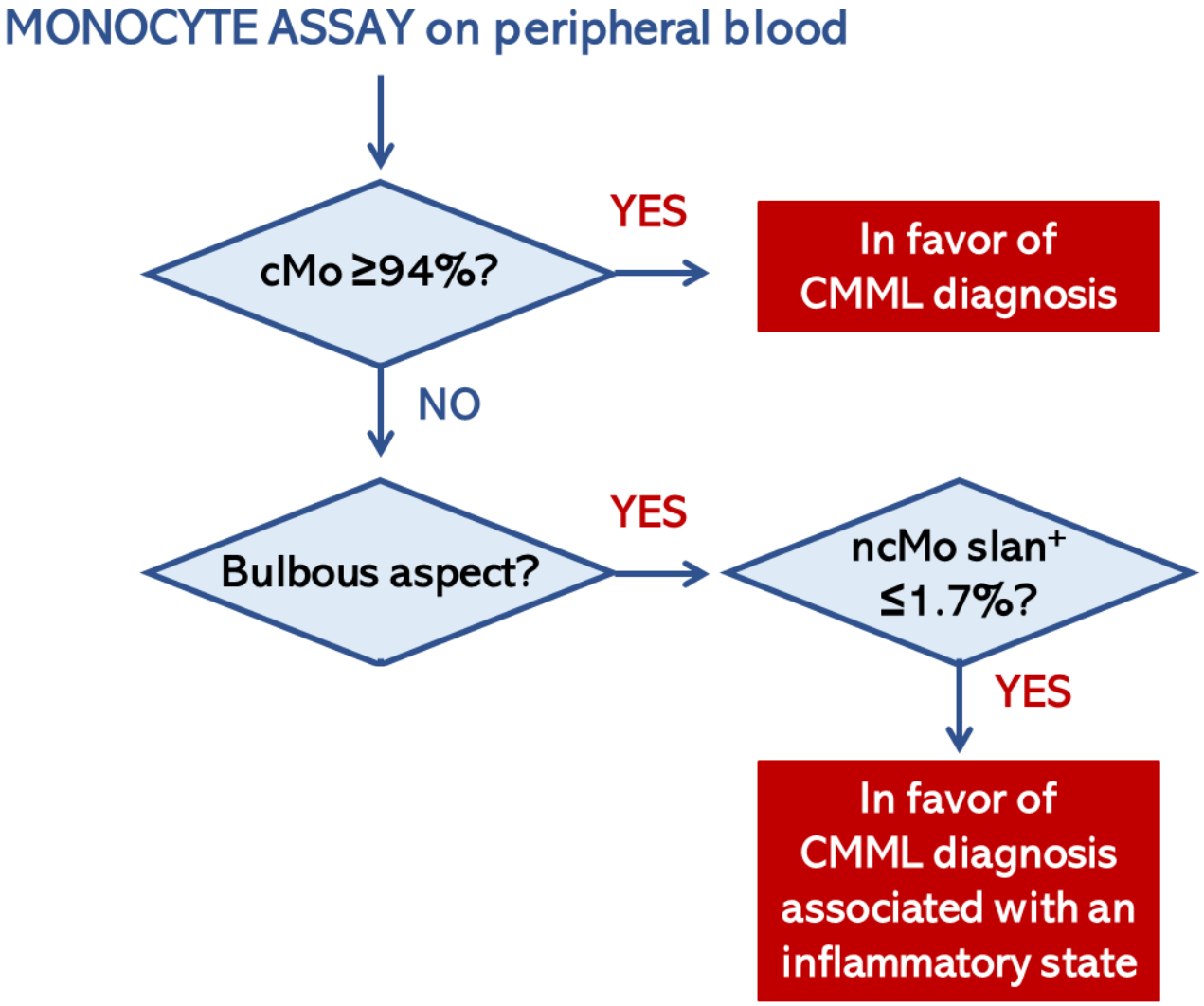


Tarfi, Badaoui et al, Haematologica 2019

Tarfi, Kern et al, Cyt PartB 2024

Algorithm for CMML diagnosis

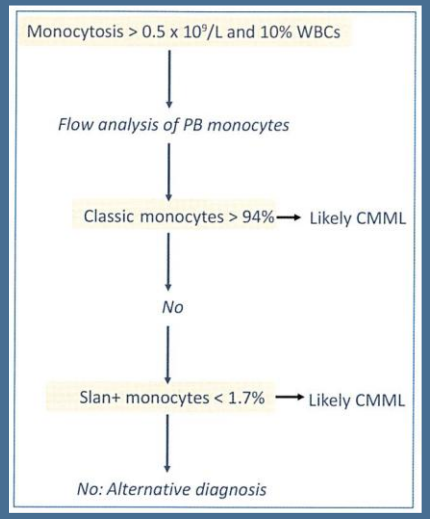


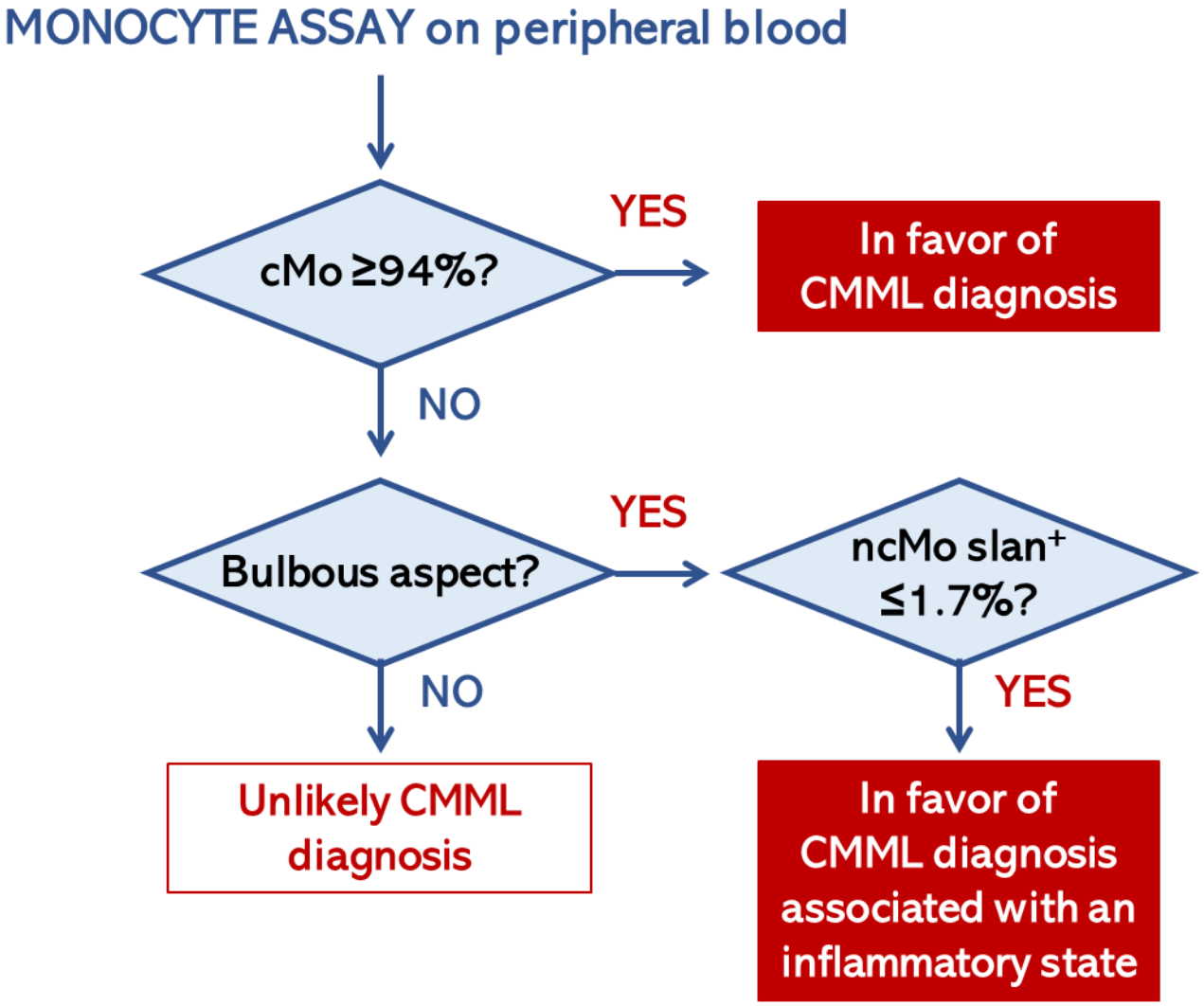


Tarfi, Badaoui et al, Haematologica 2019

Tarfi, Kern et al, Cyt PartB 2024

Algorithm for CMML diagnosis





Tarfi, Badaoui et al, Haematologica 2019

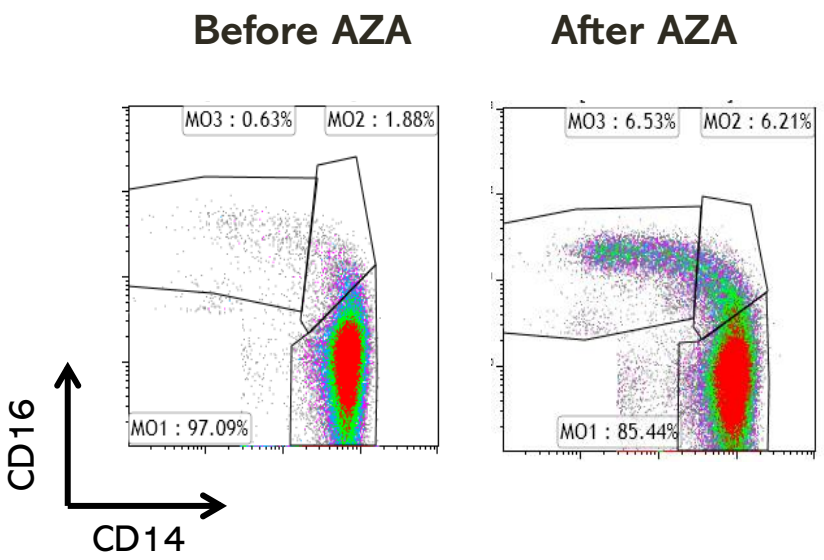
Tarfi, Kern et al, Cyt PartB 2024

INTERPRETATION PITFALLS (2)

False

cMO<94%

- CMML with inflammatory state
- Treatment by hypomethylating agents



Be careful to therapy when interpreting monocyte subset partitioning particularly for MDS patients!

SELIMOGLU-BUET, BADAOUI et al, Blood 2017

TAKE-HOME MESSAGES

- Acquire **at least 10,000 cMo** events (**200 µL** of whole blood)
- Be careful to **exclude neutrophils & NK cells** +++ **(exclusion antibody panel)**
- Use a **lyse-no-wash** protocol
- Be aware of **false positive and false negative cases:** check **treatment**, **clinical history**, **inflammatory state** before interpretation
- Our final recommendation: add **slan marker** to your panel

→ *on going study within ELN iMDS Flow group*

ACKNOWLEDGEMENTS

AP-HP. Hôpitaux universitaires Henri-Mondor

HÉMATOLOGIE

V TRAN QUANG
I SLOMA
S TARFI
B SCHELL
L LOCHER
B BADAOUI
N FREYNET




UPEC UNIVERSITÉ PARIS-EST CRÉTEIL — FACULTÉ DE SANTÉ

IMRB INSTITUT MONDOR DE RECHERCHE BIOMÉDICALE

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ÉQUIPE LABELLISÉE PAR LA LIGUE CONTRE LE CANCER

Patent GR Transfert