MONOCITOS Y CÉLULAS DENDRITICAS: implicación en el control tumoral y neoplasias de células dendríticas

CIC Centro de Investigación del Cáncer

VNiVERSiDAD D SALAMANCA
CAMPUS DE EXCELENCIA INTERNACIONAL

800 AÑOS 1218 ~ 2018

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CANCER RESEARCH UK

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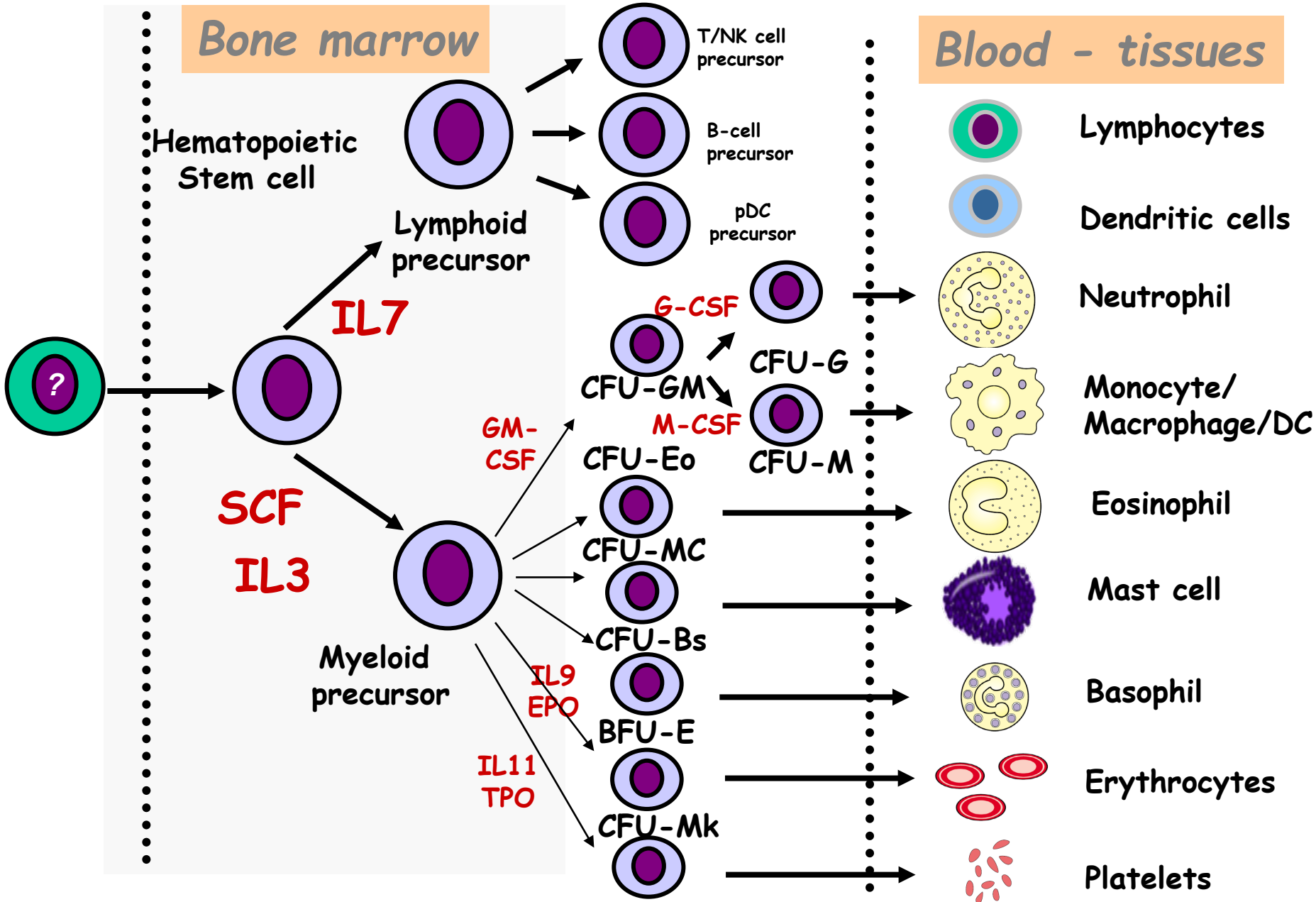
aecc Contra el Cáncer

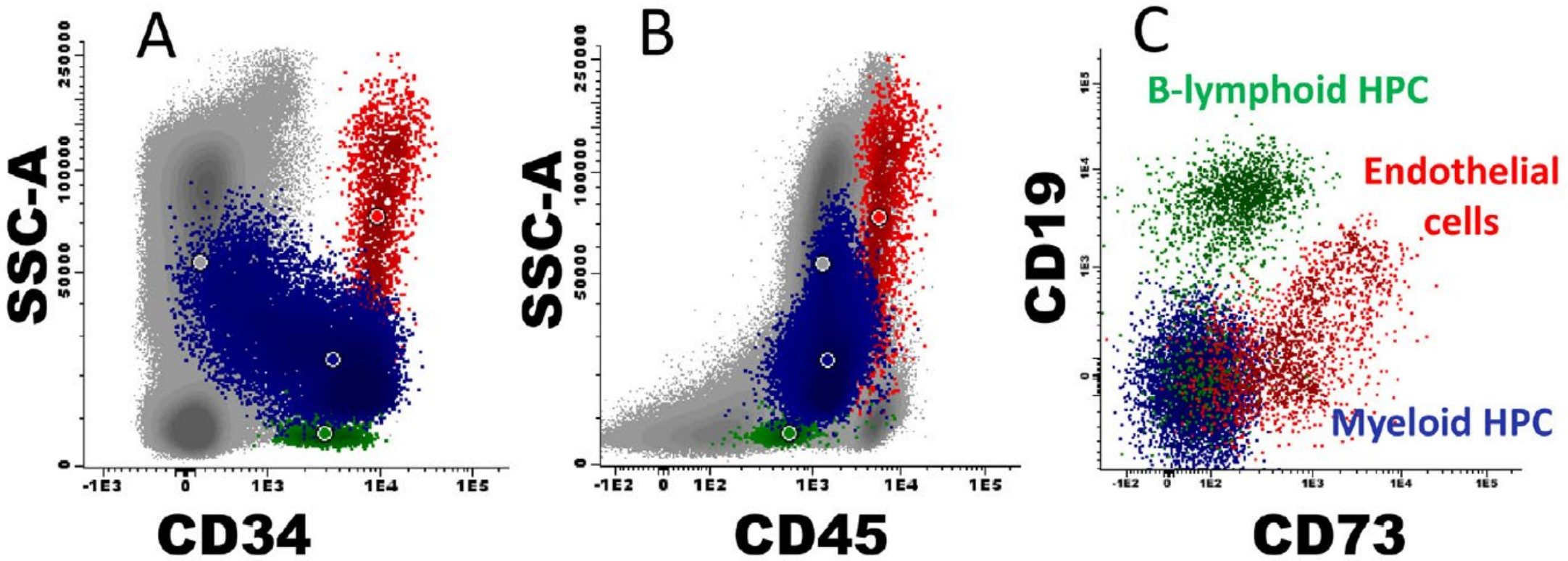
EuroFlow

6° Curso Práctico de Citometría de Flujo
Valencia, 28 de septiembre de 2023

HEMATOPOIESIS



Immunophenotypic features of human BM CD34+ HPCs vs CD34+ endothelial cells & MSCs



■ - Mesenchymal Stem Cells; ■ - CD34+ Endothelial cells; ■ - CD34+ Hematopoietic Precursors;

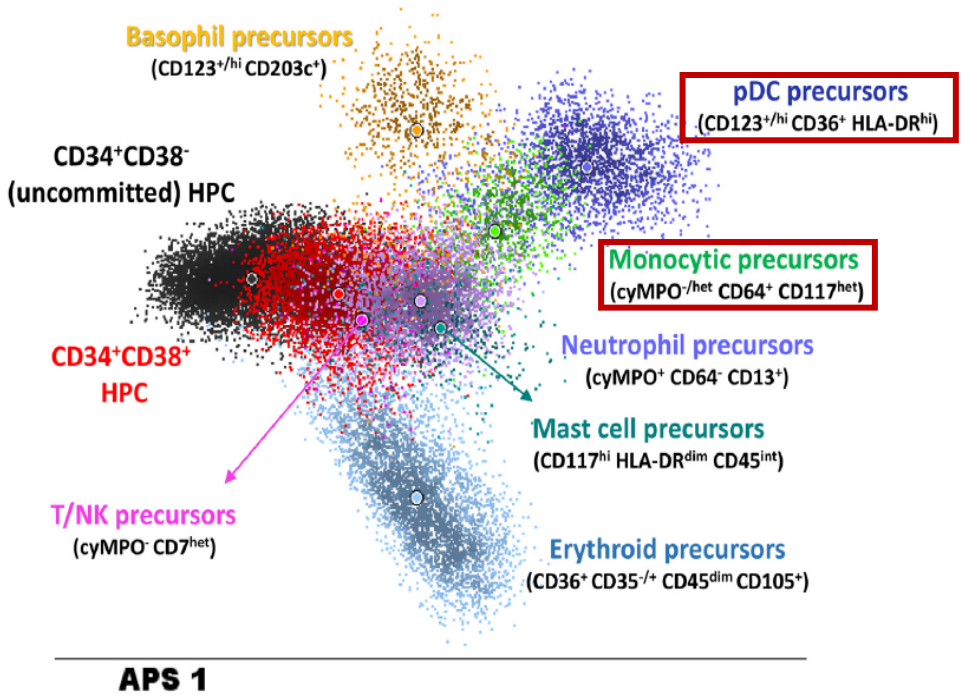
Orfao et al, J Immunol Meth 2019

IMMUNOPHENOTYPE OF CD34+ MYELOID-COMMITTED HPC

CELL LINEAGE	SSC	IMMUNOPHENOTYPE
Erythroid	stable	CD36+, CD105+, CD64-
Megakaryocytic	high	CD61+
Neutrophil	high	CyMPO+,
Eosinophil	high	CyEPO+,
Basophil	low	$CD123^{hi}$, CD203c+, $CD117^{lo}$
Monocytic	stable	CD64+
Mast cell	low	$CD117^{hi}$,
pDC	stable	CD36+, $CD123^{hi}$, $HLADR^{hi}$

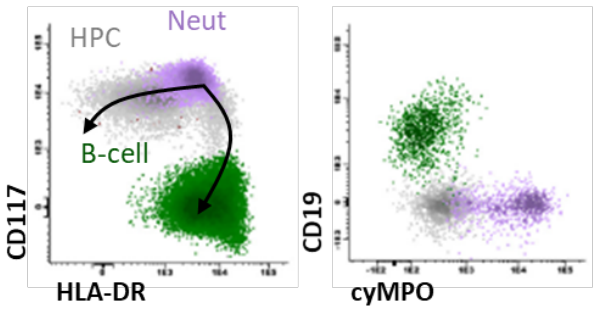
EuroFlow

HEMATOPOIETIC PRECURSOR CELLS

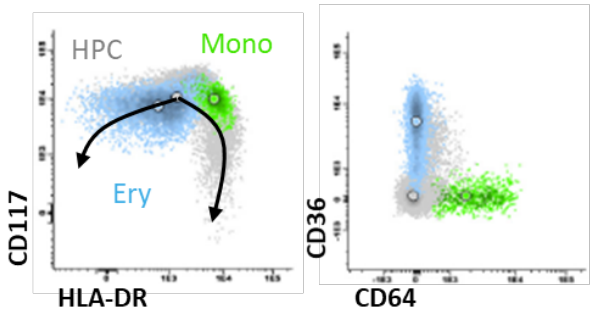


CELL LINEAGE	PHENOTYPE	FREQUENCY (% of CD34+ and/or CD117+ precursors) Median [min-max]
Myeloid precursors	*CD38+ CD45lo CD117+ HLA-DRhet*	*77% [57-85%]*
Erythroid	CD36+ CD35-/+ CD45lo CD105+ CD71+	35% [24-37%]
Neutrophil	cyMPO+ CD64- CD13+	33% [26-38%]
Monocyte	CD64+ cyMPO-/+het CD117het HLA-DRhi	22% [16-28%]
pDC	CD123+/hi HLA-DRhi CD45lo CD36+	6% [1-9%]
Basophil	CD123+/hi CD45+ CD117lo HLA-DRlo CD203c+	<1% [0-3%]
Eosinophil	cyMPO- CD15/CD65+ cyEPO+	<1%
Mast cell	CD117hi HLA-DRlo CD45int	<1%
Megakaryocyte	CD61+ CD45lo CD203clo	<1%
B-cell	*nuTdT+ cyCD79a+ CD19+*	*23% [<1-45%]*
T/NK/DC	*cyMPO- CD7het*	*12% [10-15%]*

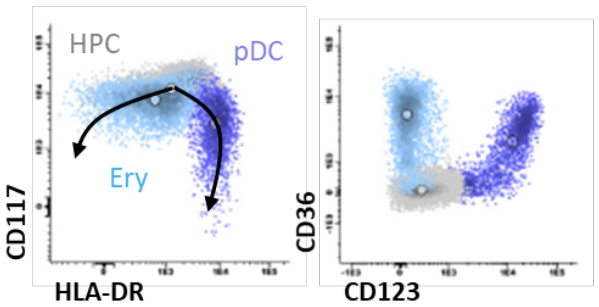
B-CELL *VS.* NEUTROPHIL



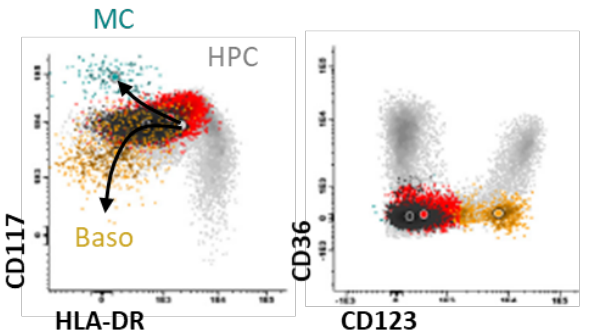
ERYTHROID *VS.* MONOCYTIC



pDC *VS.* ERYTHROID



MAST CELL *VS.* BASOPHIL

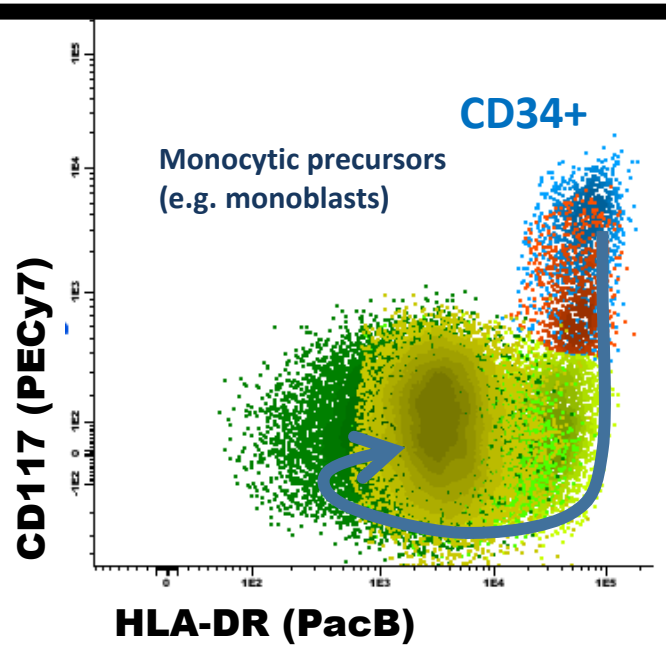


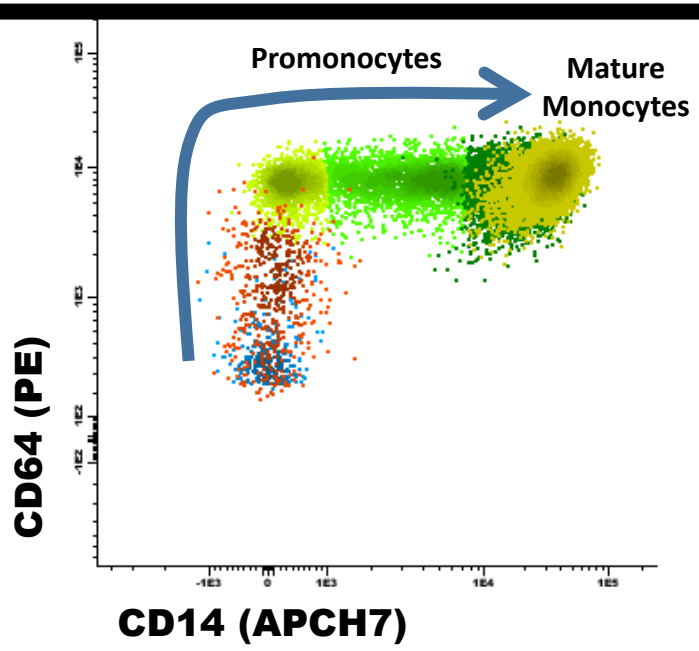
Modified from: Matarraz et al Leukemia 2008 and Orfao *et al*, JIM (2019)

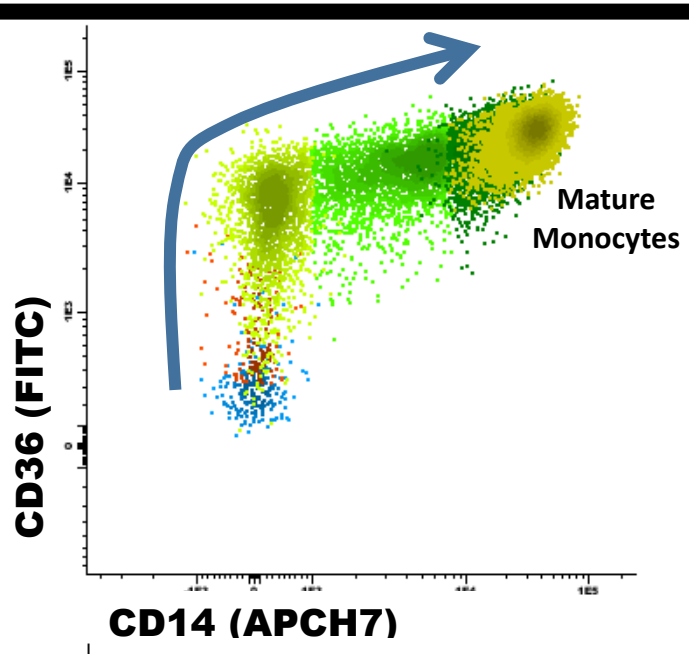
MPO, myeloperoxidase; **EPO,** eosinophil peroxidase; **TdT,** terminal deoxynucleotidyl transferase

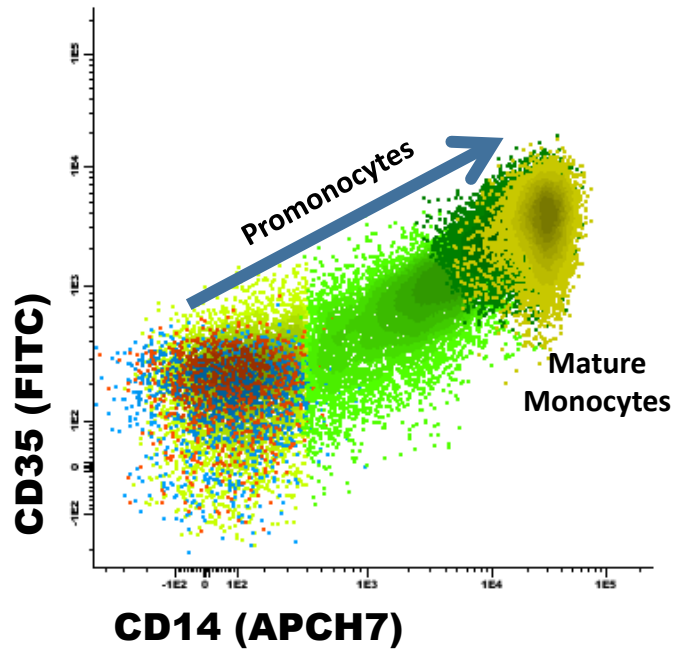
NORMAL MONOCYTIC MATURATION IN BM

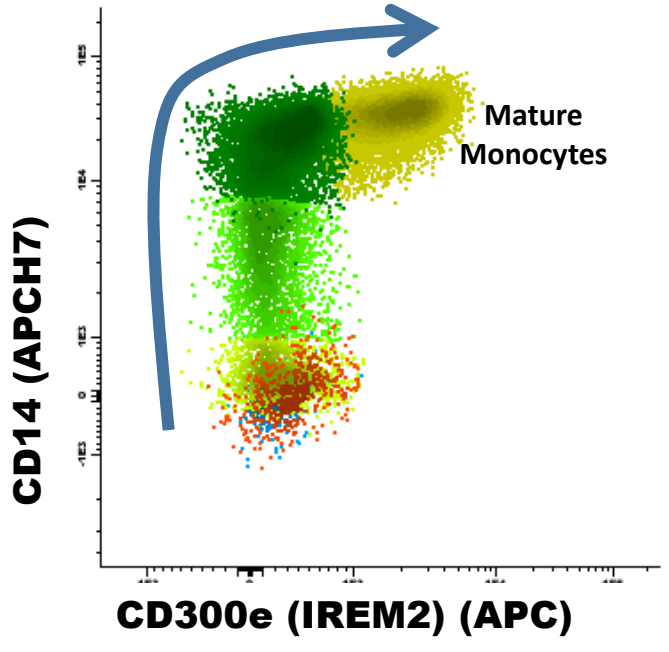
MONOCYTIC MATURATION IN BONE MARROW

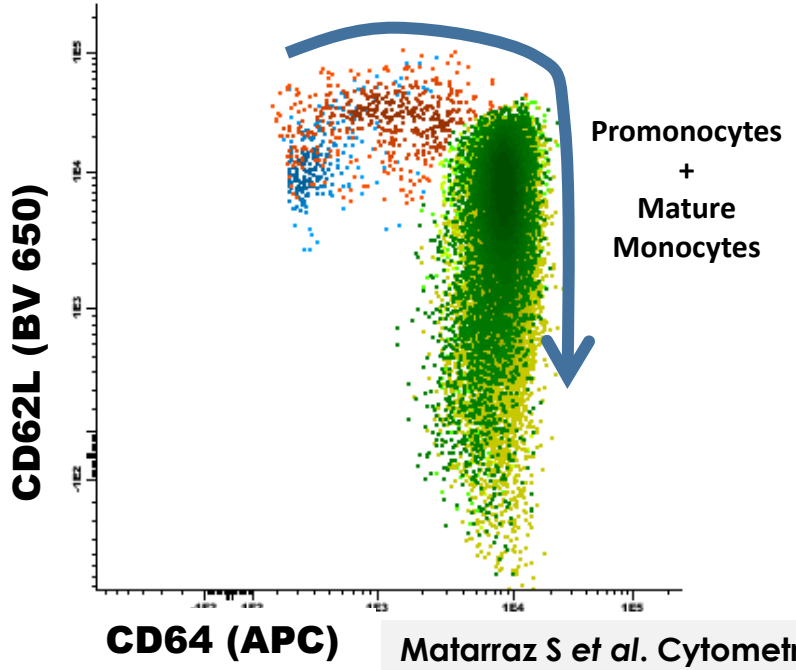






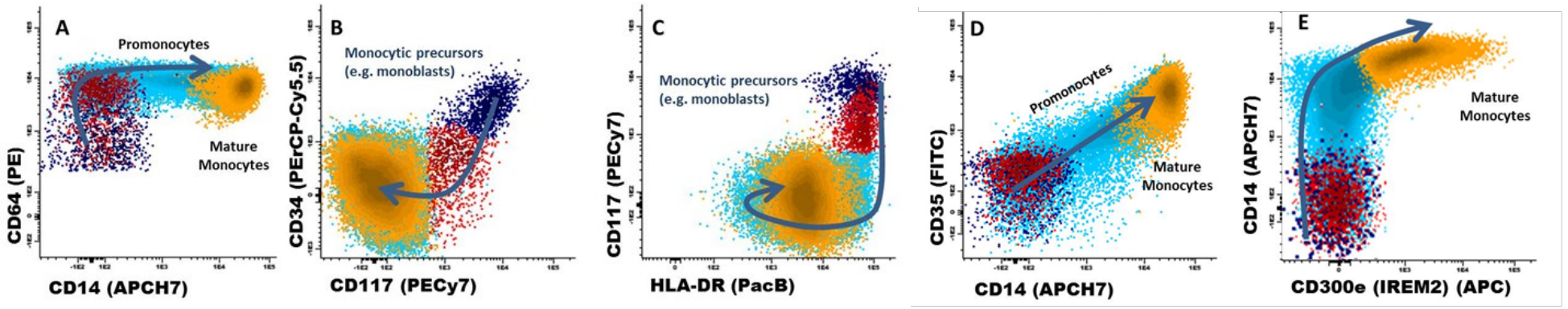






Matarraz S *et al*. Cytometry B 2015

MONOCYTIC MATURATION IN BONE MARROW



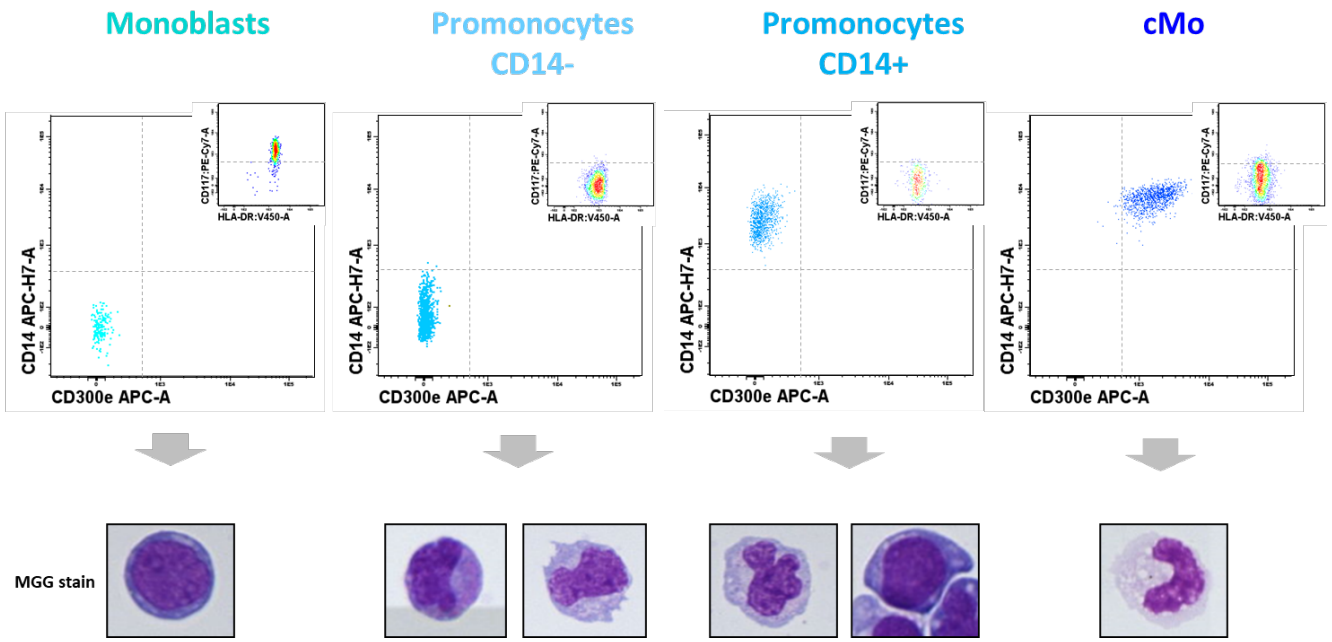
Monoblasts CD34+/CD117+

Monoblasts CD34-/CD117+

Promonocytes CD14-

Promonocytes CD14+

Mature Mo



Promonocytes

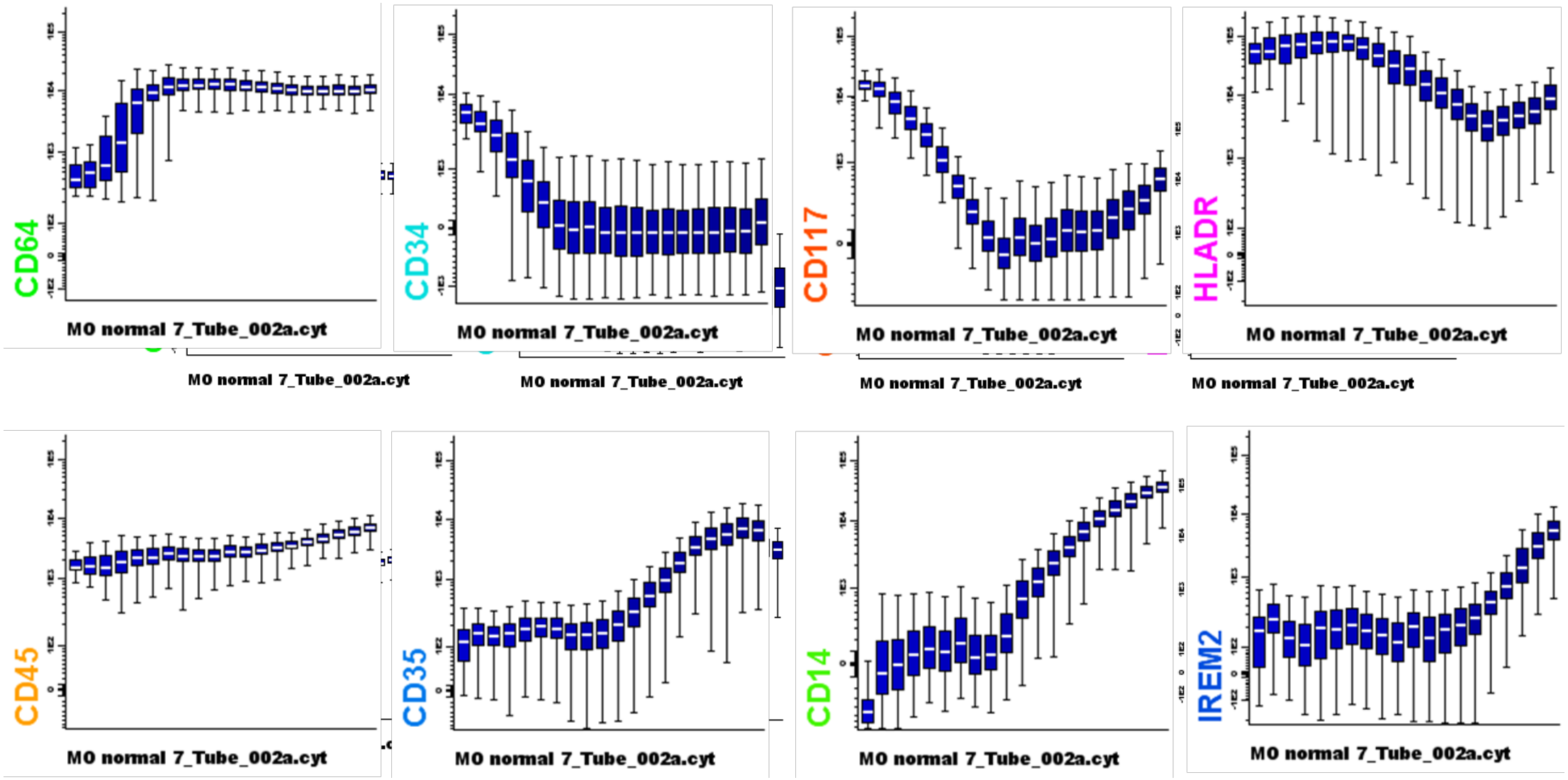
[CD34^{-}/CD117^{-}/CD14$^{-/+}$/CD300e^{-}]

↓

Morphologically **heterogeneous** population

Adapted and extended from: *Matarraz et al, Cytometry Part B (2015)*

MONOCYTIC MATURATION IN BONE MARROW

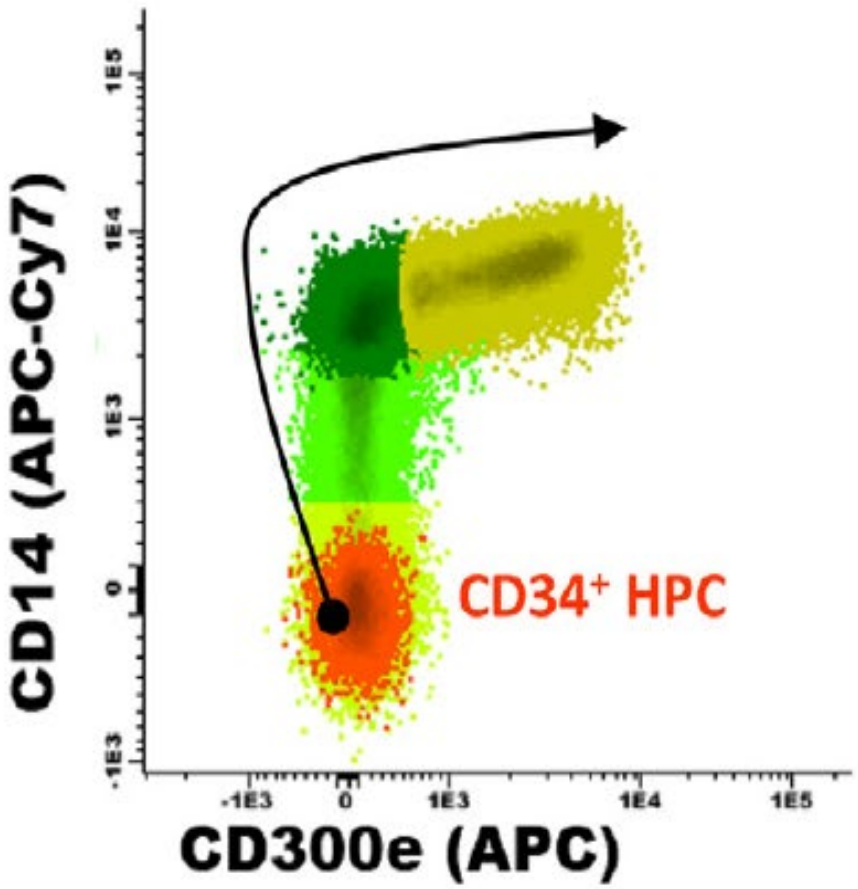


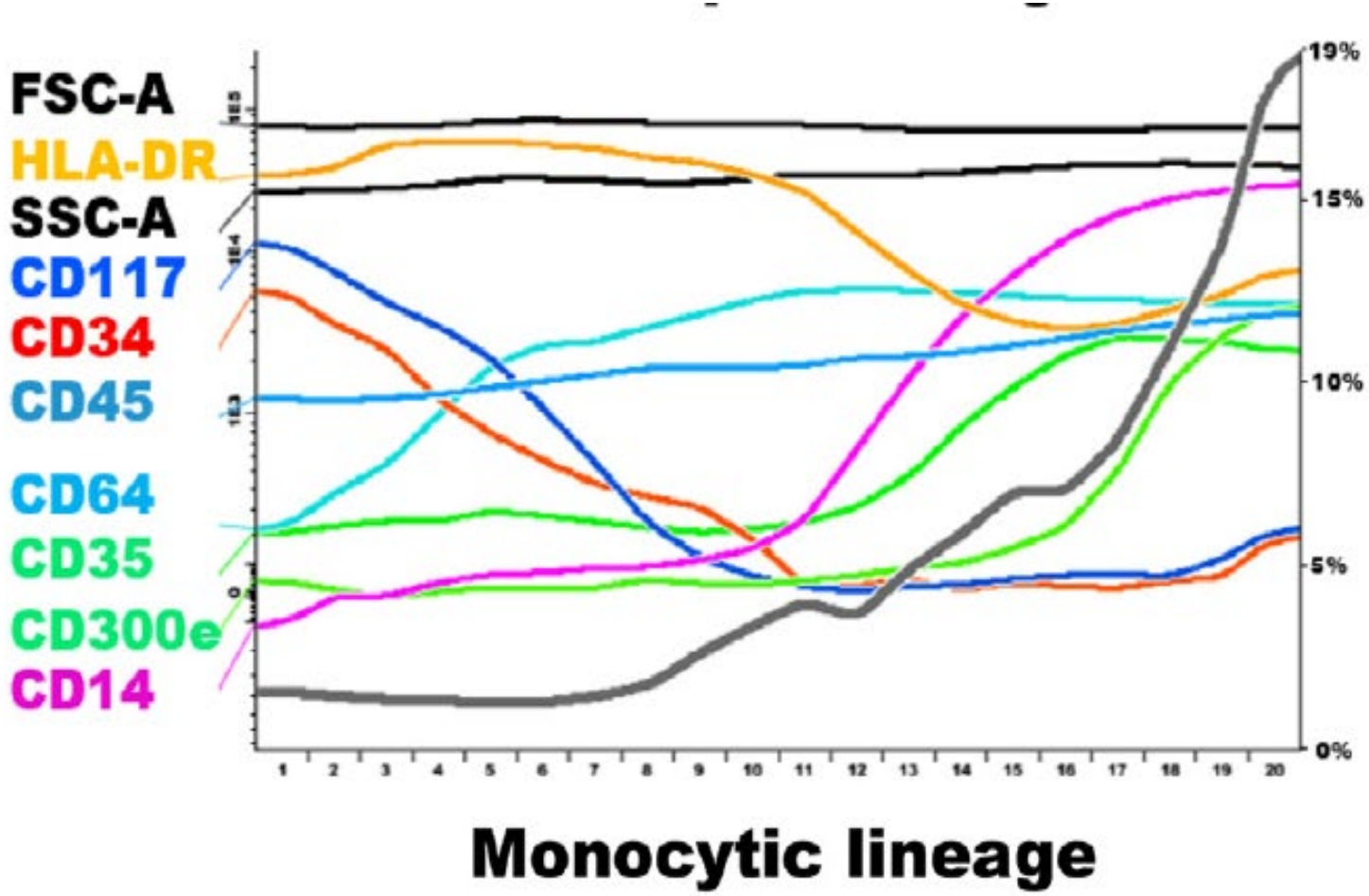
HLADR → CD64 → CD36 → CD35 → CD14 → IREM2 (CD300e)

N-DIMENSIONAL MONOCYTIC MATURATION IN NORMAL BM

EuroFlow

C

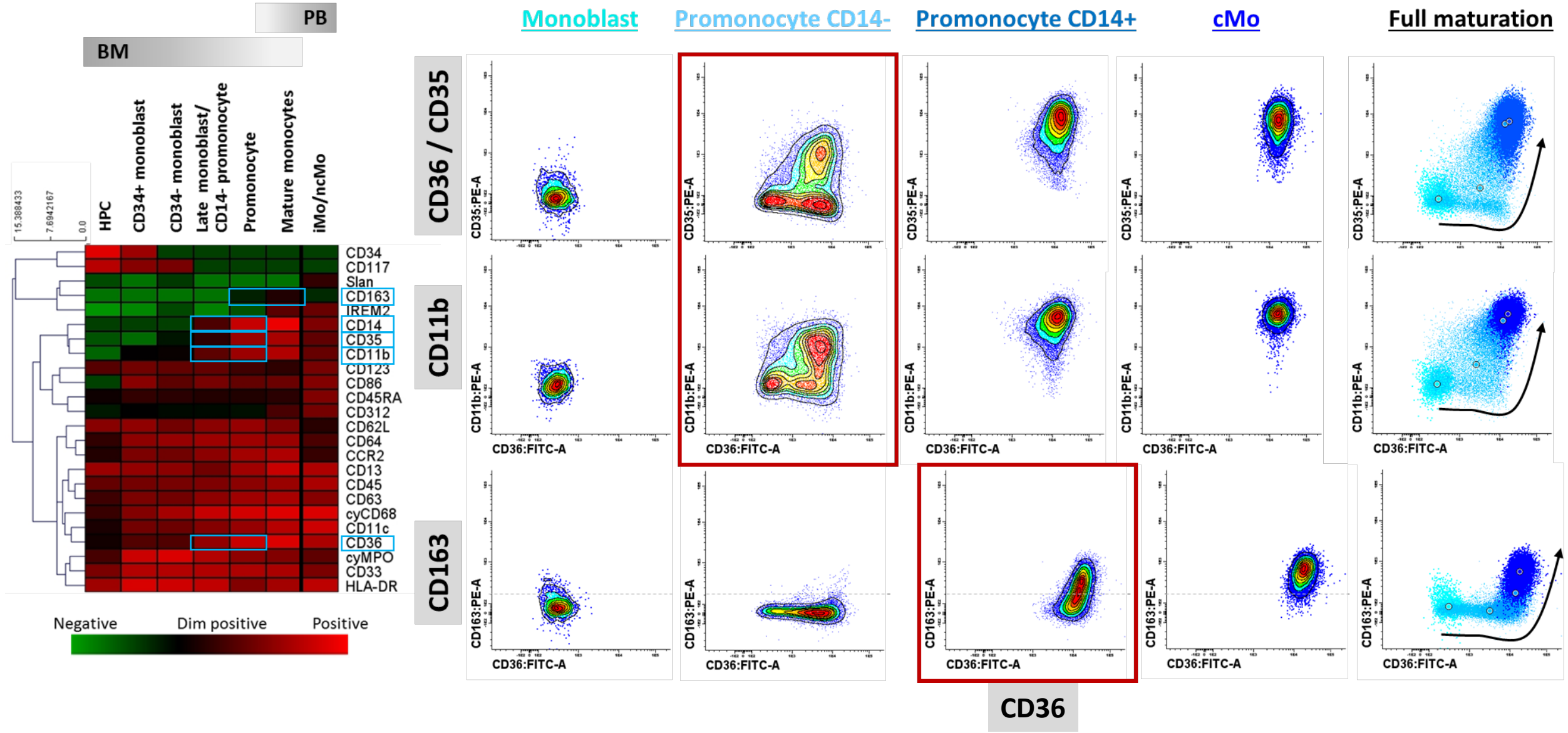




Sequential markers: CD64+ CD34- CD117- CD14+ CD35+ CD300e+

Orfao et al, J Immunol Meth 2019

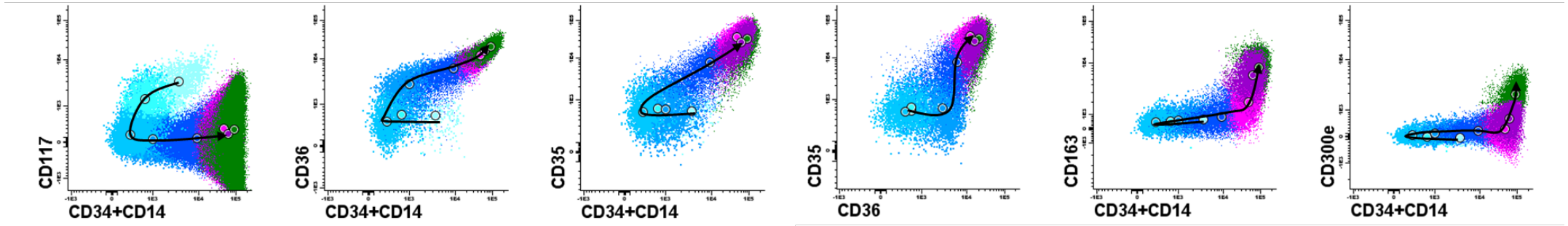
MONOCYTIC MATURATION IN BONE MARROW



In: *van der Pan et al, manuscript in preparation*

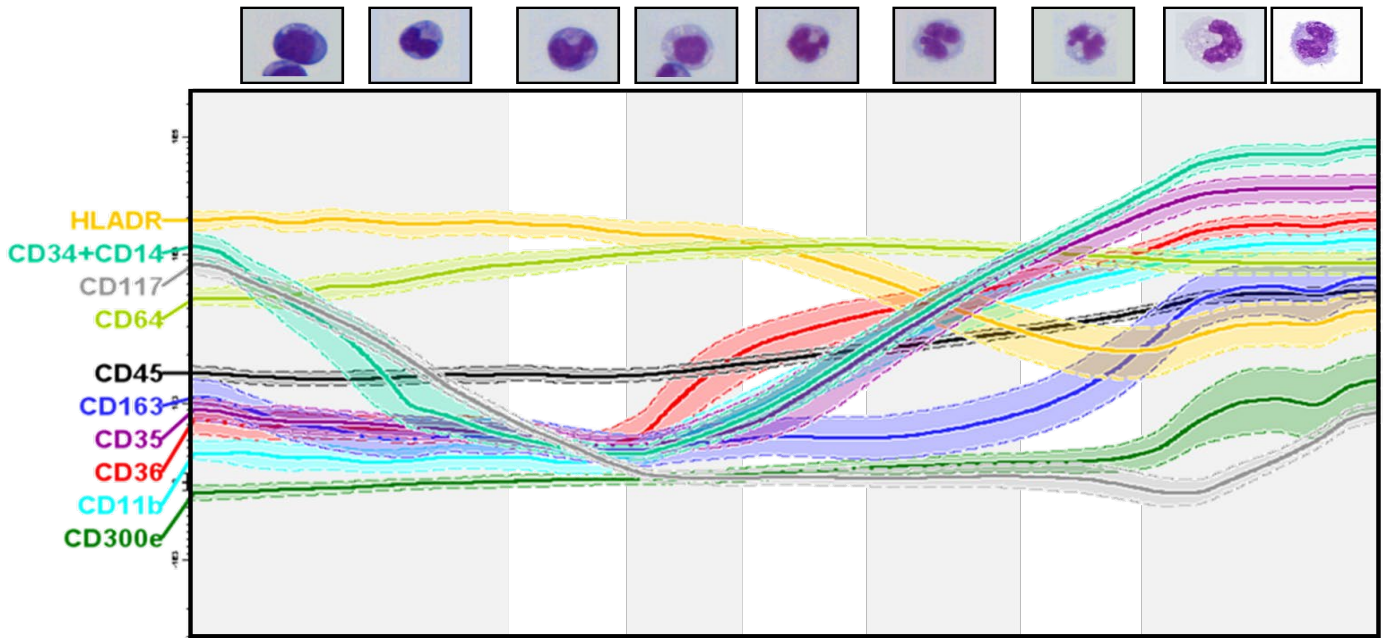
MONOCYTIC MATURATION IN BONE MARROW

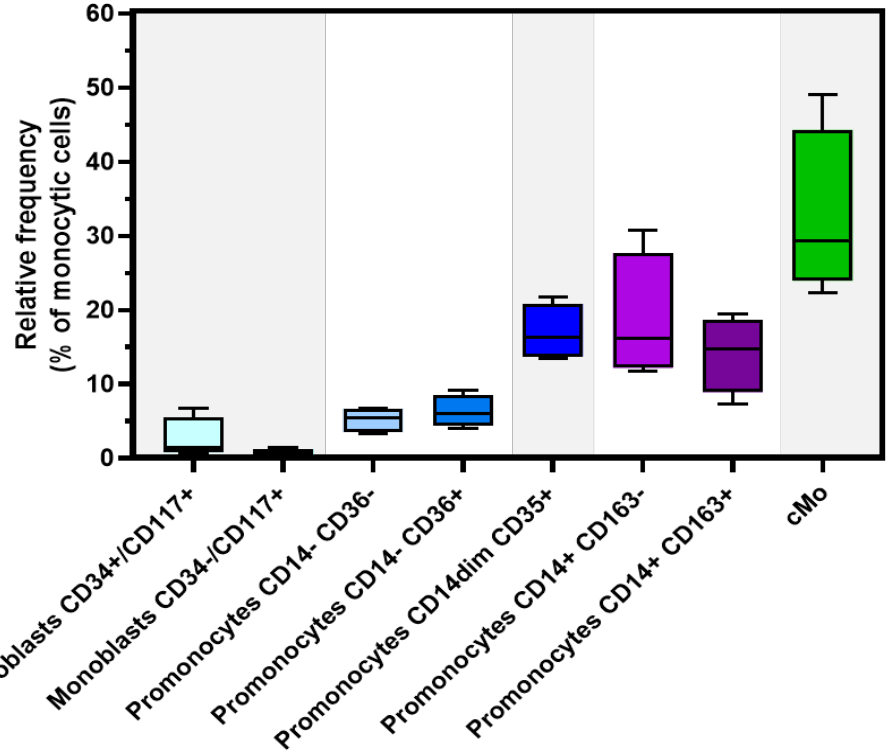
Monoblast CD34+/CD117+
Monoblast CD34-/CD117+
➧ Promonocyte CD14- CD36-
Promonocyte CD14- CD36+
➧ Promonocyte CD14dim CD35+
➧ Promonocyte CD14+ CD163-
Promonocyte CD14+ CD163+
➧ cMo



Monoblast | Promonocyte CD14^{-} | Promonocyte CD14$^{dim/+}$ | cMo

CD36^{-} CD36^{+} CD35^{+} CD163^{-} CD163^{+}





CD34- CD117- CD36+ CD35+/CD11b+/CD14^{lo} CD14+ CD163+ CD300e+

In: *van der Pan et al, manuscript in preparation*

NORMAL (PLASMACYTOID) DENDRITIC CELL MATURATION IN BM

EuroFlow

IMMUNOPHENOTYPE OF CD34+ MYELOID-COMMITTED HPC

CELL LINEAGE	SSC	IMMUNOPHENOTYPE
Erythroid	stable	CD36+, CD105+, CD64-
Megakaryocytic	high	CD61+
Neutrophil	high	CyMPO+,
Eosinophil	high	CyEPO+,
Basophil	low	$CD123^{hi}$, CD203c+, $CD117^{lo}$
Monocytic	stable	CD64+
Mast cell	low	$CD117^{hi}$,
pDC	**stable**	**CD36+, $CD123^{hi}$, $HLADR^{hi}$**

Orfao et al J Immunol Meth, 2019

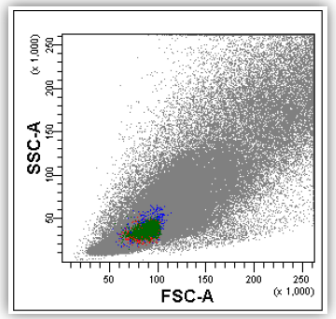
PLASMACYTOID DENDRITIC CELL MATURATION IN BONE MARROW

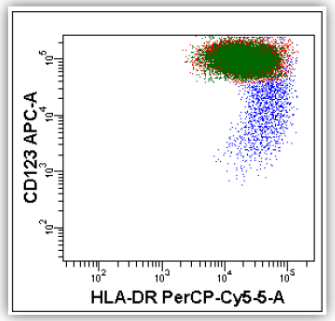
CD34+ CD38+ HPC

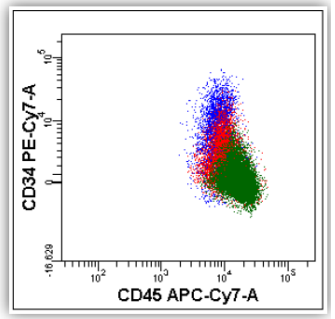
$CD34^{++}/HLA\text{-}DR^{++/+++}/CD123^{++}/CD45^{+/++}$ (Stage I)

$CD34^{+}/HLA\text{-}DR^{+/++}/CD123^{++/+++}/CD45^{+/++}$ (Stage II)

$CD34^{-}/HLA\text{-}DR^{++}/CD123^{++/+++}/CD45^{++}$ (Stage III)



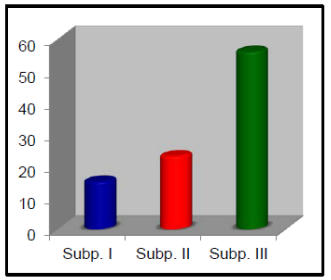


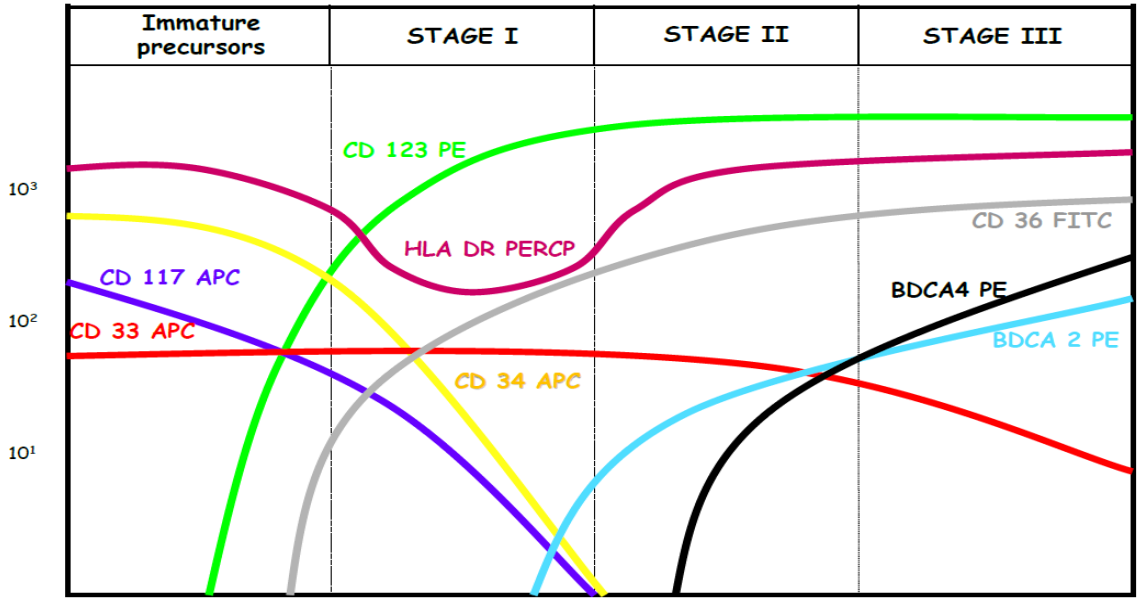


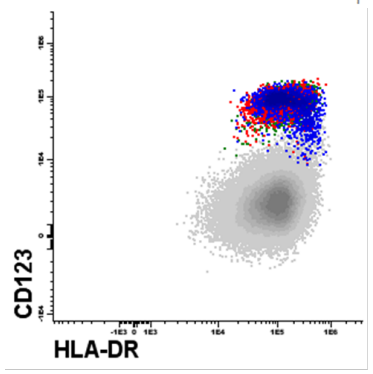
Total pre-pDC
(0.28%±0.13% of BM nucleated cells)

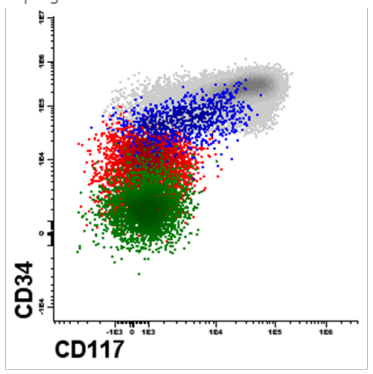
	Stage I (Immature)	Stage II (Intermediate)	Stage III (Mature)
% of BM nucleated cells	0.03±0.01*	0.08±0.03	0.17±0.09
% of pre-CDp	15±6	23±6	56±11

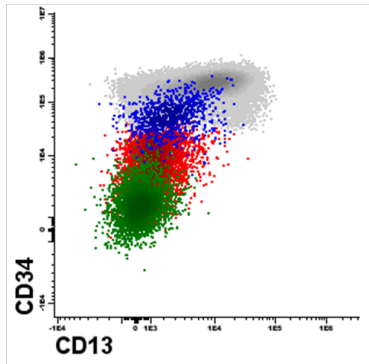
* 3.4%±0.7% of all CD34+ hematopoietic progenitor cells

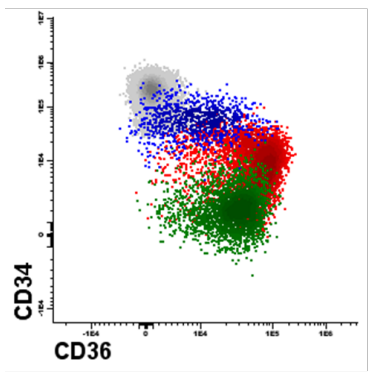


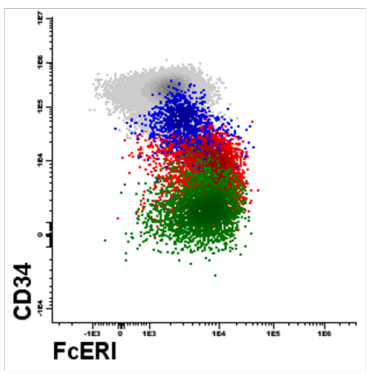


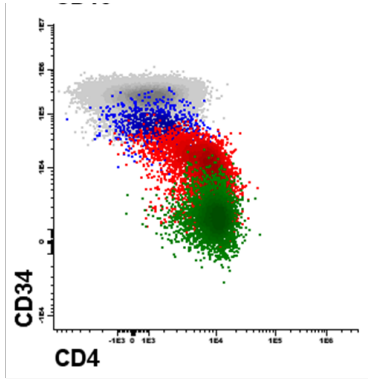






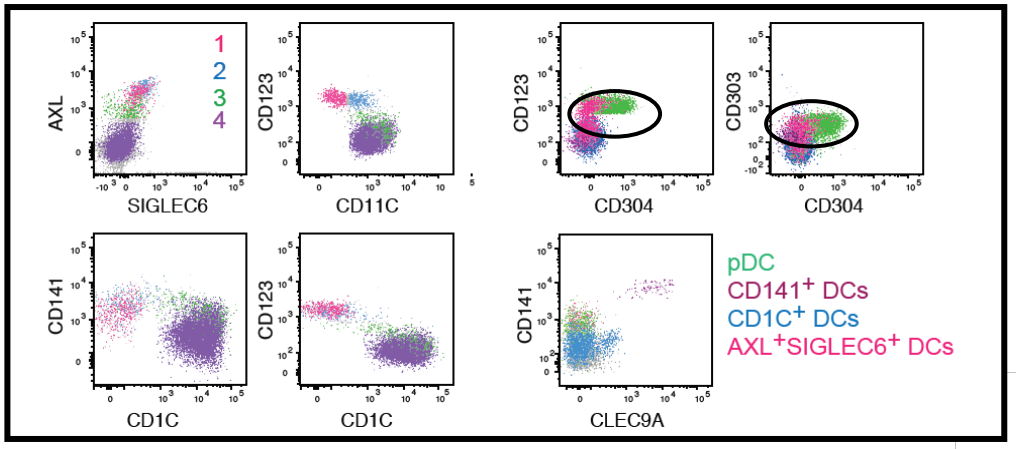




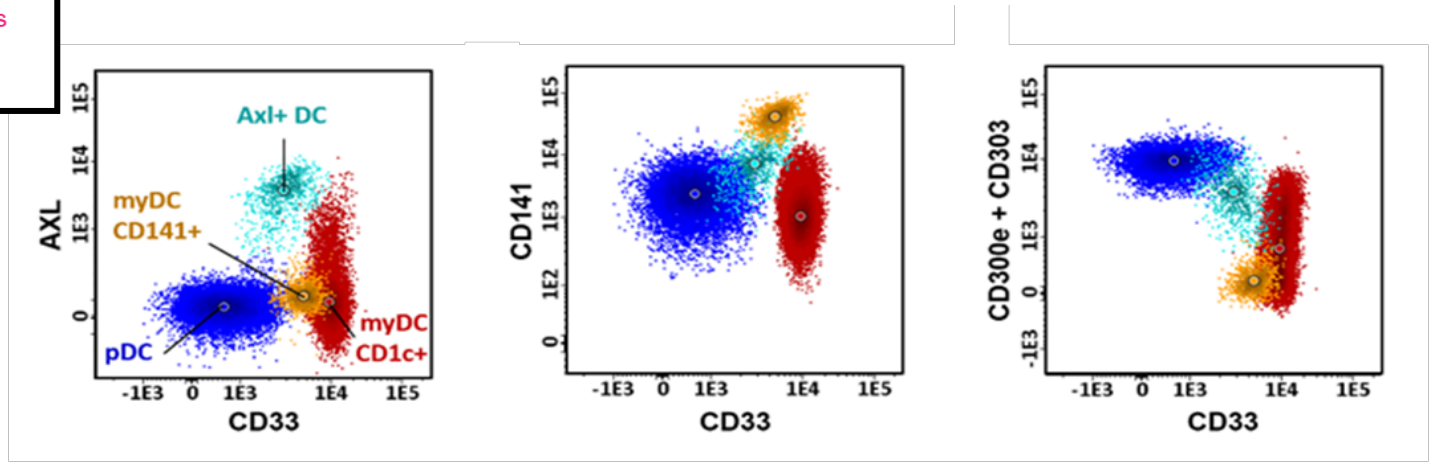


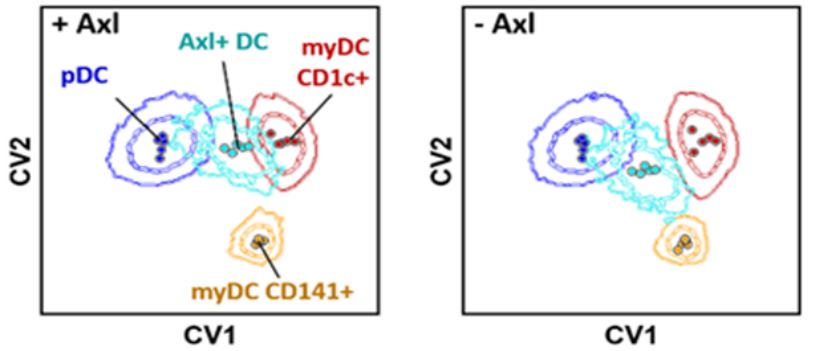
Adapted from: *Martín-Martín et al, Transfusion (2010)*

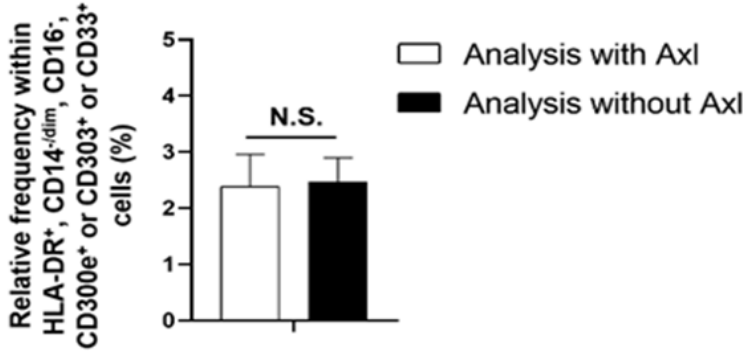
AXL+ DENDRITIC CELLS IN THE CLASSICAL pDC GATE (BLOOD)



The classical CD123^{hi} HLADR^{hi} pDC gate includes another population of AXL+ DC







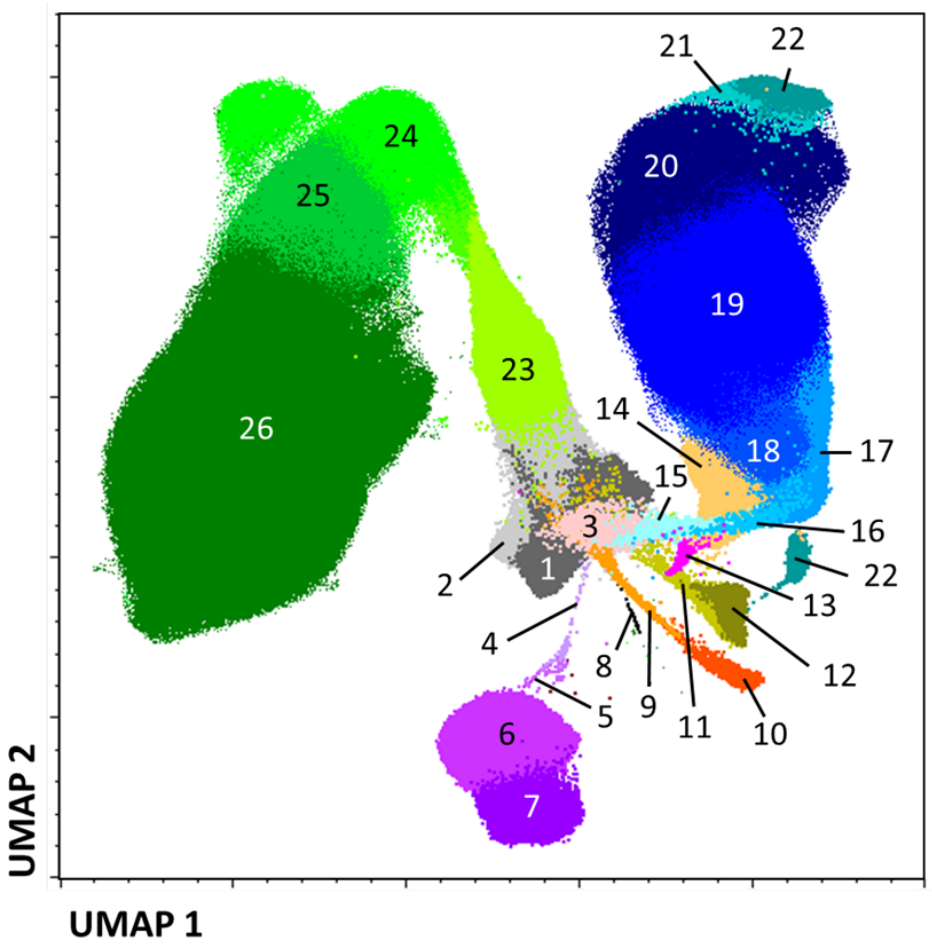
In: *Villani et al, Science 2017; van der Pan et al, Front Immunol 2023*

NORMAL (MYELOID) DENDRITIC CELL MATURATION IN BM

MONOCYTE AND DENDRITIC CELL POPULATIONS IN BONE MARROW

HIGH SENSITIVITY NGF + 28 COLOR PANEL

[Bone marrow mononuclear cells]



HPC
- 1 Hematopoietic stem cells (HSC)
- 2 Other hematopoietic precursor cells (HPC)

Neutrophil lineage
- 3 Myeloblasts
- 4 Promyelocytes
- 5 Myelocytes
- 6 Metamyelocytes
- 7 Band + segmented neutrophils

- 8 Mast cells

Basophils
- 9 Basophil precursor cells
- 10 Mature basophils

Dendritic cells
- 11 pDC precursor cells
- 12 Mature pDC
- 13 CD141^{+} myDC
- 14 CD1c^{+} myDC

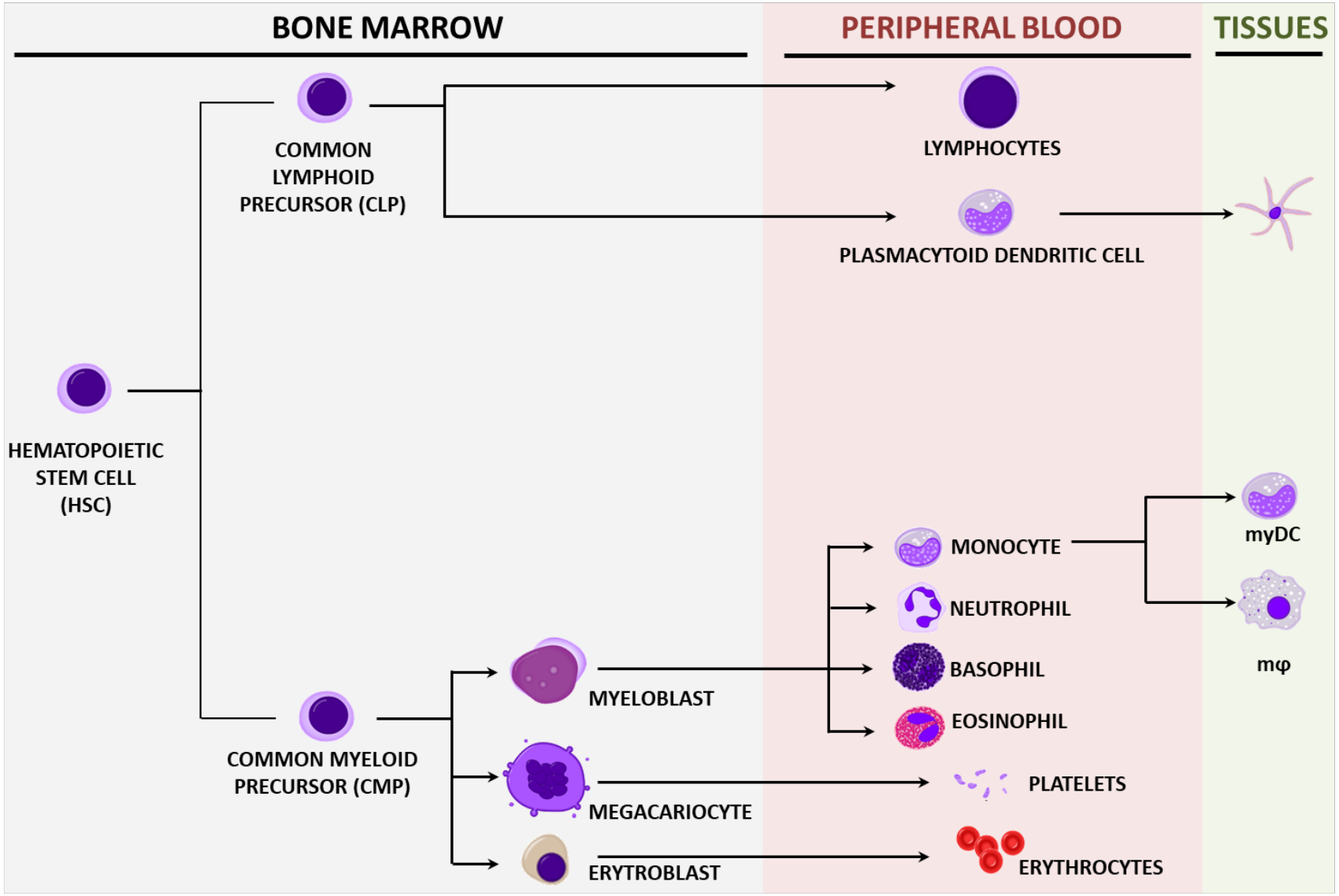
Monocytic lineage
- 15 Monoblasts CD34^{+}/CD117^{+}
- 16 Monoblasts CD34^{-}/CD117^{+}
- 17 Promonocytes CD14^{-}
- 18 Promonocytes CD14^{lo}
- 19 Promonocytes CD14^{hi}
- 20 cMo
- 21 iMo
- 22 ncMo

Etythroid lineage
- 23 Pro-erythroblast CD34^{+} CD117^{+} HLA-DR^{+} CD105^{lo}
- 24 Pro-erythroblast CD34^{-} CD117^{+} HLA-DR^{lo} CD105^{+}
- 25 Erythroblast CD34^{-} CD117^{-} HLA-DR^{-} CD105^{+}
- 26 Erythroblast CD34^{-} CD117^{-} HLA-DR^{-} CD105^{-}

Responsible scientists: *Cristina Teodosio, Kirsten Canté, Frank Staal*

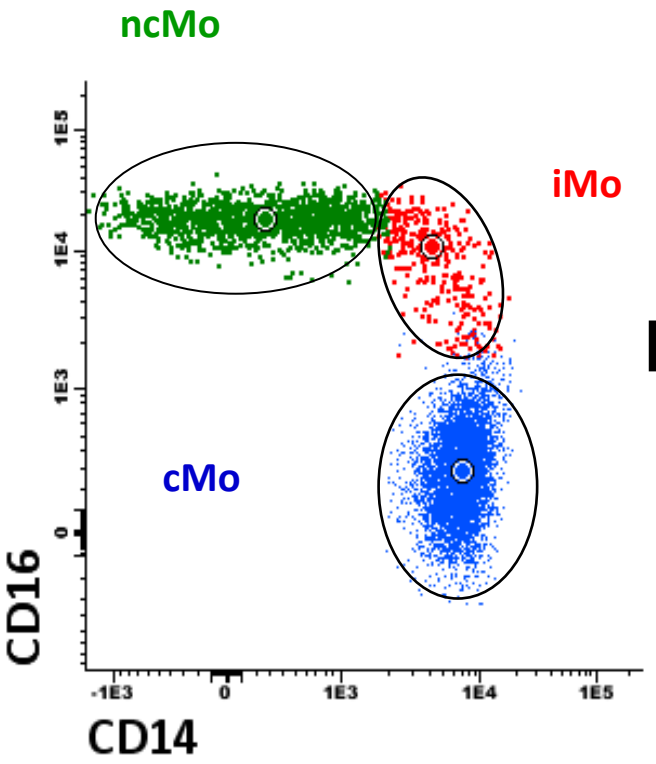
pDC, plasmacytoid dendritic cells; *myDC,* myeloid dendritic cells; *cMo,* classical monocytes; *iMo,* intermediate monocytes; *ncMo,* non-classical monocytes

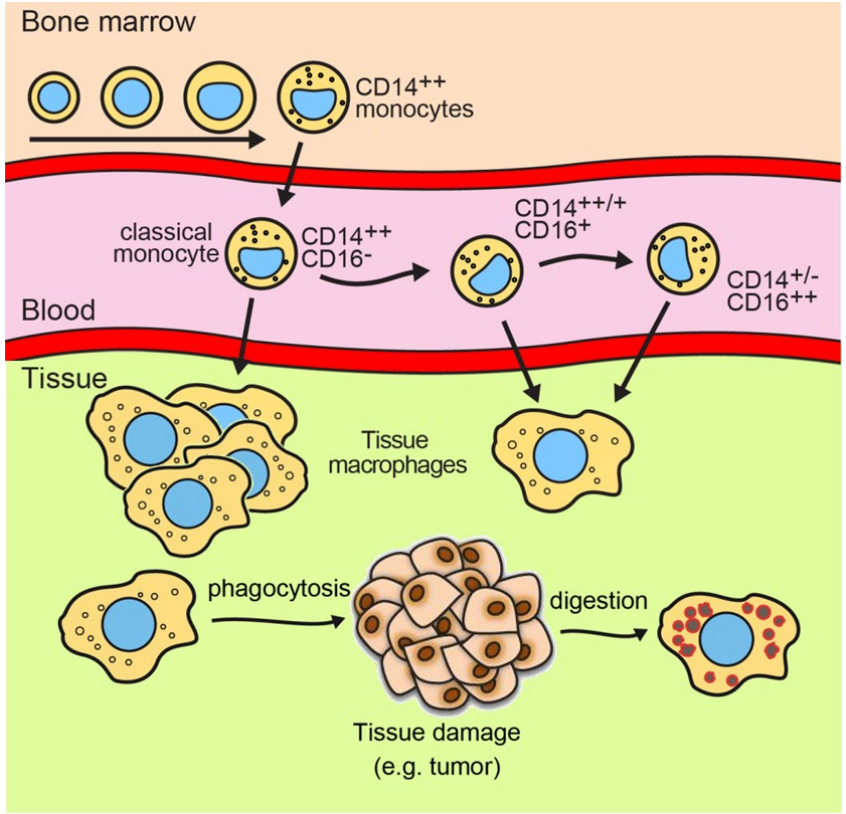
BONE MARROW MYELOPOIESIS



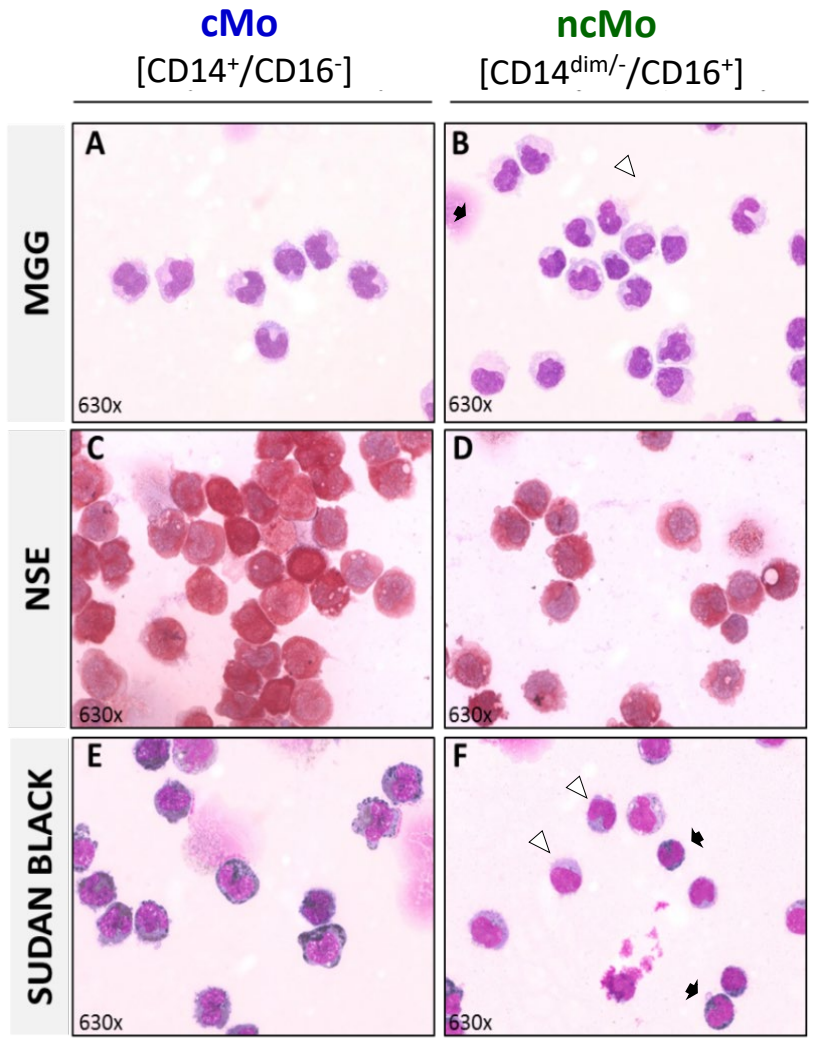
MONOCYTE AND DENDRITIC CELL POPULATIONS CIRCULATING IN PB

MONOCYTE POPULATIONS IN PERIPHERAL BLOOD





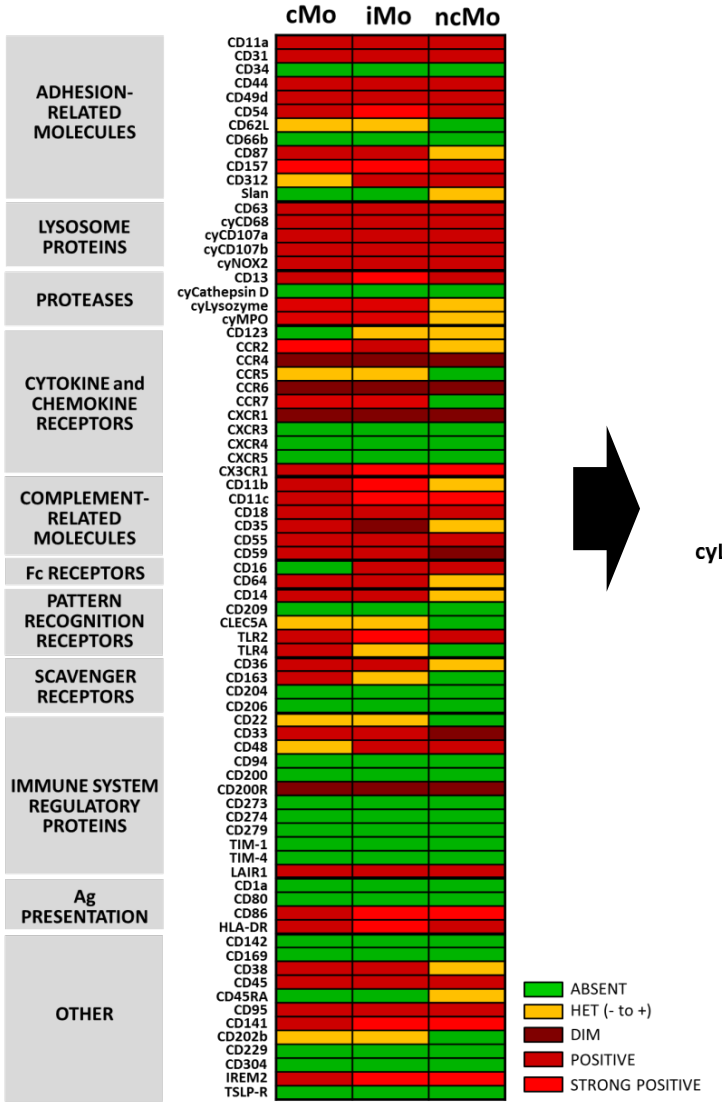
Adapted from: Geissmann *et al.*, Science 2010; Van de Veerdonk and Netea, Immunity 2010; Ziegler-Heitbrock *et al.*, Blood 2010.



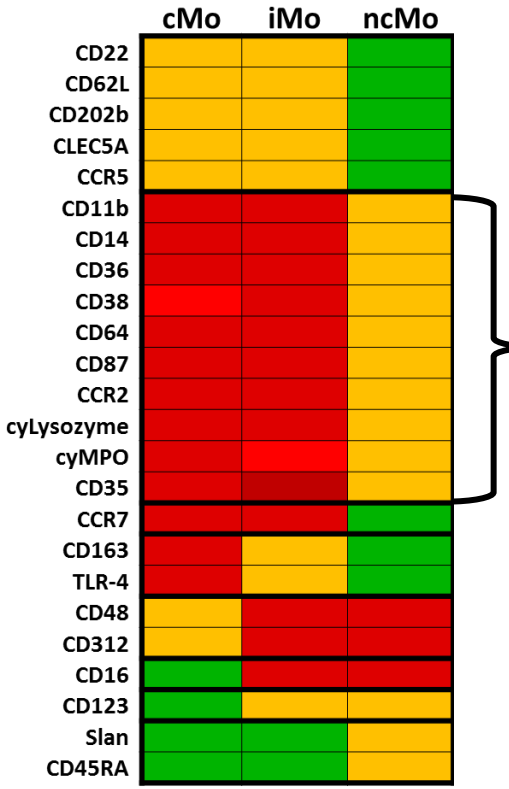
Heterogeneous populations

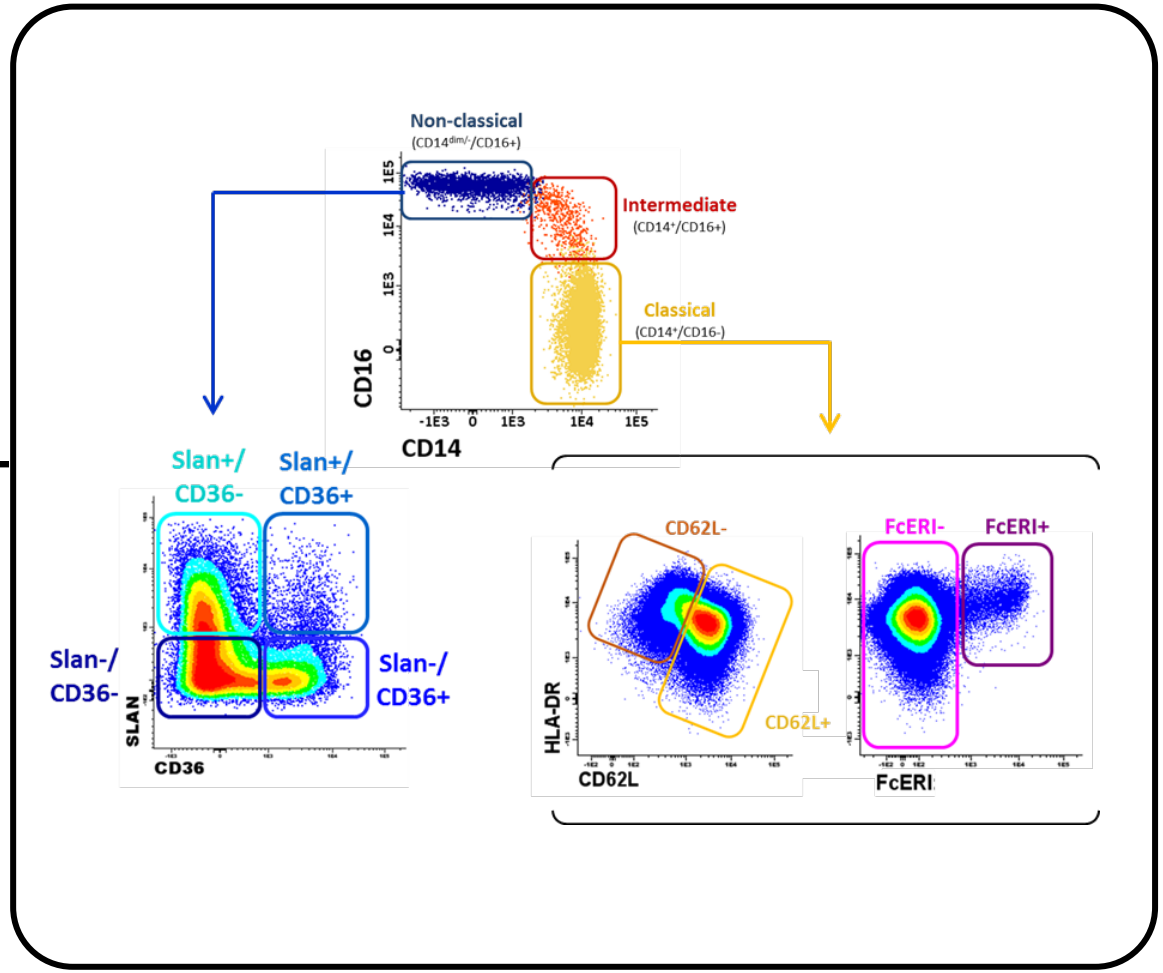
MONOCYTE POPULATIONS IN PERIPHERAL BLOOD

- **77 proteins** evaluated in healthy donors:



Markers with heterogenous expression pattern



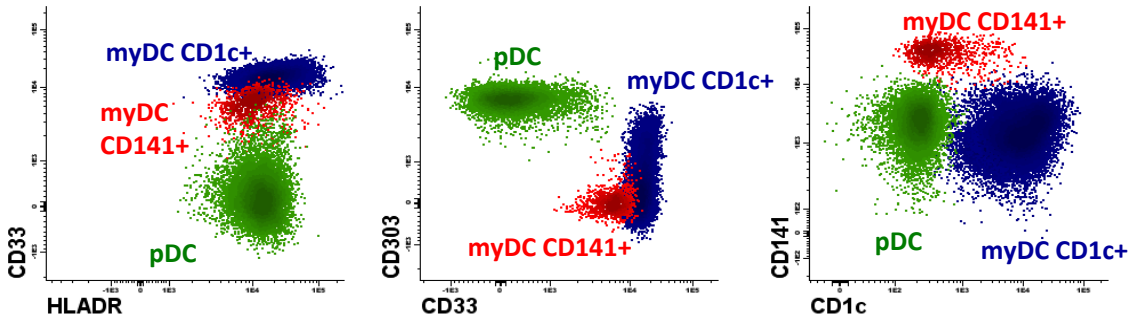


In: *Damasceno et al, J Allergy Clin Immunol 2018*
van der Pan et al, Front Immunol 2022

DENDRITIC CELL POPULATIONS IN BLOOD

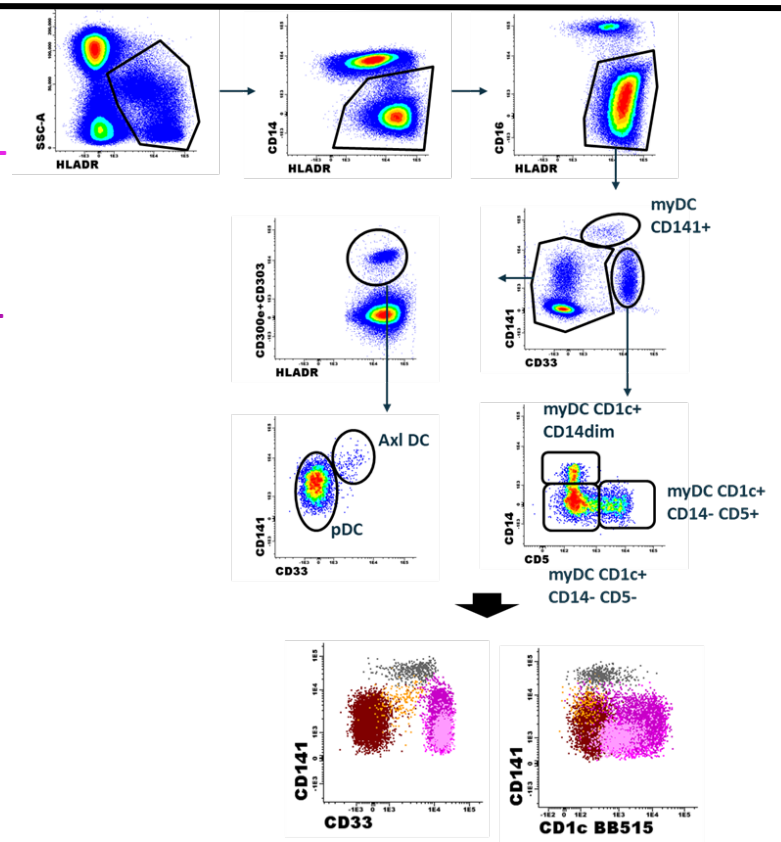
Original classification

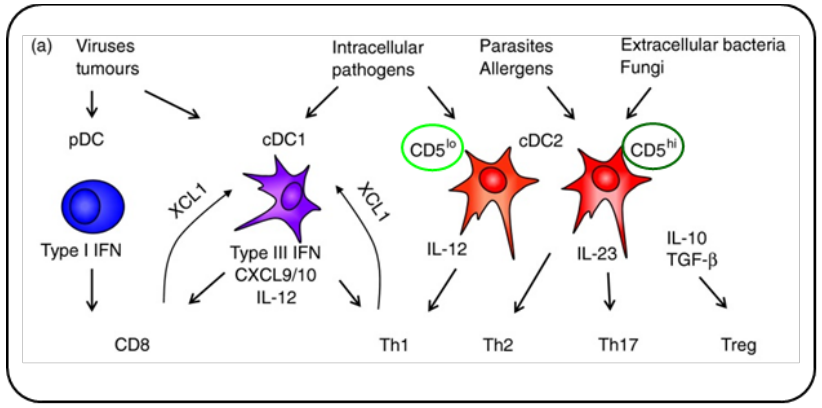
- pDC
- myDC CD141+
- myDC CD1c+



New dendritic cell populations

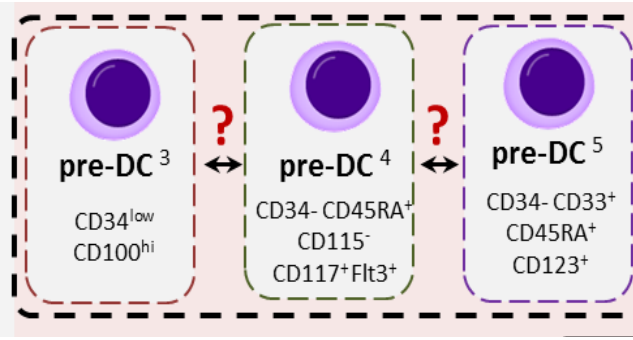
- myDC CD1c+ CD14^{dim}
- myDC CD1c+ CD14^{-}
  - myDC CD1c+ CD14^{-}/ CD5-
  - myDC CD1c+ CD14^{-}/CD5+





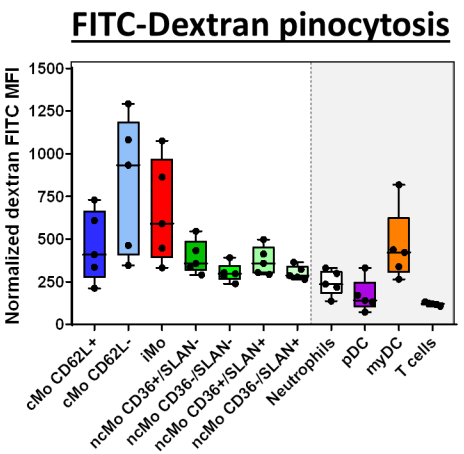
In: Collin et al. Immunology (2018)

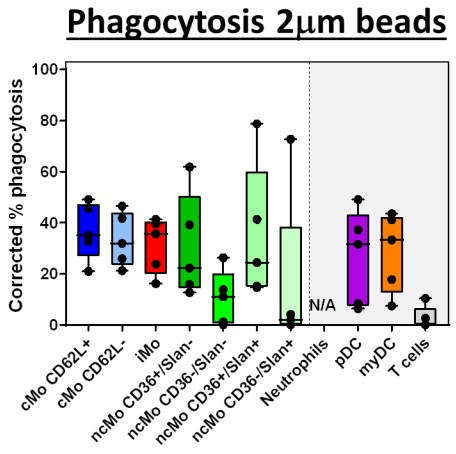
Myeloid dendritic cell precursor?

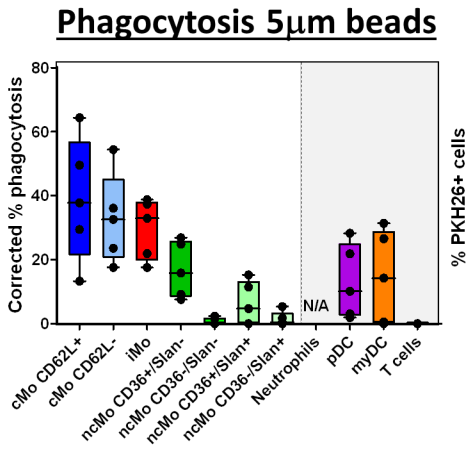


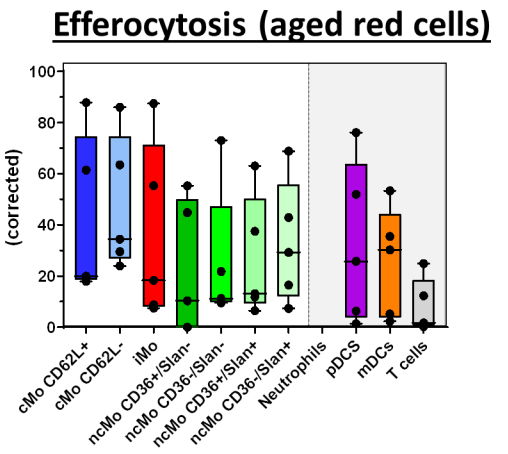
Villani et al, Science (2017); Collin et al. Immunology (2018); Yin et al. J Immunol (2017)

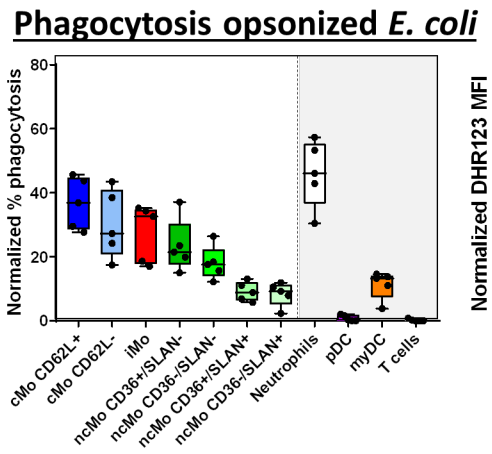
MONOCYTE POPULATIONS IN PERIPHERAL BLOOD

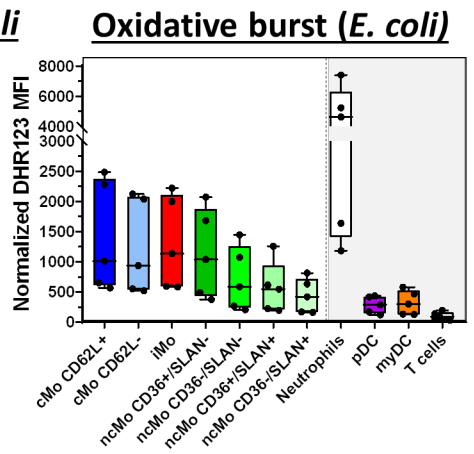


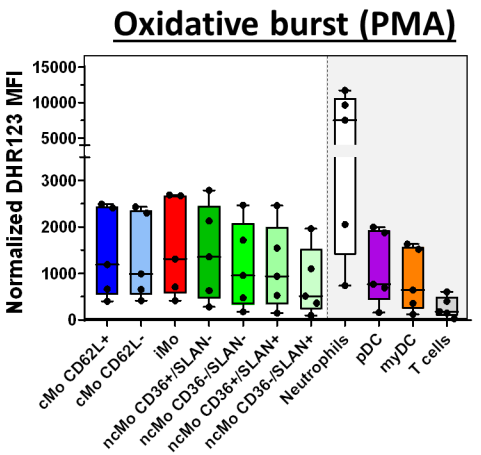


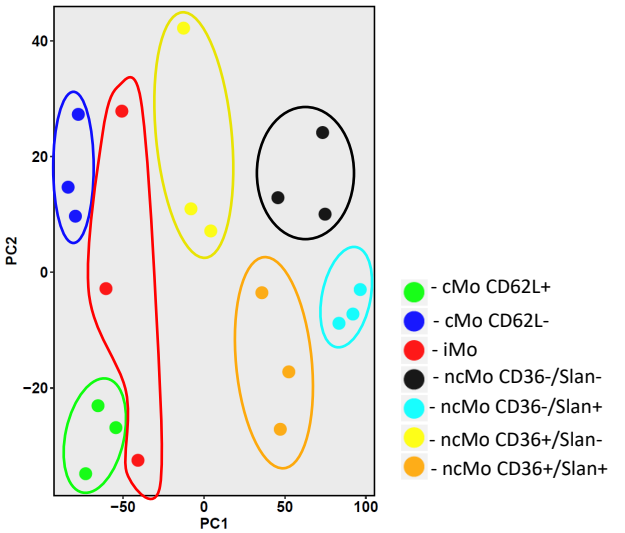












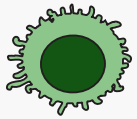
✓ **Monocytic (sub)pobulations exhibit unique functional profiles**

In: *van der Pan et al, manuscript in preparation*

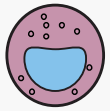
Monocyte and Dendritic cell populations: HISTORICAL PERSPECTIVE

2011

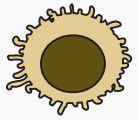
Classical monocytes (cMo)
[CD14^{hi}/CD16^{-}]



Plasmacytoid dendritic cells (pDC)

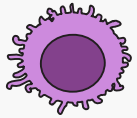


Intermediate monocytes (iMo)
[CD14^{hi}/CD16^{+}]



CD141^{+} myeloid dendritic cells (myDC CD141+; cDC1)

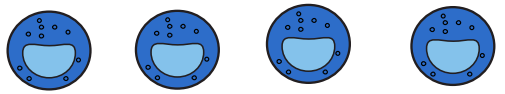
Non- classical monocytes (ncMo)
[CD14$^{-/dim}$/CD16^{+}]



CD1c^{+} myeloid dendritic cells (myDC CD1c+, cDC2)

2021

cMo

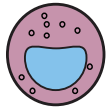


FcεRI^{+} CD9^{+} CD93^{hi} CD93^{lo}

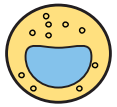
Hamers et al, Arterioscler Thromb Vasc Biol (2019)

FcεRI^{+} CD62L^{+} CD62L^{-}

Damasceno et al, Cancers (Basel) (2021)



iMo



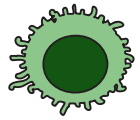
ncMo

Slan^{-} Slan^{+} CD9^{-} Slan^{+} CD9^{+}

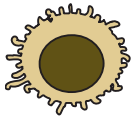
Hamers et al, Arterioscler Thromb Vasc Biol (2019)

CD36^{+} Slan^{-} CD36^{-} Slan^{-} CD36^{+} Slan^{+} CD36^{-} Slan^{+}

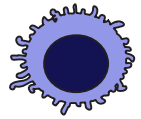
Damasceno et al, Cancers (Basel) (2021)



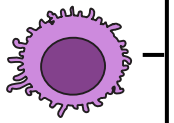
pDC



myDC CD141+



Axl DC
Villani et al, Science (2017)



myDC CD1c+

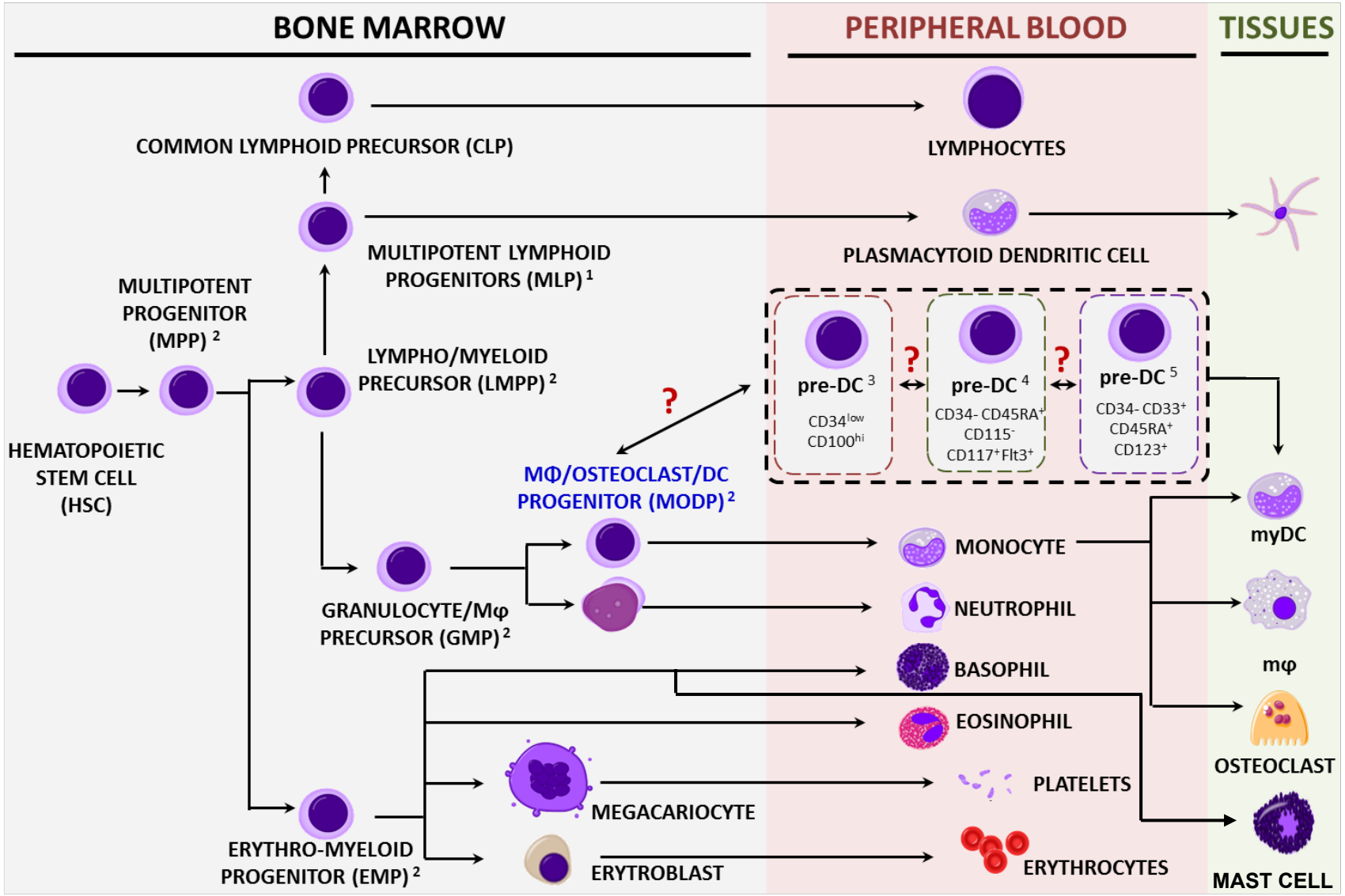
CD14^{dim} CD14^{-}

Villani et al, Science (2017)

CD5^{-} CD5^{+}

Yin et al, J Immunol (2017)

MYELOID CELL POPULATIONS IN BONE MARROW



[1]Helft *et al*, Cell Reports (2017) [2]Xiao *et al*, Blood advances (2017) [3]Villani *et al*, Science (2017) [4]Ma *et al*, BMC Mol Cell Biol (2019) [5]See *et al*, Science (2017)

Immunophenotypic profile of monocytes and dendritic cells in blood

POPULATION		CD141	CD5	CD192	CD62L	HLA-DR	CD16	CD1c	CD36	FcERI	SLAN	CD34	CD33	CD300e	CD303	CD45	CD14
Eosinophils#		-	-	-	+	-	-	-	-*	- (-/+)	-	-	dim	-	-	+	-
Neutrophils#	Mature	-/+	-	-/+	++	-	++	-	-*	-	-	-	dim	-	-	+	-
	Immature CD62L-	-	-	-	-	-	-	-	-*	-	-	-	+	-	-	+	-
	Immature CD62L+	-	-	-	+	-	dim	-	-*	-	-	-	+	-	-	+	-
Basophils		-	-	+	++	-	-	-	-	++	-	-	+	-	-	dim	-
cMo	CD62L+/FcERI-	dim	-	+	+	+	-	dim	+	-	-	-	++	dim	-	+	+
	CD62L+/FcERI+	dim	-	+	+	+	-	dim	+	+	-	-	++	dim	-	+	+
	CD62L-/FcERI-	dim	-	+	-	++	-	dim	+	-	-	-	++	+	-	+	+
	CD62L-/FcERI+	dim	-	+	-	++	-	dim	+	+	-	-	++	+	-	+	+
iMo		+	-	+	-/dim	++	+	dim	+	-	-	-	++	+	-	+	+
ncMo	CD36+/Slan-	+	-	-/+	-	+	+	dim	+	-	-	-	++	++	-	++	dim
	CD36-/Slan-	+	-	-	-	+	+	dim	-	-	+	-	++	++	-	++	-
	CD36+/Slan+	+	-	-	-	+	+	dim	+	-	-	-	+	++	-	++	-
	CD36-/Slan+	+	-	-	-	+	+	dim	-	-	+	-	+	++	-	++	-
pDC		+	-	+	-/+	++	-	-	+	dim/+	-	-	-	-	+	+	-
Axl DC		+	-/+	+	dim/+	++	-	-	dim	-/+	-	-	+	-	+	+	-
myDC CD1c+	CD14dim	dim	-	+	-/+	++	-	dim	+	+	-	-	++	-/+	-	+	dim
	CD14-/CD5-	dim	-	+	-/+	++	-	+	+	+	-	-	++	-/+	-	+	-
	CD14-/CD5+	+	+	+	-/+	++	-	+	+	+	-	-	++	-/+	-	+	-
myDC CD141+		++	dim	+	+	++	-	-	-*	-	-	-	++	-	-	+	-
M-MDSC		-	-	-/dim	+	-/dim	-/+	dim	-/dim	-	-	-	+	-/dim	-	+	dim/+
HPC		-	-	-	-/+	+	-	-	-*	-/dim	-	+	-/+	-	-	dim	-
Pre-DC		-	-	dim	+	++	-	-	-*	-	-	dim	-/+	-	-	dim	-

*Some unspecific staining may be observed due to platelets bound to leukocytes
AutoFL for some detectors, specially those corresponding to the violet and blue lasers.

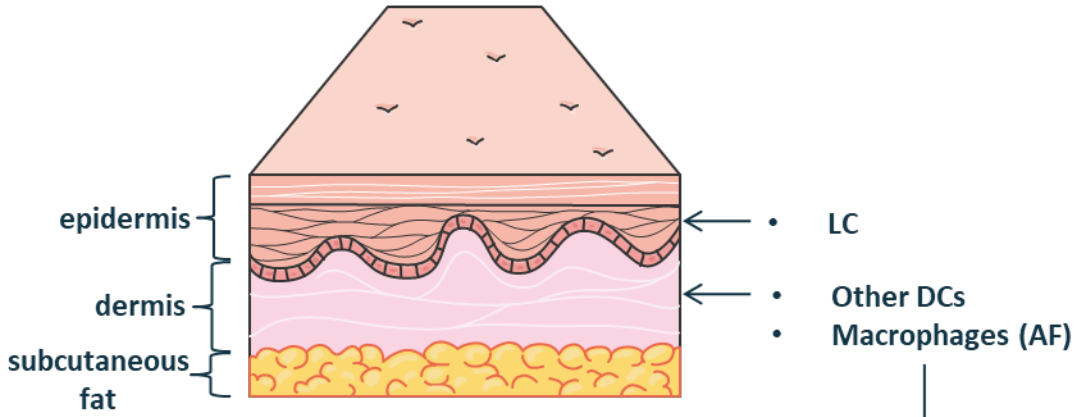
Slide prepared by Cristina Teodósio

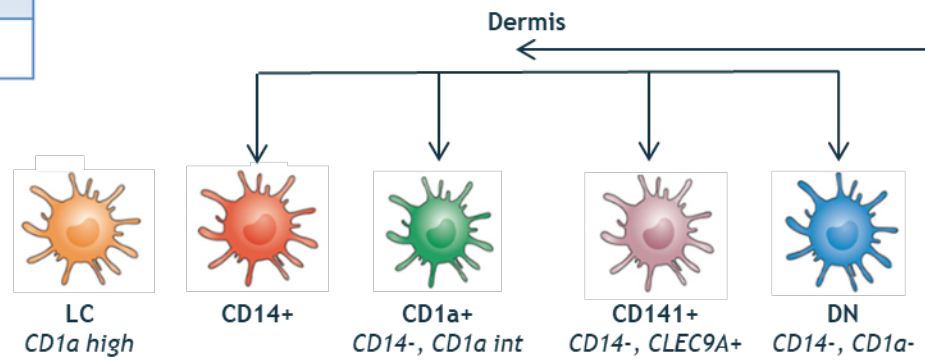
TISSUE MACROPHAGES, DENDRITIC CELL AND MAST CELL POPULATIONS

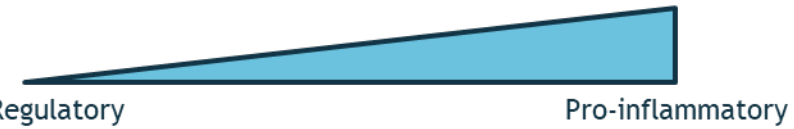
TISSUE MACROPHAGES AND DENDRITIC CELLSS

SKIN

Cell type	Subsets
Dendritic cell	CD141+ DC
	CD14+ DC
	CD1a+ DC
	CD14- CD1a- DC (DN)
	Langerhans cell (LC)
Macrophage	-







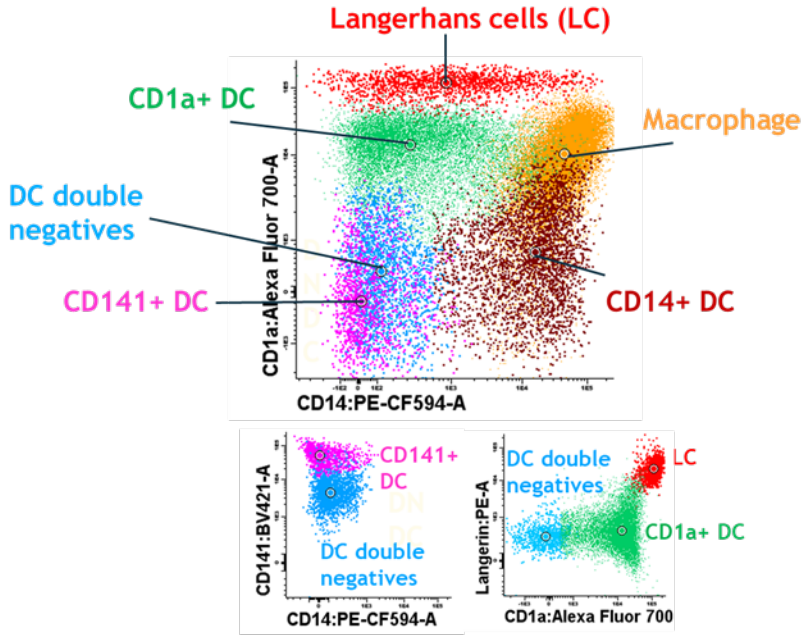


Figure by B.M.F. Winkel, dep. Parasitology, LUMC

MONITORING MONOCYTE - MACROPHAGE, DENDRITIC CELL AND MAST CELL POPULATIONS

Monitoring Innate Myeloid Cells (IMC)

INNATE MYELOID CELLS

✓ Express receptors to monitor and **sense microenvironmental changes**

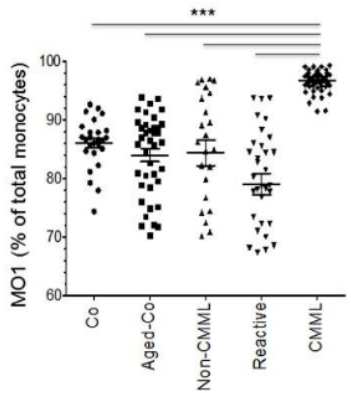
✓Responsible for **intra-tissue scanning** and elimination of debris and apoptotic cells

✓ Key players in the **initiation of immune responses**

✓ **Production and recruitment** reflect **disturbance** of body **homeostasis**

MONITORING HOMEOSTASIS IMBALANCE IN PATIENT CARE

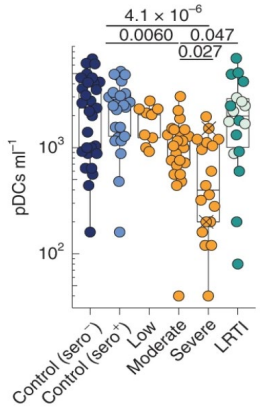
Hemato-oncology



Frequency of cMo → differential diagnosis of chronic myelomonocytic leukemia (CMML)

In: Selimoglu-Buet *et al,* Blood (2015)

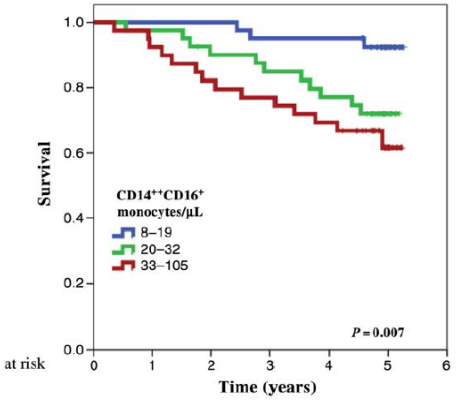
Infection



Decreased PB pDCs → severe disease in COVID-19

In: Laing *et al*, Nature (2020)

Chronic kidney disease



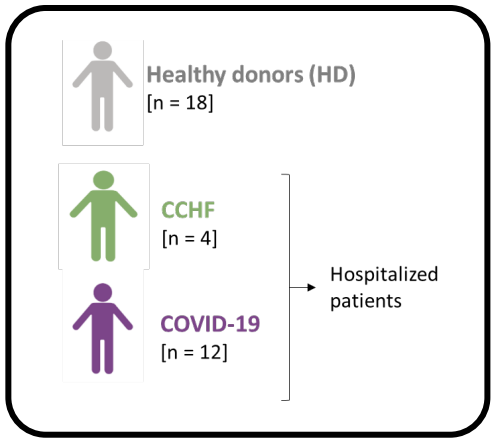
High absolute counts of iMo (CD14⁺⁺CD16⁺) → poor survival

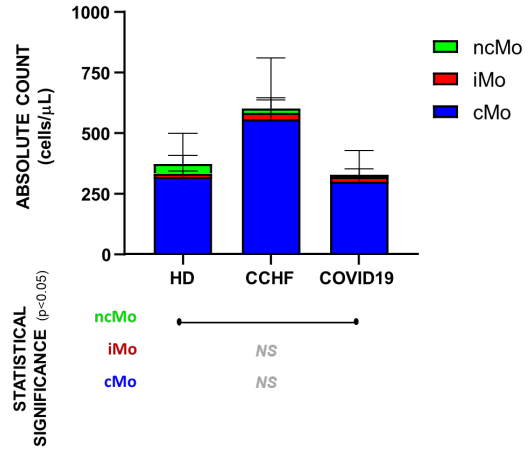
In: Rogacev *et al,* Eur Heart J (2011)

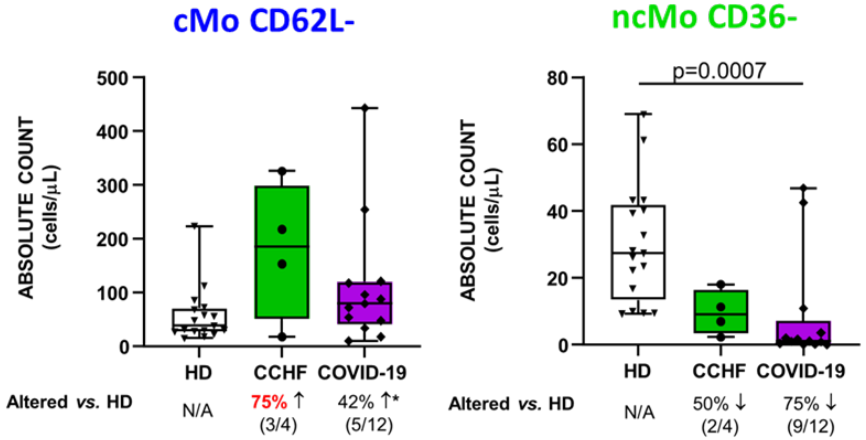
ROLE OF MONITORING INNATE MYELOID CELLS (IMC)

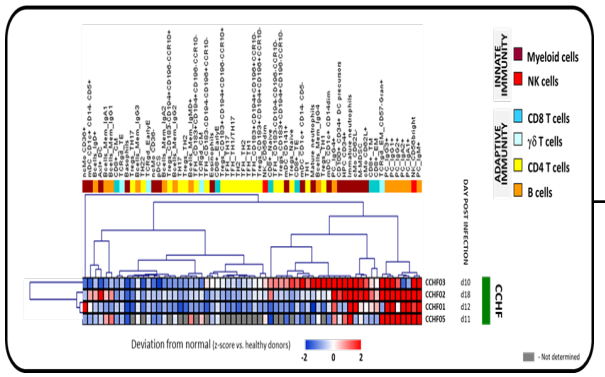
IMMUNE RESPONSE IN INFECTION: PRIMARY RESPONSE TO VIRUS

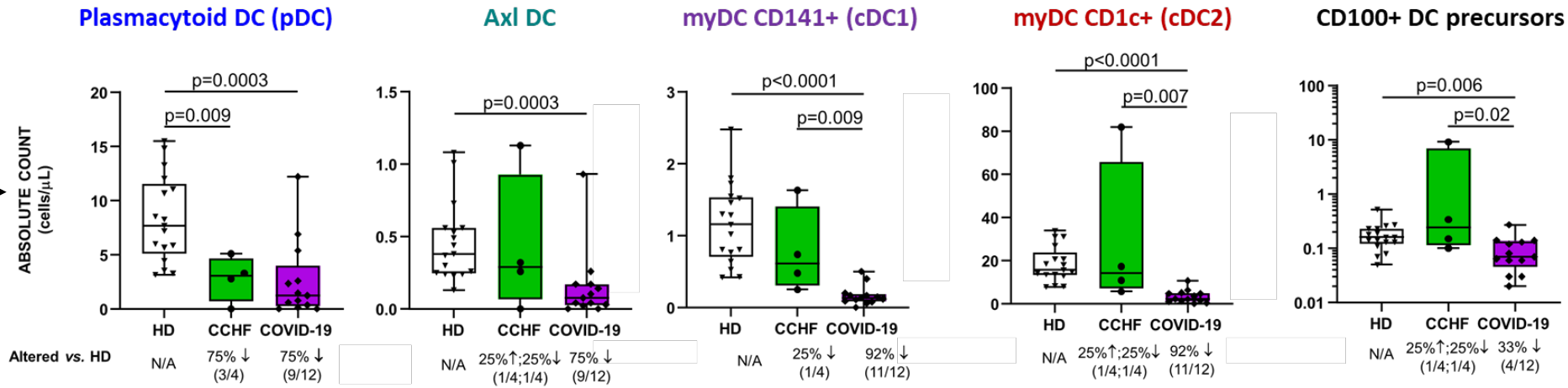
✓ **COVID-19 *vs.* Crimean-Congo Haemorrhagic Fever (CCHF)**







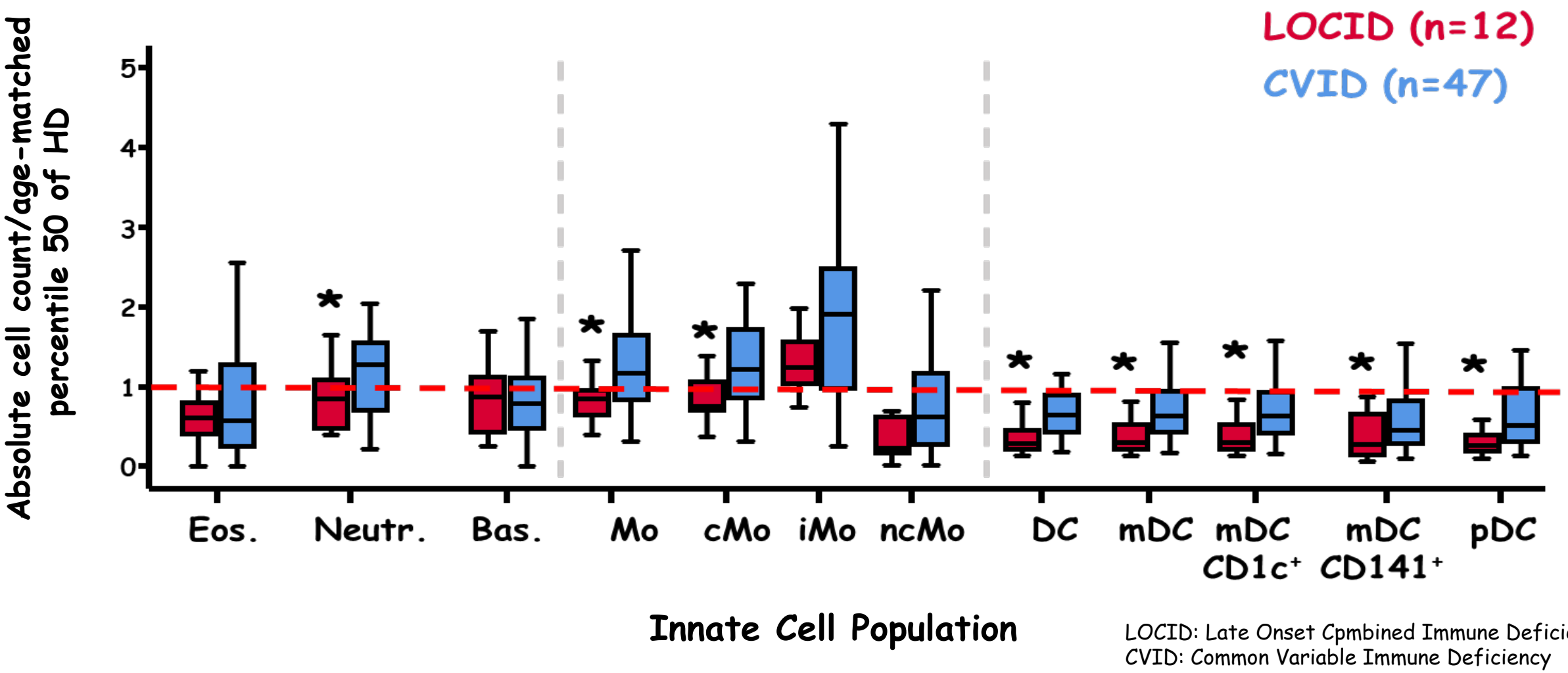




In: *Criado & Teodosio et al, manuscript in preparation*

ROLE OF MONITORING INNATE MYELOID CELLS (IMC)

BLOOD MONOCYTE AND DENDRITIC CELL LEVELS IN PRIMARY IMMUNODEFICIENCY



LOCID: Late Onset Cpmbined Immune Deficiency
CVID: Common Variable Immune Deficiency

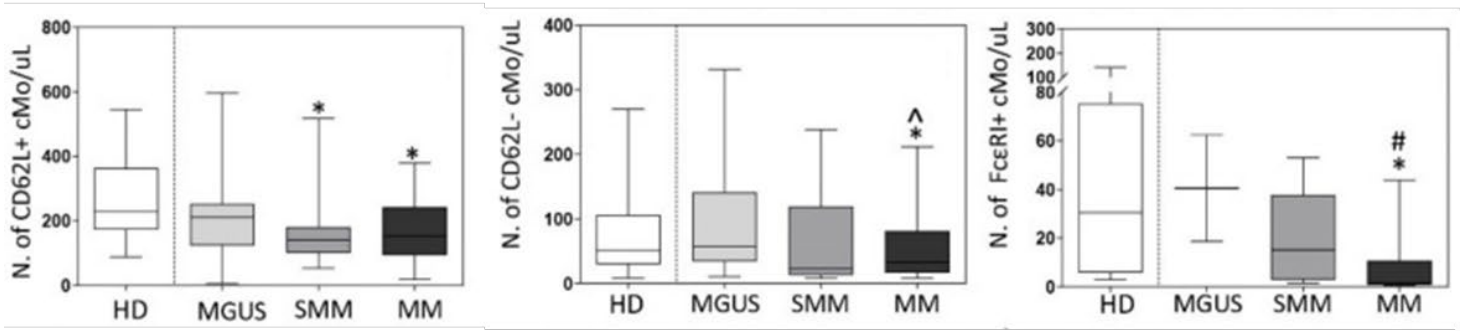
pDC, plasmacytoid dendritic cells; *myDC,* myeloid dendritic cells; *HPC,* hematopoietic precursor cell; *cMo,* classical monocytes; *iMo,* intermediate monocytes; *ncMo,* non-classical monocytes

In: *Torres et al, manuscript in preparation*

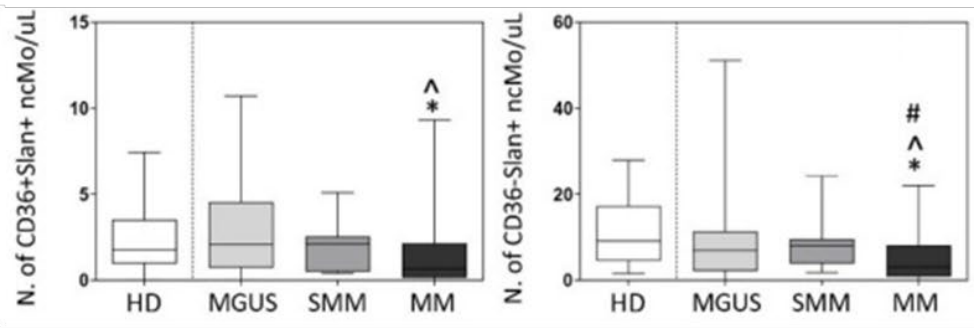
ROLE OF MONITORING INNATE MYEOLOID CELLS (IMC)

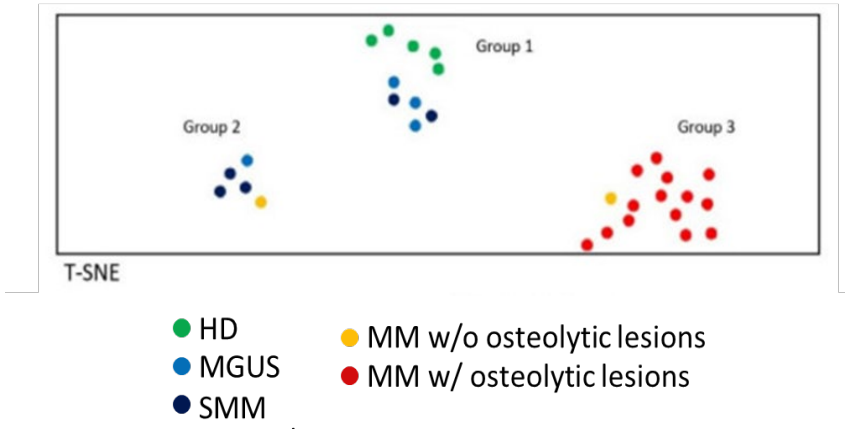
MONITORING IMC IN HEMATO-ONCOLOGY: MULTIPLE MYELOMA

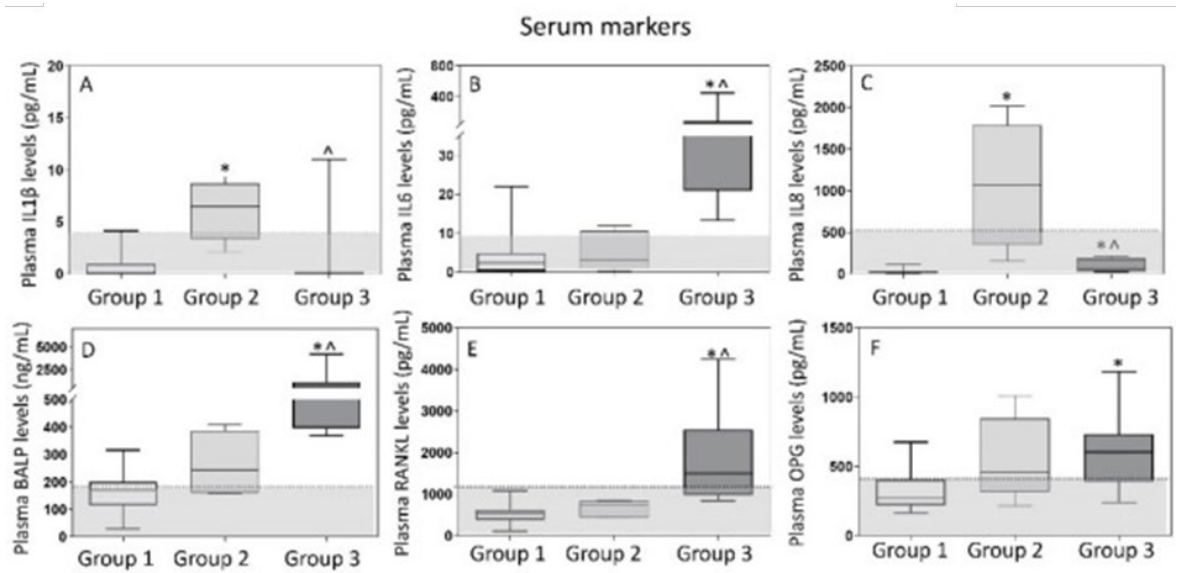
cMo subsets

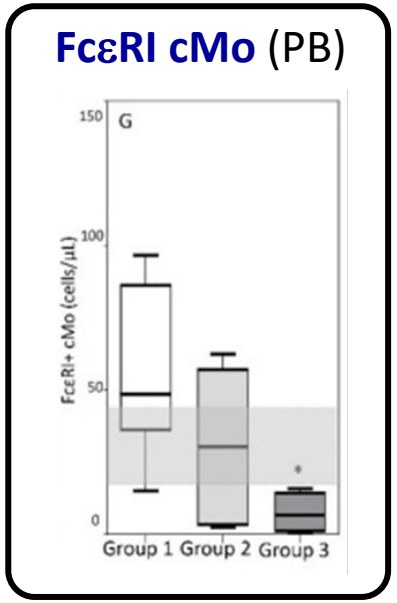


ncMo subsets





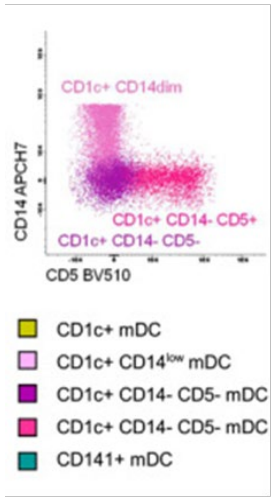


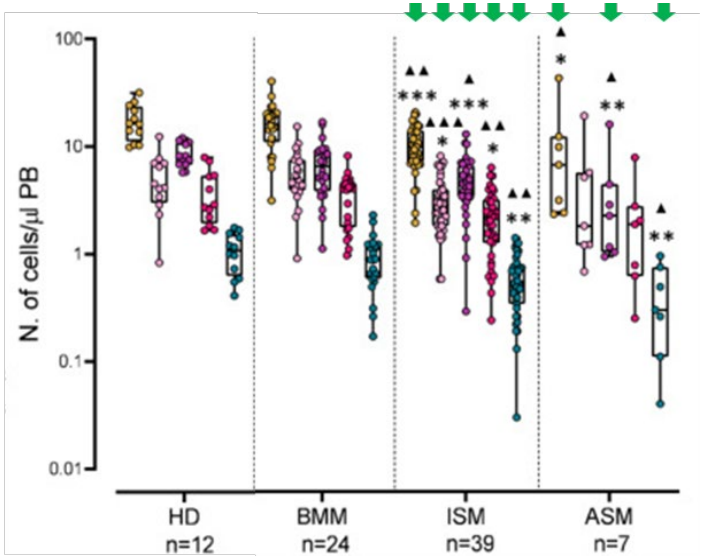


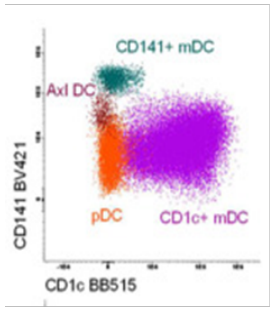
cMo, classical monocytes; ncMo, non-classical monocytes; HD, healthy donor; MGUS, Monoclonal gammopathy of undetermined significance; SMM, Smoldering multiple myeloma; MM, multiple myeloma; p<0.05 *vs* * HD, ^ MGUS, # SMM

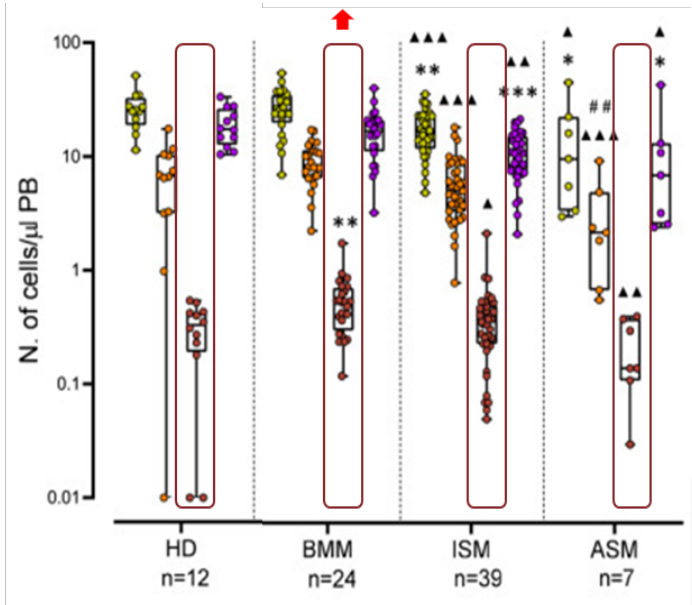
In: *Damasceno et al, Cancers (Basel) (2021)*

MONITORING INNATE MYELOID CELLS IN SYSTEMIC MASTOCYTOSIS





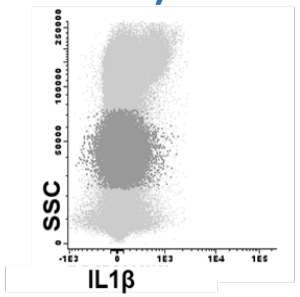


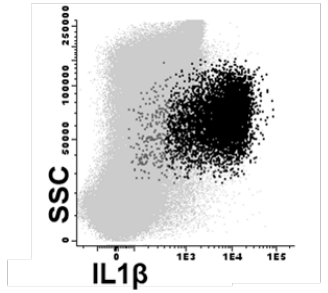


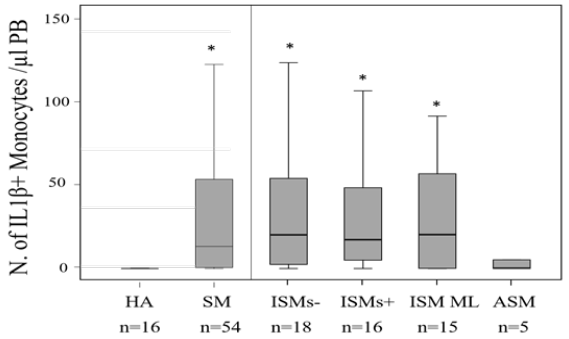
Total DC
pDC
Axl DC
mDC

Healthy adults | Systemic mastocytosis

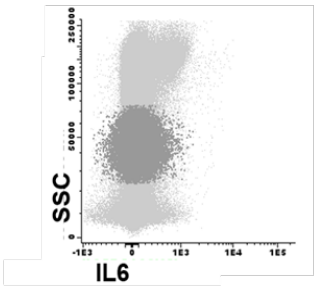
IL 1β

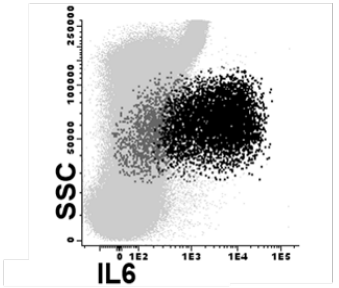


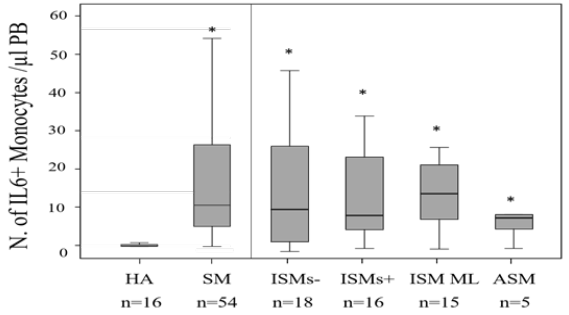




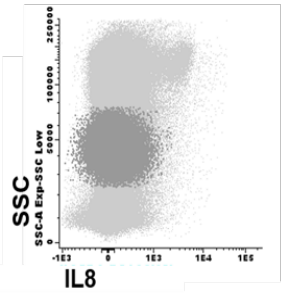
IL 6

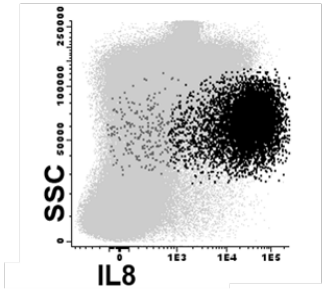


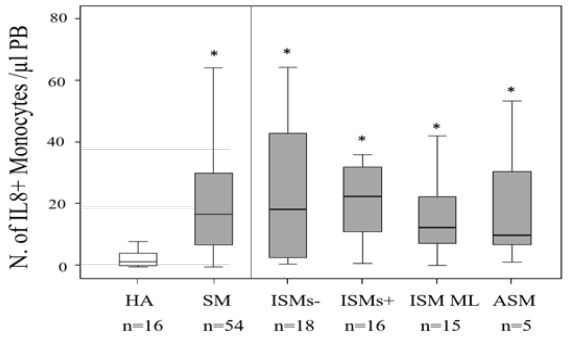




IL 8







- **ISM** and **ASM** patients → **decreased** number of myDC subsets in blood
- **Expansion of AXL⁺ DC** → in **BMM**.

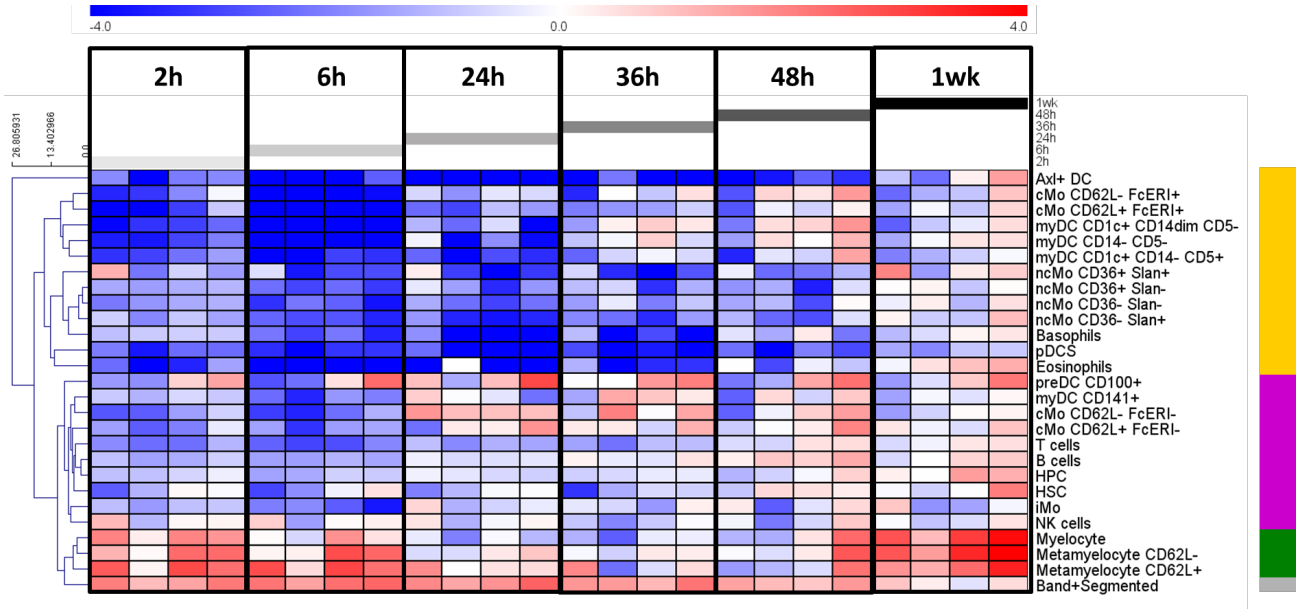
pDC, plasmacytoid dendritic cells; *myDC,* myeloid dendritic cells; *HD,* healthy donor; *BMM,* bone marrow mastocytosis; *ISM,* indolent systemic mastocytosis; *ASM,* aggressive systemic mastocytosis

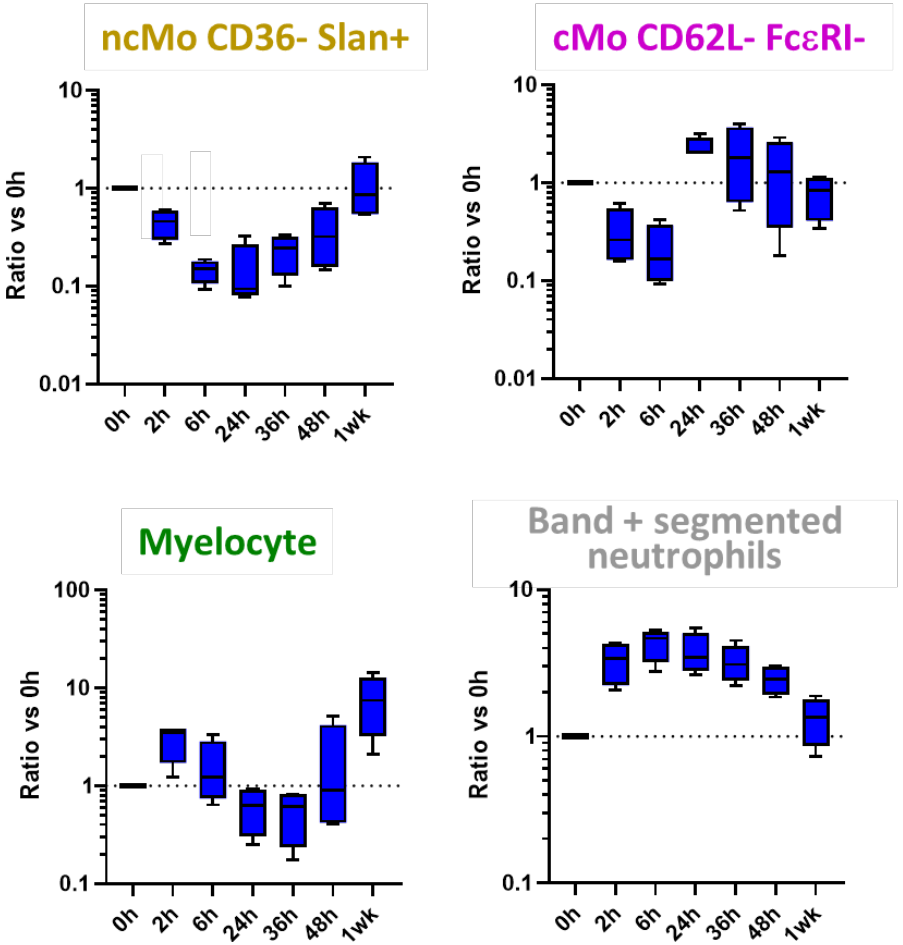
Modified from: *Pérez-Pons et al, Clin Transl Allergy (2022)*

ROLE OF MONITORING INNATE MYELOID CELLS (IMC)

MONITORING RESPONSE TO TISSUE DAMAGE

Leucocyte kinetics in PB after Total Hip Replacement (THR)





pDC, plasmacytoid dendritic cells; *myDC,* myeloid dendritic cells; *HPC,* hematopoietic precursor cell; *cMo,* classical monocytes; *iMo,* intermediate monocytes; *ncMo,* non-classical monocytes

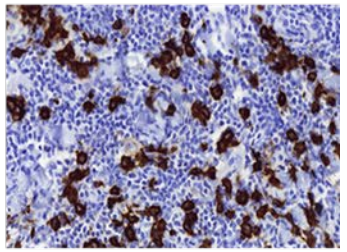
In: *van den Bossche et al, manuscript in preparation*

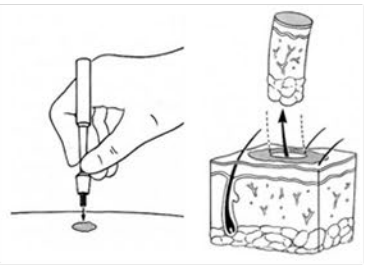
NEOPLASTIC MONOCYTIC, DENDRITIC CELL AND MAST CELL POPULATIONS

NEOPLASTIC COUNTERPART OF TISSUE DENDRITIC CELLS

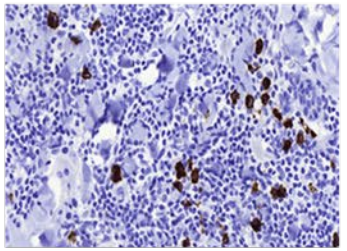
Langerhans HISTIOCYTOSIS

CD1a

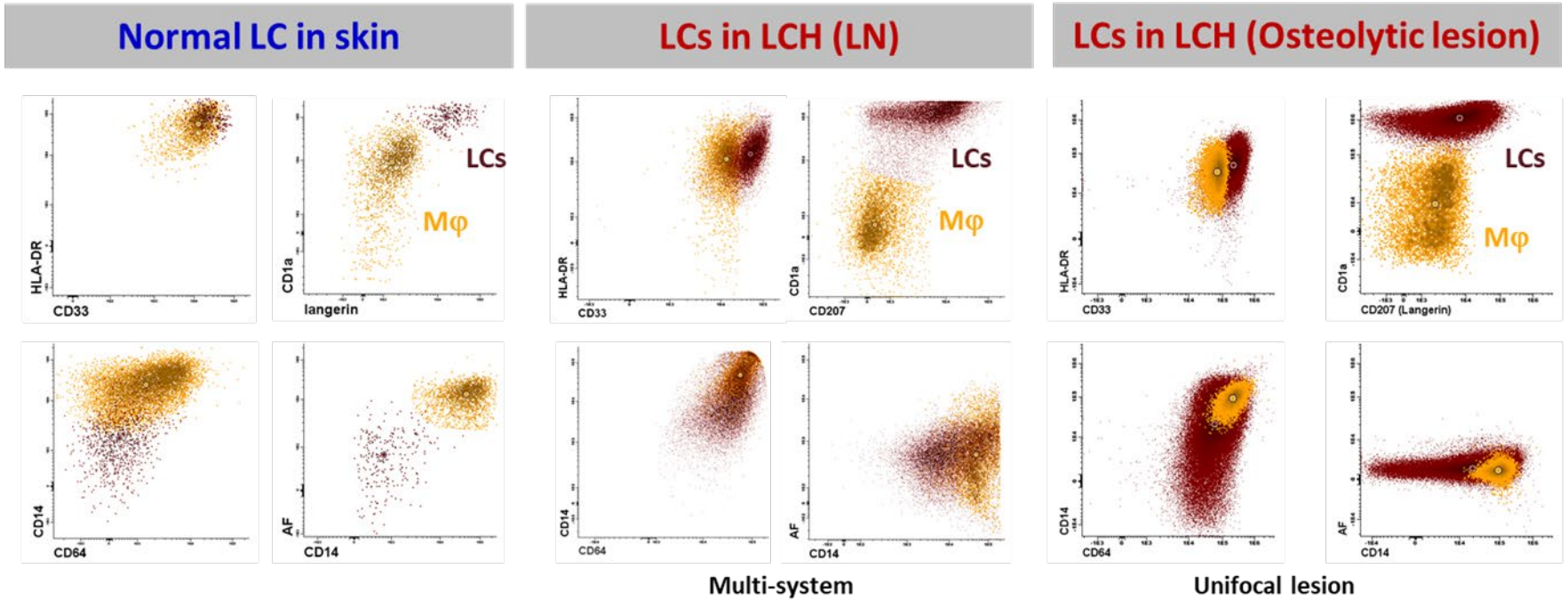




CD207



- Clonal expansión of **Langerhans cells**
- Cell lesions expressingan LC-associated markers **(CD1a, CD207)**
- **Heterogeneous clinical presentation** (unifocal, multifocal bone, multi-systemic)

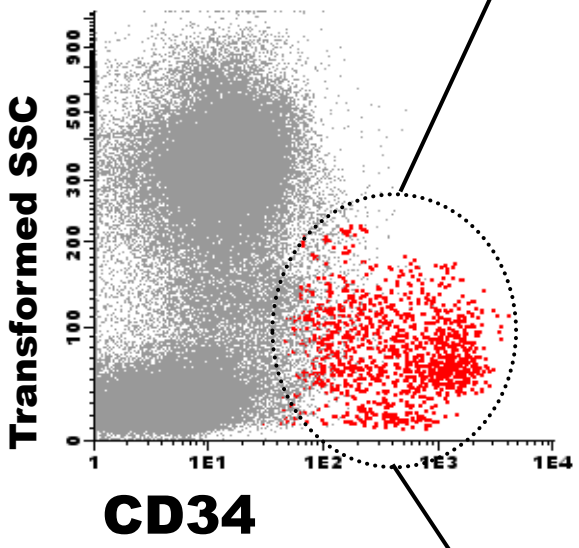


Monocytic alterations in **MDS**

Abnormal cell distribution	**Frequency**
Maturation blockades	56%
Abnormal antigen expression patterns	
Abnormal granularity (SSC)	30%
Abnormal CD45	23%
Abnormal distribution of immature/mature cells	47%
Abnormal CD33	3%
Abnormal HLA-DR	10%
Abnormal CD11b/HLA-DR pattern	10%-29%
Asynchronous antigen expression	
Expression of CD34	12%
Abnormal CD14	20%
Abnormal CD13	39%
Abnormal CD36	31%
Abnormal CD64	23%
Abnormal CD15	33%
Expression of lineage infidelity markers	
Lineage infidelity CD2	9%
Lineage infidelity CD5	2%
Lineage infidelity CD7	3%
Lineage infidelity CD19	2%
Overexpression of CD56	15%

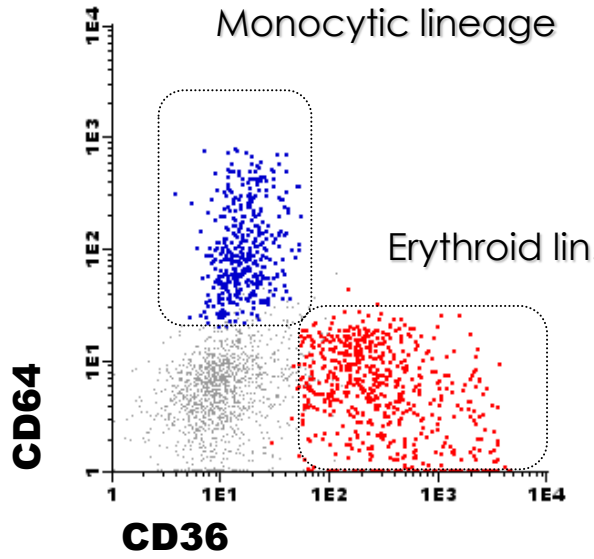
Stetler-Stevenson, Blood 2001
Ogata, Blood 2002
Wells, Blood 2003
Malcovati, Leukemia 2005
Benesch, Hematology 2007
Matarraz, Leukemia 2008
Stachursky, Leuk Res 2008
Van de Loosdrecht, Blood 2008
Subirá, Transl Res 2008
Kern, Cancer 2010
Matarraz, Cytometry 2010
Kern, Leuk Lymph 2011
Westers, Leukemia 2012
Matarraz, Cytometry B 2015
Harrington, Am J Clin Pathol 2016

Monocytic differentiation in normal BM



Cell lineage	Phenotype	Frequency Normal BM (%)
Neutrophil	CyMPO^{+}, CD13^{hi}	31 (12-39)
B-Lymphoid	nTdT^{+}, cyCD79a^{+}, CD19^{+}	23 (<1-45)
Erythroid	CD36^{+}, CD64^{-}, CD45^{dim}, CD105^{+}	15 (5-35)
pDC	CD123^{hi}, HLA-DR^{hi}, CD36^{+}	6 (1-15)
Monocytic	**CyMPO^{-}, CD64^{+}, HLA-DR^{+}, CD117^{dim}**	5 (3-15)
Basophil	CD123^{hi}, HLA-DR^{dim}, CD117^{dim}, CD45^{hi}, CD203c^{+}	<1 (<1-3)
Megakaryocytic	CD61^{+}, CD45^{dim}	<1
Eosinophil	CyMPO^{-}, CD15/65^{+}, CyPEo^{+}	<1
Mast cell	CD117^{hi}, HLA-DR^{dim}, CD45^{hi}	<1

Gated CD34+ BM cells



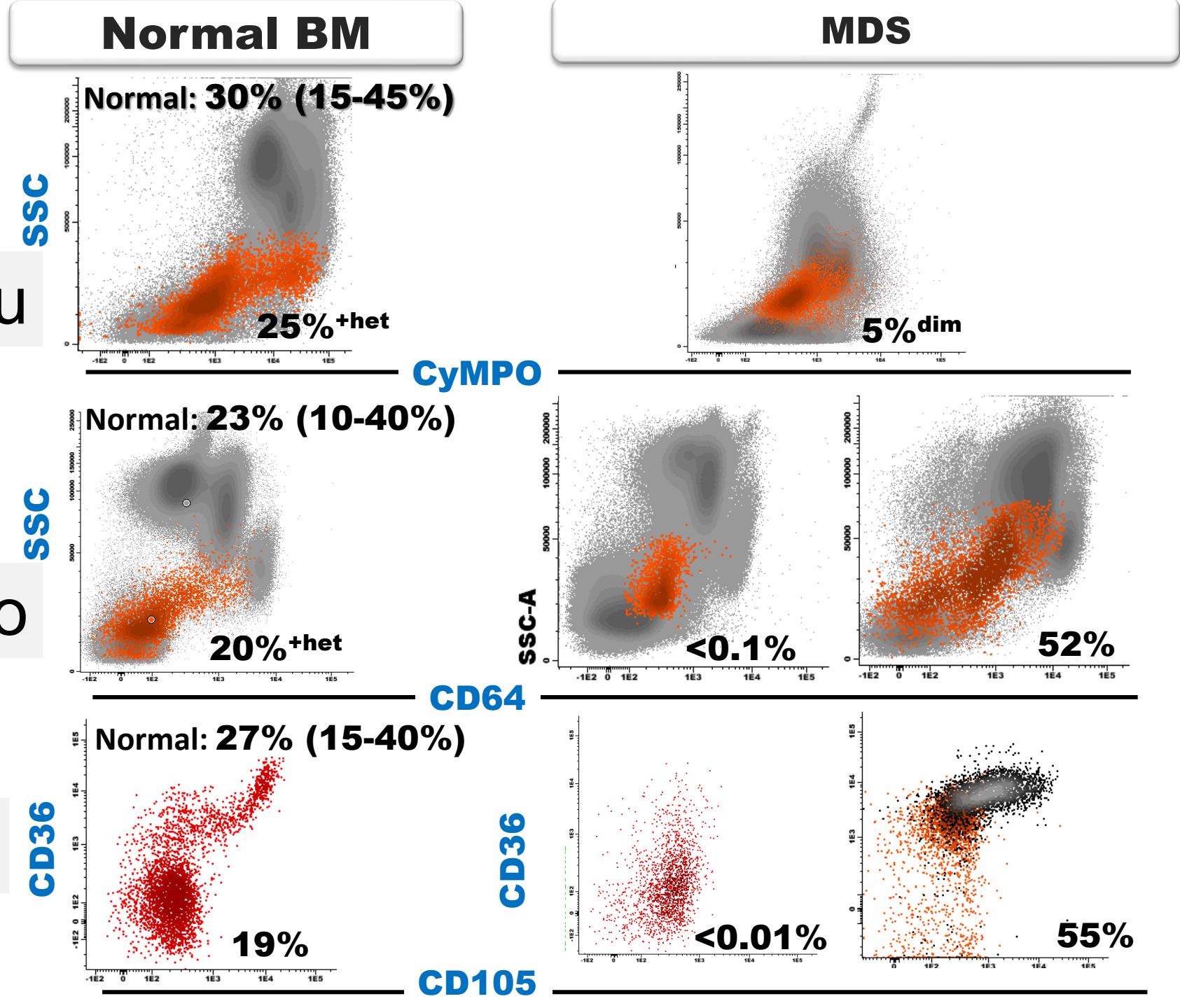
CD64: high-affinity IgG receptor FcγRI
(van der Poel *et al, J. Immunol 2011*)

Matarraz et al, Leukemia 2008

CD34⁺ cells in MDS

- ✓ Distribution (total %)
- ✓ **Major Lineage commitment**

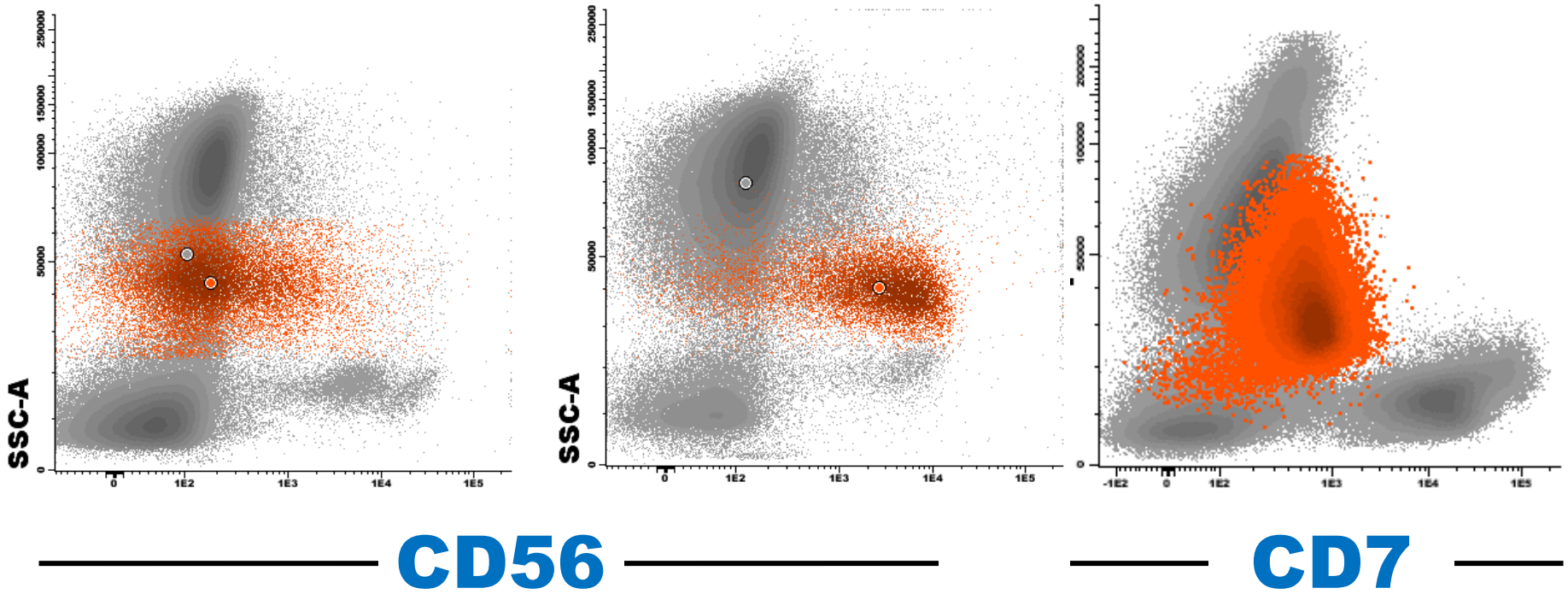
  (relative %, CV)



Monocytic L. in MDS

CD56 and CD2

- ✓ Matutation blockades (relative %)
- ✓ **Lineage infidelity**



CD56 and CD2 expression is more frequent in **CMML vs. MDS**

(47% and 56% vs. 18% and 16%, respectively)

Subirá D, Trans Res 2008
Matarraz, Cytometry B 2017

Altered monocytic patterns in CMML vs AMML

Monocytic maturation ↓

Cell distribution and phenotype	CMML	Monoblastic leukemia	Monocytic leukemia
↓ CyMPO	**40%**	**90%**	**70%**
↓ %CD36+ cells	20%	**90%**	0%
↓ %CD11b+ cells	**70%**	**100%**	28%
↓ %CD15+ cells	-	**70%**	0%
↓ %CD35+ cells	10%	**90%**	0%
↓ %CD14+ cells	**30%**	**100%**	0%
↓ %CD300e+ cells	10%	**100%**	10%

Matarraz S, *Cytometry B* 2015

Monocytic lineage infidelity in CMML vs AMML

Aberrant phenotype	CMML	Monoblastic leukemia	Monocytic leukemia
CD34+	0%	**45%**	**43%**
CD16	50%	0%	60%
CD19 (partial)	0%	**55%**	**43%**
CD7 (partial)	20%	45%	15%
NuTdT	0%	0%	20%
CD56*	**70%**	**70%**	**80%**
NG2 (7.1)	**0%**	**70%**	**60%**

Matarraz S, *Cytometry B* 2015

Unique immunophenotypic features of CD34+ HPC in AML^{NPM1}

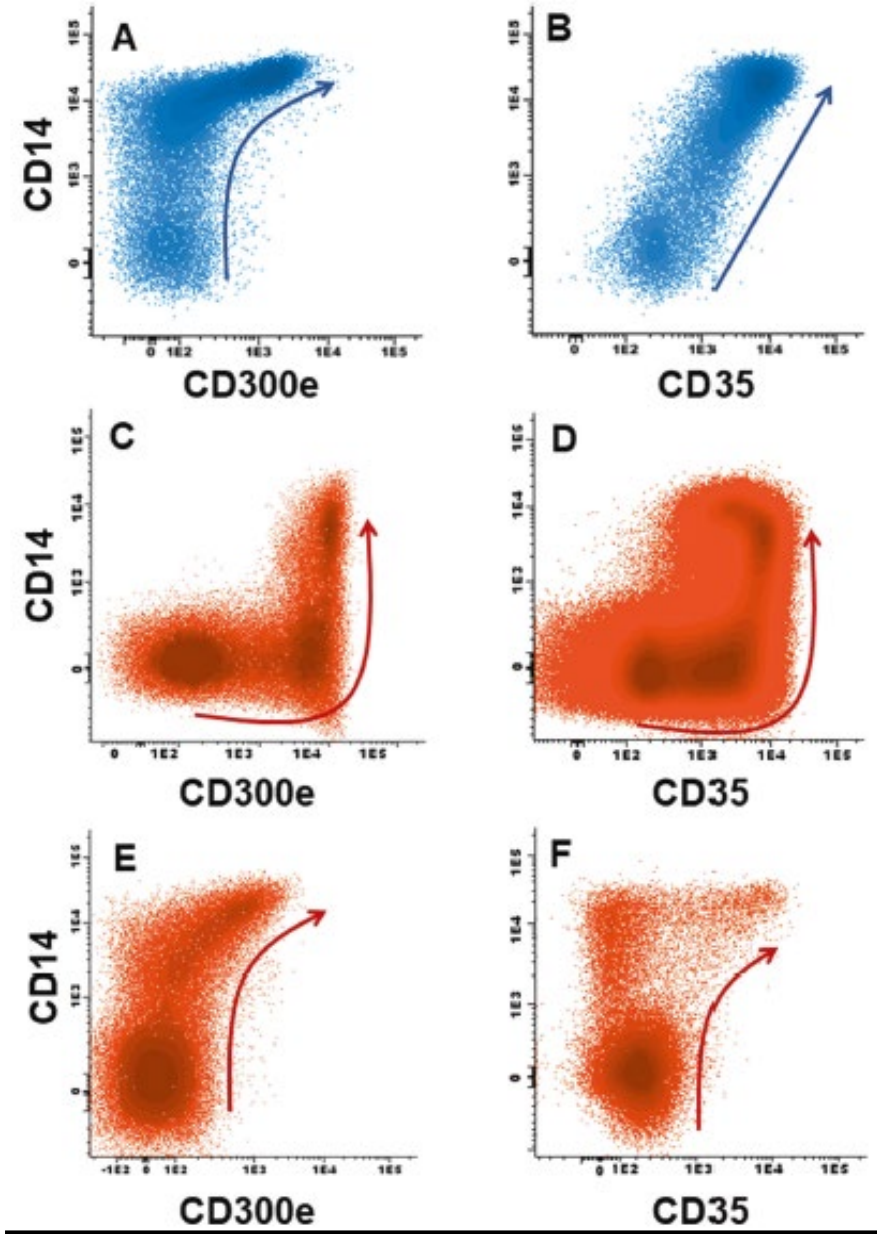


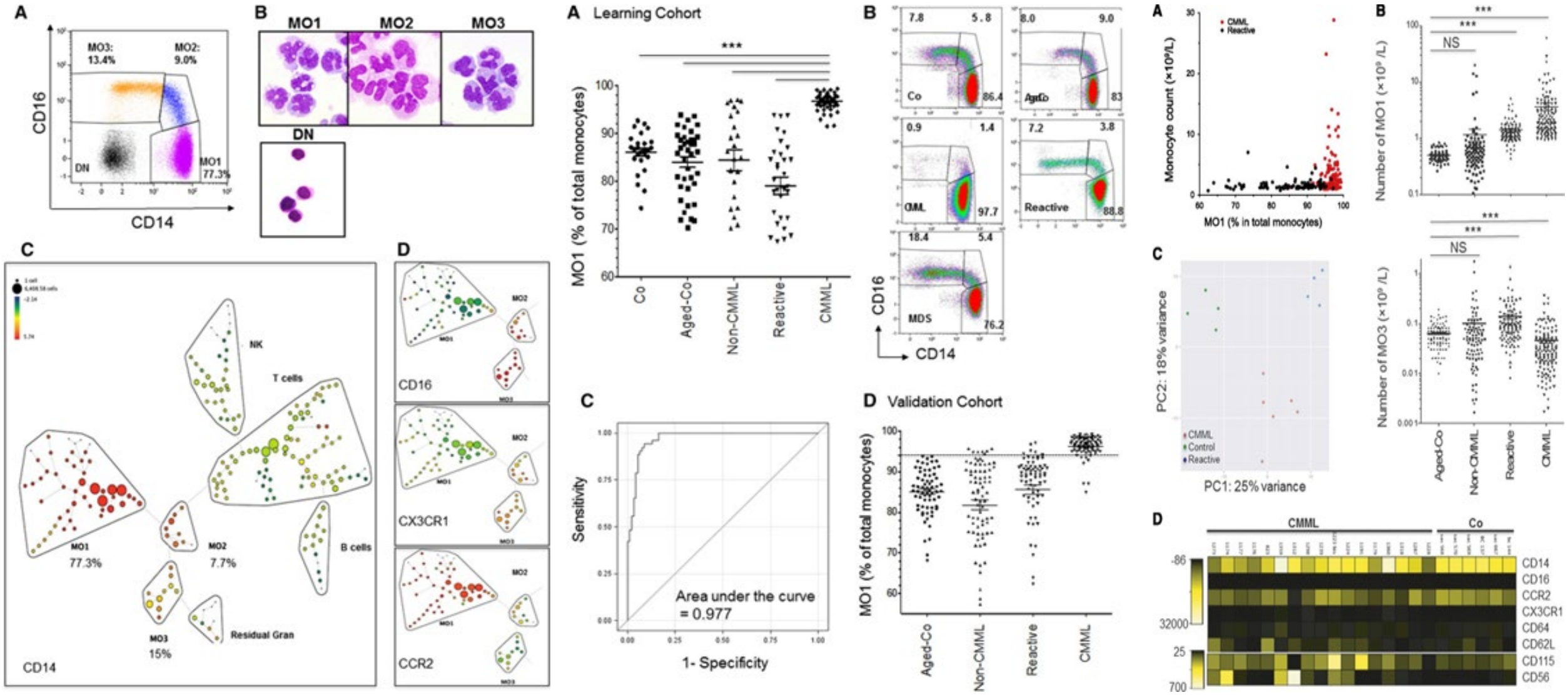
Table 3. Univariate and multivariate logistic regression analysis of immunophenotypic patterns associated with *NPM1* mutation among leukemic cell subsets from AML patients

Variables and leukemic cell subsets	Univariate analysis				Multivariate analysis			
		95% CI				95% CI		
	OR	Lower	Upper	p-value	OR	Lower	Upper	p-value
CD34+ and/or CD117+HLADR+ leukemia cells								
<26.5% of all leukemia cells	2.085	1.088	4.000	**0.02**				
CD34+ (<35%)	22.957	11.075	47.585	**<0.001**	**15.220**	**6.841**	**33861**	**<0.001**
CD33 (>96%)	6.528	3.368	12.652	**<0.001**	**3.035**	**1.264**	**7.288**	**0.01**
CD105 (<9.5%)	2.647	1.405	4.987	**0.003**				
HLA-DR (<97%)	2.427	1.353	4.543	**0.003**	**2.592**	**1.122**	**5.988**	**0.02**
CD15 (>6.6%)	3.460	1.692	7.076	**0.001**				
CD7 (>3%)	6.439	3.389	12.234	**<0.001**	**4.712**	**2.024**	**10.973**	**<0.001**
CD56 negative	3.579	1.396	9.173	**0.008**				
NuTdT negative	4.775	1.893	12.049	**<0.001**				
Leukemic cells with neutrophil differentiation								
>21.5% of all leukemia cells	3.494	1.142	10.694	**0.02**	-	-	-	-
CD34 (<5%)	10.933	5.031	23.761	**<0.001**	**12.903**	**4.597**	**36.216**	**<0.001**
CD71 (<70%)	4.653	2.240	9.665	**<0.001**	**4.269**	**1.534**	**11.881**	**0.005**
CD105 (>3%)	7.468	3.424	16.287	**<0.001**	**6.232**	**2.240**	**17.340**	**<0.001**
CD64 (<30%)	9.167	4.332	19.441	**<0.001**	**6.339**	**2.366**	**16.981**	**<0.001**
CD13 (<92%)	7.003	3.338	14.668	**<0.001**	-	-	-	-
CD56 (>5%)	4.889	1.766	13.531	**0.002**	-	-	-	-
Leukemic cells with monocytic differentiation								
Asynchronous CD300e and/or CD35	56.320	28.891	109.790	**<0.001**	**616.785**	**62.241**	**6112.08**	**<0.001**
Asynchronous CD300e	123.2	43.5	348.7	**<0.001**				
Asynchronous CD35	31.7	16.6	60.2	**<0.001**				
CD34+ (<3.8%)	116.250	15.199	889.122	**<0.001**	**519.029**	**27.601**	**9760.2**	**<0.001**
CD117 (<5.9%)	3.505	1.705	6.847	**0.001**	-	-	-	-
CD13 (<77%)	14.525	6.414	32.892	**<0.001**	-	-	-	-
CD123 (>82.8%)	2.935	1.516	5.681	**0.001**	**9.208**	**1.013**	**83.70**	**0.05**
CD15+ (>77%)	7.788	3.640	16.663	**<0.001**	-	-	-	-
CD36 (>87%)	4.062	2.067	7.983	**<0.001**	-	-	-	-

OR, odds ratio; CI, confidence interval.

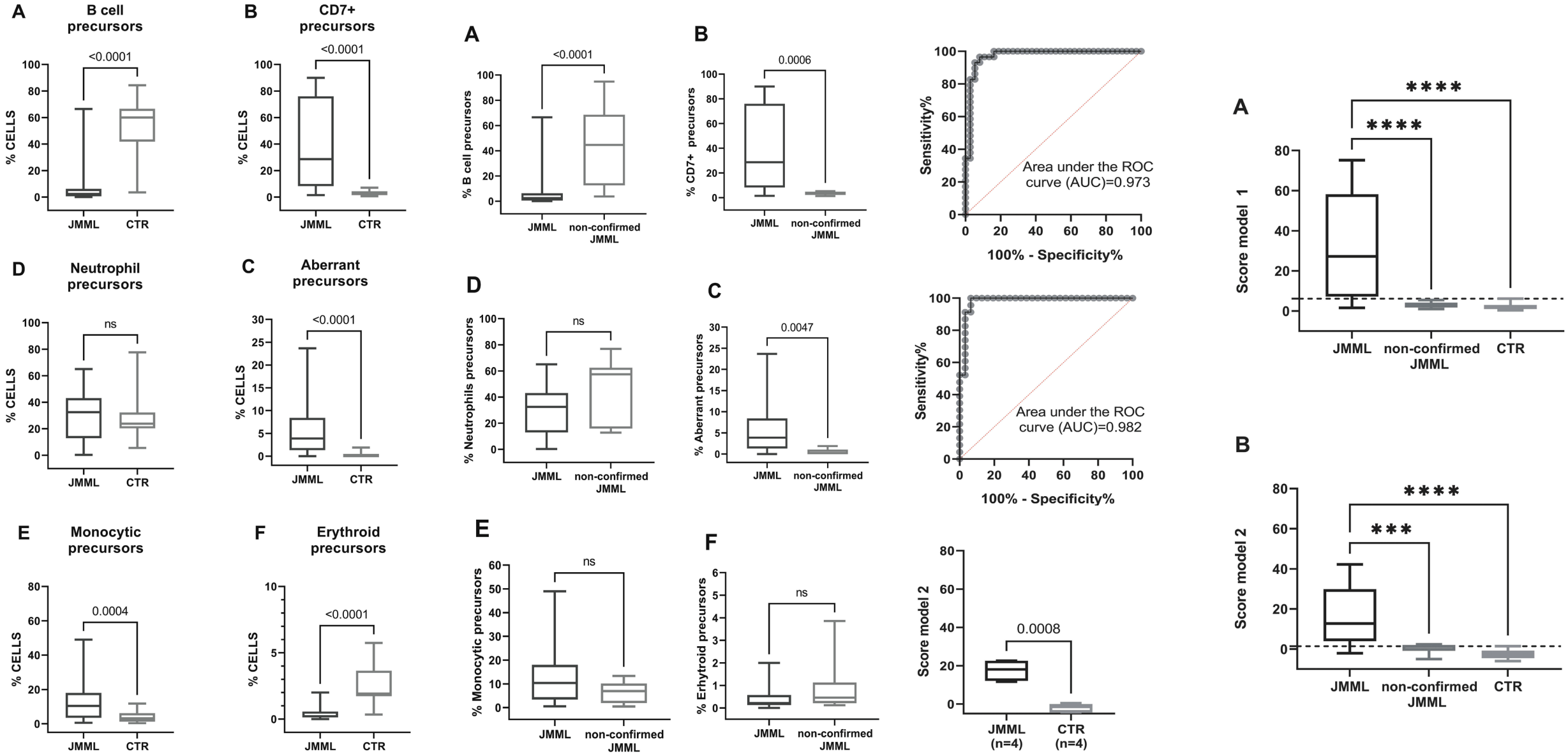
Matarraz et al, Blood Cancer J, 2023

Unique immunophenotypic features of blood monocytes in CMML



Selimoglu-Buet et al , Blood 125: 36118-26, 2015

Unique immunophenotypic features of CD34+ HPC in JMML



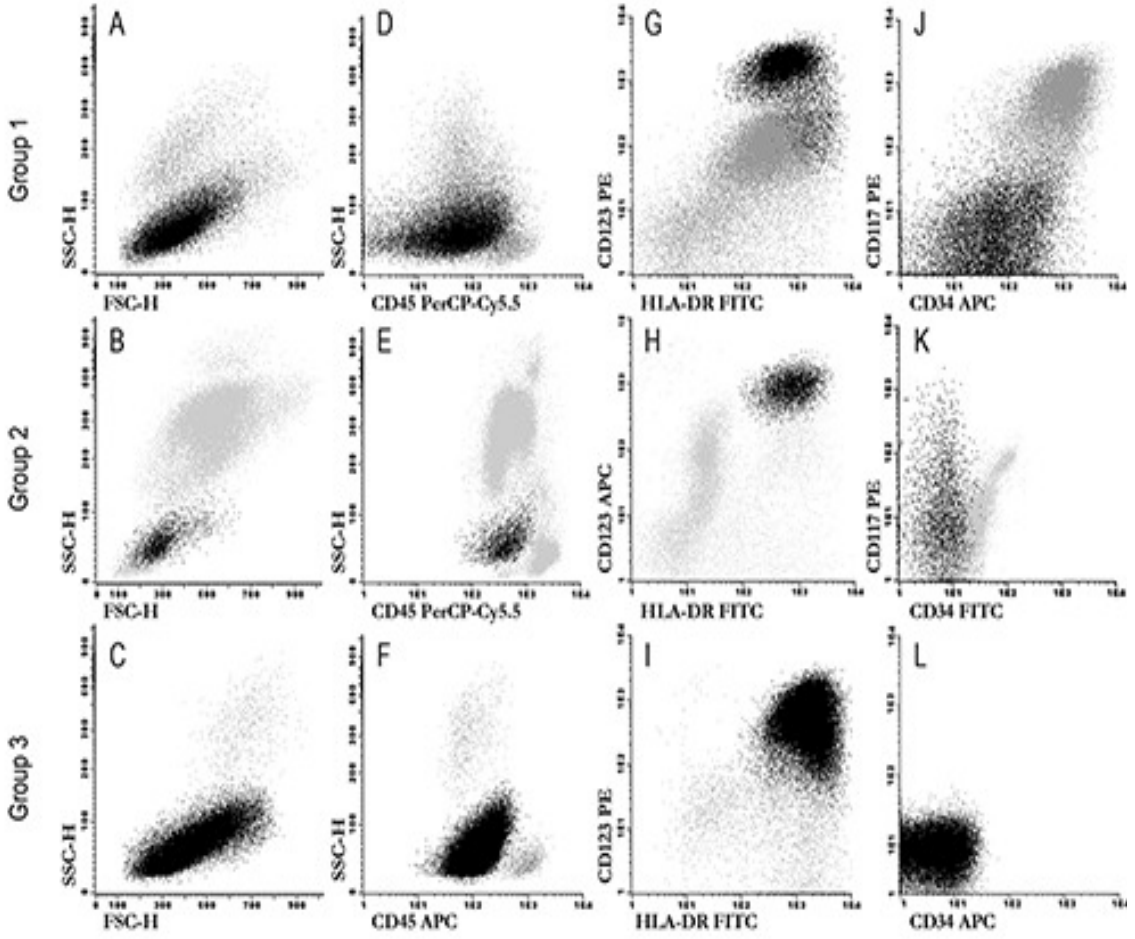
Bugarin et al, Haematologica 2023

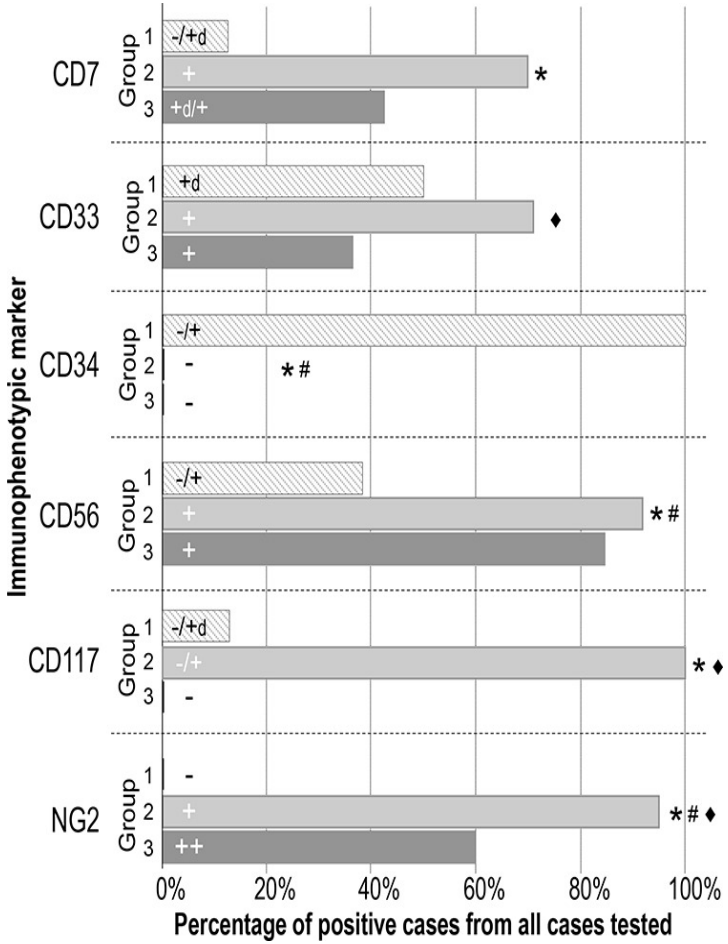
Plasmacytoid dendritic cell neoplasm

Immature leukemia/ lymphoma cells
[CD34+ in a fraction of neoplastic cells]

Intermediate leukemia/ lymphoma cells
[CD34-/CD117 in a fraction of neoplastic cells]

Mature leukemia/lymphoma cells
[CD34-/CD117-]



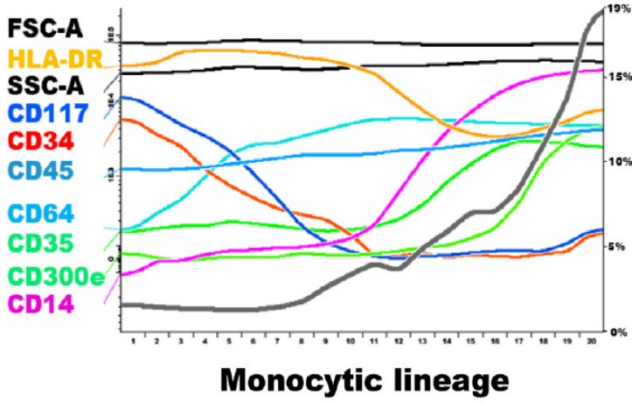


Martin Martin et al, Oncotarget 2015

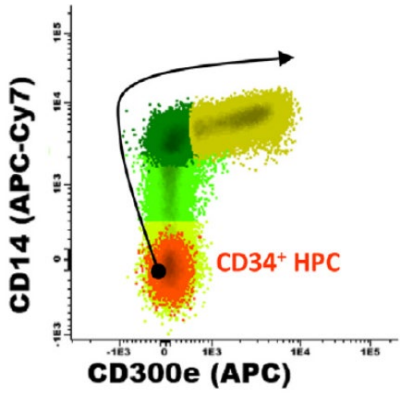
Myeloid neoplasm (MDS) with NPM1 mutation

Maturing neutrophils and monocytes

Monocytic maturation in normal BM

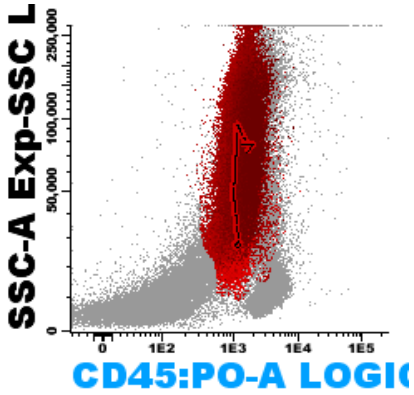


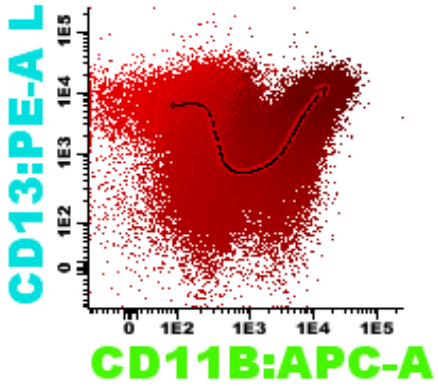
Sequential markers: CD64+ CD34- CD117- CD14+ CD35+ CD300e+

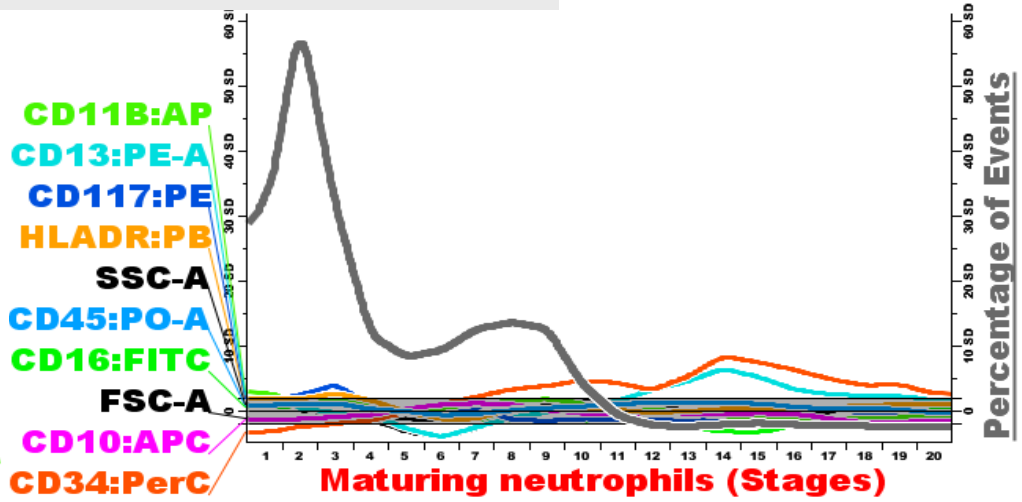


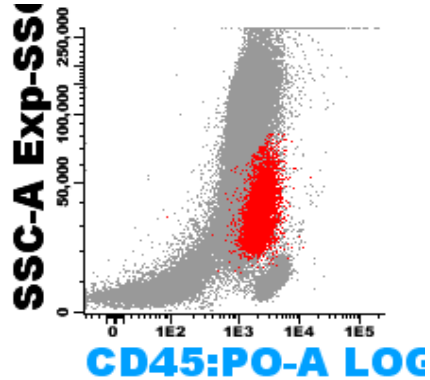
Orfao et al, J Immunol Meth 2019

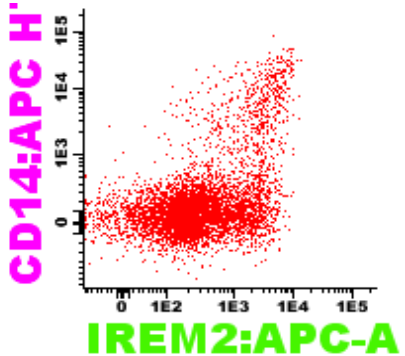
Altered monocytic maturation in AML/MDS

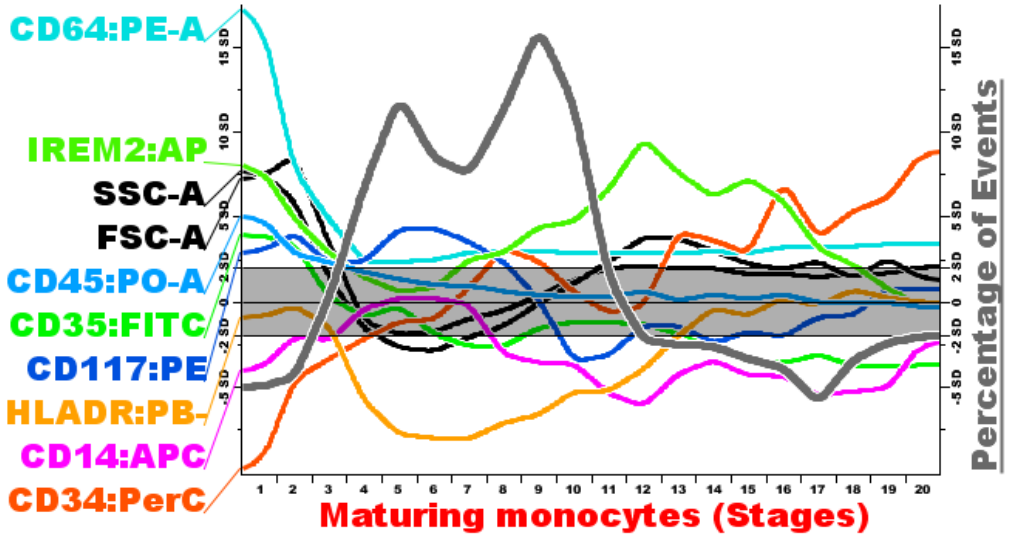












CONCLUDING REMARKS:

- Optimized multi-color antibody combinations have been proposed which facilitate assessment of the normal monocytic and dendritic cell compartments in human BM, PB and other tissues.

- Important advances have been made in the identification and understanding of the normal monocytic, DC and MC maturation pathways in different tissue compartments.

- All the above has highlighted the existence of multiple distinct subpopulations of monocytes and dendritic cells in human blood which can be simultaneously assessed.

- Such increased knowledge about the normal B-cell and T-cell maturation pathways provides the basis for monitoring specific alterations of these cell populations in multiple disease conditions aberrant protein expression profiles in their neoplastic counterparts.

Acknowledgements & Collaborations in Immune Monitoring

VNiVERSiDAD D SALAMANCA

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Martin Perez-Andres
Elena Blanco
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Luzalba Sanoja-Flores
Daniela Damasceno
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Vitor Botafogo
Quentin Lecrevisse
Lourdes Martín
Sara Gutiérrez
María Herrero
Alejandro Hernandez
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Sheila Mateos
Alberto Orfao

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Miriam López
Maria Victoria Mateos
Norma Gutiérrez
Marcos González

Junta de Castilla y León

Sacyl

LUMC Leiden University Medical Center

Department of Immunology

Cristina Teodosio
Magda Berkowska
Pieter van der Pol
Indu Khatri
Wouter van den Bossche
Kyra van der Pan
Annieck Diks
Gita Naber
Alita van der Sluijs
Sandra de Bruin
Sandra Vloemans
Rick Groenland
Bas de Mooij
Marieke Bitter
Bart Lubbers
Châtelaine Voets
Jacques JM van Dongen

peri scope

WP5 Task 5.6

USAL, Salamanca, ES
LUMC, Leiden, NL
RIVM, Bilthoven, NL
RUMC, Nijmegen, NL
UTU, Turku, FI
UOXF, Oxford, UK
US, Southampton, UK
MRC, Gambia, GM
ULB, Brussels, BE

CANCER RESEARCH UK

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WP5

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Franco Locatelli
Sara Gorashian
Ignacio Criado
Silvia Zaninelli
Alessandro Rambaldi
Chiara Magnani
Chiara Buracchi
Giuseppe Gaipa
Andrea Biondi

erc European Research Council

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MUCHAS GRACIAS