DISSECTION OF THE MYELOID HEMATOPOIETIC COMPARTMENT BY NEXT GENERATION FLOW CYTOMETRY

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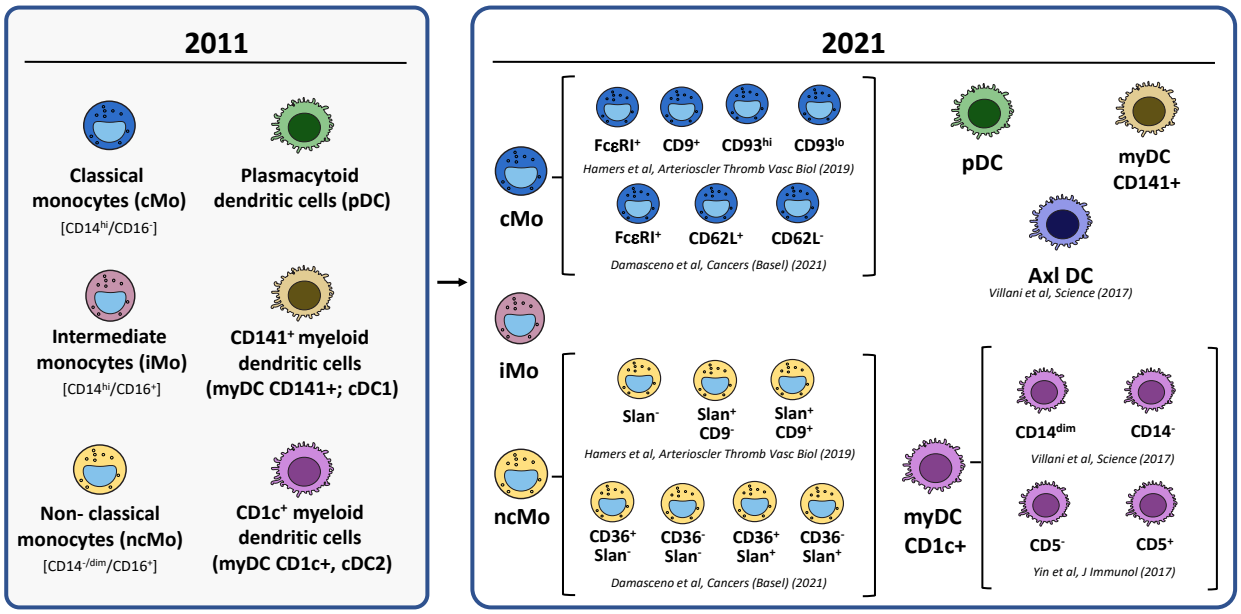
HISTORICAL PERSPECTIVE



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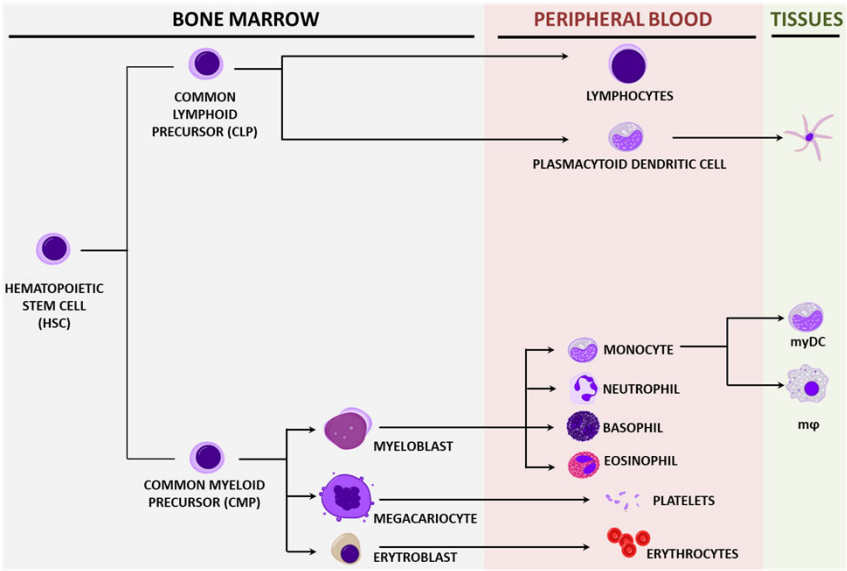
MYELOPOIESIS



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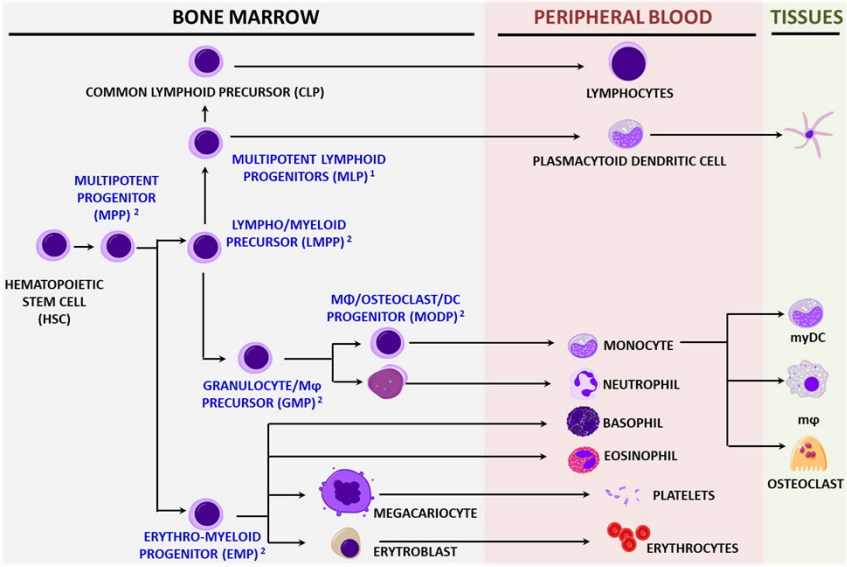
MYELOPOIESIS



[1]Helft *et al*, Cell Reports (2017) [2]Xiao *et al*, Blood advances (2017)

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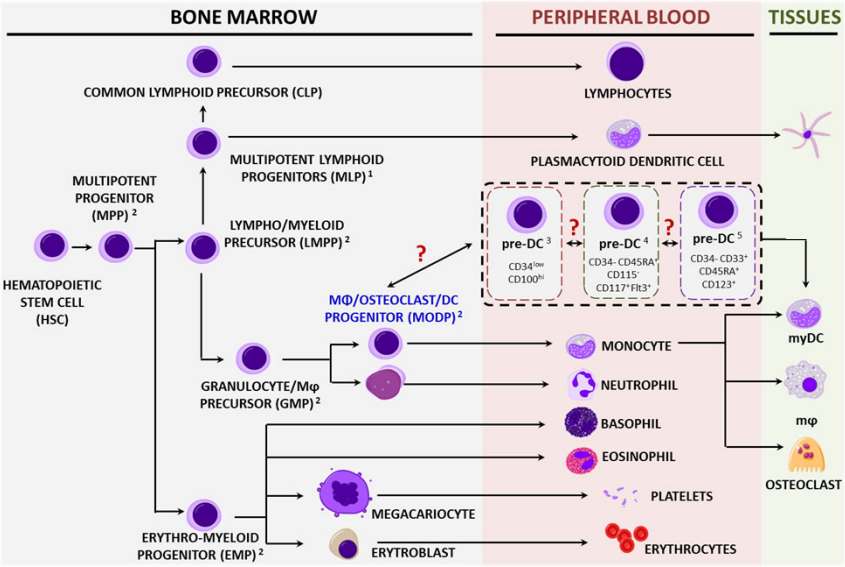
MYELOPOIESIS



[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)

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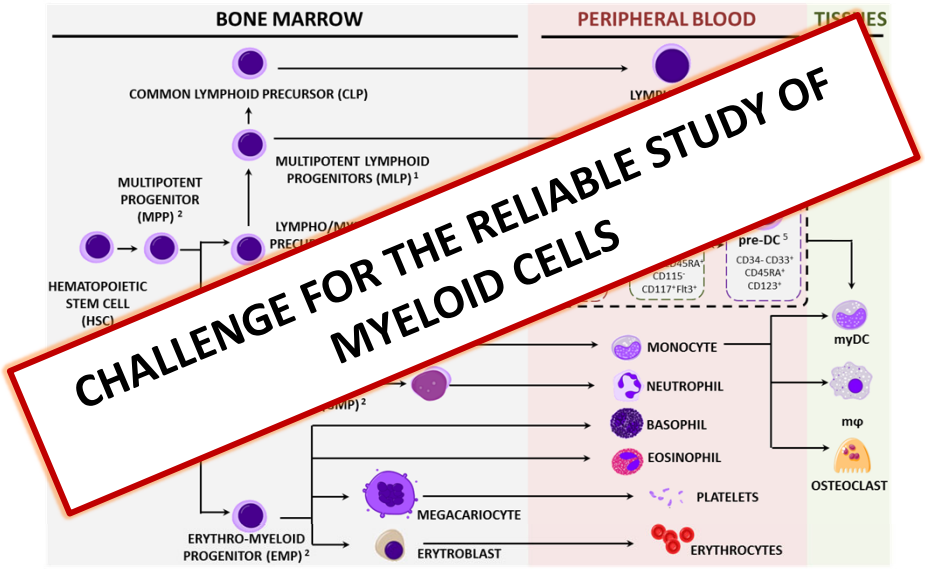
MYELOPOIESIS



[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)

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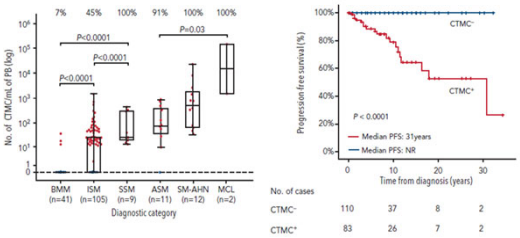
NEXT GENERATION FLOW CYTOMETRY (NGF)

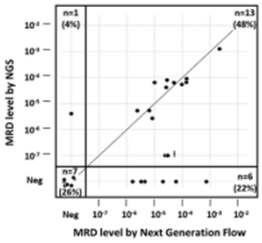
NGF = STANDARDIZATION + HIGH SENSITIVITY

Detection of circulating tumor mast cells (CTMC) in PB from mastocytosis patients



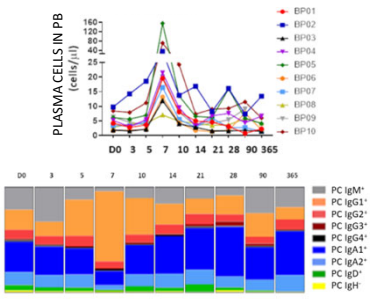
In: Henriques *et al*. Blood 2022

Sensitivity of flow cytometry *vs.* NGS for detection of MRD in MM



In: Flores-Montero *et al*. Leukemia 2017

Monitoring immune responses (PB circulating plasma cells) to vaccination (Tdap; Boostrix)



In: Diks *et al*, Front Immunol (2021)

✓ **Role in the dissection of the myeloid hematopoietic compartment**

BMM, bone marrow mastocytosis; *SM,* systemic mastocytosis; *ISM,* indolent SM; *SSM,* smouldering SM; *ASM,* aggressive SM; *SM-AHN,* SM associated with another hematologic neoplasm; *MCL,* mast cell leukemia

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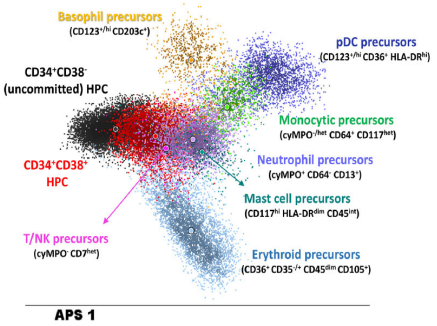
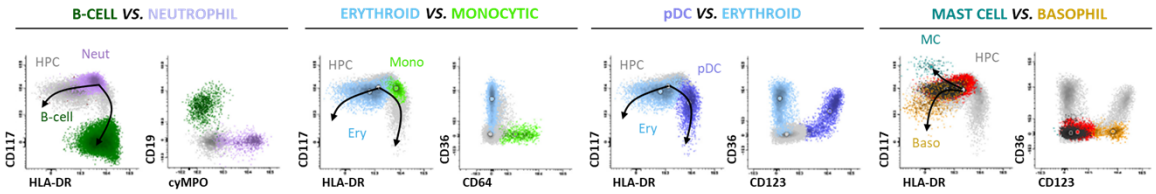
HEMATOPOIETIC PRECURSOR CELLS

CELL LINEAGE	PHENOTYPE	FREQUENCY (% of CD34+ and/or CD117+ precursors) Median [min-max]
Myeloid precursors	*CD38⁺ CD45^{lo} CD117⁺ HLA-DR^{het}*	*77% [57-85%]*
Erythroid	CD36⁺ CD35$^{-/+}$ CD45^{lo} CD105⁺ CD71⁺	35% [24-37%]
Neutrophil	cyMPO⁺ CD64⁻ CD13⁺	33% [26-38%]
Monocyte	CD64⁺ cyMPO$^{-/+het}$ CD117^{het} HLA-DR^{hi}	22% [16-28%]
pDC	CD123$^{+/hi}$ HLA-DR^{hi} CD45^{lo} CD36⁺	6% [1-9%]
Basophil	CD123$^{+/hi}$ CD45⁺ CD117^{lo} HLA-DR^{lo} CD203c⁺	<1% [0-3%]
Eosinophil	cyMPO⁻ CD15/CD65⁺ cyEPO⁺	<1%
Mast cell	CD117^{hi} HLA-DR^{lo} CD45^{int}	<1%
Megakaryocyte	CD61⁺ CD45^{lo} CD203c^{lo}	<1%
B-cell	*nuTdT⁺ cyCD79α⁺ CD19⁺*	*23% [<1-45%]*
T/NK/DC	*cyMPO⁻ CD7^{het}*	*12% [10-15%]*





Modified from: Orfao *et al*, JIM (2019)

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

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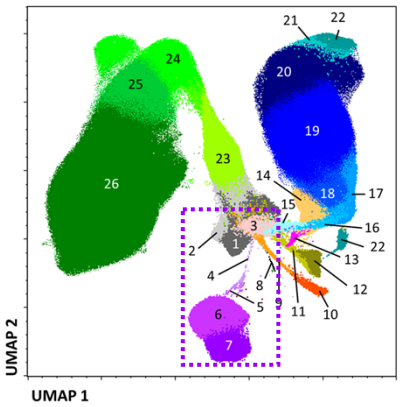
MYELOID HEMATOPOIETIC CELL COMPARTMENT: CURRENT LANDSCAPE

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

Erythroid 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

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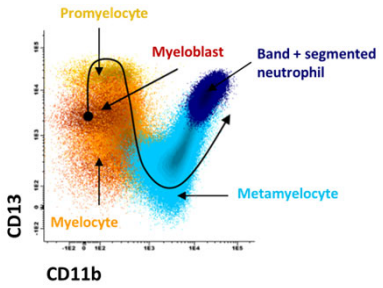
NEUTROPHIL MATURATION IN BONE MARROW



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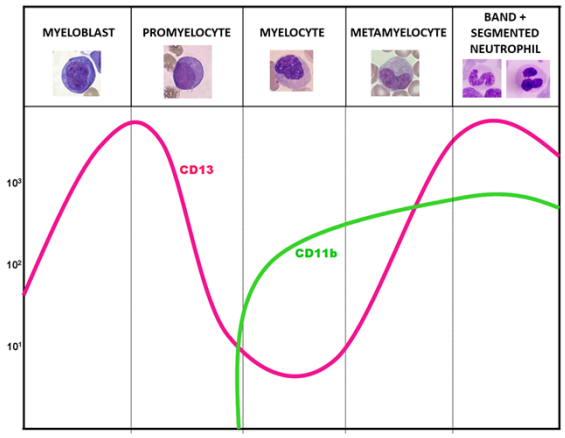

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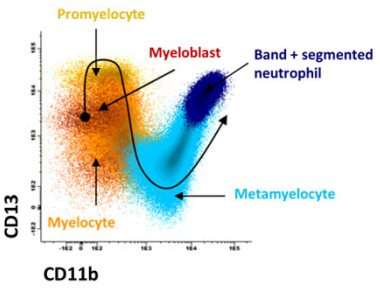
NEUTROPHIL MATURATION IN BONE MARROW



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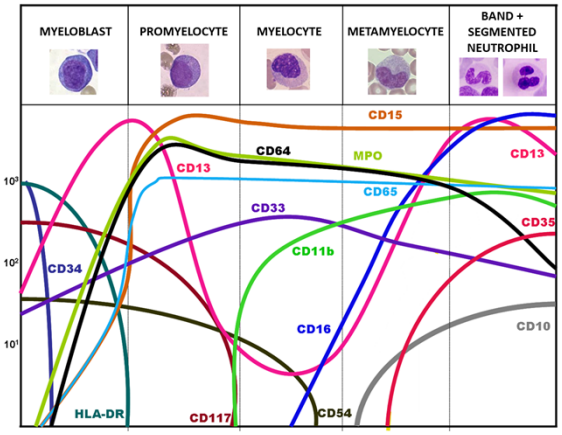

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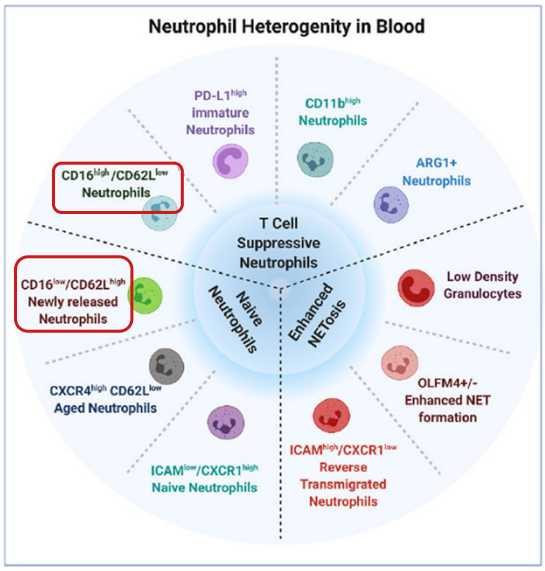
NEUTROPHIL HETEROGENEITY IN PERIPHERAL BLOOD



In: Rawat *et al*, Mol Aspects Med (2021)

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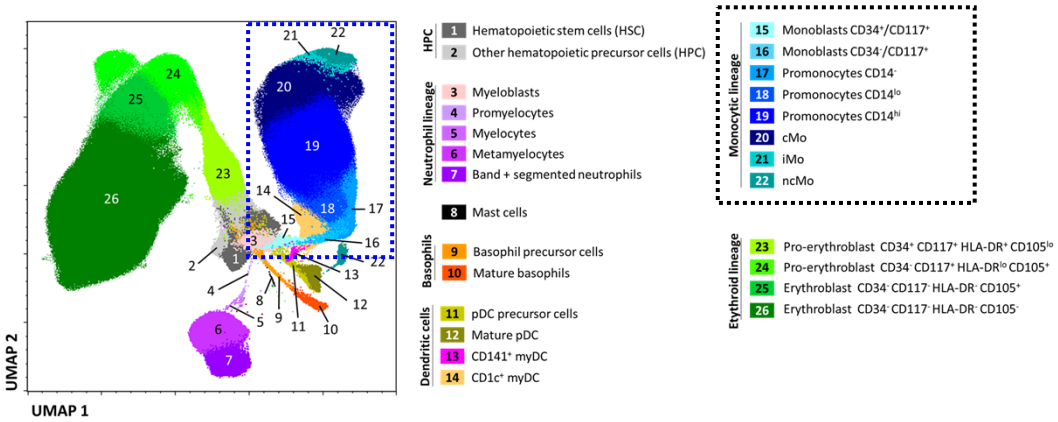
MYELOID HEMATOPOIETIC CELL COMPARTMENT: CURRENT LANDSCAPE

HIGH SENSITIVITY NGF + 28 COLOR PANEL
[Bone marrow mononuclear cells]



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

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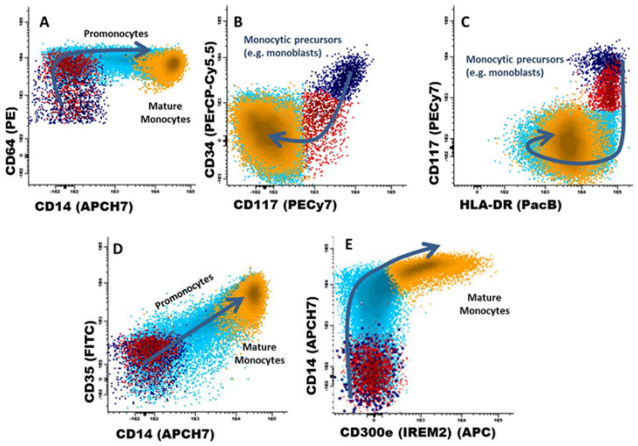
MONOCYTIC MATURATION IN BONE MARROW



Monoblasts CD34+/CD117+
Monoblasts CD34-/CD117+
Promonocytes CD14-
Promonocytes CD14+
cMo

Adapted from: *Matarraz et al, Cytometry Part B (2014)*

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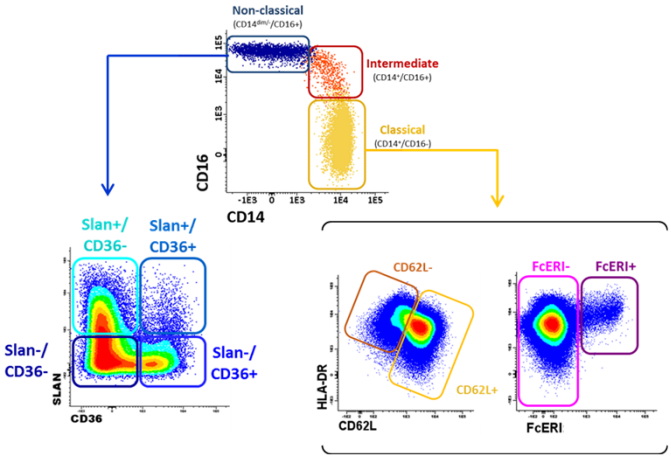
MONOCYTIC POPULATIONS IN PERIPHERAL BLOOD



In: *Damasceno et al, Cancers (Basel) (2021)*
van der Pan et al, manuscript in preparation

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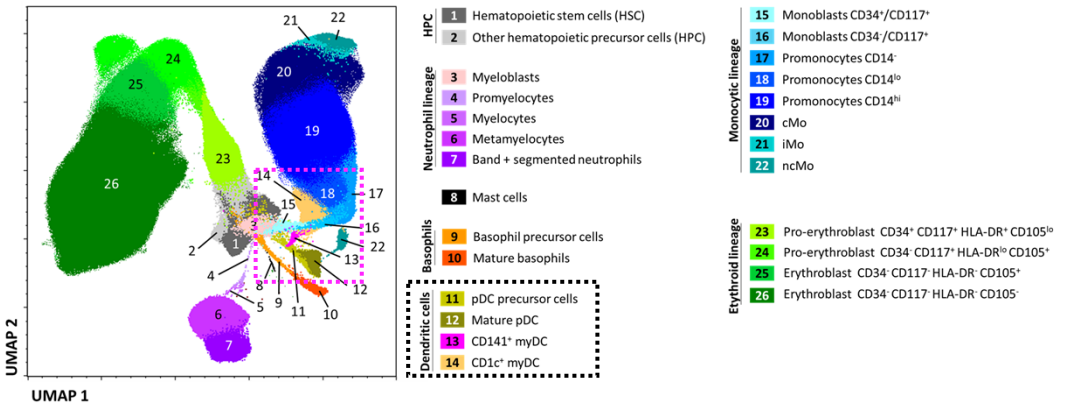
MYELOID HEMATOPOIETIC CELL COMPARTMENT: CURRENT LANDSCAPE

HIGH SENSITIVITY NGF + 28 COLOR PANEL
[Bone marrow mononuclear cells]



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

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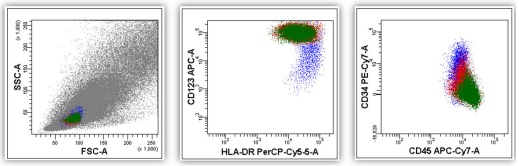
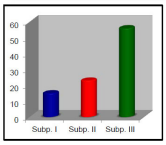
PLASMACYTOID DENDRITIC CELL MATURATION

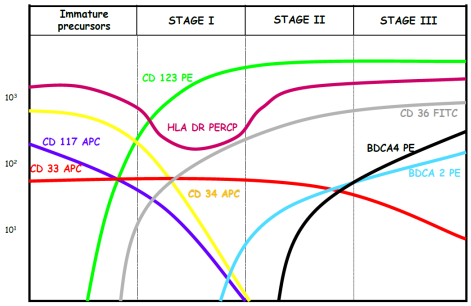


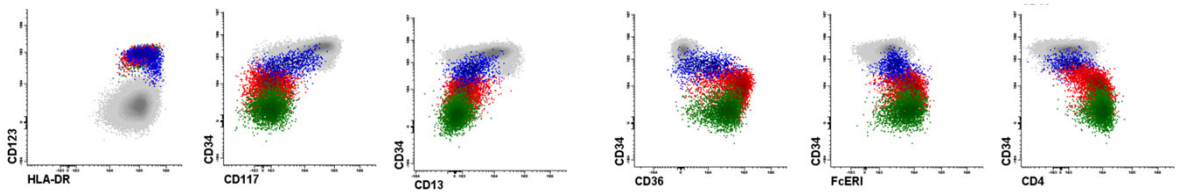
Total pre-pDC
(0.28%±0.13% of BM nucleated cells)

	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)*

CD34+ CD38+ HPC | CD34^{++}/HLA-DR$^{++/+++}$/CD123^{++}/CD45$^{+/++}$ (Stage I) | CD34^{+}/HLA-DR$^{+/++}$/CD123$^{++/+++}$/CD45$^{+/++}$ (Stage II) | CD34^{-}/HLA-DR^{++}/CD123$^{++/+++}$/CD45^{++} (Stage III)

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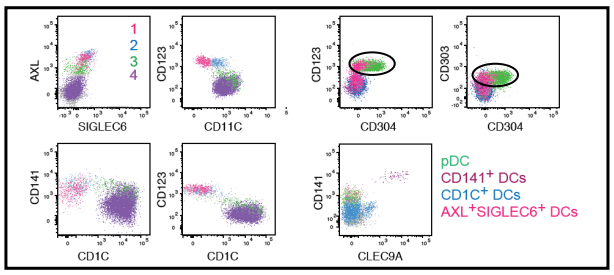
AXL+ DENDRITIC CELL



✓Identification of **pDC** based exclusively on the expression of **CD123 or CD303**

↓

Contamination with Axl^{+} dendritic cells

↓

- ✓ **Mixed gene expresssion profile** [pDC and myDC]
- ✓ Increased ability to **induce T cell proliferation**
- ✓ Reduced **IFNα production** in response to stimulus via **TLR9**

In: *Villani et al, Science (2017)*
van der Pan et al, manuscript in preparation

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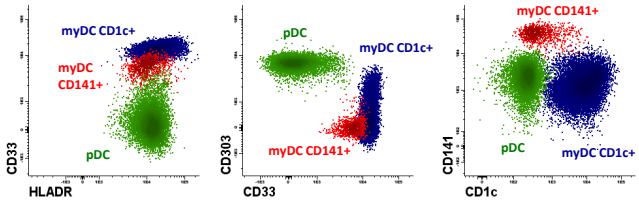
DENDRITIC CELL POPULATIONS IN PERIPHERAL BLOOD

Original classification

- pDC
- myDC CD141+
- myDC CD1c+



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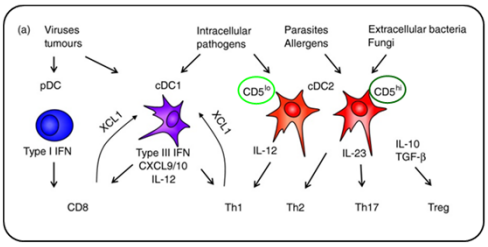
DENDRITIC CELL POPULATIONS IN PERIPHERAL BLOOD

Original classification | **New dendritic cell populations**

- pDC
- myDC CD141+
- myDC CD1c+
  - myDC CD1c+ CD14^{dim}
  - myDC CD1c+ CD14^{-}
    - myDC CD1c+ CD14^{-}/ CD5-
    - myDC CD1c+ CD14^{-}/CD5+



In: Collin et al. Immunology (2018)

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

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CONCLUSIONS

- ✓ Study of the **myeloid hematopoietic compartment** is **challenging**
- ✓ **NGF** has the potential to provide the level of **standardization and sensitivity** required to address those challenges

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- Better understanding of **normal maturation pathways**
- Unravelling **population heterogeneity**
- Establishing a the **frame of reference for different applications** (e.g., hemato-oncology, study of immune responses, tumor immunology, etc.)

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ACKNOWLEDGEMENTS

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