RELEVANCIA DEL SISTEMA INMUNE EN LAS HEMOPATÍAS MALIGNAS

CIC Centro de Investigación del Cáncer

VNiVERSiDAD D SALAMANCA
CAMPUS DE EXCELENCIA INTERNACIONAL

800 AÑOS 1218 - 2018

IBSAL Instituto de Investigación Biomédica de Salamanca

CANCER RESEARCH UK

CANCER RESEARCH CENTER IBSAL, UNIVERSITY & UNIVERSITY HOSPITAL OF SALAMANCA

aecc Contra el Cáncer

EuroFlow

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

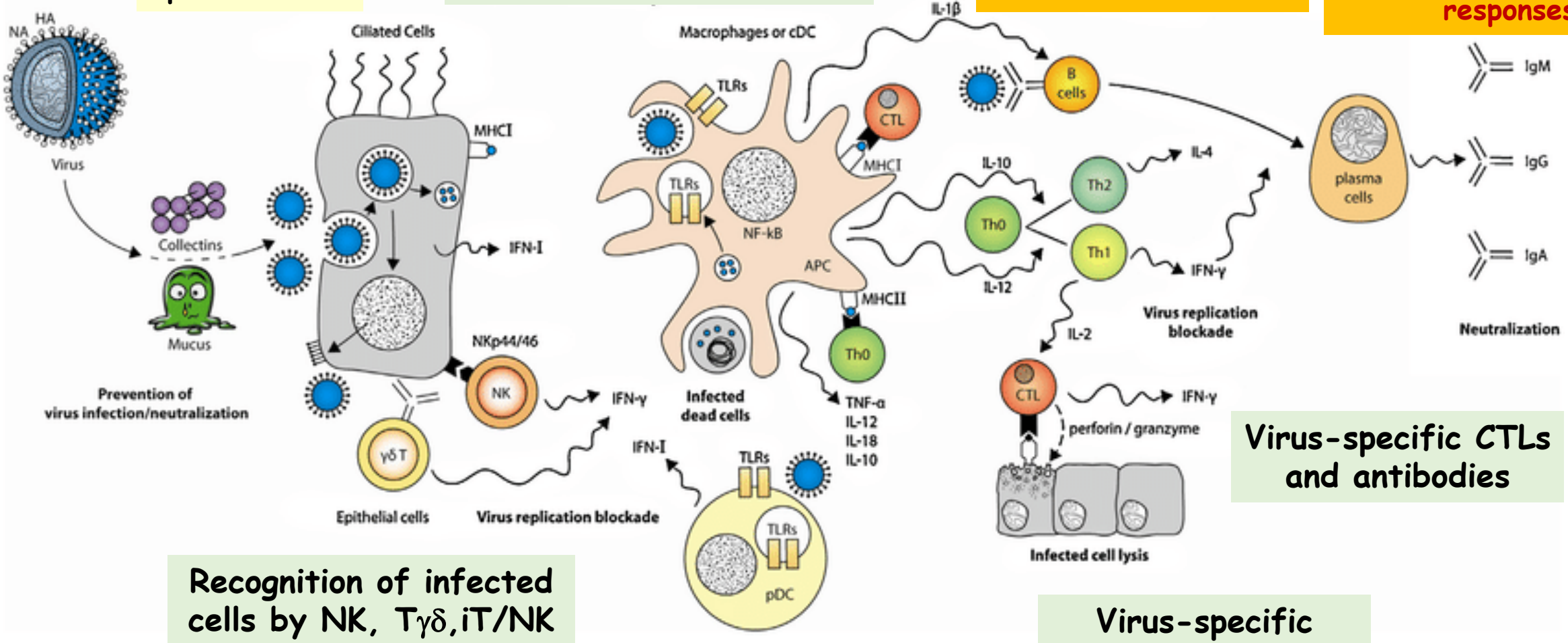
MUCOSAL IMMUNE RESPONSE IN COVID-19 PATIENTS

Infection of epithelial cells

Lysis and Phagocytosis of infected epithelial cells and increased Ag-presentation

Chemoattraction of immune cells: local inflammation

Migration to regional lymphoid tissues and adaptative immune responses



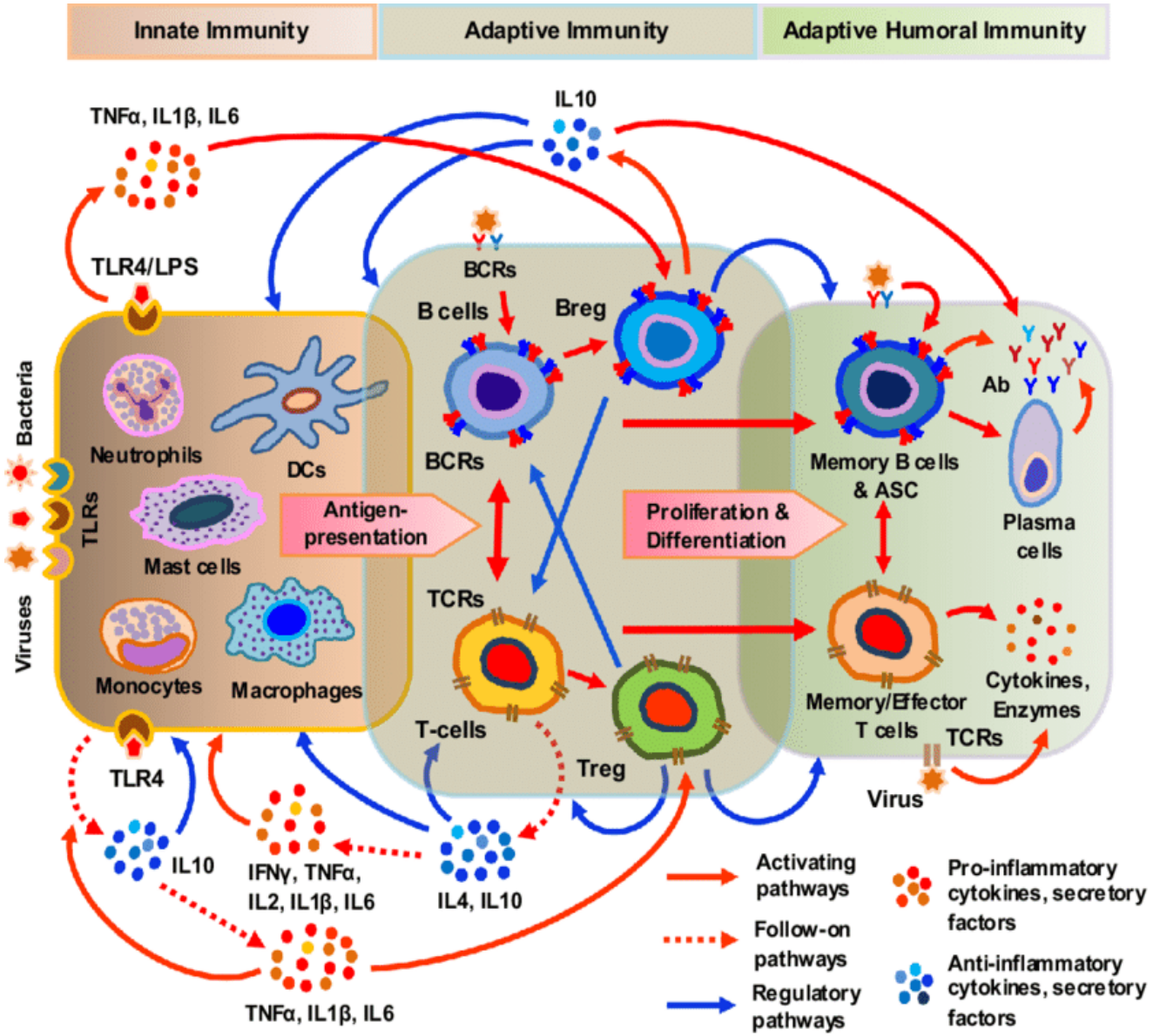
Virus-specific CTLs and antibodies

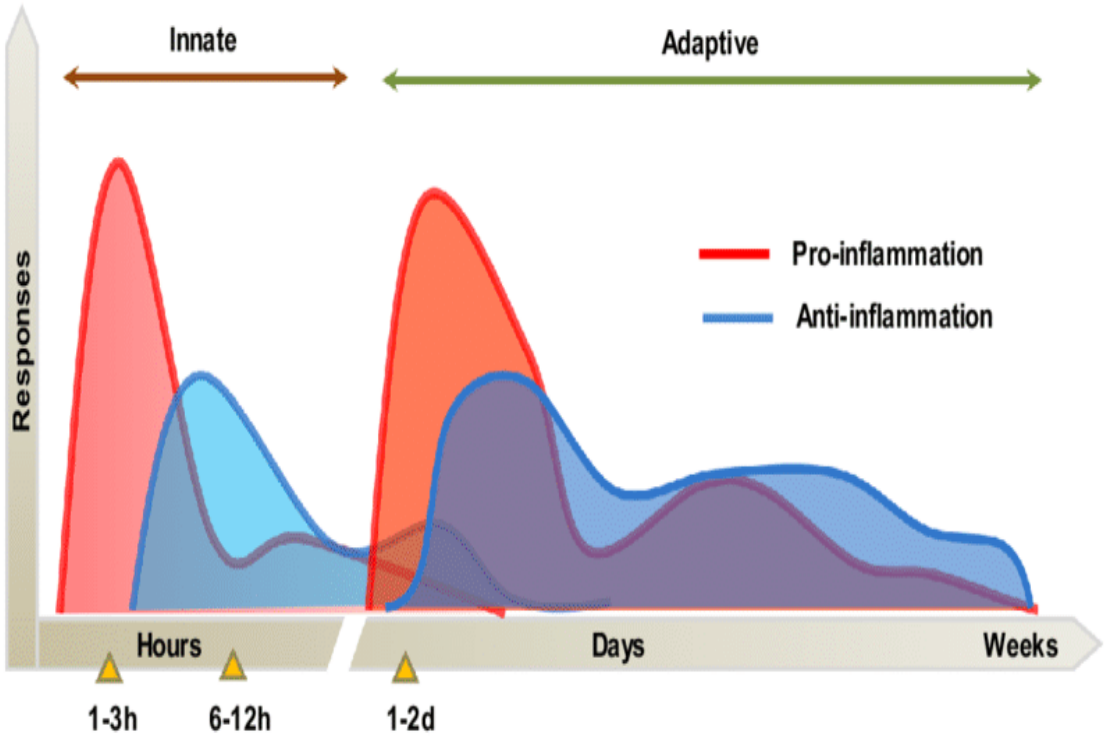
Recognition of infected cells by NK, Tγδ,iT/NK and MAITcells + natural antibodies

Activation of Macrophages & DCs

Virus-specific effector responses and tissue repair

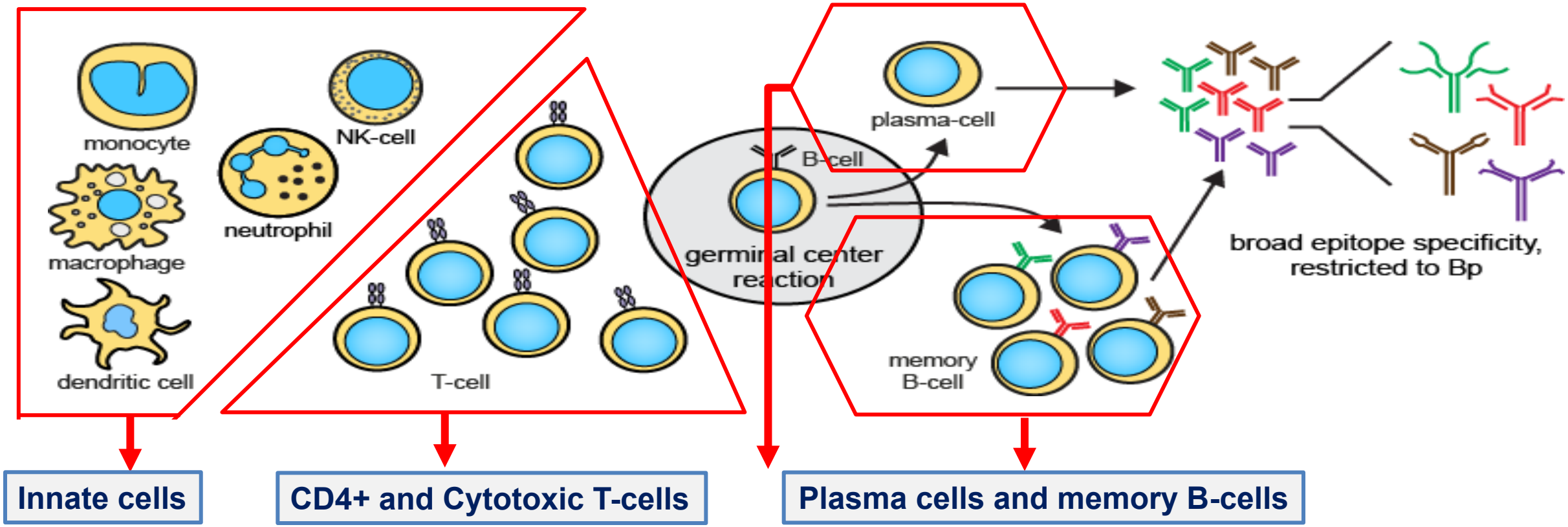
THE KINETICS OF THE IMMUNE RESPONSE





Nguyen TG, Inflammation Research, 69: 813-824, 2020

Immune response monitoring

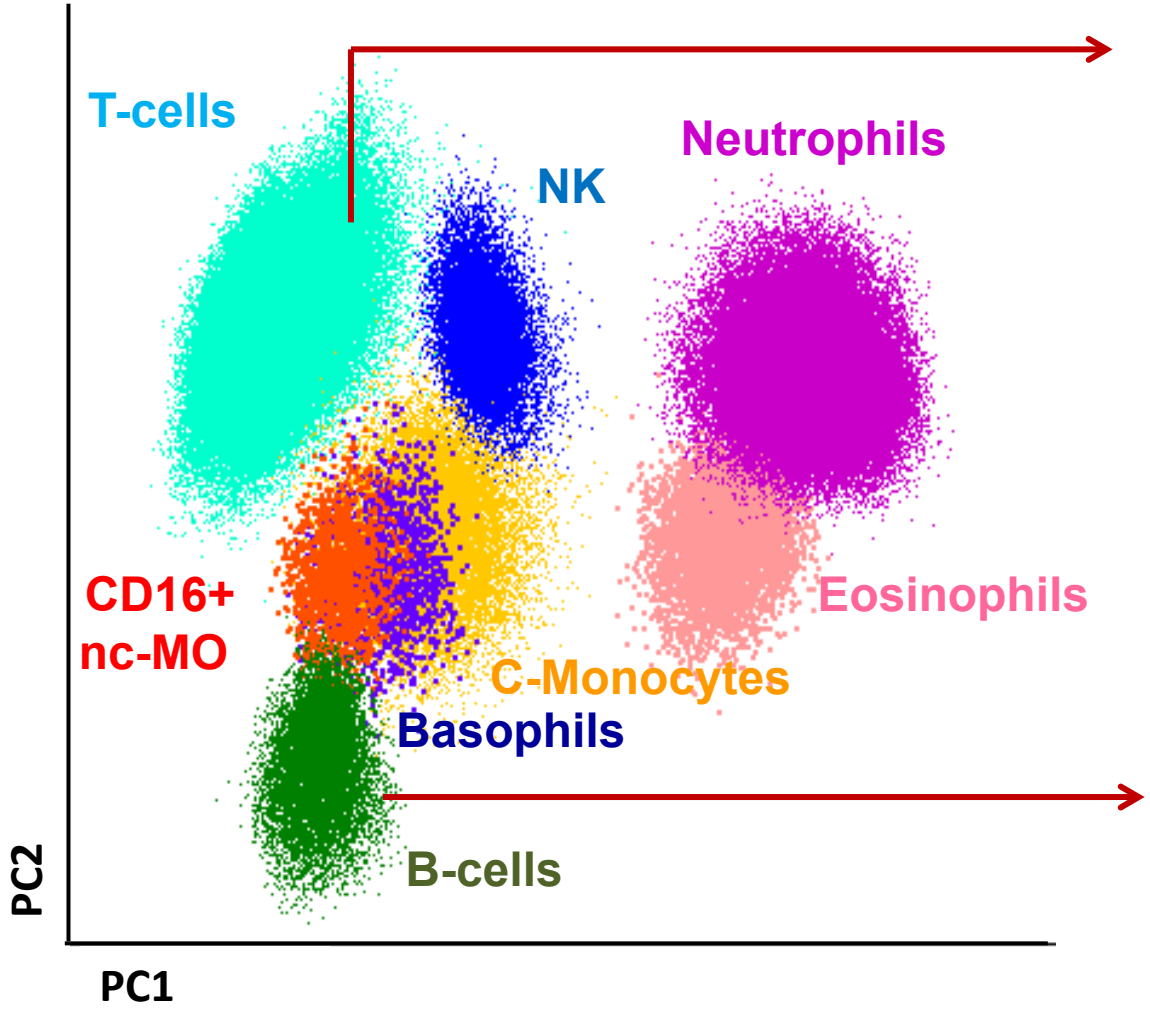


EuroFlow-based next generation flowcytometry in four 13 to 14-color tubes:

- 14-color monocyte-macrophage and dendritic cells (DC): **completed**
- 14-color CD4+ T-cell populations: **completed**
- 13-color CD8+ T-cell subsets and NK-cells: **completed**
- 14-color Immature, Memory B-cells, plasma cells, IGH isotypes: **completed**

European Patent 119646NL00, 2019

Identification of major subsets of leukocytes subsets in blood with the EuroFlow PID screening tube



Responsible scientists: Mirjam van der Burg & Martin Perez

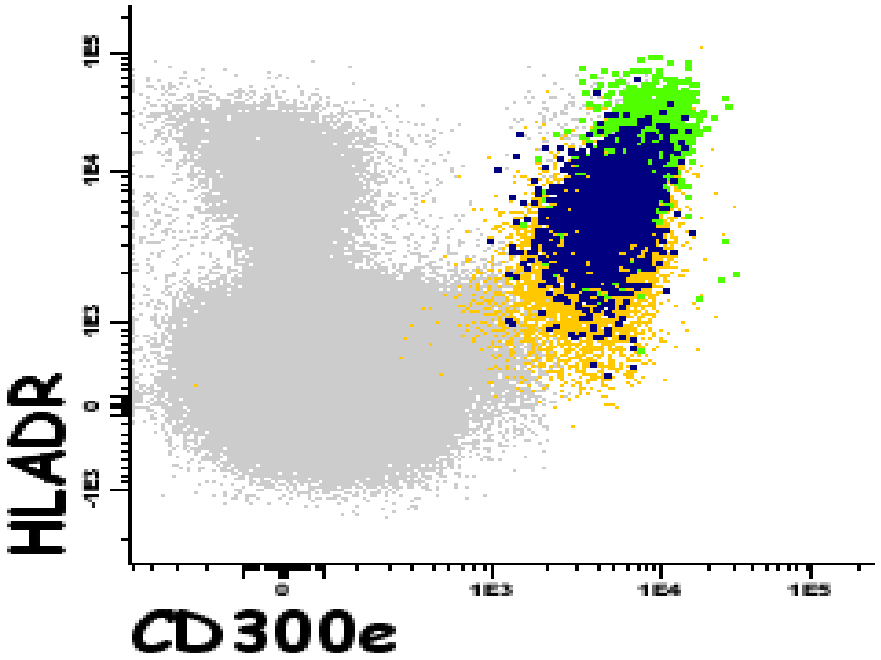
Van der Burg et al, Front Immunol 2019

PHENOTYPIC IDENTIFICATION OF CIRCULATING PB MONOCYTES AND DCs

EuroFlow

peri scope

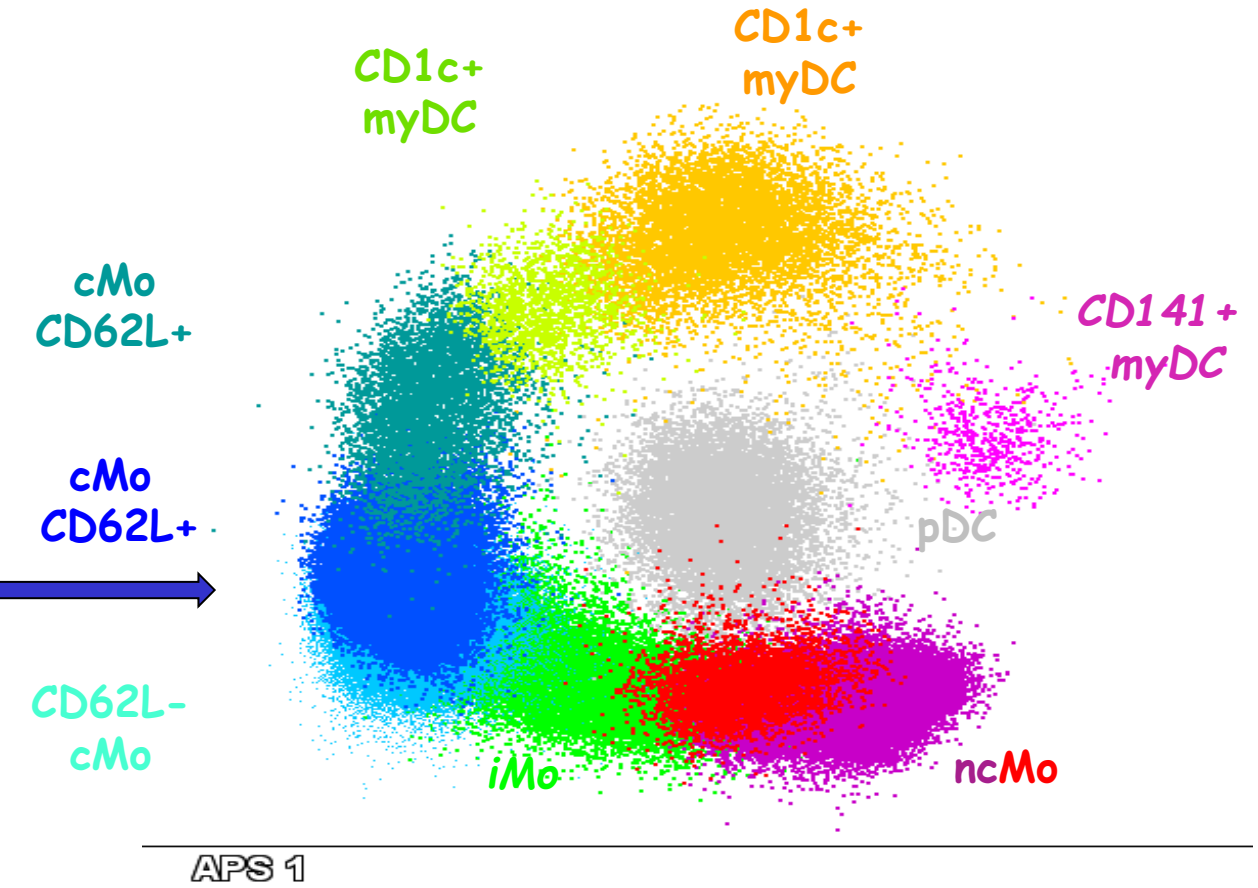
Identification



~23 subsets of monocytes and dendritic cells in normal blood

Subsetting

BM

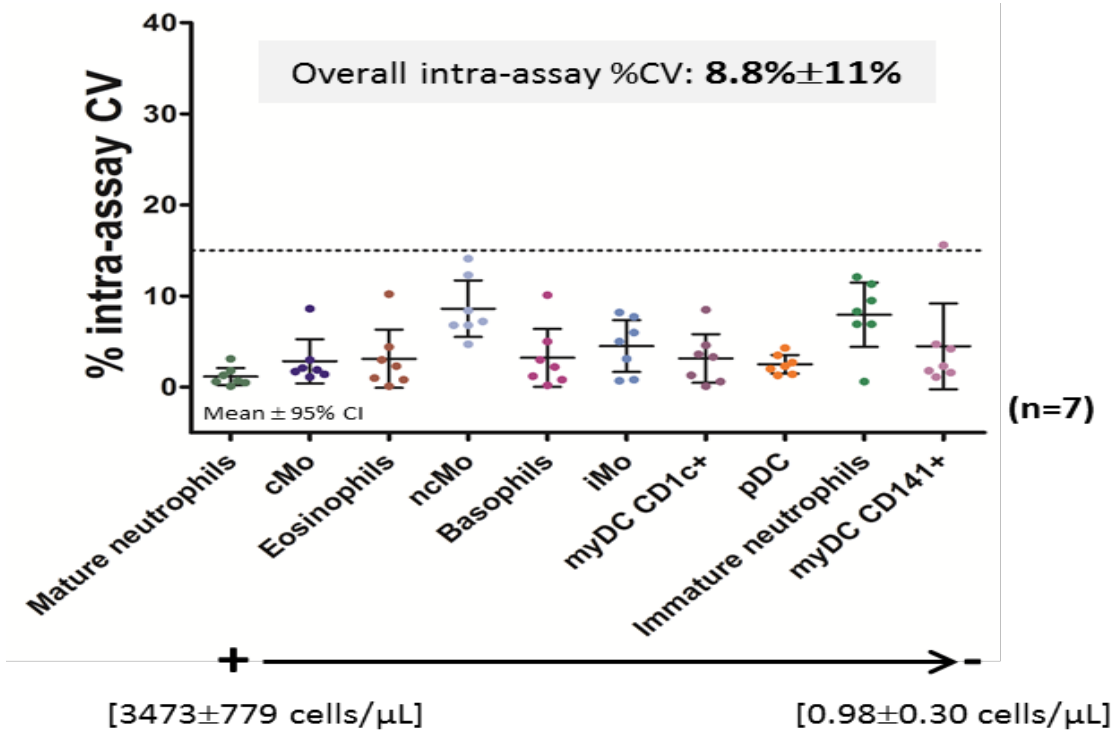


Damasceno et al, J Allergy Clin Immunol, 2019

Validation of the Monocyte-DC Tube

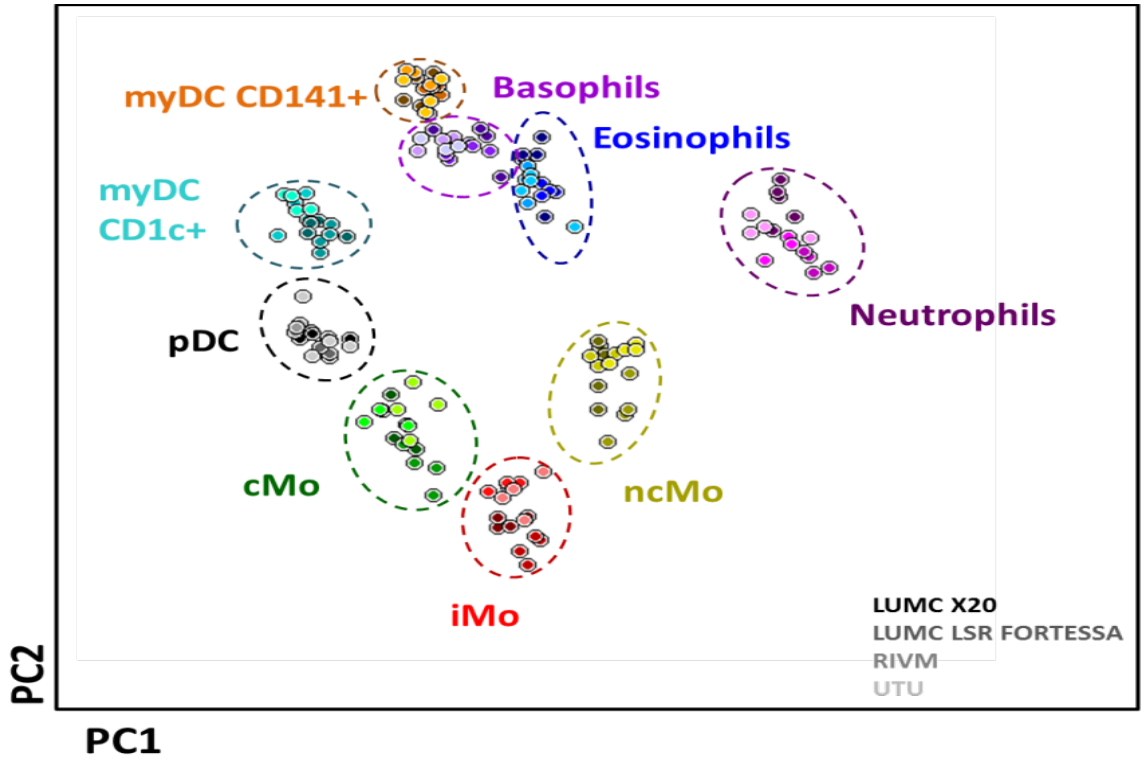
INTRA ASSAY VARIABILITY

(n=7; 2 replicates/sample; 2 technicians)



REPRODUCIBILITY (Multicentric study)

(n=16; 3 centers; 4 different cytometers)

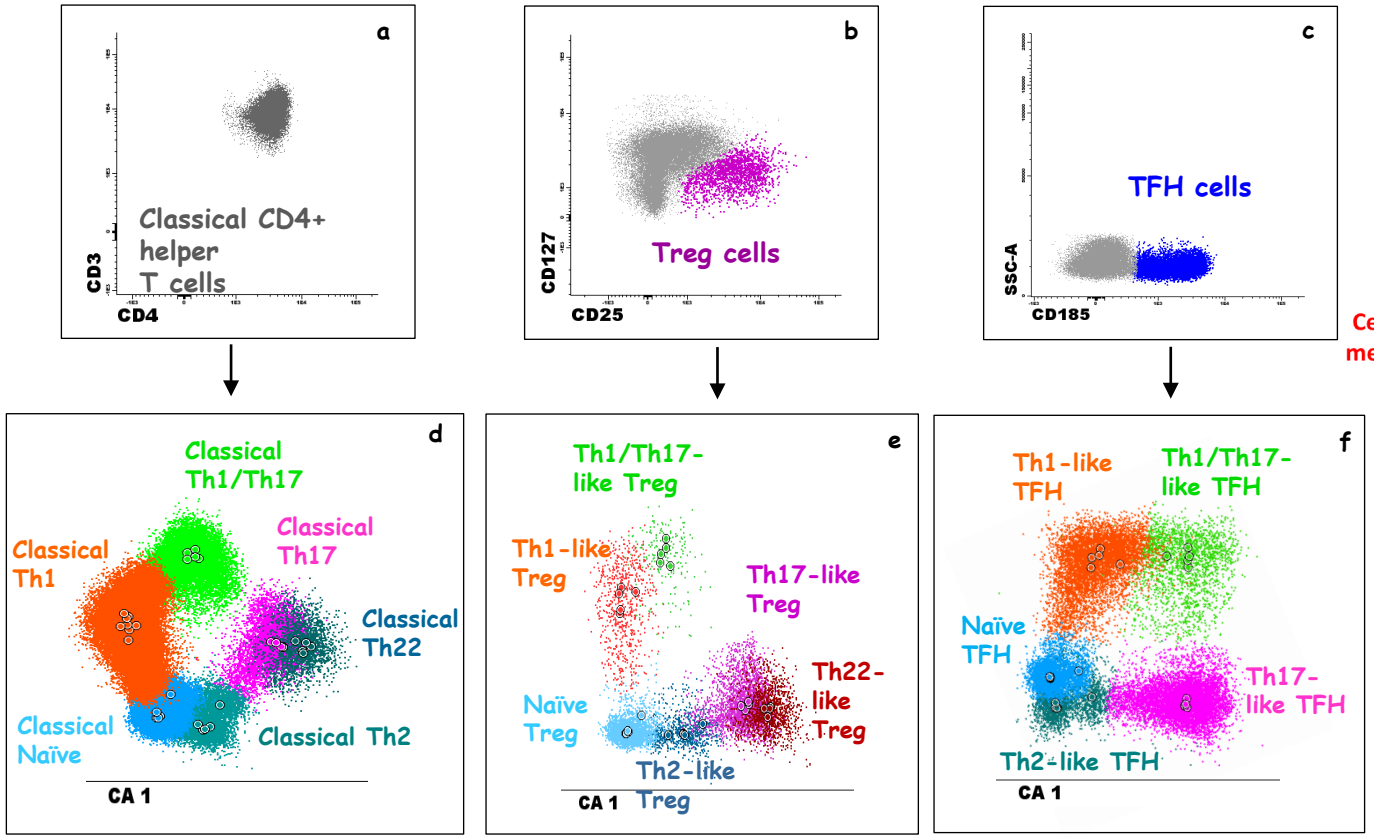


Damasceno et al J Allergy Clin Immunol 2019; van der Pan et al Front Immunol 2022

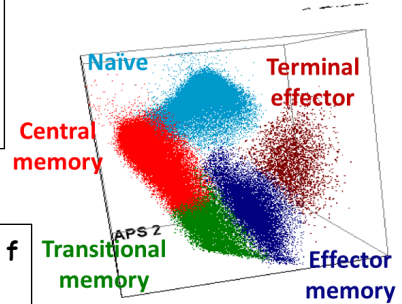
Dissection of CD4+ T-cell compartment

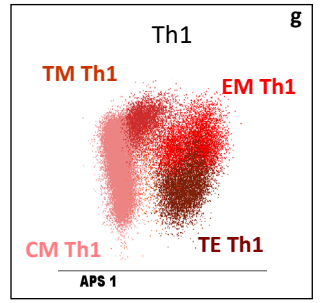
Total Th, Treg and TFH identification
(CD3, CD4, CD25, CD45, CD127, CD185)

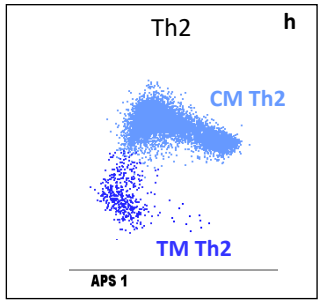
Total CD4+ T cells

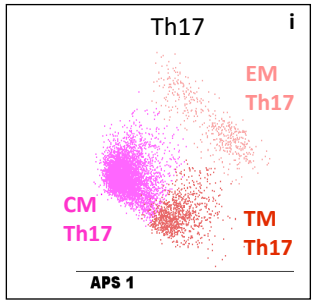


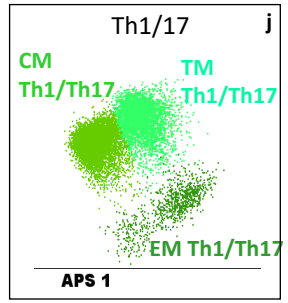
Th maturation stages
(CD27, CD45RA, CD62L)

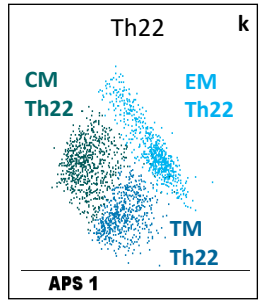












Naive vs fuctional Th Treg and TFH subsets

~89 to 190 CD4+ T-cell subsets in (200-1000 µL of) blood

Botafogo et al. Front Immunol, 2020

A total of ~85 different CD4+ T-cell subsets identified by the "EUROFLOW CD4+ T-cell tube"

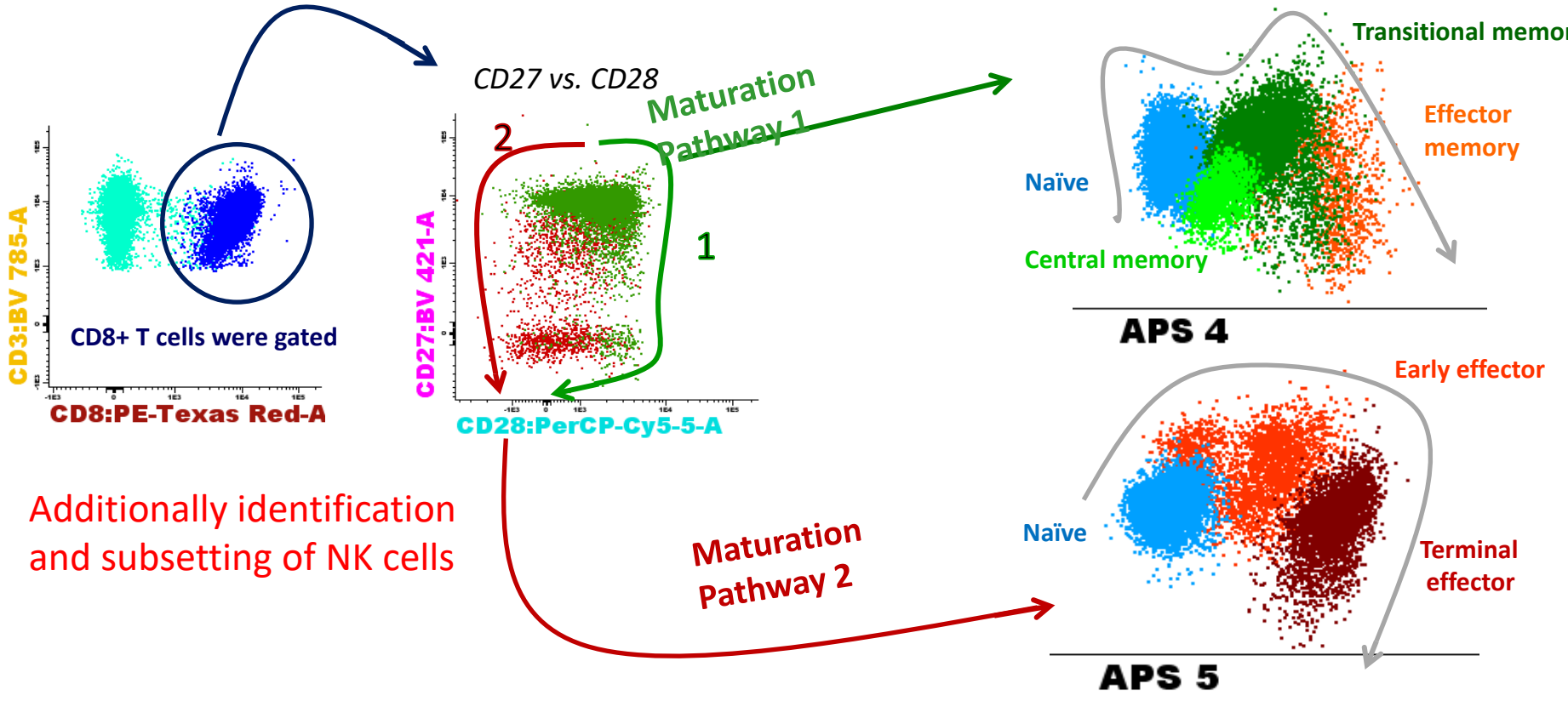
Major CD4 T-cell subsets	Maturation stage	Functional T-cell subsets
"Classical" CD4+ T cells (~ 40 cell subsets)	Naïve Central memory Transitional memory Effector memory Terminal effector	TH1 TH2 TH17 TH22 TH1/TH17 Other TH CD4+ T cell subsets*
Regulatory T cells (CD127lo/CD25hi) (~ 25 cell subsets)	Naïve Central memory Transitional memory Effector memory Terminal effector	Treg-TH1-like Treg-TH2-like Treg-TH17-like Treg-TH22-like Treg-TH1/TH17-like Other Treg-TH CD4+ T cell subsets*
Follicular helper T cells (CXCR5+) (~ 20 cell subsets)	Naïve Central memory Transitional memory Effector memory Terminal effector	T follicular regulatory cells (Tfr) TFH1 (or Tfh-Th1-like) TFH2 (or Tfh-Th2-like) TFH17 (or Tfh-Th17-like) Other TregH CD4+ T cell subsets*

Responsible scientists: Julia Almeida, Vitor Botafogo et al.

* Between 4 and 5 TH subsets other than the classical ones

Dissection of CD8+ T-cell compartment

Color codes: general identification of T and NK cells,, functional markers,, maturation markers, activation marker



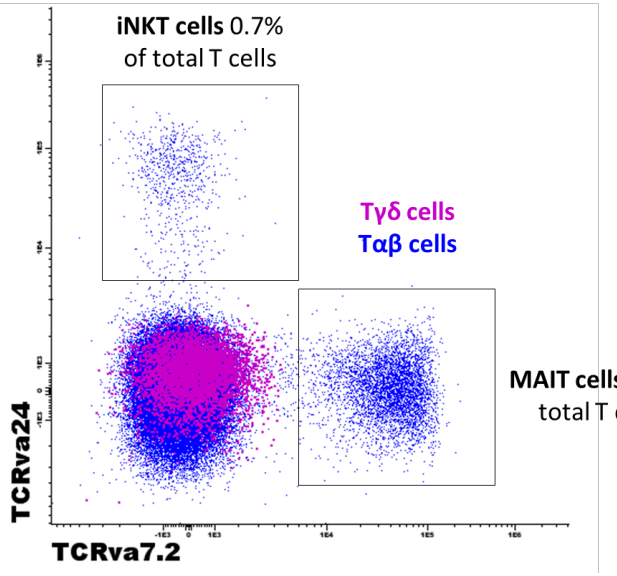
Additionally identification and subsetting of NK cells

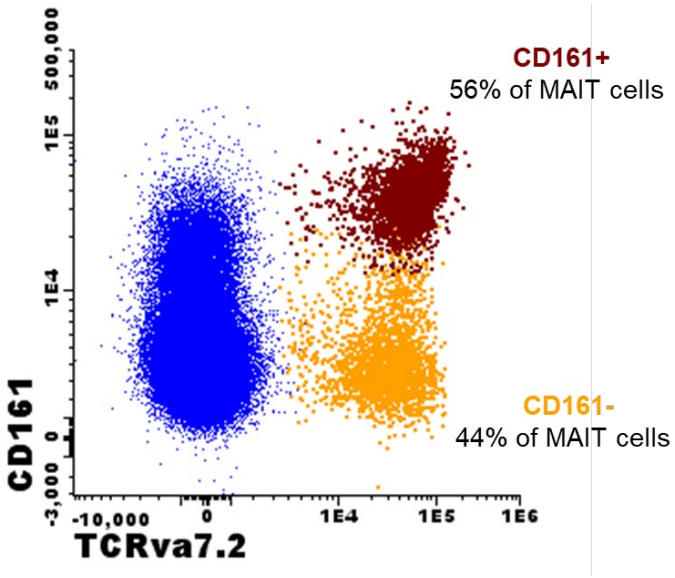
13-color combination: ~45 T CD8+ and NK cell subsets in blood

Responsible scientists: Julia Almeida, Vitor Botafogo et al.

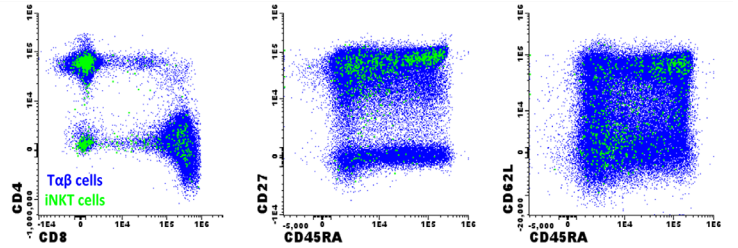
Innate T-cell subsets in normal adult blood

Innate NK/T cells and Mucosa-Associated Innate T cells

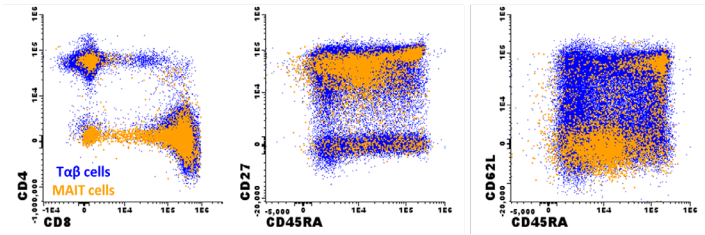




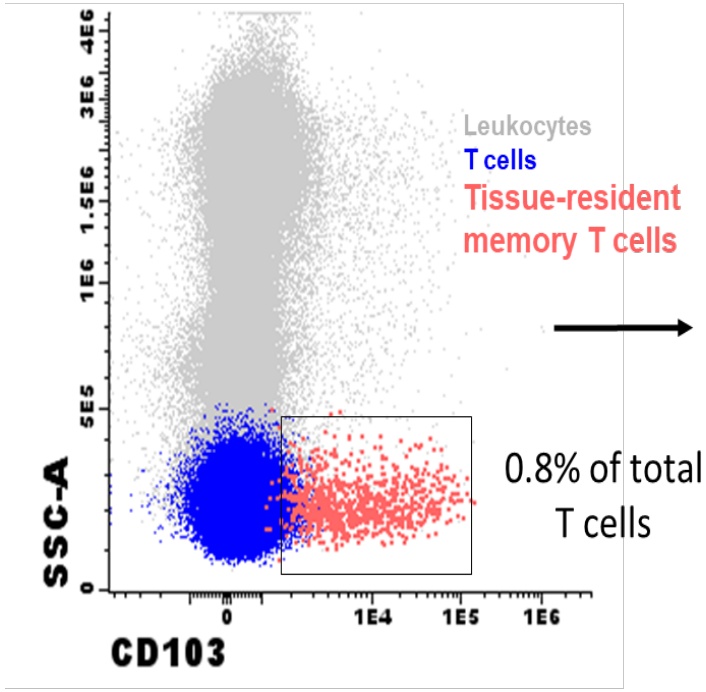
Most iNKT cells are CD4+ CD8- with a naïve or central/transitional memory phenotype

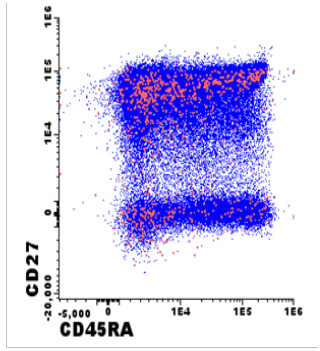


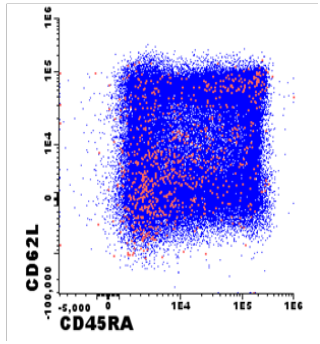
Most iNKT cells are CD8+ CD4- with a naïve or central/transitional memory phenotype



Circulating Tissue-Resident T cells



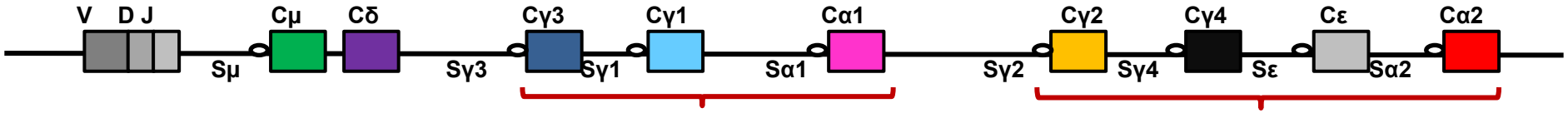




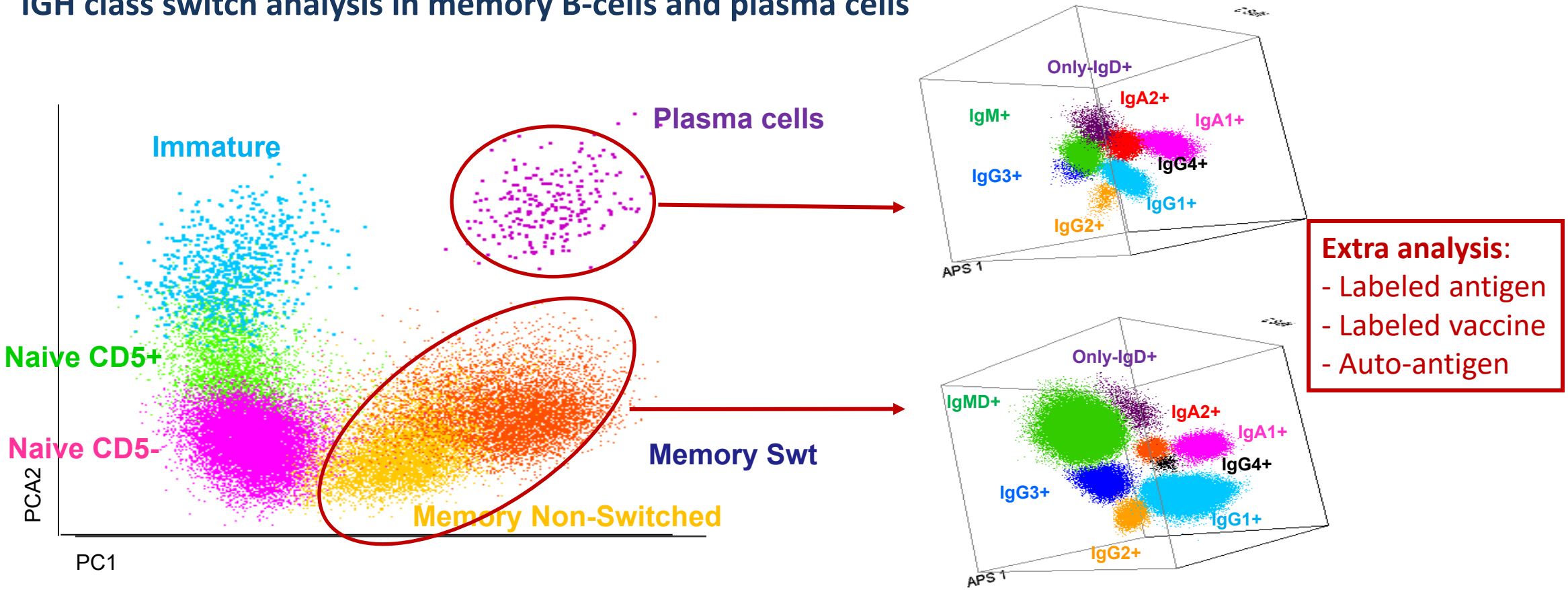
Slide prepared by Javier Morán-Plata and Julia Almeida

IDENTIFICATION OF PB SUBSETS OF NORMAL B-CELLS & PLASMA CELLS

IGH isotypes



IGH class switch analysis in memory B-cells and plasma cells



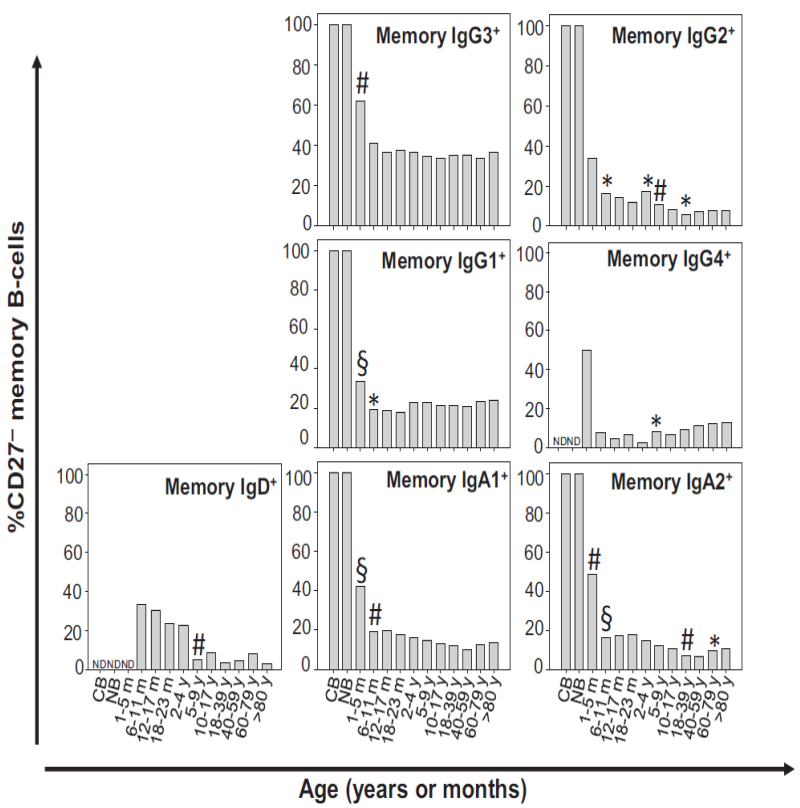
Extra analysis:
- Labeled antigen
- Labeled vaccine
- Auto-antigen

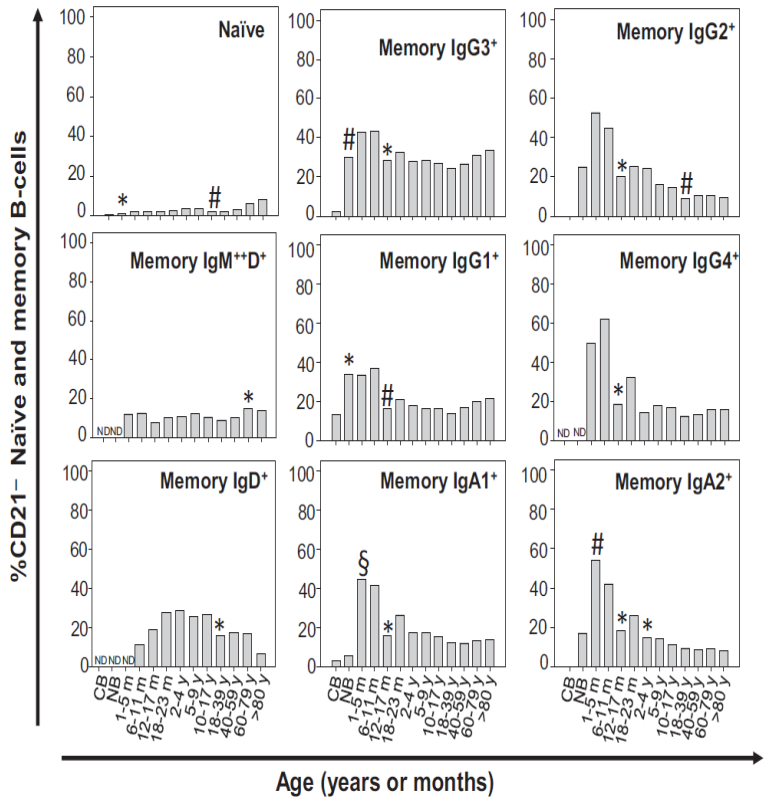
14 colour- combination → ~120 populations

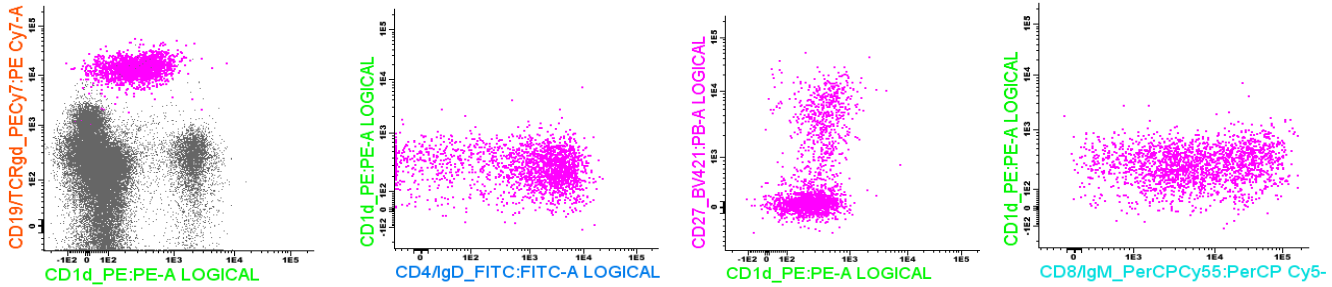
Responsible scientists: Alberto Orfao , Martin Perez, Elena Blanco

EuroFlow

Distribution of minor mature B-cell subsets in blood through life

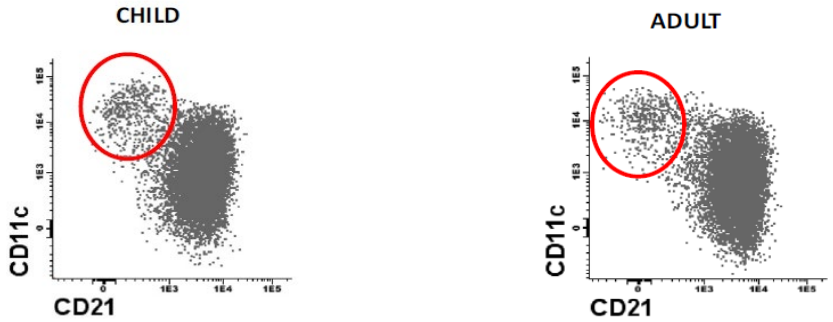






CD21-/CD11c+ MBC: FcRL4+FcRL5+Tbet^{hi}

Condition	Termed	Location	Phenotype in PB
Health			
Healthy individuals	Tissue-resident	Tonsil	CD27–IgD–CD38– CD11c+
Healthy individuals	Tissue restricted	PB BM Spleen	CD27+/–IgD– CD38lowCD11c+
CVID	CD21–/low	PB SLO BAL	CD27–IgD+ IgM+CD38lowCD11c+
SLE	CD11^{hi} Tbet^{hi}	PB Kidneys	CD27– CD38lowCD11c^{hi}
SLE	DN2	PB	CD27– IgD- CD11c+ CXCR5-
Established RA	CD21–/low	PB SF	CD27– IgD-
Malaria (*P. falciparum* and *P. vivax*)	Atypical	PB	CD27–CD11c+ CXCR5–
HIV	Exhausted, tissue-like	PB	CD27– CD11c+
Primary Sjögren syndrome		PB	CD27– CD38lowCD11c+
Systemic sclerosis		PB	CD38lowCD11c+
Crohn's disease		PB Gut	Tbet+
Multiple sclerosis	CD21low	PB Cerebrospinal fluid	CD11c+
HBV	Atypical	PB Liver	CD27– CD11c+
HCV	Tissue-like	PB	CD27–CD11c+
COVID-19	Atypical/DN2	PB	CD27–CD11c+



Blanco et al, J Allergy Clin Immunol 2018;
Gjertsson et al, Clin Exp Immunol, 2022

Condensed IMM tubes into a single >40-markers tube (38-color 56 marker tube)

EuroFlow

T_H-Cell subsets

TCRαβCD4$^+$CD8$^-$
Treg (Regulatory T-Cell)
TFH (Follicular T-Cells)

CCR/CXCR expression:

CD183	CD194	CD196	CCR10

Cytotoxic T-cell subsets

TCRαβCD8$^+$CD4$^-$
TCRγδ (CD3^{hi} and CD3^{lo})
TCRαβCD8$^+$CD4^{lo}

Others T-cell sub

TCRαβ TRCγδ$^-$CD8$^{-/lo}$
NKT (and iNKT) ce
MAIT cells
TRM-Like (CD103$^+$)

At different maturation stages:

Naïve	CM	TM	EM	TE

βC1 expression

NK-cell subsets

CD16$^{-/lo}$CD56^{bright},
CD16$^+$CD56^{dim} NK cells
CD16$^{+/++}$CD56$^-$ NK cells

Innate Lymphoid Cells (ILC)

CD45^{++}/NKp80$^-$/ CD3$^-$/CD19$^-$
(CD127$^{+/lo}$

B-cell subsets

Transitional B Cells
Naive B-Cells
Memory B-Cells
Plasma Cells (PC)

IgH Subclasess expression

IgG1	IgG2	IgG3	IgG4

IgA1	IgA2

Kappa/ Lambda expression

Monocytes (Mo)

cMo (Classical Monocytes)
iMo (Intermediate Monocytes)
ncMo (Non-Classical Monocytes)

Funtional Stages:

	FcERI	Slan

cells (Dc)

Myeloid Dendritic Cells (MyDc)
Plasmocytoid Dendritic Cells (pDc)
Axl Dendritic Cells

Others IMC

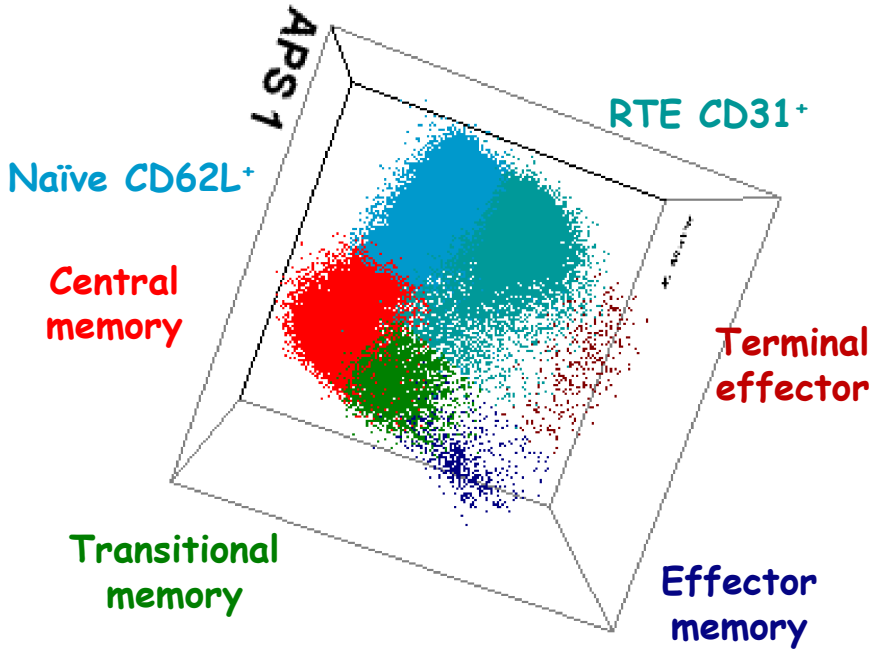
Eosinophils
Neutrophils
Basophils
HPC
M-MDSC

≈1,000 cell populations

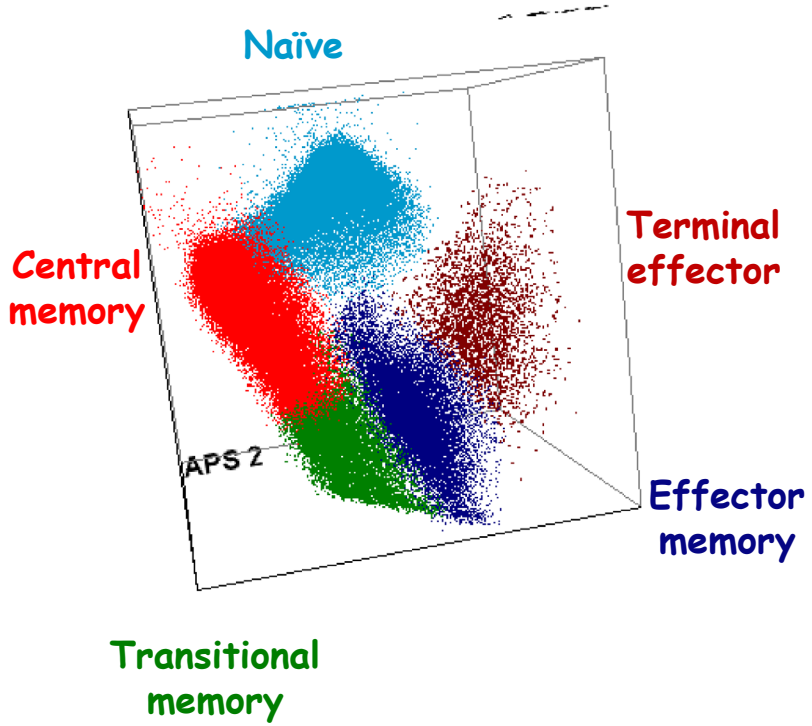
AGE-REFERENCE RANGES OF IMMUNE CELLS IN HUMAN BLOOD

CD4+ T-cell maturation pathway in healthy controls

Children (n=3); age 1-6 years



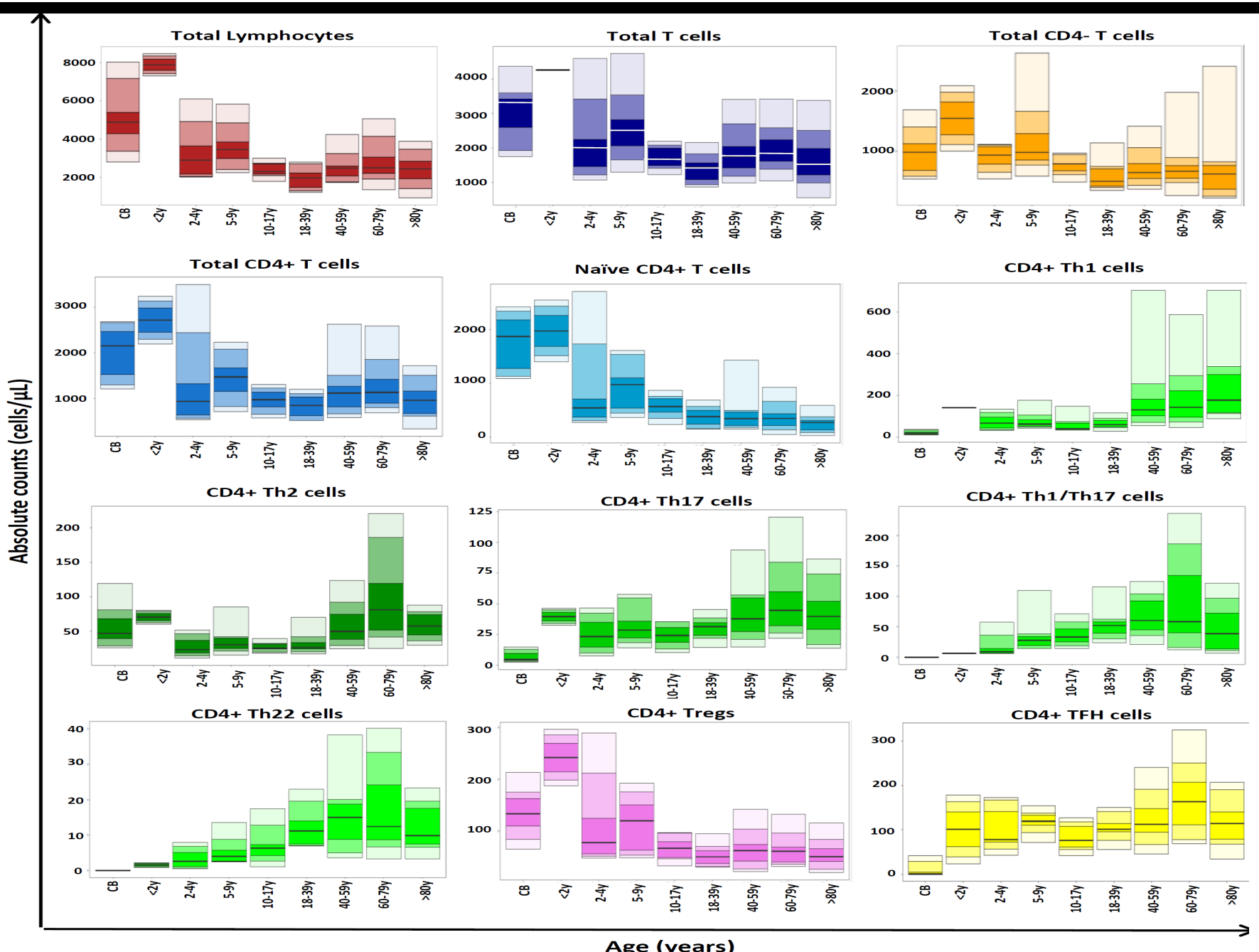
Adults (n=3); age 39-68 years



EuroFlow

Botafogo et al., Front Immunol, 2020

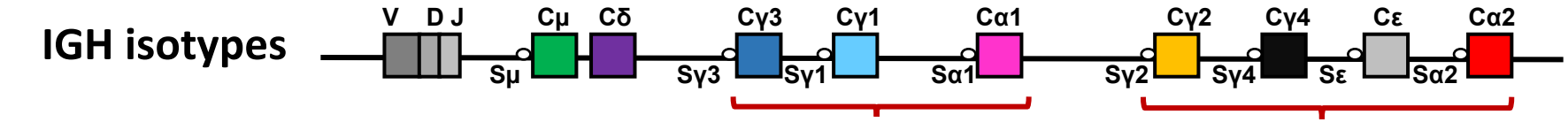
Normal distribution of CD4+ T-cell functional subsets through life

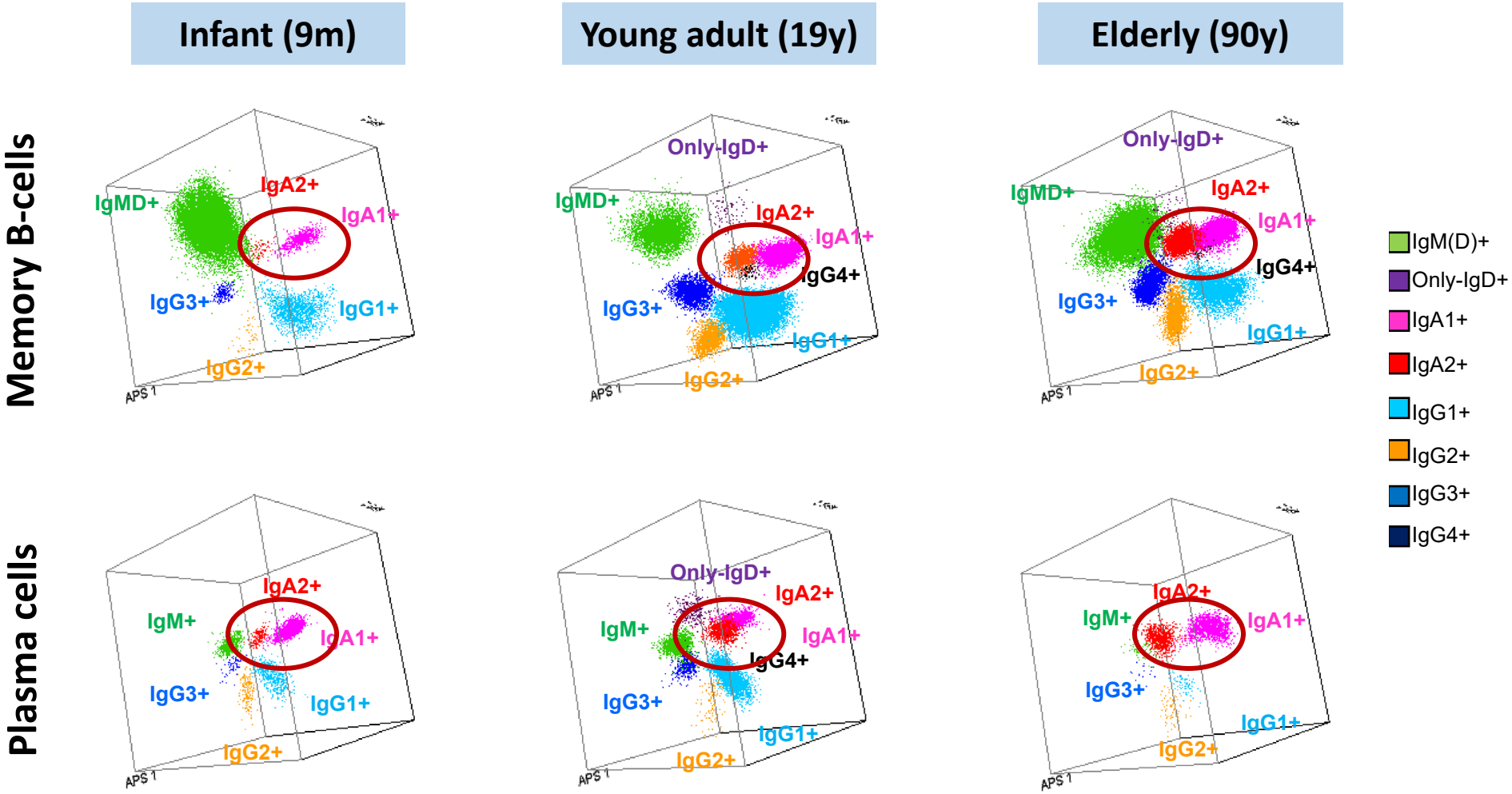


EuroFlow

Botafogo et al. Front Immunol, 2020

Distribution of normal PB B-cell and plasma cell subsets through life

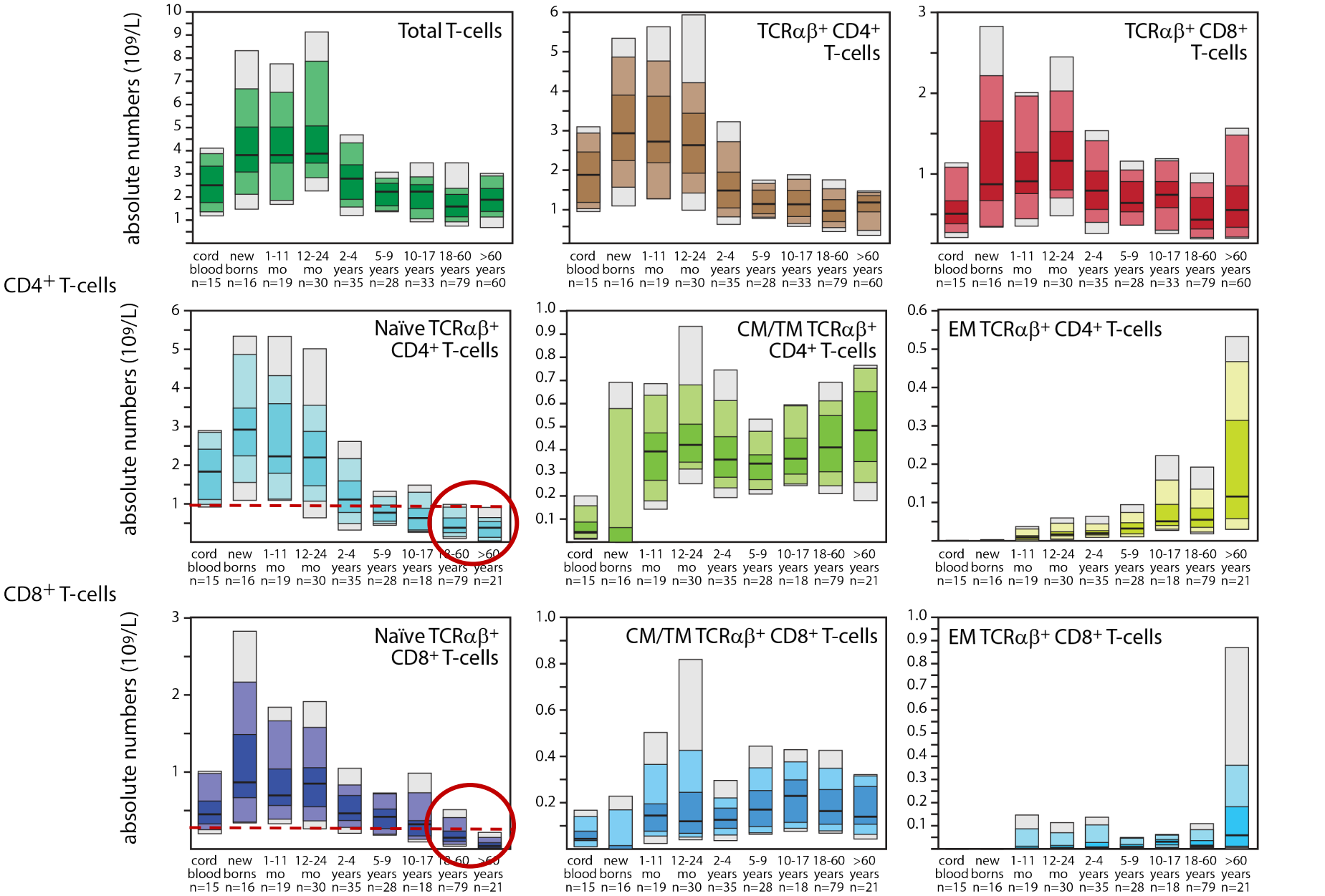




EuroFlow

Blanco et al., JACI 2018; 141: 2208-2219

Age-related reference values of blood T-cell subsets



EuroFlow PID report, J.J.M. van Dongen et al. Frontiers Immunol 2019;10, article 1271

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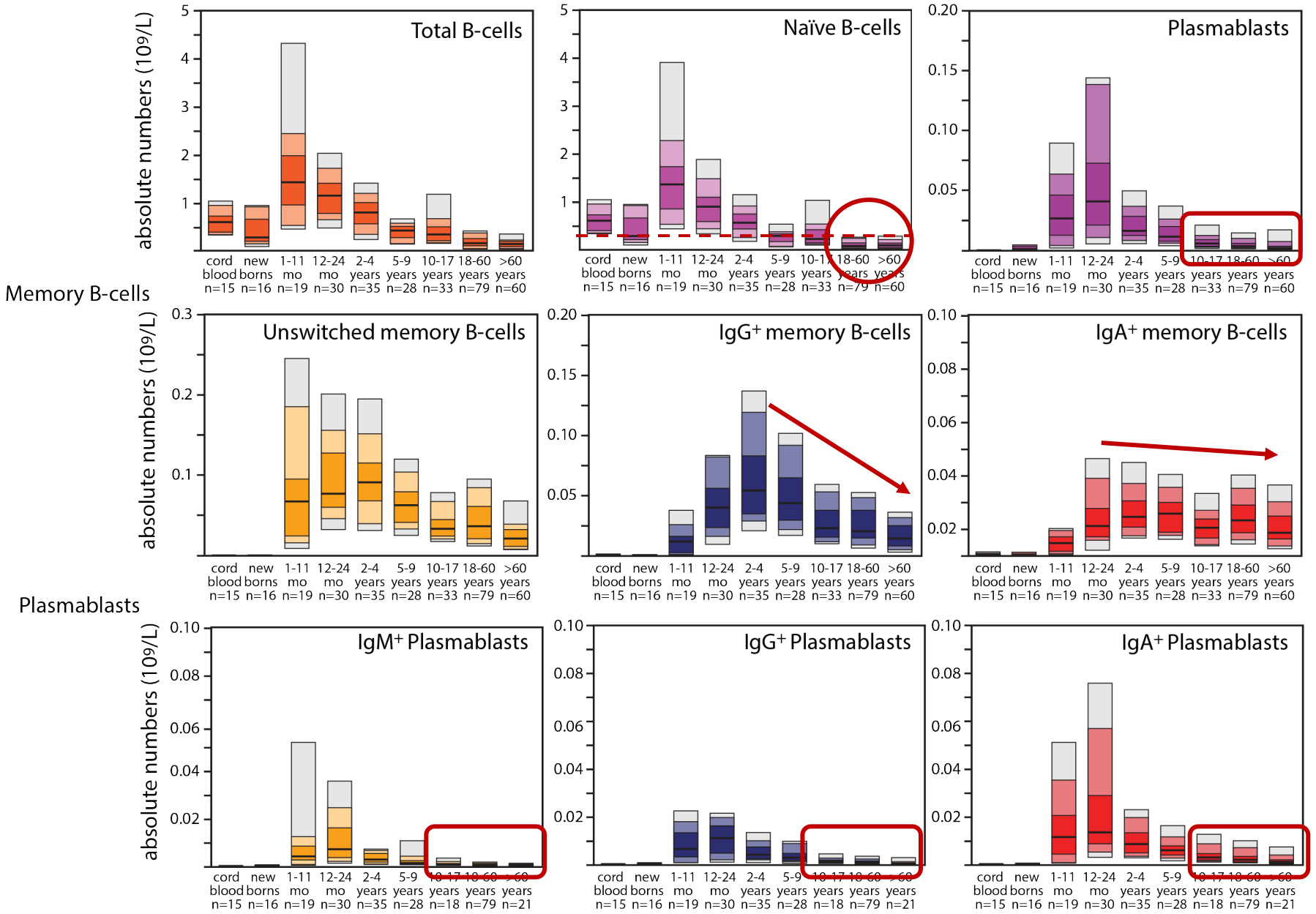
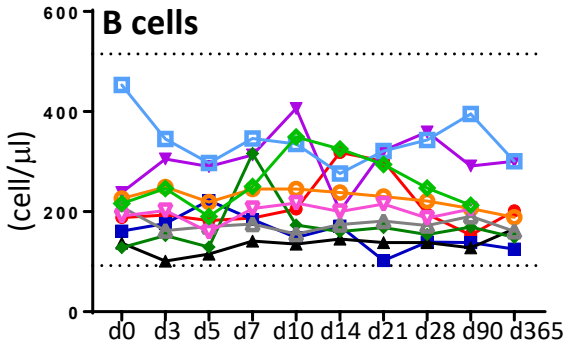
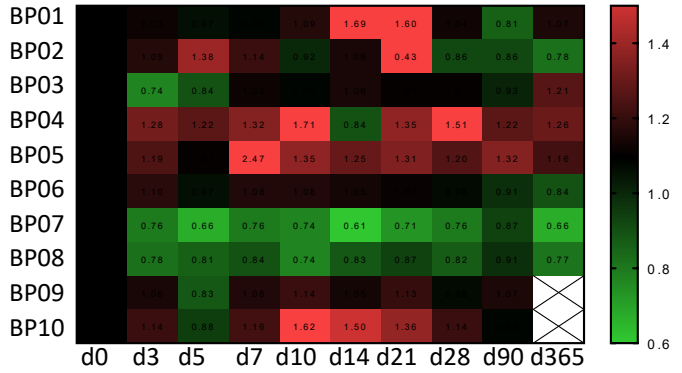


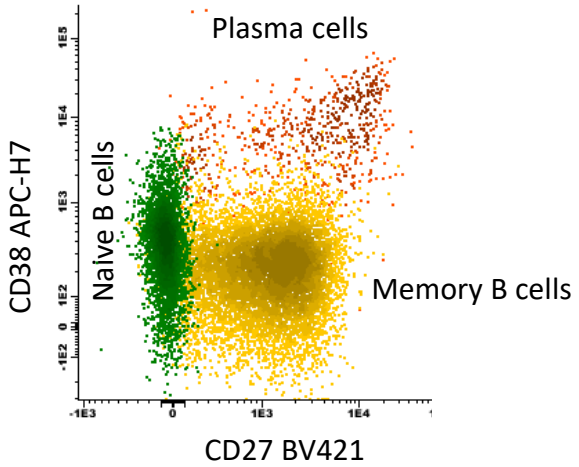
EuroFlow PID report, J.J.M. van Dongen et al. Frontiers Immunol 2019;10, article 1271

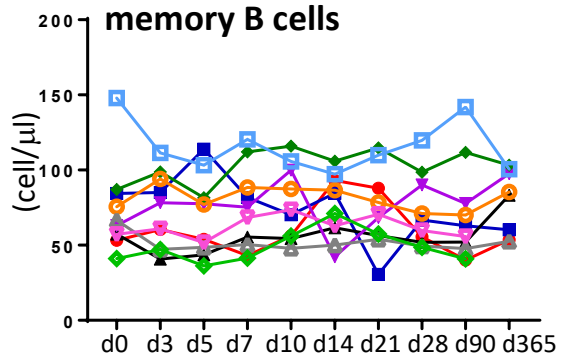
IMMUNE CELL KINETICS IN BLOOD DURING IMMUNE RESPONSES

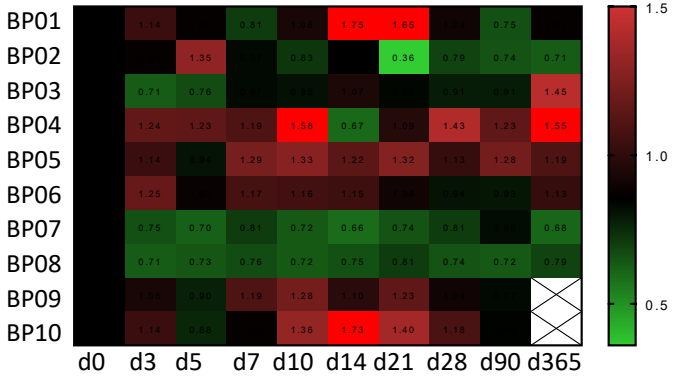
KINETICS OF MAJOR B-CELL SUBSETS AFTER BOOSTER VACCINATION

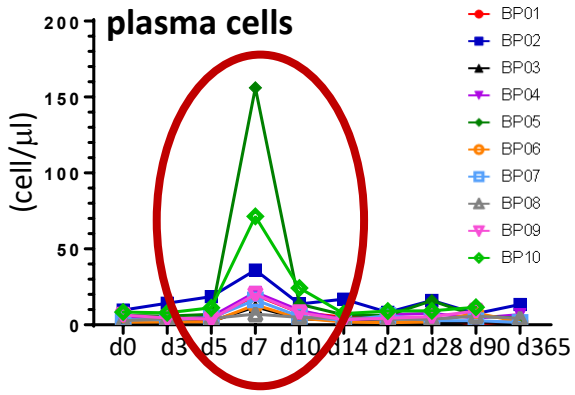


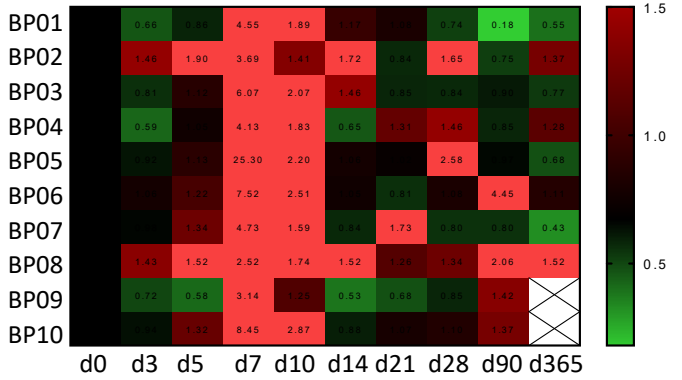








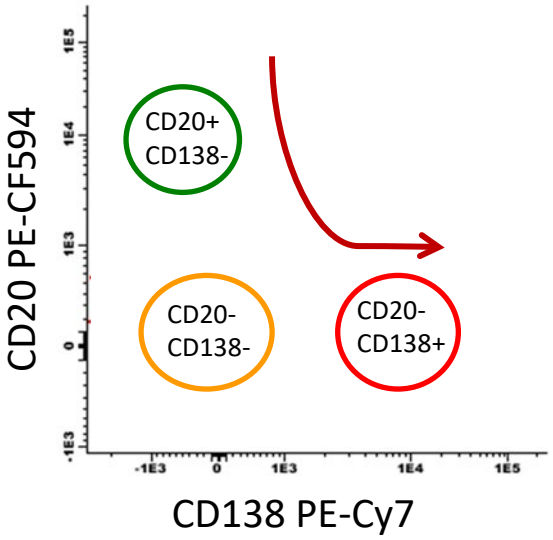


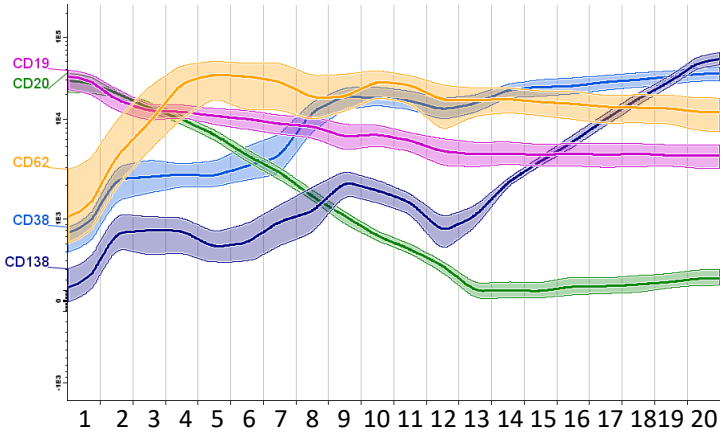


Increase in PC numbers at d7 (d5-d10; consistent in all donors; up to **25x**)

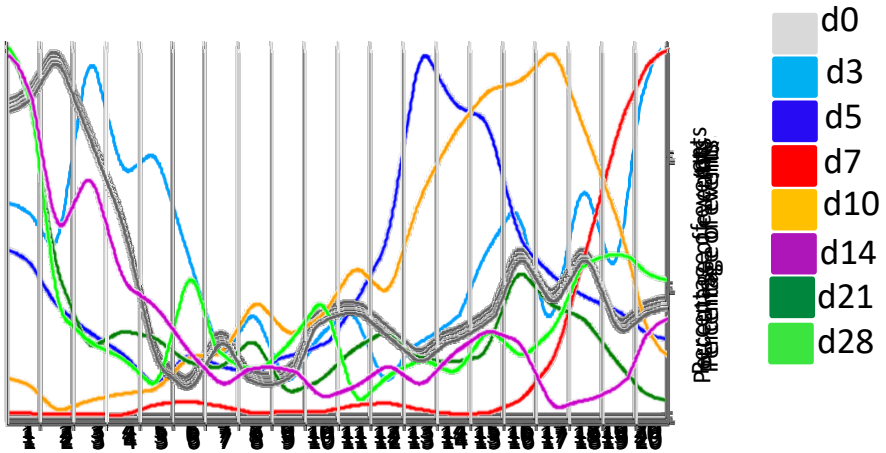
A. Diks, M. Berkowska, J.J.M.van Dongen et al. Frontiers Immunol 2021; 12, Art. 666953

Plasma cell maturation after booster





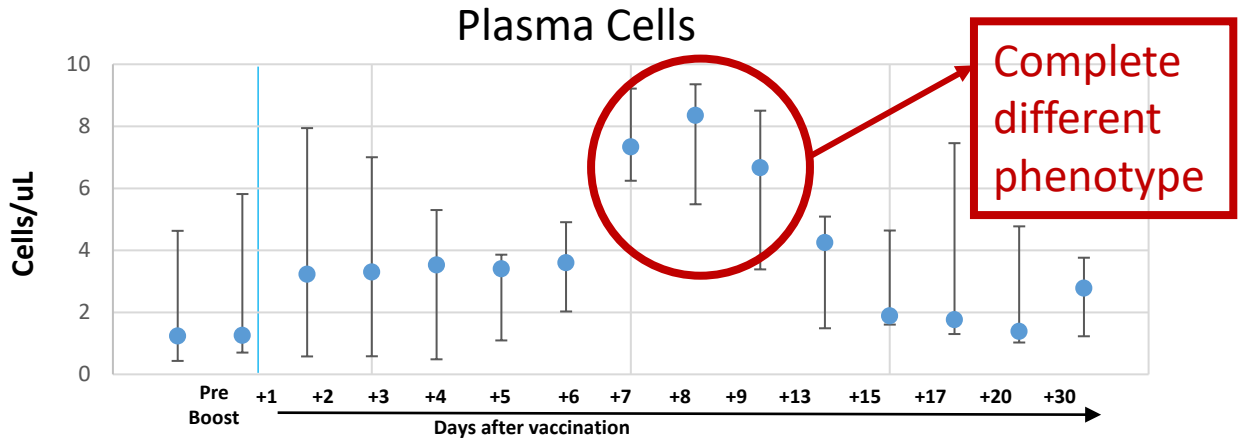
Expression of CD38/ CD138/ CD62L increases during PC maturation
Expression of CD19/CD20 decreases during PC maturation

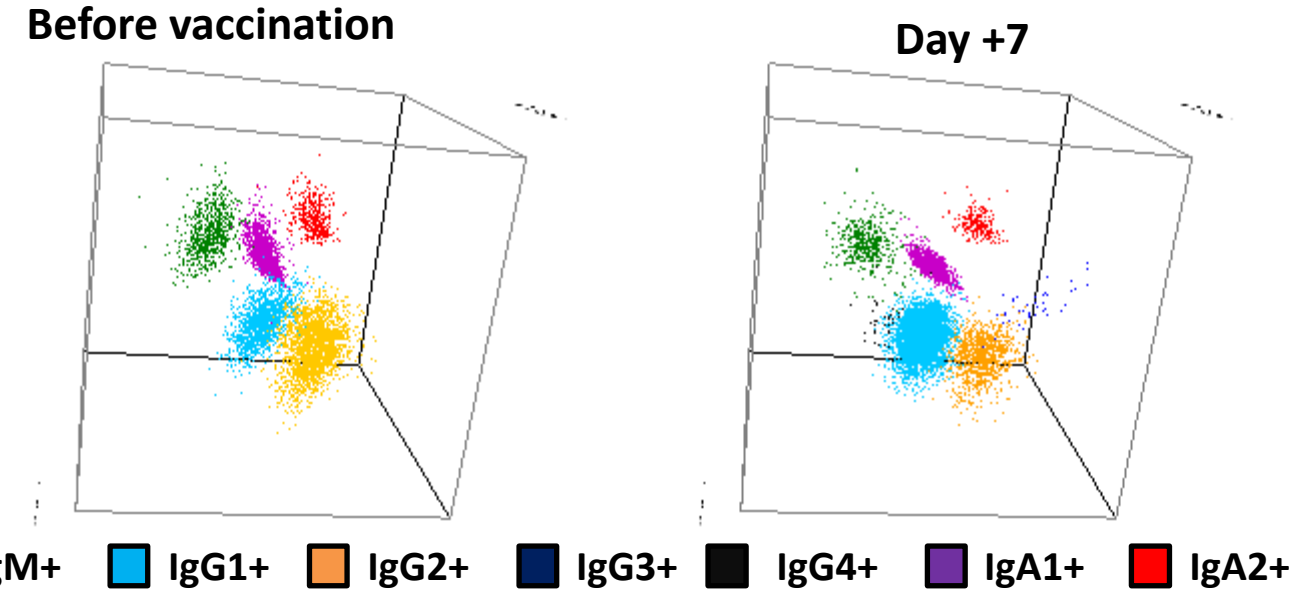


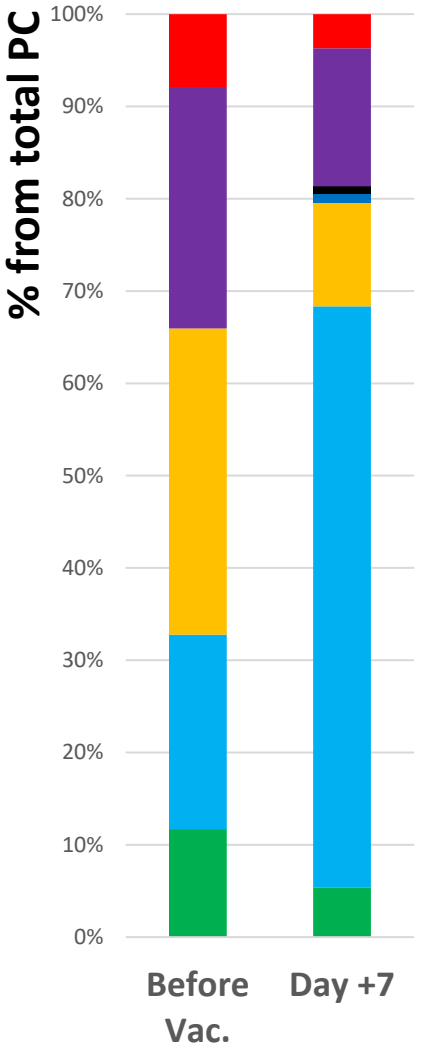
Plasma cells mature in time after booster
Most mature plasma cells are found at day 7

Responsible scientists: Magda Berkowska, Annieck Diks, Jacques J.M. van Dongen

B-cells in context of vaccination

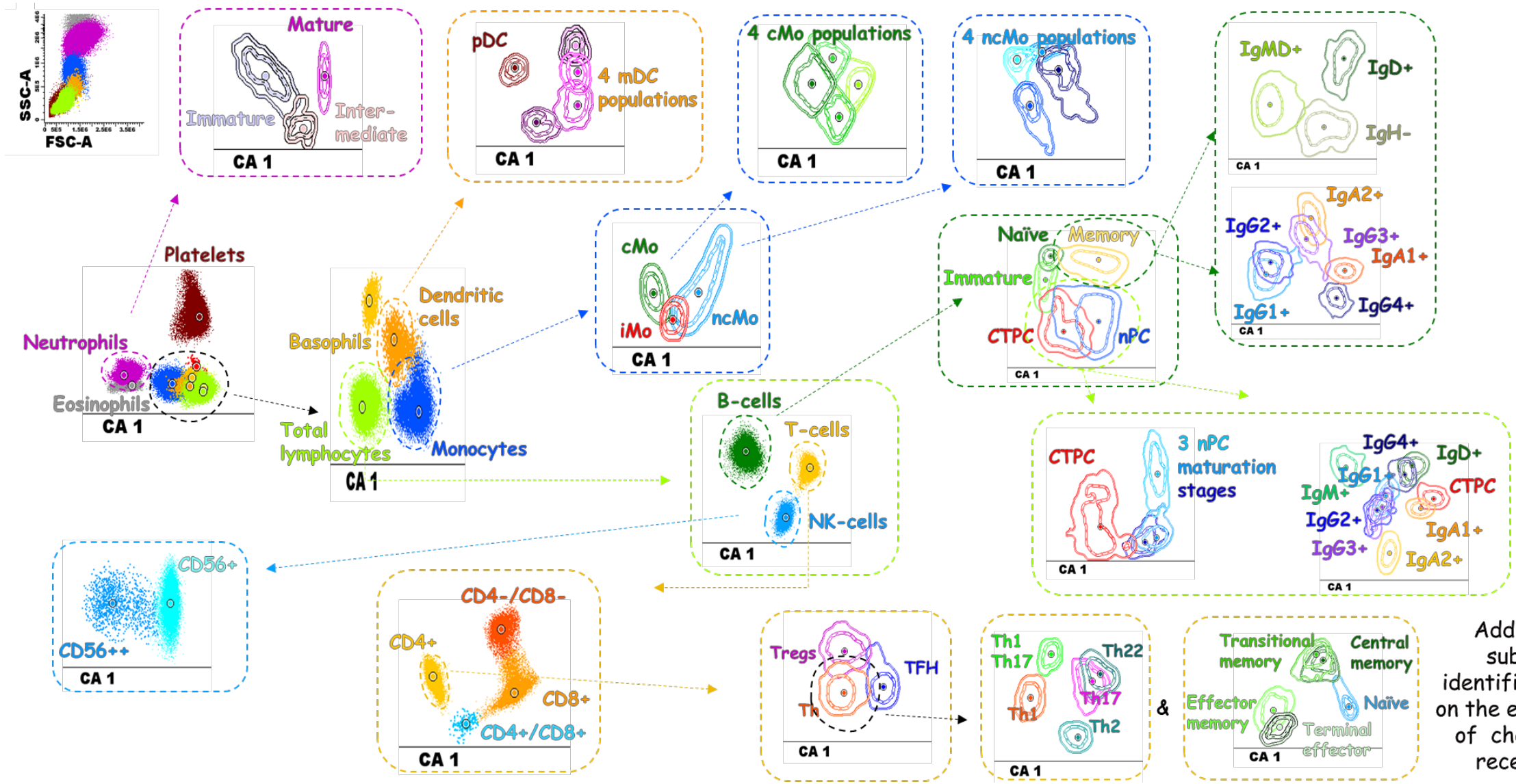






Responsible scientists: Alberto Orfao , Martin Perez, Elena Blanco

EuroFlow Immune monitoring of blood leukocyte subsets



DC, dendritic cells; pDC, plasmacytoid DC; mDC, myeloid DC; Mo, monocytes; cMO, classic Mo, iMo, intermediate Mo; ncMo, non classic Mo; PC, plasma cells; CTPC, circulating tumor PC; nPC, normal PC; Th, helper T-cells; Tregs, regulatory T-cells; TFH, T follicular helper cells

Condensed IMM tubes into a single >40-markers tube (38-color 53 marker tube)

EuroFlow

T_H-Cell subsets

$TCR\alpha\beta CD4^+CD8^-$
Treg (Regulatory T-Cell)
TFH (Follicular T-Cells)

CCR/CXCR expression:

CD183	CD194	CD196	CCR10

Cytotoxic T-cell subsets

$TCR\alpha\beta CD8^+CD4^-$
$TCR\gamma\delta$ ($CD3^{hi}$ and $CD3^{lo}$)
$TCR\alpha\beta CD8^+CD4^{lo}$

Other T-cell subsets

$TCR\alpha\beta$ $TRC\gamma\delta^-CD8^{-/low}CD4^-$
NKT (and iNKT) cells
MAIT cells
TRM-Like ($CD103^+$)

At different maturation stages:

Naïve	CM	TM	EM	TE

βC1 expression

NK-cell subsets

$CD16^{-/lo}CD56^{bright}$,
$CD16^+CD56^{dim}$ NK cells
$CD16^{+/++}CD56^-$ NK cells

Innate Lymphoid Cells (ILC)

$CD45^{++}$/$NKp80^-$/ $CD3^-$/$CD19^-$
($CD127^{+/low}$)

B-Cells

Inmature/ Transitional B Cells
Naive B-Cells
Memory B-Cells
Plasma Cells (PC)

IgH Subclasess expression

IgG1	IgG2	IgG3	IgG4
IgA1		IgA2	

Kappa/ Lambda expression

Monocytes (Mo)

cMo (Classical Monocytes)
iMo (Intermediate Monocytes)
ncMo (Non-Classical Monocytes)

Funtional Stages:

CD36	CD62L	FcERI	Slan

Dendritic cells (Dc)

Myeloid Dendritic Cells (MyDc)
Plasmocytoid Dendritic Cells (pDc)
Axl Dendritic Cells

Others IMC

Eosinophils
Neutrophils
Basophils
HPC
M-MDSC

≈1,000 populations

Botafogo et al Front Immunol 2020
Van der Pan et al Front Immunol 2023

How can flow cytometric immune monitoring be performed: easy, fast, and reproducible

EuroFlow

1. **Speed** of cellular analyses:
   - At least ≥ 30,000 cells per second with limited abort rates.
   - At least 5 to > 10 million cells (in < 0.2 ml) acquired in approximately 5 minutes.
2. Number of fluorochromes: 20 to 25 colors, preferably ≥ 25 colors
3. No complex requirements for compensation matrices
4. Stability over time within and between instruments.
5. Comparability between instruments at different sites
6. Automation: autoloader for tubes and/or for plates.

CONCLUDING REMARKS:

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

- Important advances have been made in the identification and understanding of the normal innate, B and T cell maturation pathways in different tissue compartments.

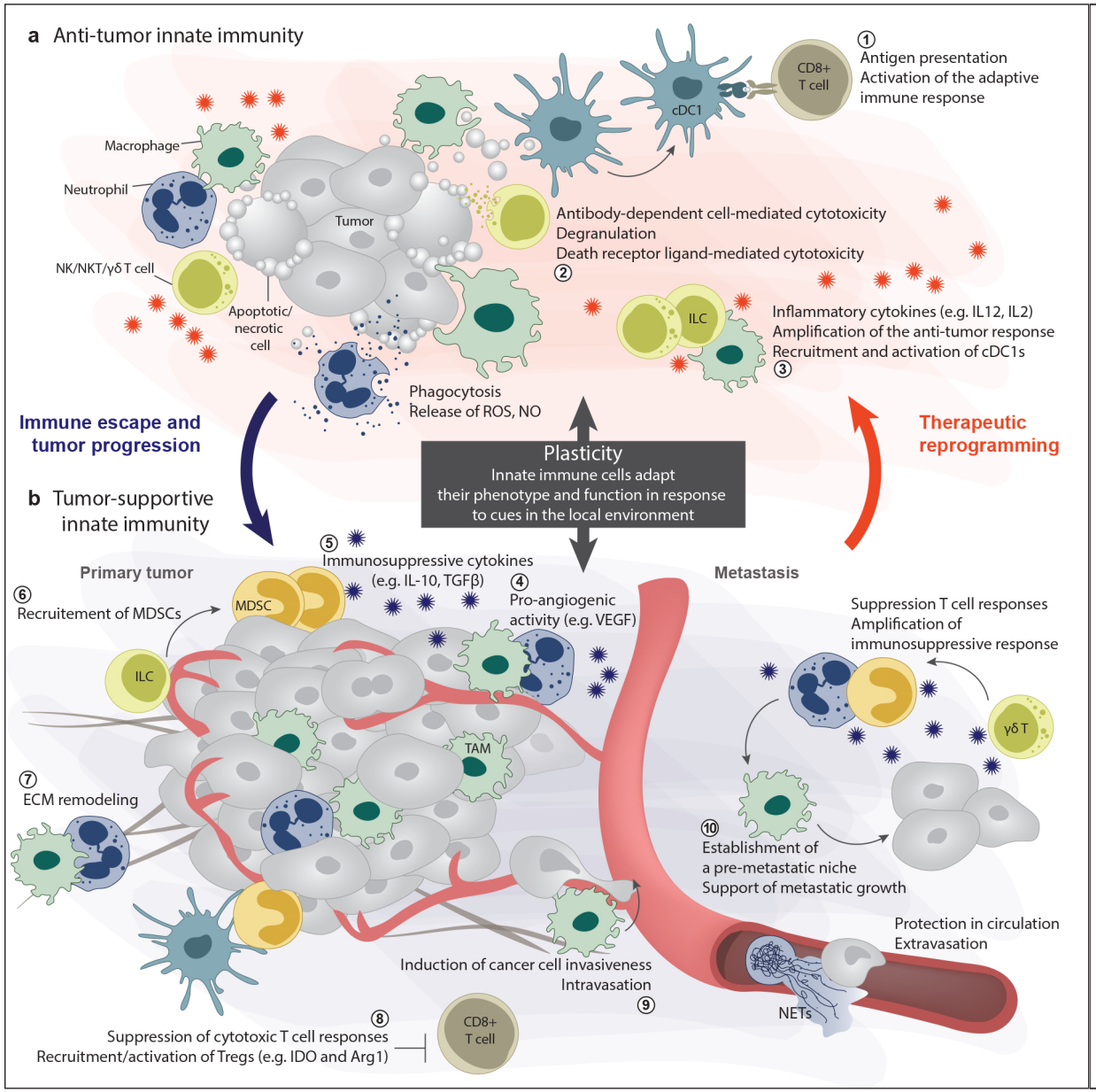
- All the above has highlighted the existence of hundred of distinct innate myeloid cell, T-cell and B-cell populations, in human blood which can be simultaneously assessed.

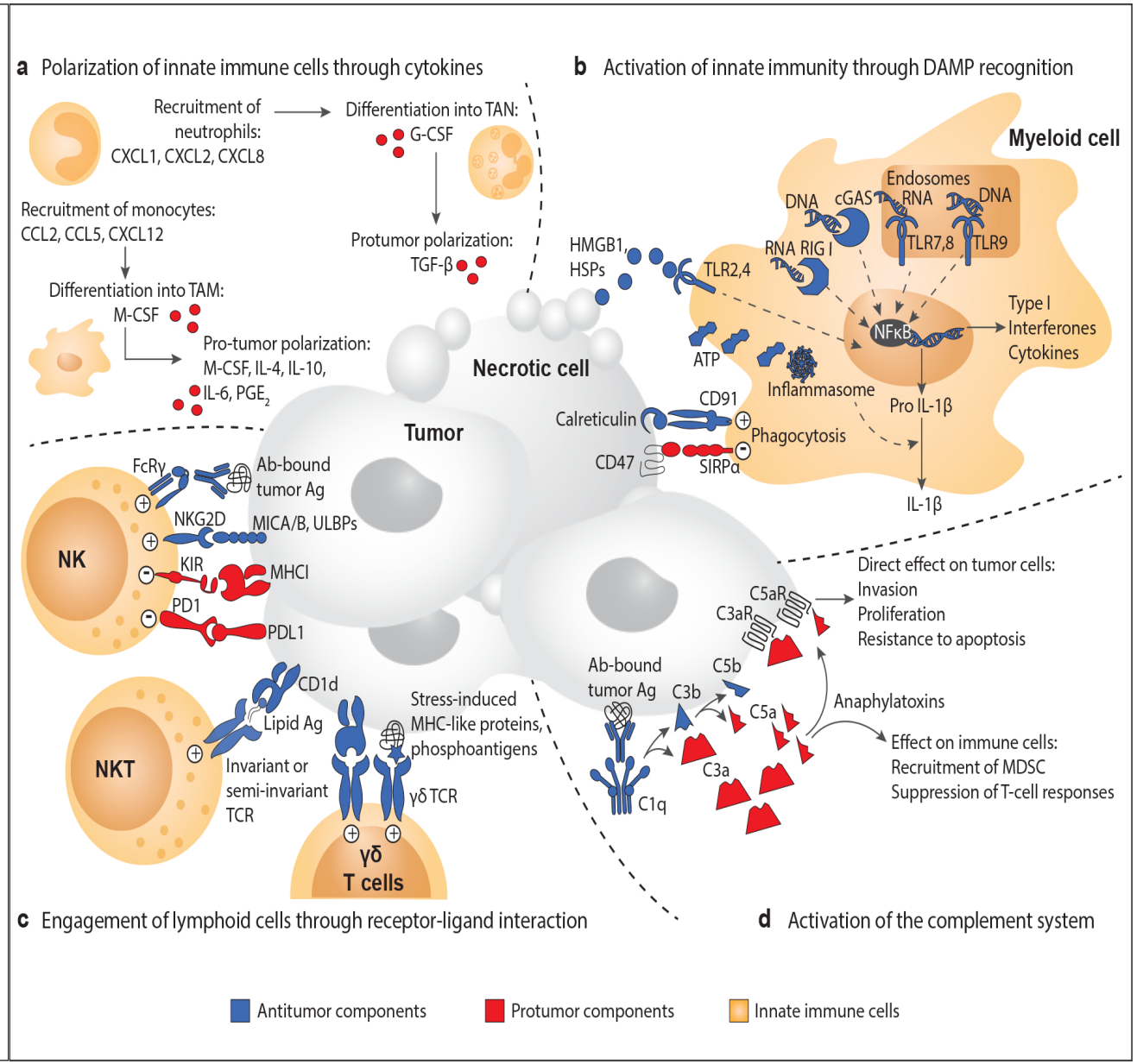
- Important age-related differences are confirmed, which point out the dramatic changes that occur in PB in the first months of life, also contributing to a better understanding of the innate, B-cell and T-cell homeostasis.

- Such increased knowledge about the normal B-cell and T-cell maturation pathways provides the basis for a comprehensive identification and classification of PID and for immune monitoring in cancer patients.

- Recent technological developments allow fast and automated all-in-one monitoring of immunotherapy in cancer patients.

IMMUNE RESPONSE IN CANCER PATIENTS



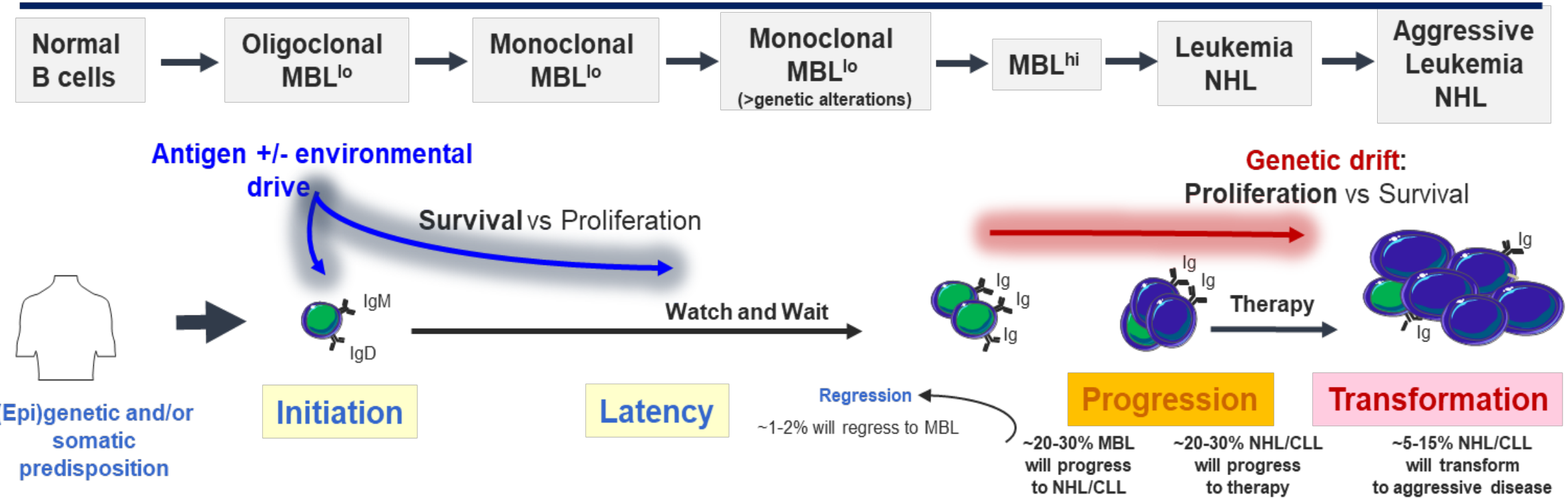


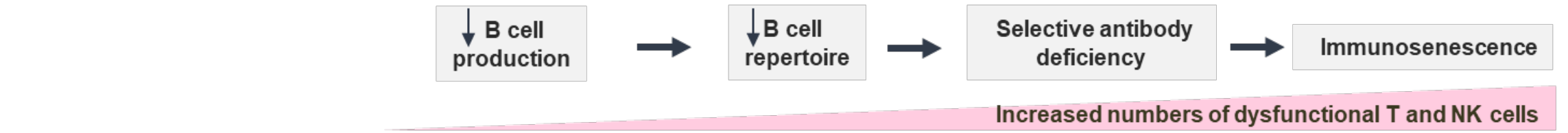
Maiorino et al, Ann Rev Pathol, 2022

ALTERED BLOOD IMMUNE CELL PROFILES IN HM:

The MBL and SM models

Natural history of MBL is affected by environmental (antigen) and intrinsic (immune) factors





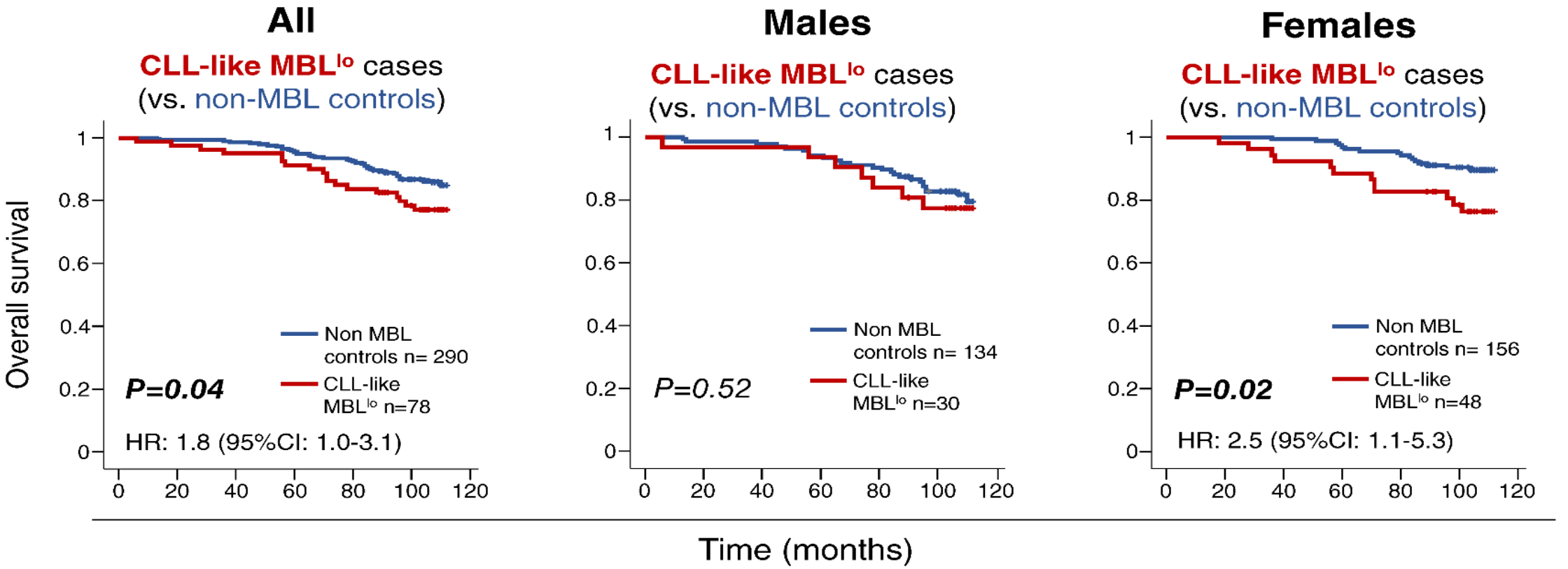
Severe infections + second neoplasias / premature deaths (2/1000 per year)

MBL ~3-14% of adults (>40y) ⟶ 509.590 NHL/CLL in 2018

ECRIN M3
Early Cancer Research Initiative Network

Slide prepared by Francesco Forconi

MBL^{lo} in healthy subjects is associated with shorter survival



No significant differences in OS at 3y
Lamb et al, BMJ Open, 2021; 11: e041296

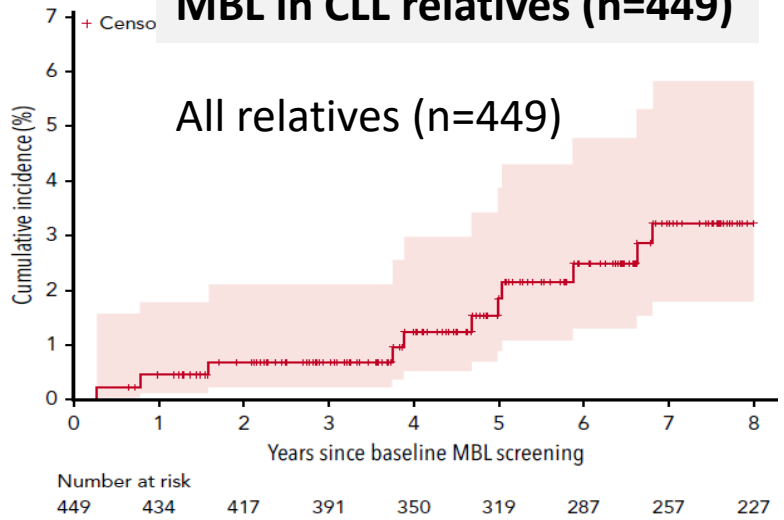
	Cardiovascular disease	Cancer#	Infection	Other#
CLL-like MBL^{lo}	29%	36%	21%	14%
General population*	33%	26%	1.4%	39.6%

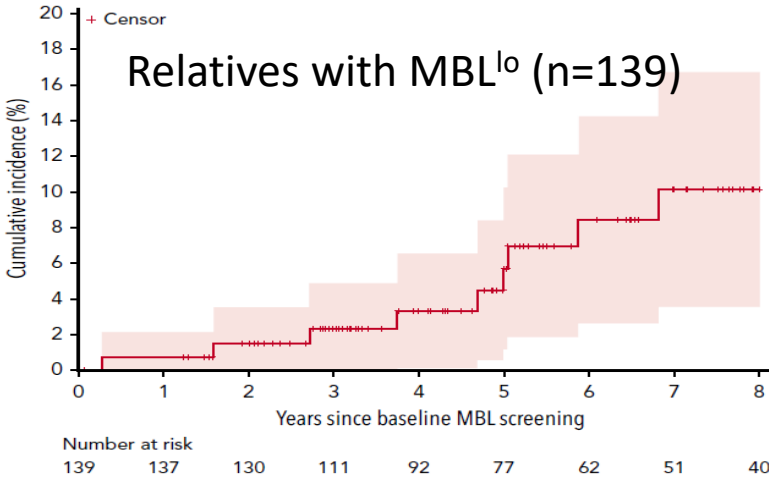
*Data obtained from INE databases.
#Infection was the direct cause of death in one individual in these groups.

Criado et al, Haematologica, 2018; 103: 1198-208

MBL^{lo} in a screening population and CLL relatives: progression to CLL

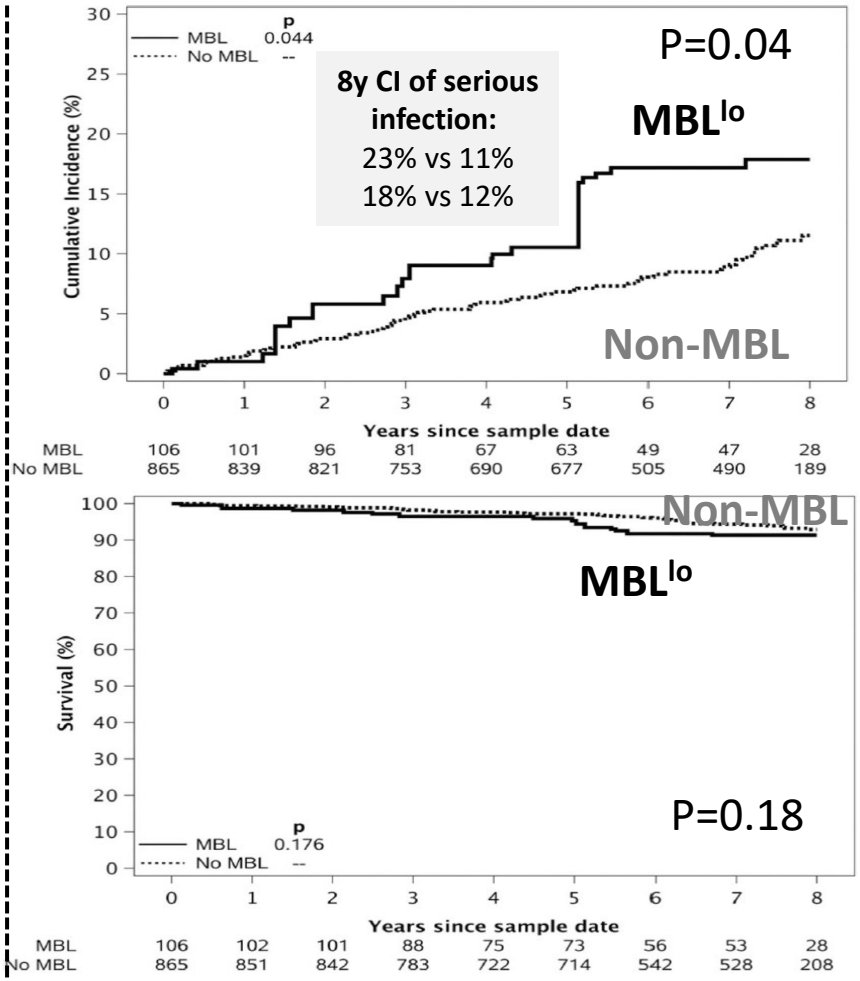
MBL in CLL relatives (n=449)



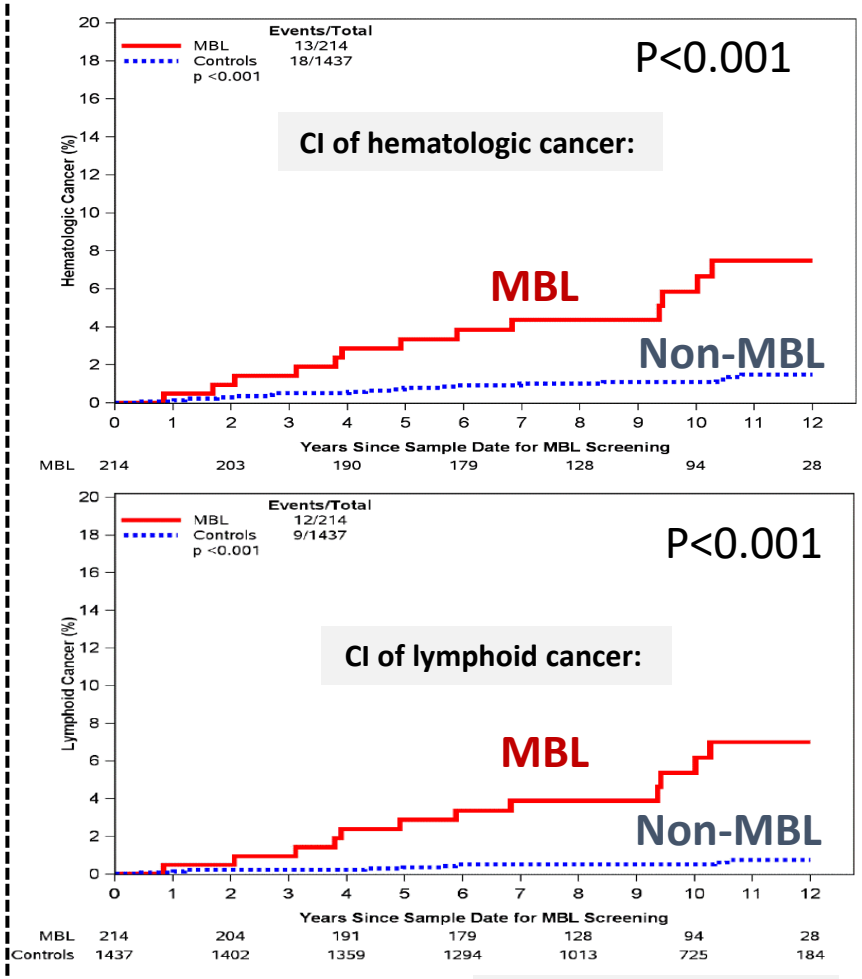


Slager et al, Blood, 2021; 137: 2046-56

MBL in the general population (n=449)



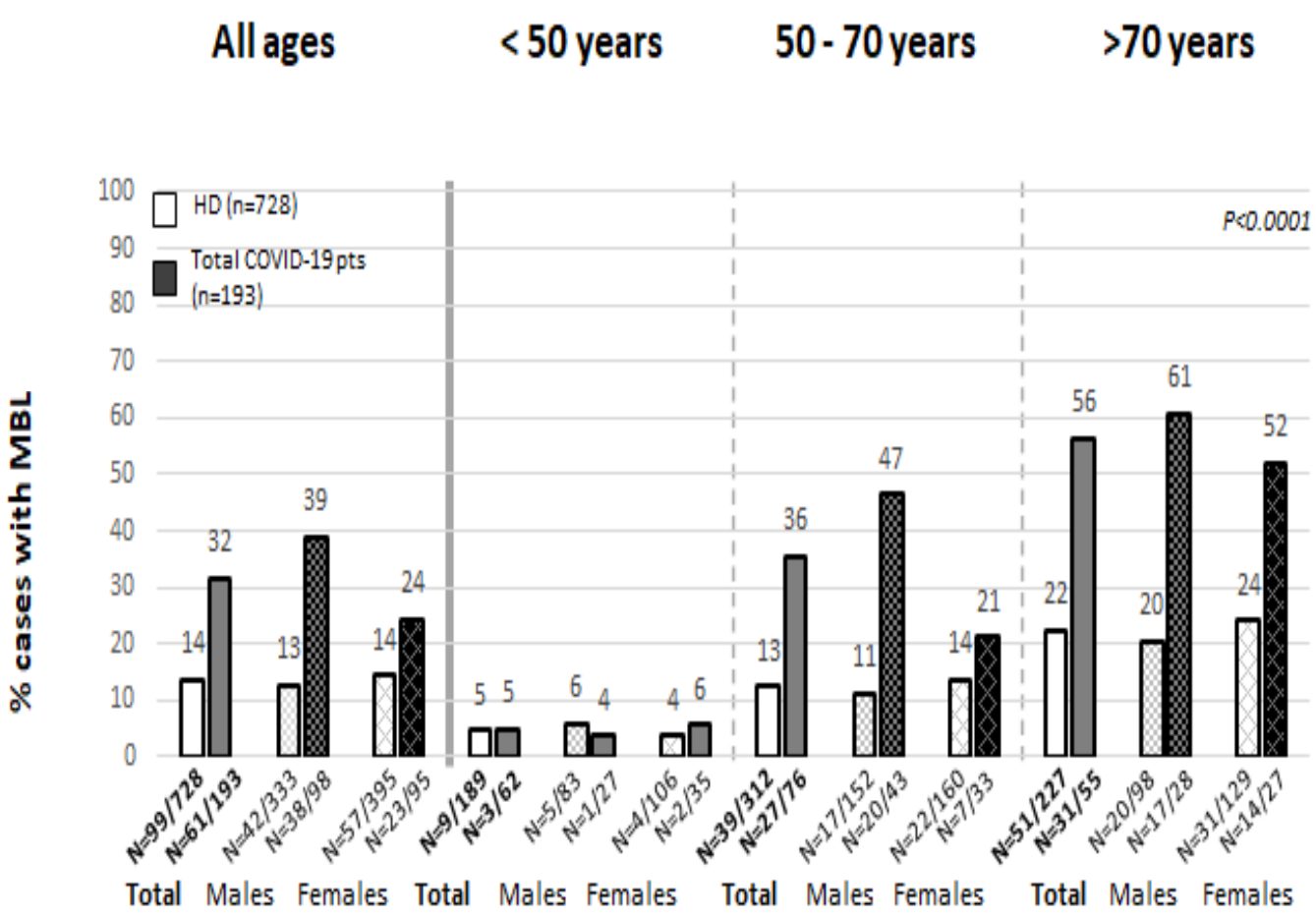
Shanafelt et al, Leukemia, 2021; 35: 239-44



Slager et al, Blood, 2022

MBL^{lo} among relatives of familial CLL patients show higher rates of progression to CLL (5.7% at 5 years follow-up), severe infections and hematologic (lymphoid) cancer

PREVALENCE OF MBL^{lo} IN (HOSPITALIZED) COVID-19 PATIENTS vs THE GENERAL POPULATION



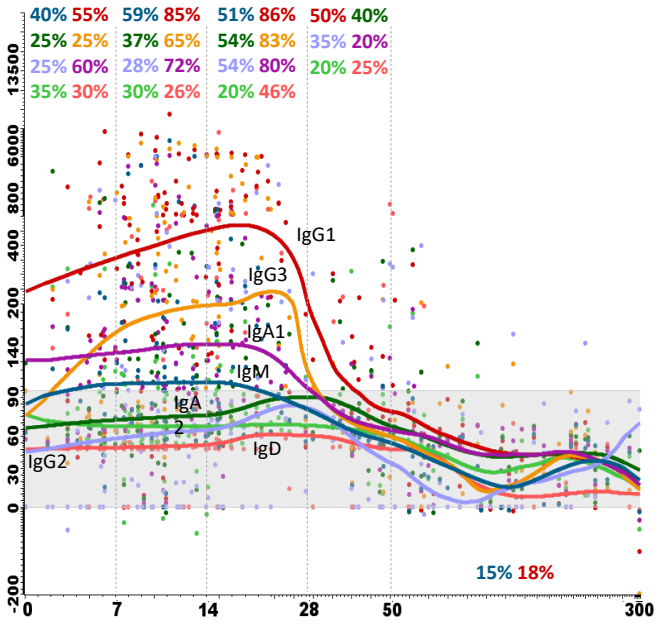
Prediction of mild vs severe COVID-19

Variables	Univariate analysis			Multivariate analysis	
	Non-hospitalized	Hospitalized	P-value	OR (95%CI)	P-value
Sex (male)	42/114 (37%)	91/135 (67%)	<0.0001	2.83 (1.29 – 6.21)	0.01
Dyspnea	36/107 (33%)	95/134 (71%)	<0.0001	4.88 (2.17 – 10.93)	<0.0001
Fever	56/114 (49%)	104/135 (77%)	<0.0001	3.71 (1.52 – 9.06)	0.004
Presence of MBL^{lo}	18/114 (16%)	53/135 (39%)	<0.0001	2.97 (1.19 – 7.42)	0.02
Anti-SARS-CoV-2 IgA ≥24 AU/mL	61/114 (54%)	112/134 (84%)	<0.0001	5.36 (2.13 – 13.52)	<0.0001
Eosinophils <20/µL	17/114 (15%)	71/135 (53%)	<0.0001	6.16 (2.37 – 16.04)	<0.0001
Neutrophils >6000/µL	14/114 (12%)	55/135 (41%)	<0.0001	4.09 (1.48 – 11.3)	0.007
B-cells <100/µL	18/114 (16%)	63/135 (47%)	<0.0001	3.6 (1.3 – 9.99)	0.01
NK cells <150/µL	32/114 (28%)	54/135 (40%)	0.05	3.14 (1.21 – 8.13)	0.02

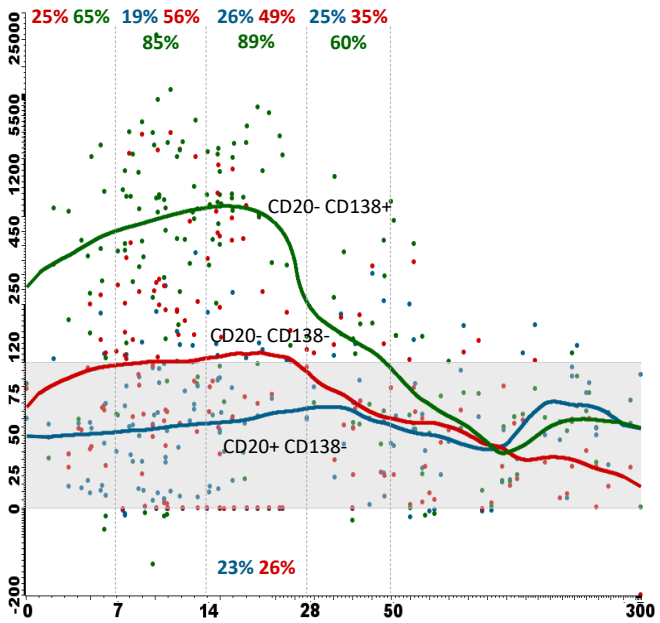
Oliva-Ariza et al, Blood 2022

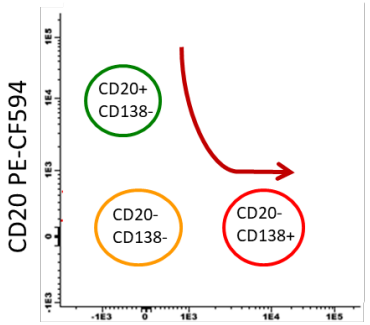
PLASMA CELL KINETICS IN BLOOD OF MBL^{lo} VS NON-MBL PATIENTS DURING AND AFTER COVID-19

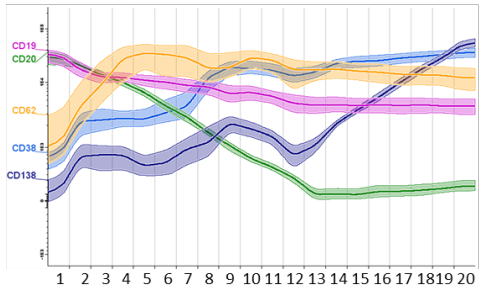
Subsets of plasma cells by IgH subclass

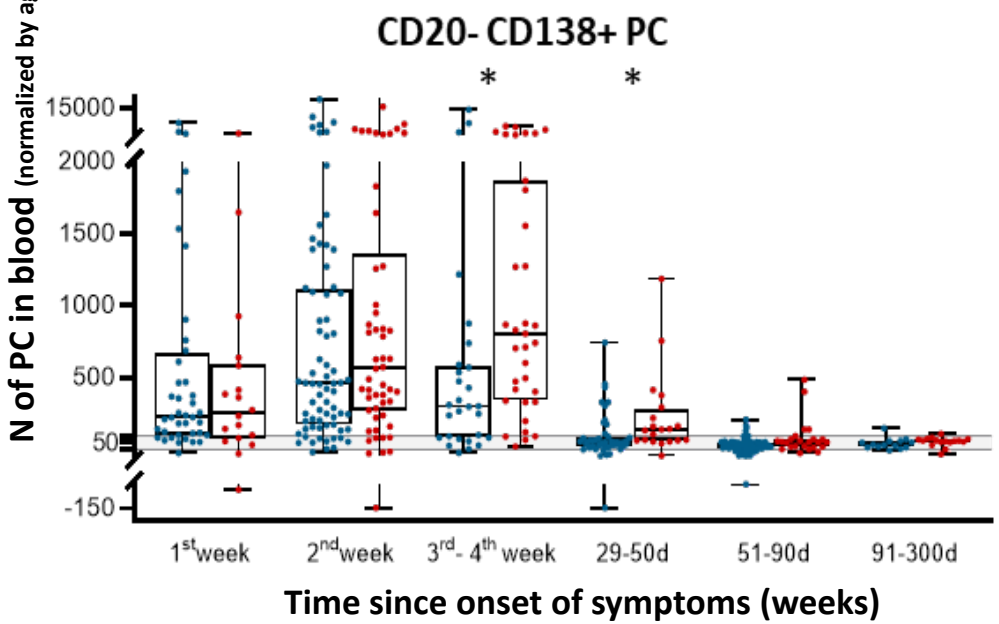


Maturation-associated subsets of plasma cells









Delayed plasma cell peak in blood of MBL^{lo} vs non-MBL is at the expense of more mature IgG1, IgG3 and IgA1 PC

Oliva-Ariza et al, Am J Hematol 2023

ECRIN M3
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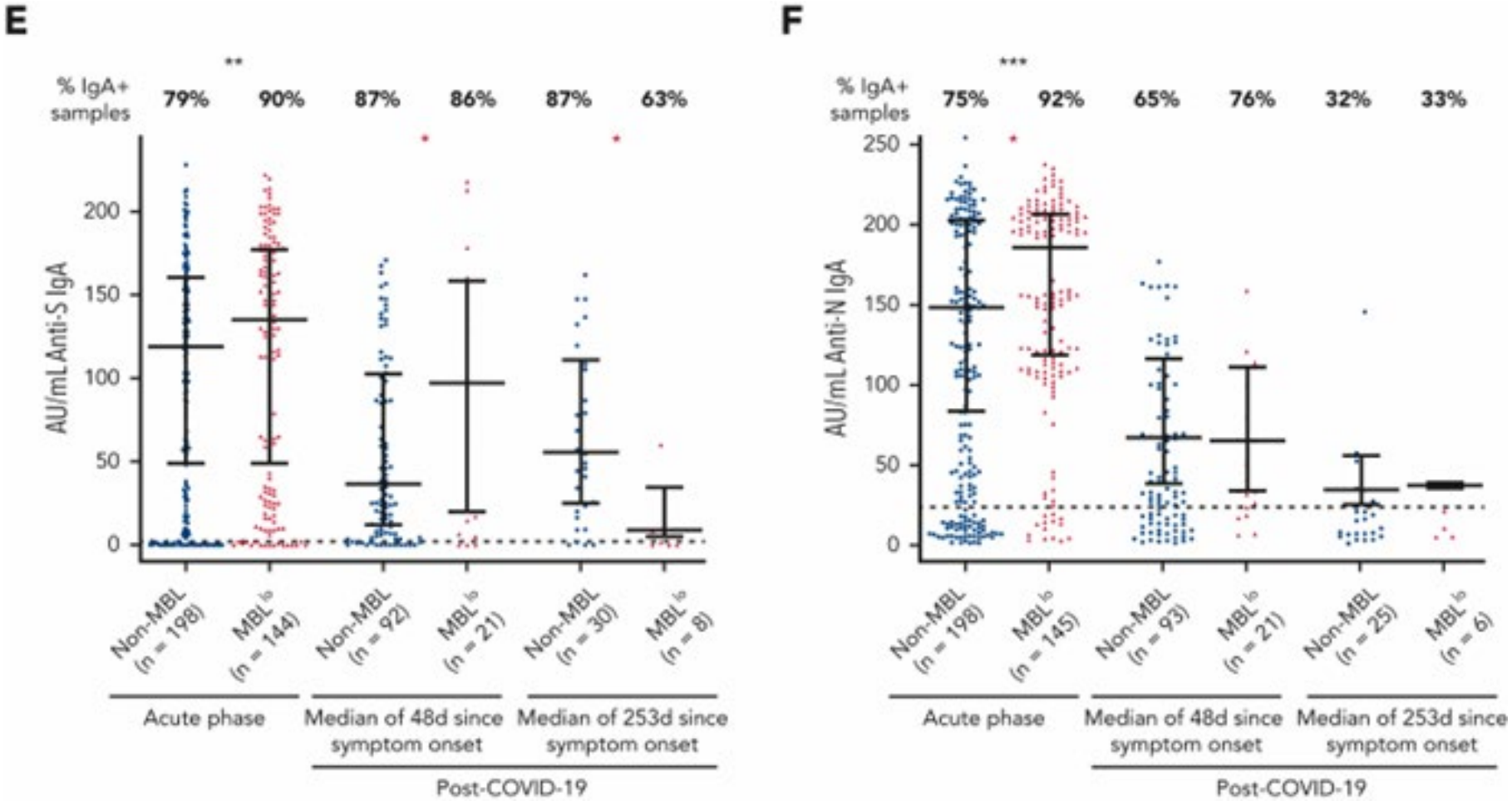
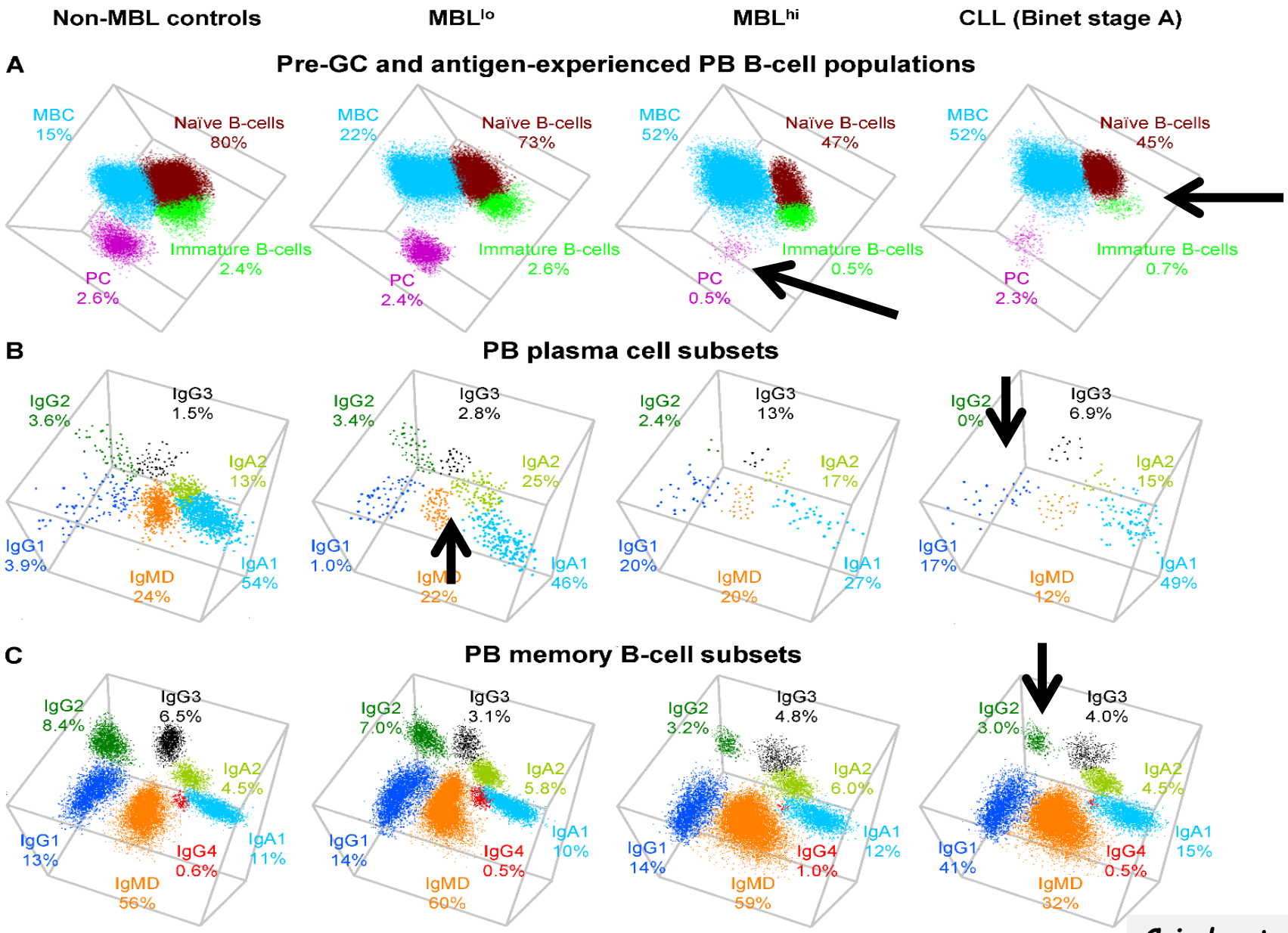
ANTI-SARS-CoV-2 ANTIBODY LEVELS IN MBL^{lo} VS NON-MBL PATIENTS DURING AND AFTER COVID-19



Delayed plasma cell peak in blood of MBL^{lo} vs non-MBL patients during COVID-19 is associated with decreased pre-germinal center B cell counts

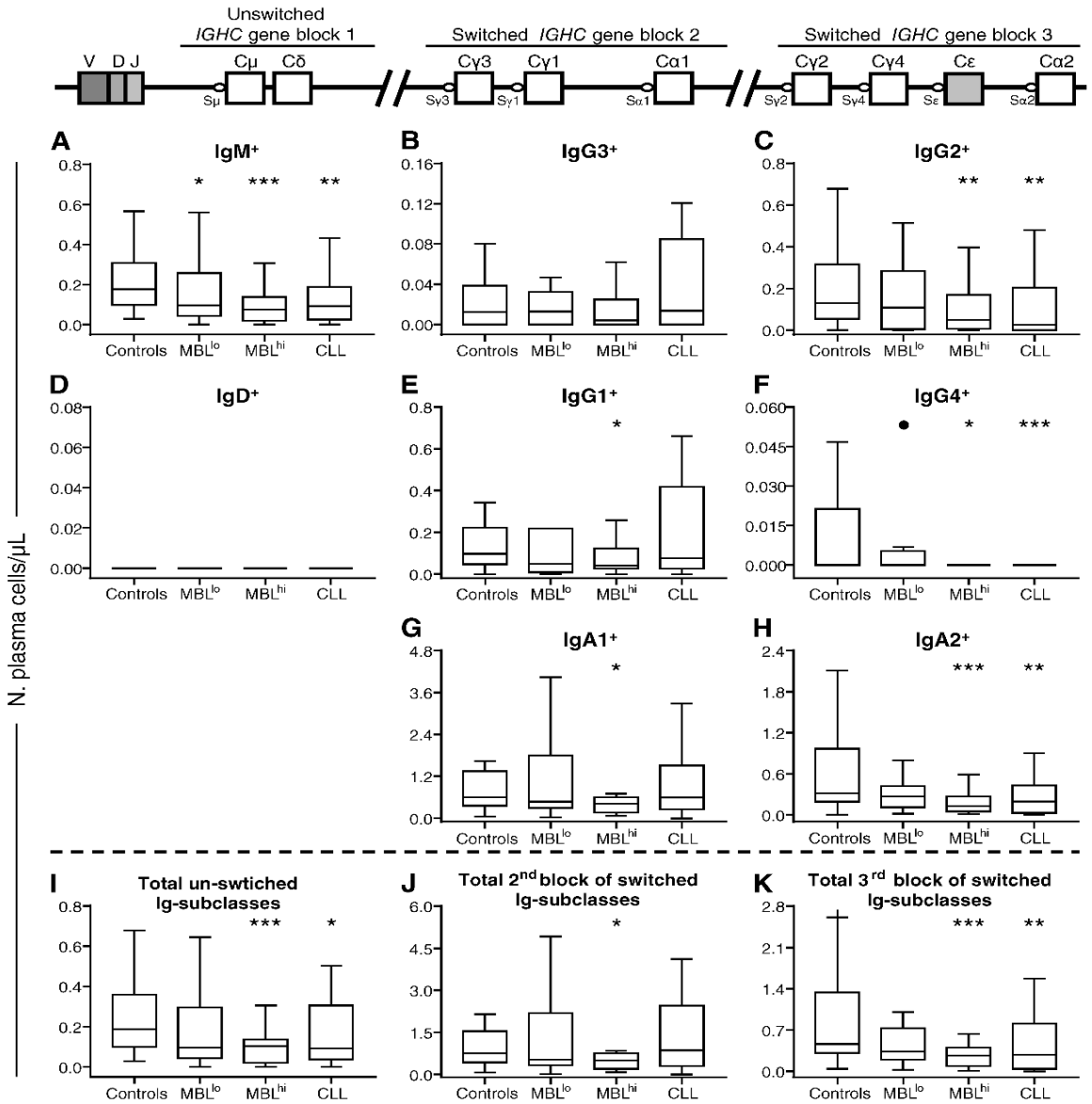
Oliva-Ariza et al, Am J Hematol 2023

DISTRIBUTION OF PB B CELL SUBSETS EXPRESSING DISTINCT IG ISOTYPES AND SUBCLASSES IN MBL^{LO} vs MBL^{HI} vs STAGE A CLL



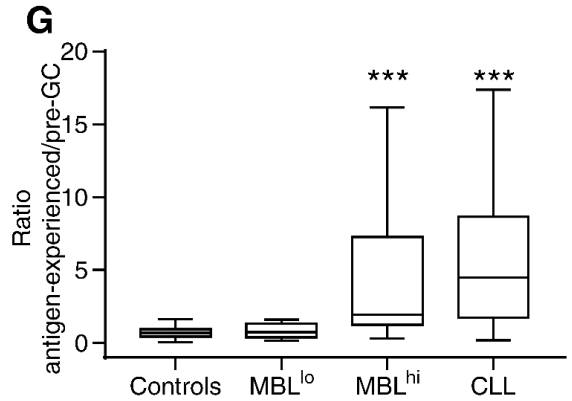
Criado et al, Leukemia 2018

Progressively altered B cell and plasma cell subsets from MBL to CLL



*P-value <0.05, ** P-value <0.01, ***P-value ≤0.001 vs. Controls

Decreased B-cell production
with a potentially
narrower B-cell repertoire

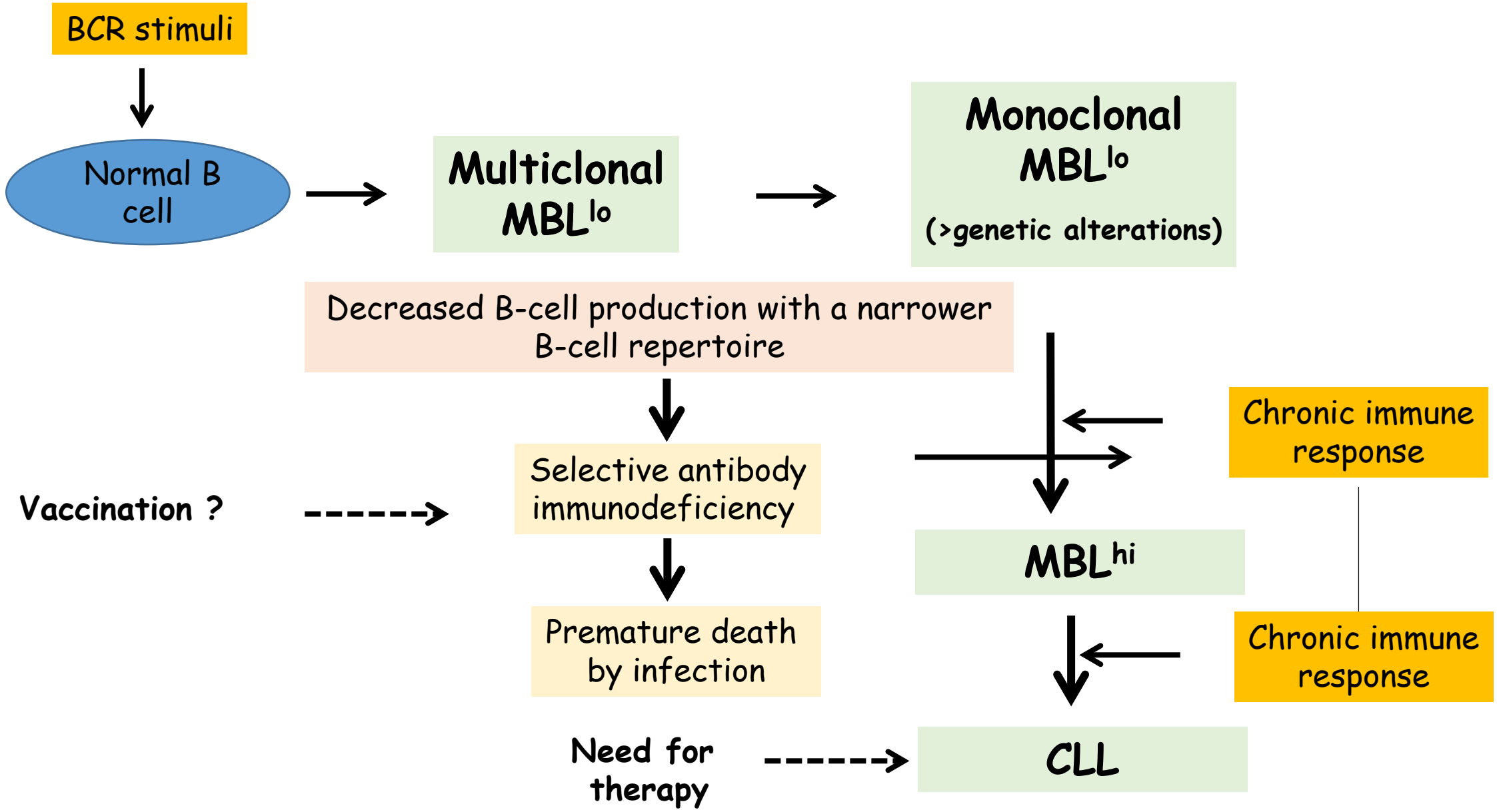


Sequential decrease in:

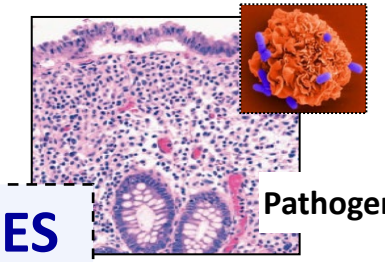
i) IgM+ PC in MBL^{lo},
ii) all PC subsets in MBL^{hi},
iii) but only IgG$_{2+4}$, IgA$_2$ in stage A CLL

Criado et al, Leukemia 2108, 32: 2701-5

MBL vs CLL: B-cell response status



NORMAL MAST CELL FUNCTION



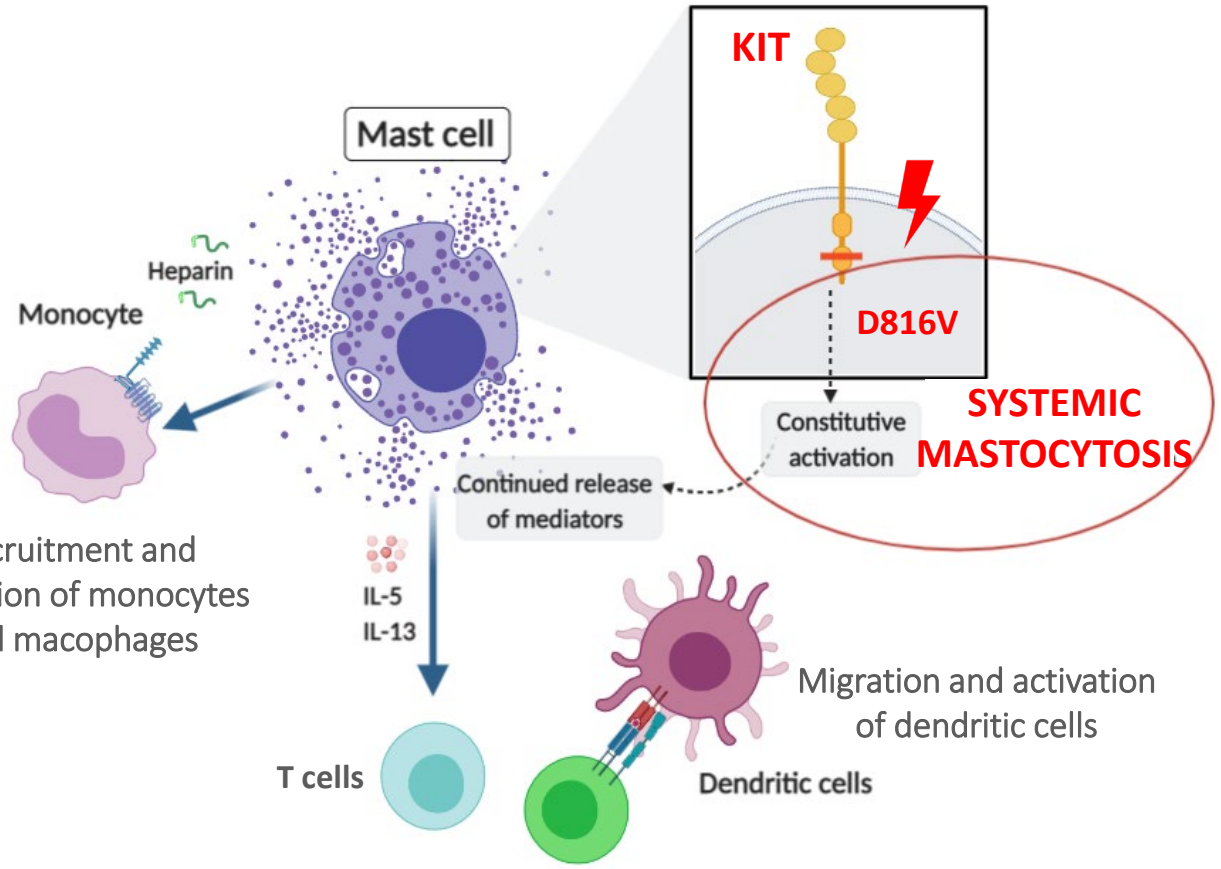
TISSUES

Pathogen exposure

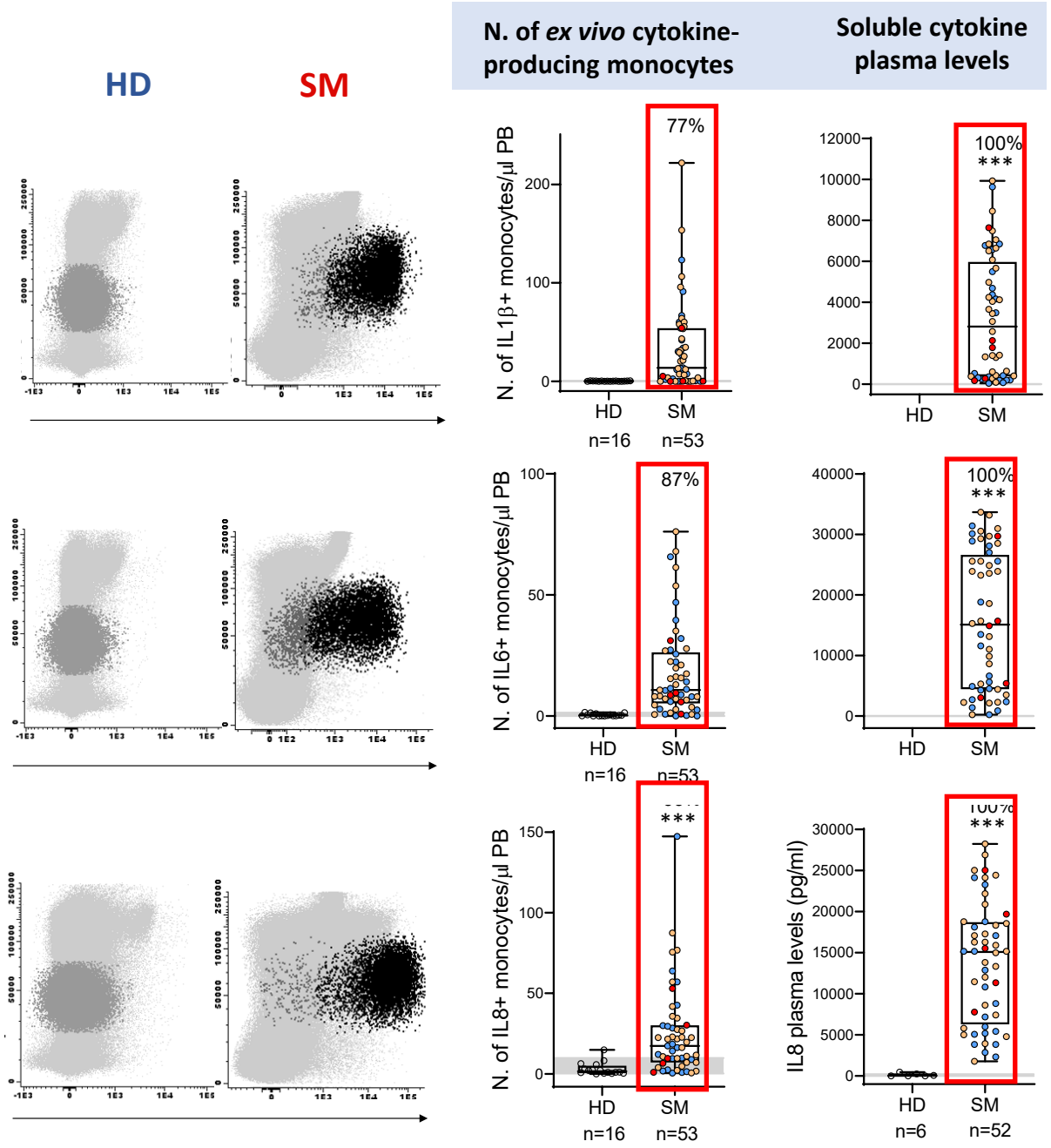
IMMUNE RESPONSE

Inflammation

- ✓ **Production** and **release**:
  - **Lipid mediadors;**
  - **Cytokines;**
  - **Chemokines;**
  - **Angiogenic growth factors;**
- ✓ **Release** of **preformed mediadors**:
  - **Histamine;**
  - **Heparin;**
  - **Proteases, etc;**



Spontaneous *ex vivo* cytokine production by blood monocytes in SM and cytokines plasma levels



↑ spontaneous production of IL1β, IL6, IL8, TNFα of monocytes in parallel with ↑ IL1β, IL6, IL8, TNFα and IL10 plasma levels in SM

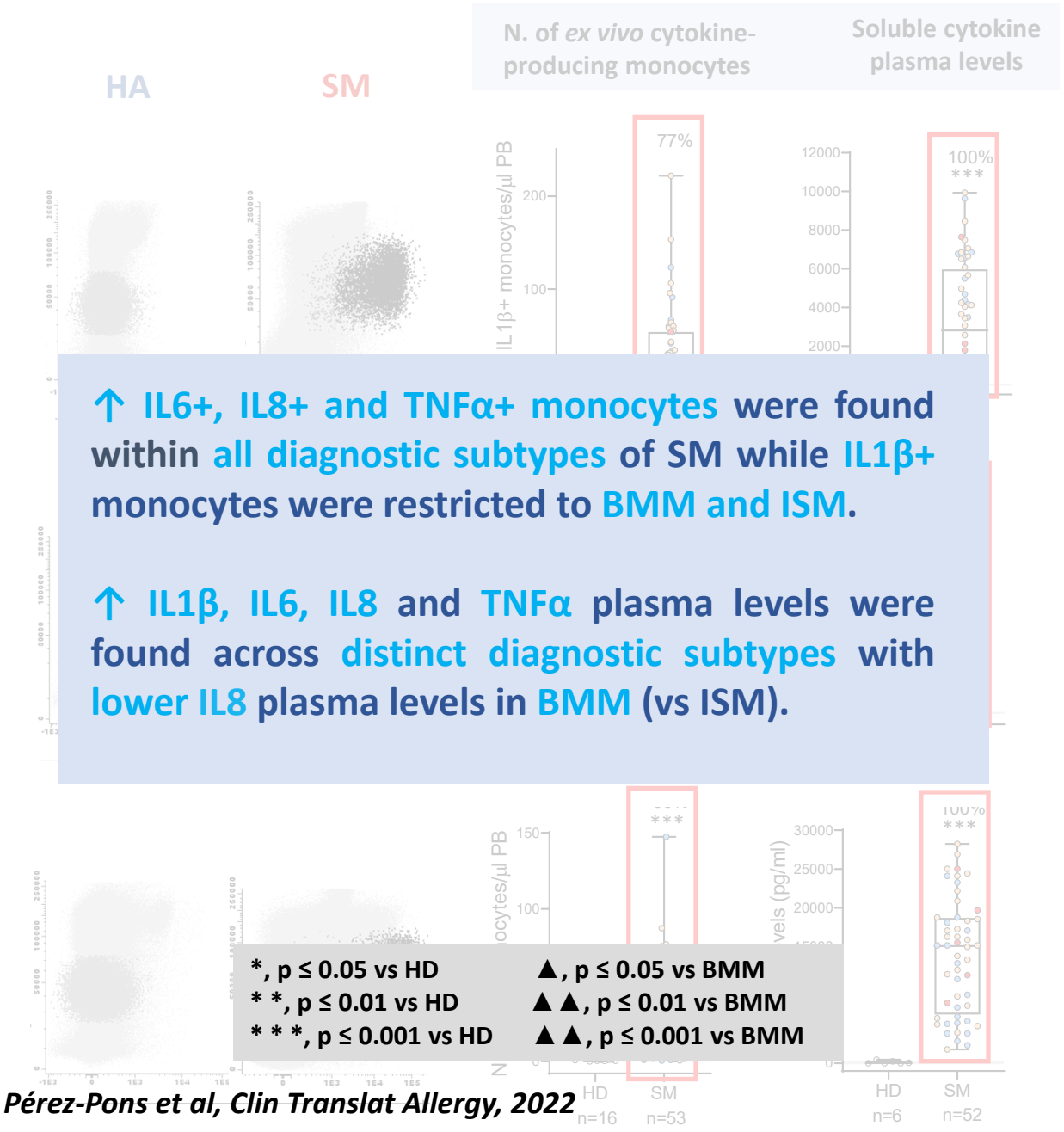
Due not only to constitutive activation of tumor MC but also functional activation of circulating blood monocytes

Pérez-Pons et al, Clin Translat Allergy, 2022

*, p ≤ 0.05 vs HA * *, p ≤ 0.01 vs HA * * *, p ≤ 0.001 vs HA

** Percentage values indicate the percentage of SM patients above percentile 95 of HD

Spontaneous *ex vivo* cytokine production by blood monocytes and cytokines plasma levels in distinct subtypes of SM

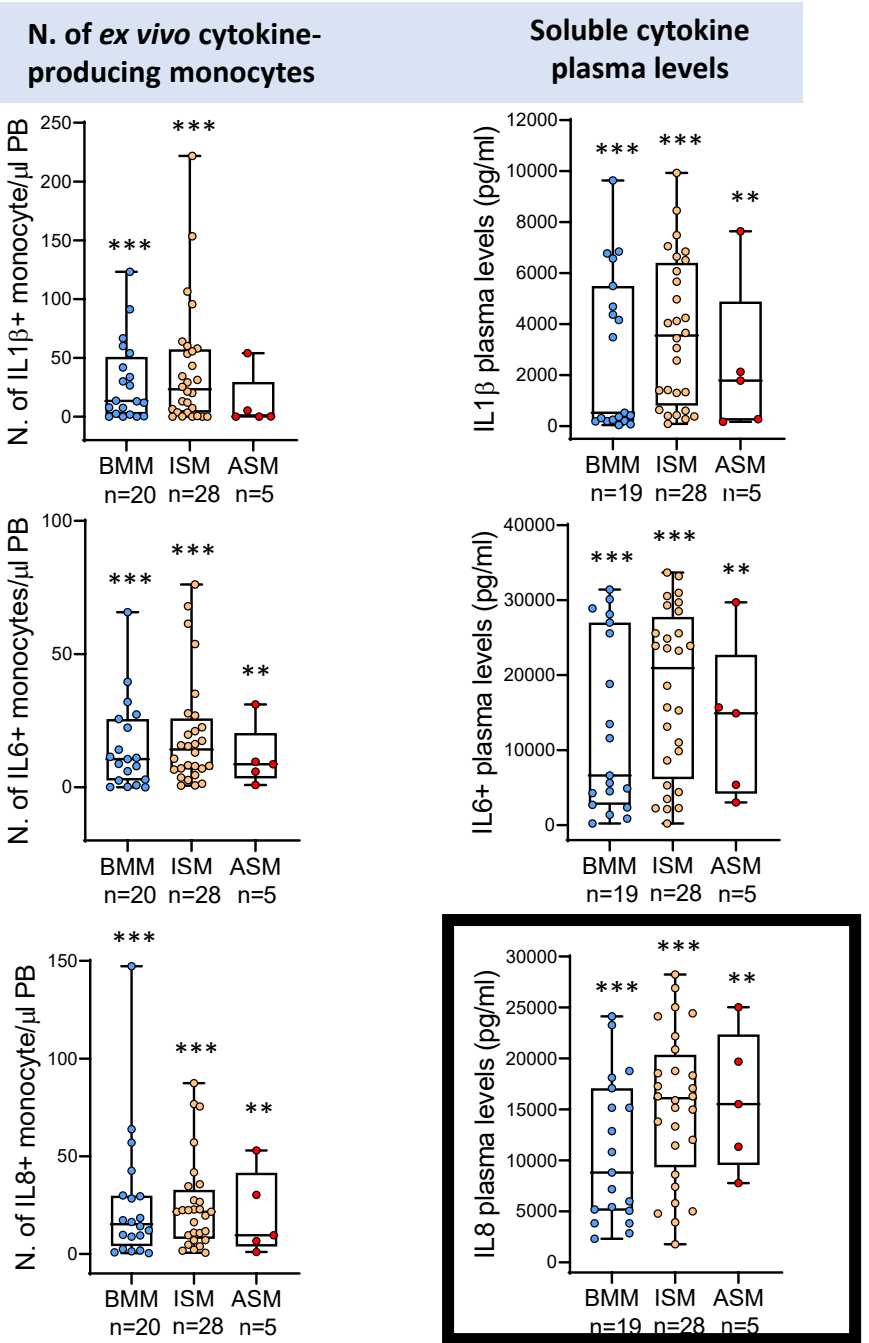


↑ IL6+, IL8+ and TNFα+ monocytes were found within all diagnostic subtypes of SM while IL1β+ monocytes were restricted to BMM and ISM.

↑ IL1β, IL6, IL8 and TNFα plasma levels were found across distinct diagnostic subtypes with lower IL8 plasma levels in BMM (vs ISM).

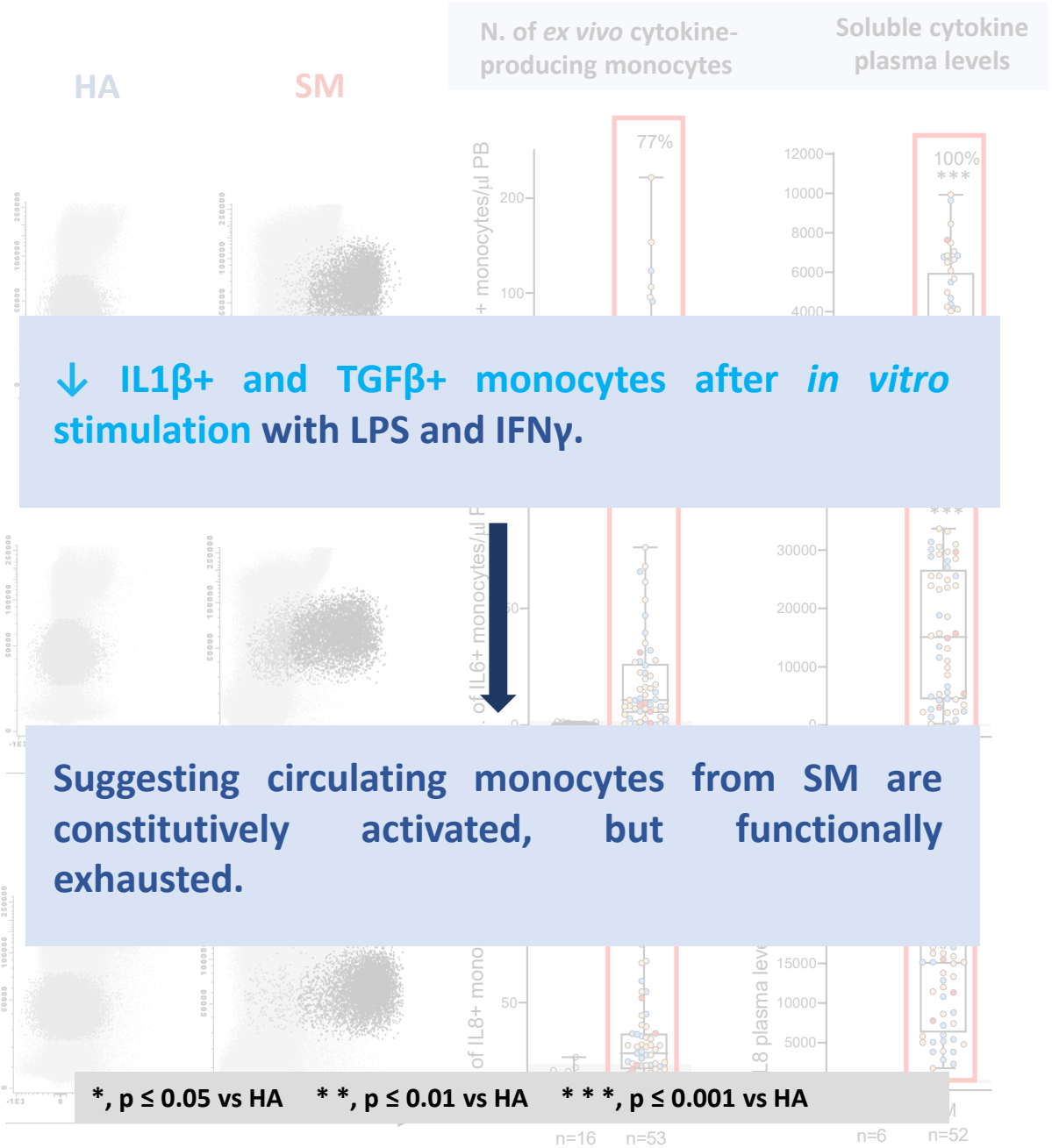
*, p ≤ 0.05 vs HD
* *, p ≤ 0.01 vs HD
* * *, p ≤ 0.001 vs HD

▲, p ≤ 0.05 vs BMM
▲▲, p ≤ 0.01 vs BMM
▲▲, p ≤ 0.001 vs BMM



Pérez-Pons et al, Clin Translat Allergy, 2022

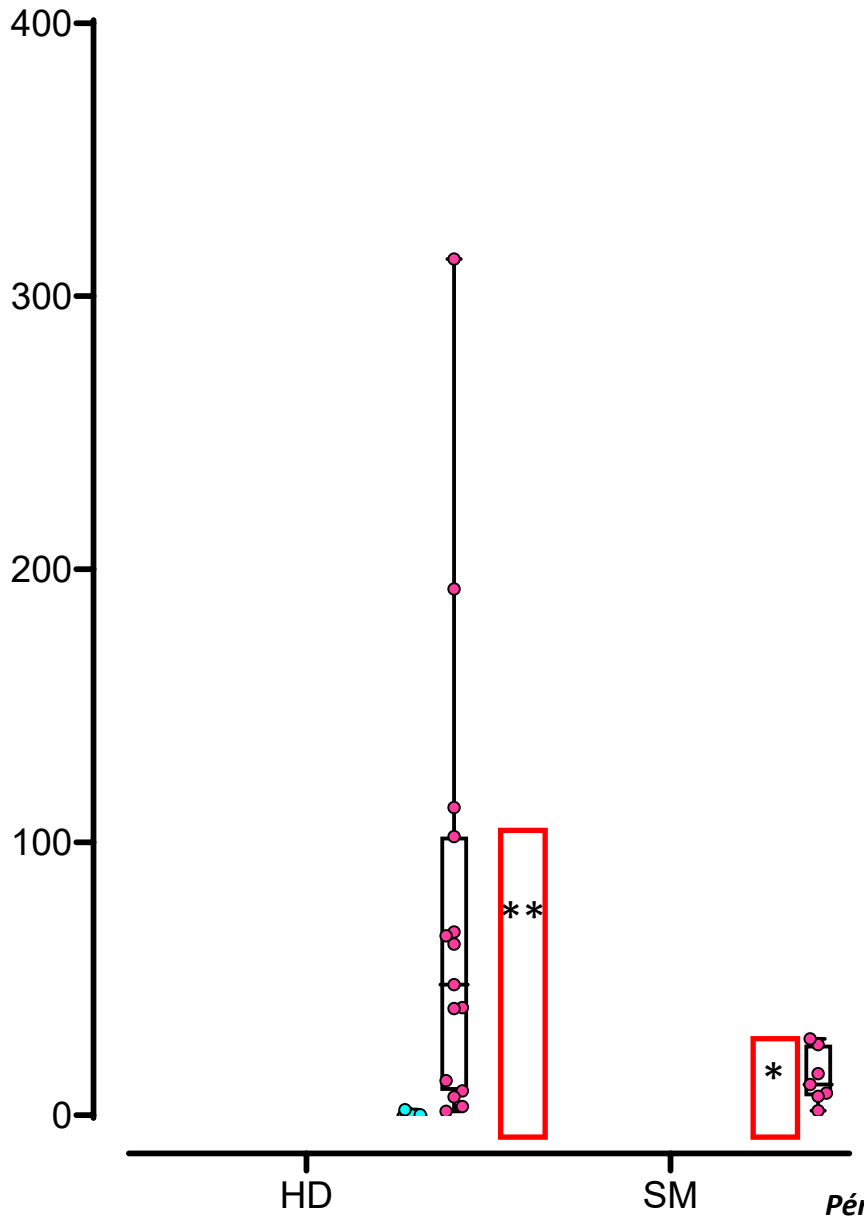
Cytokine-producing monocytes after *in vitro* stimulation of blood samples with LPS plus IFNγ in SM



↓ IL1β+ and TGFβ+ monocytes after *in vitro* stimulation with LPS and IFNγ.

Suggesting circulating monocytes from SM are constitutively activated, but functionally exhausted.

*, p ≤ 0.05 vs HA * *, p ≤ 0.01 vs HA * * *, p ≤ 0.001 vs HA

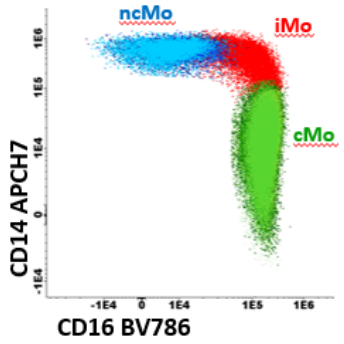


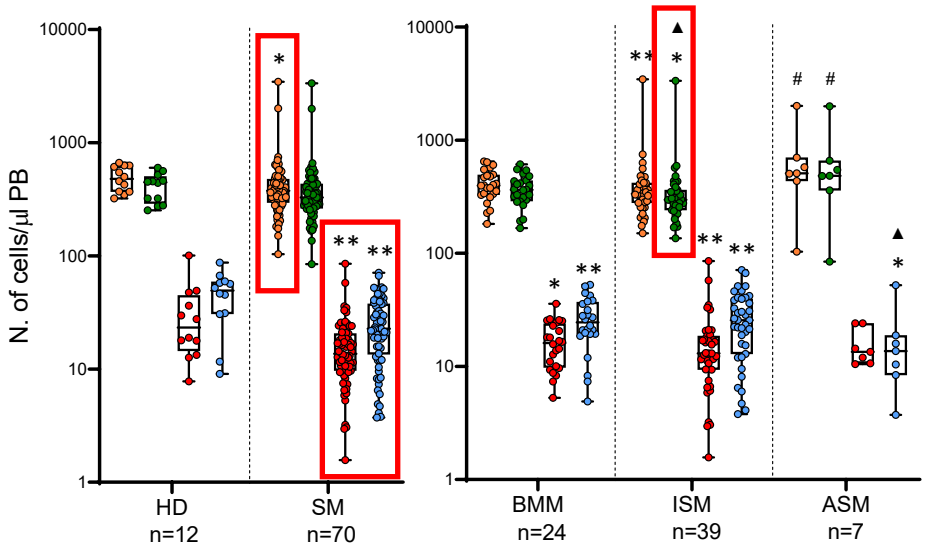
Pérez-Pons et al, Clin Translat Allergy, 2022

Distribution of distinct populations of monocytes in blood of SM patients

Total monocytes

Total monocytes
cMo (Classical monocyte)
iMo (Intermediate monocyte)
ncMo (Non-classical monocyte)





↓ Total monocytes in SM at the expense of intermediate monocytes and non-classical monocytes.
↓ Classical monocytes in ISM vs HD and also between BMM and ASM.

*, p ≤ 0.05 vs HD ▲, p ≤ 0.05 vs BMM #, p ≤ 0.05 vs ISM
* *, p ≤ 0.01 vs HD ▲▲, p ≤ 0.01 vs BMM # #, p ≤ 0.01 vs ISM
* * *, p ≤ 0.001 vs HD ▲▲, p ≤ 0.001 vs BMM # # #, p ≤ 0.001 vs ISM

↓ cMo in ISM vs BMM at the expense of the CD62L+ FcεRI+, while ↑CD62L- FcεRI- in ASM vs BMM.

↓ ncMo at the expense of SLAN+ subsets, more pronouncedly decreased in ASM than BMM, in association with ↓ SLAN+CD36-.

Pérez-Pons et al,
Clin Translat Allergy, 2022

Distribution of distinct populations of dendritic cells in blood of SM patients

Dendritic cells (DC)

CD34+ DCp
(DC precursors
CD100+)

pDC

mDC CD141+
(cDC1, BDCA-3)

mDC CD1c+
(cDC2, BDCA-1)

new Axl+ DC
(Siglec6+)

T-cell proliferation

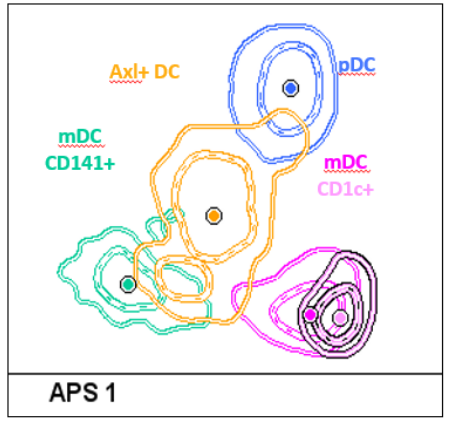
Mixed GEP
(pDC and mDC)

new myDC CD1c+
CD14^{low}
"Inflammatory"

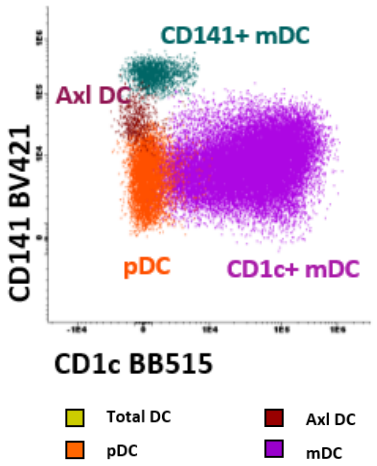
new myDC CD1c+
CD14^{-}
"Non-Inflammatory"

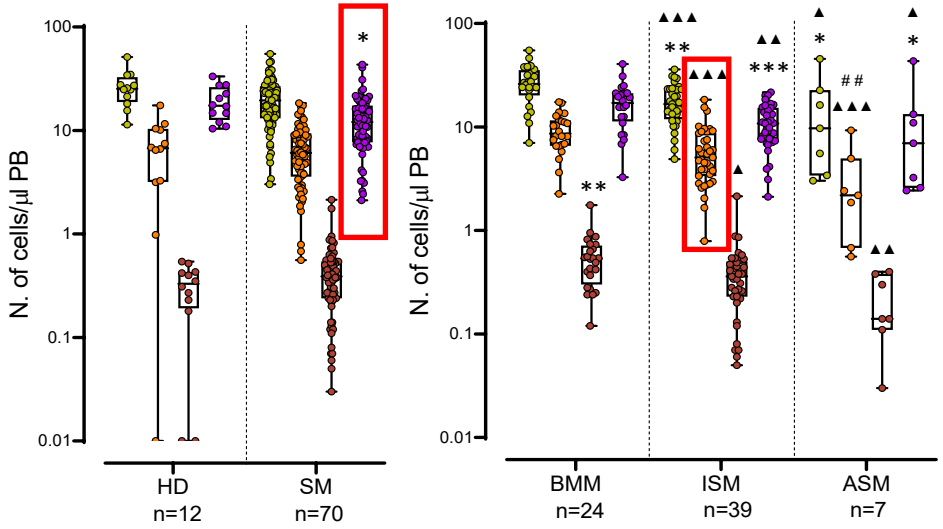
new myDC CD1c+
CD14^{-}/ CD5-

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



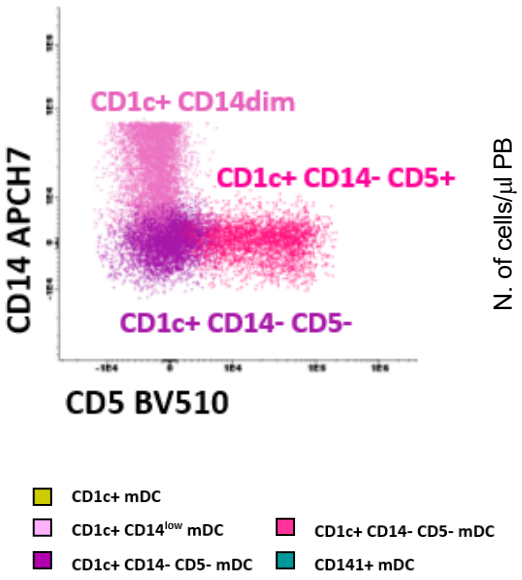
Adapted from: Villani et al, Science, 2017; Collin et al. Immunology 2018

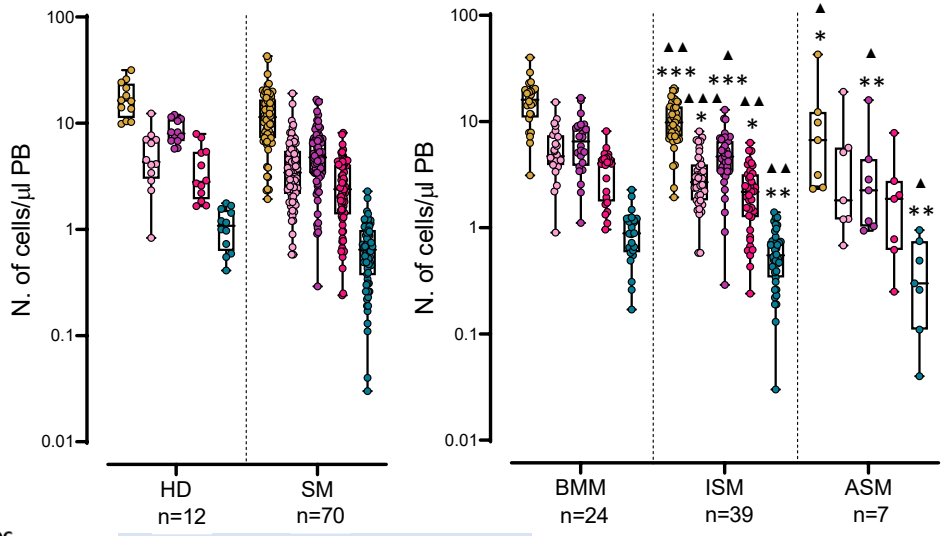




↓ Myeloid DC in SM.

↓ total DC and mDC in ISM and ASM.
Plasmacytoid DC ↓ from BMM to ISM and ASM.
↑ Axl DC were increased only in BMM cases.

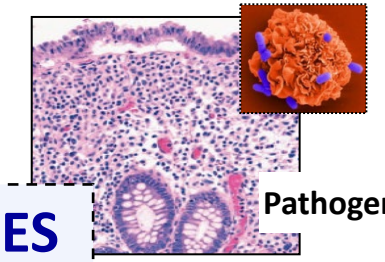




↓ Subsets of mDC in SM.
↓ Subsets of mDC in ISM and ASM.

Pérez-Pons et al,
Clin Translat Allergy, 2022

NORMAL MAST CELL FUNCTION



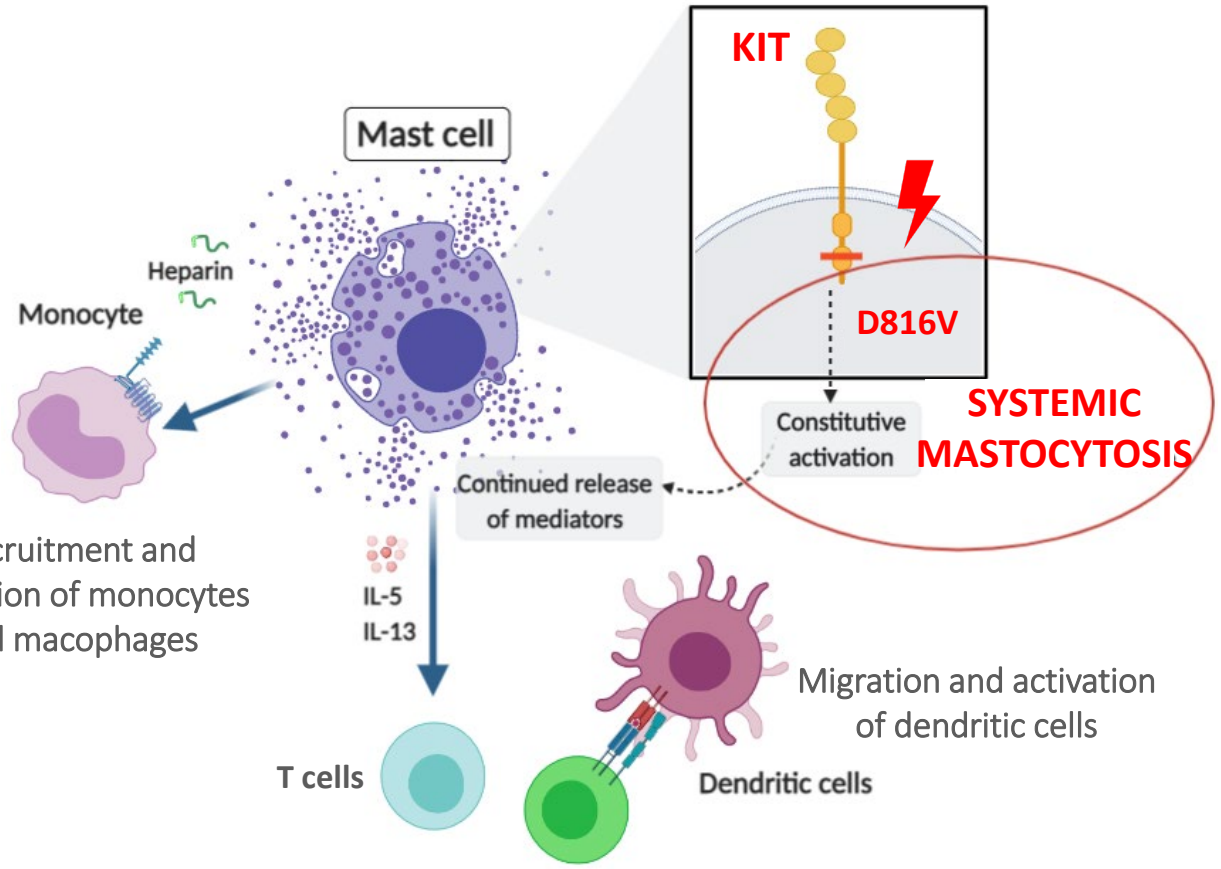
TISSUES

Pathogen exposure

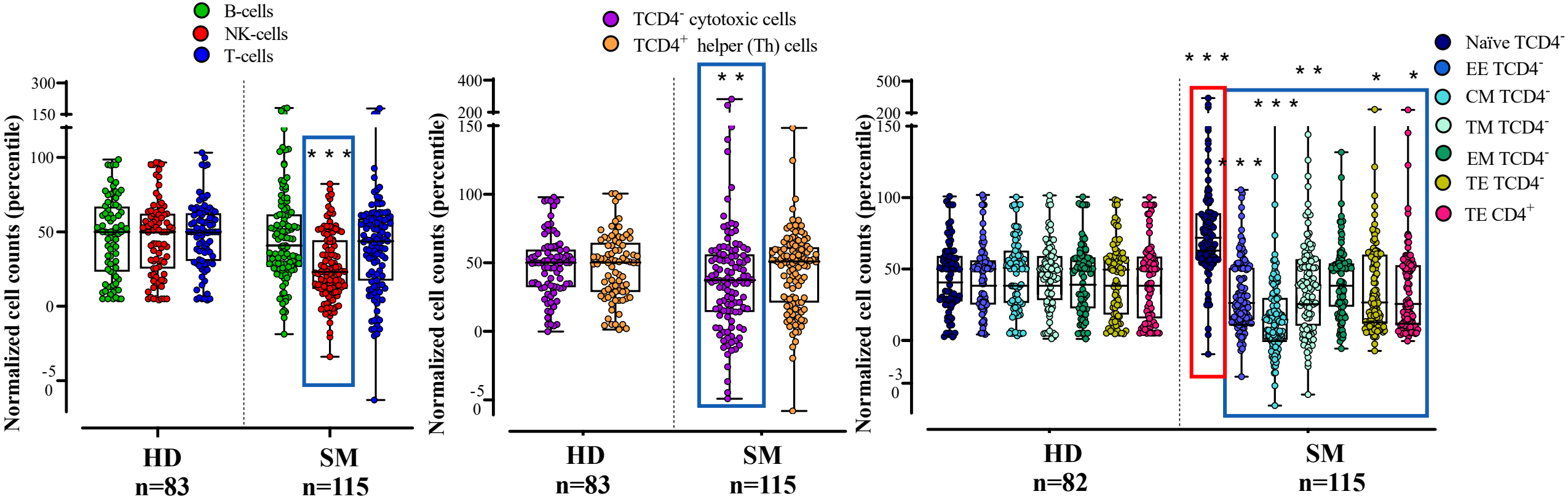
IMMUNE RESPONSE

Inflammation

- ✓ **Production** and **release**:
  - **Lipid mediadors;**
  - **Cytokines;**
  - **Chemokines;**
  - **Angiogenic growth factors;**
- ✓ **Release** of **preformed mediadors**:
  - **Histamine;**
  - **Heparin;**
  - **Proteases, etc;**



Distribution of NK-cell and T and B lymphocyte in blood

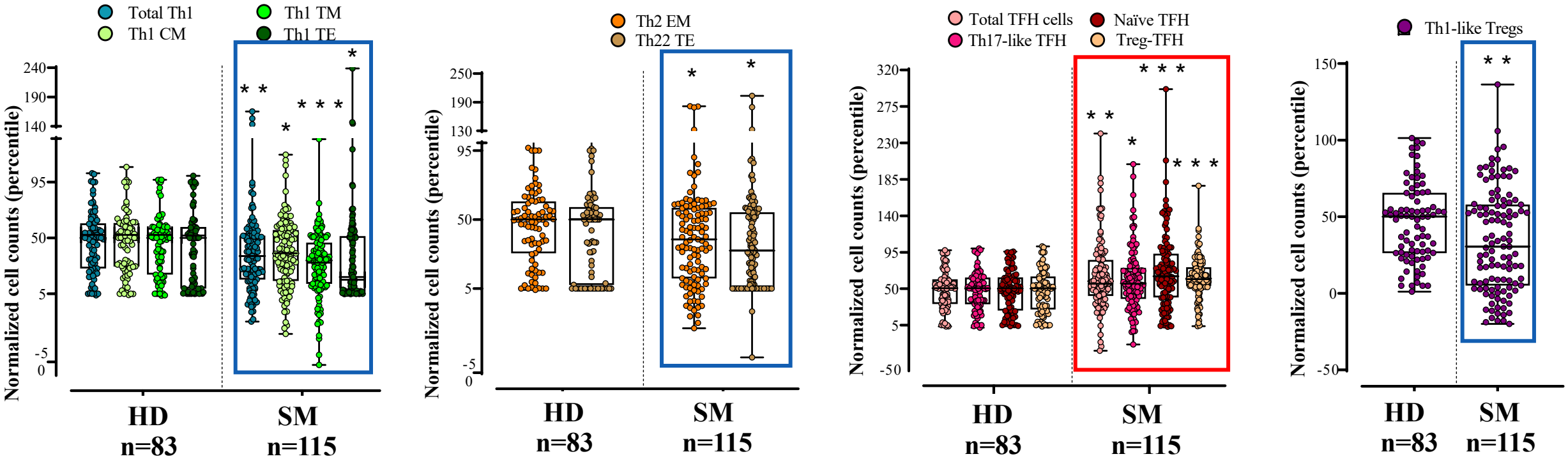


Decreased NK-cell and (most) CD4- cytotoxic T cell subsets in blood of SM patients, together with **increased naïve CD4- cytotoxic T cells** vs HD

EE, early effector; CM, central memory; TM, transitional memory; EM, effector memory; TE, terminal effector

Pérez-Pons et al, Allergy, 2023

Distribution of CD4⁺ classical Th, TFH and T-reg cell subsets in blood



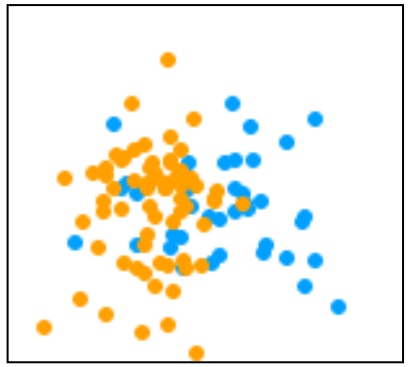
Decreased values of Th1 cells and the subsets of Th2 EM, Th22 TE y Th1-Treg cells together with **increased TFH** cell subsets in blood of SM patients vs HD

CM, central memory; TM, transitional memory; EM, effector memory; TE, terminal effector; TFH, folicular T helper; Treg, regulatory T cells

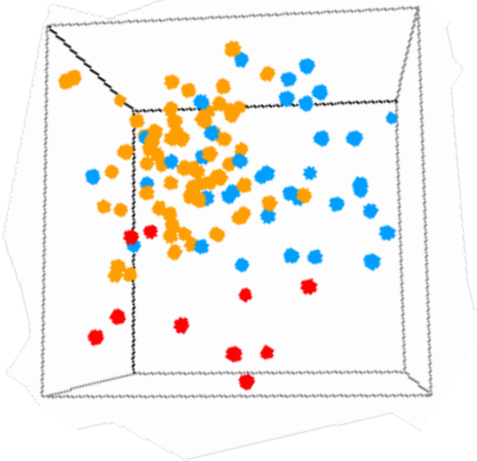
Pérez-Pons et al, Allergy, 2023

The blood immune profile of distinct diagnostic subtypes of SM

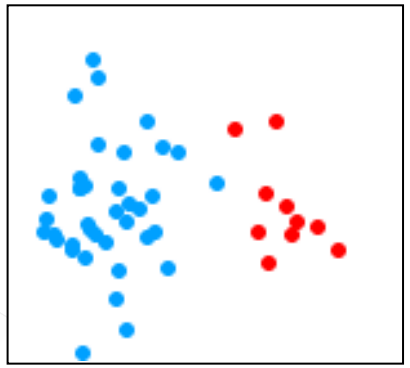
BMM vs ISM



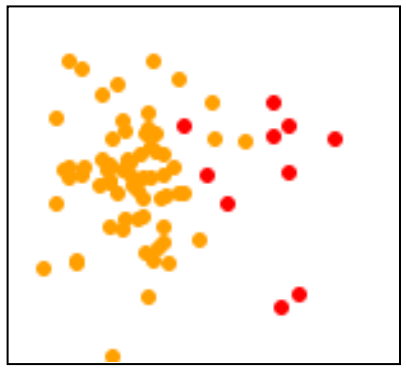
BMM vs ISM vs ASM



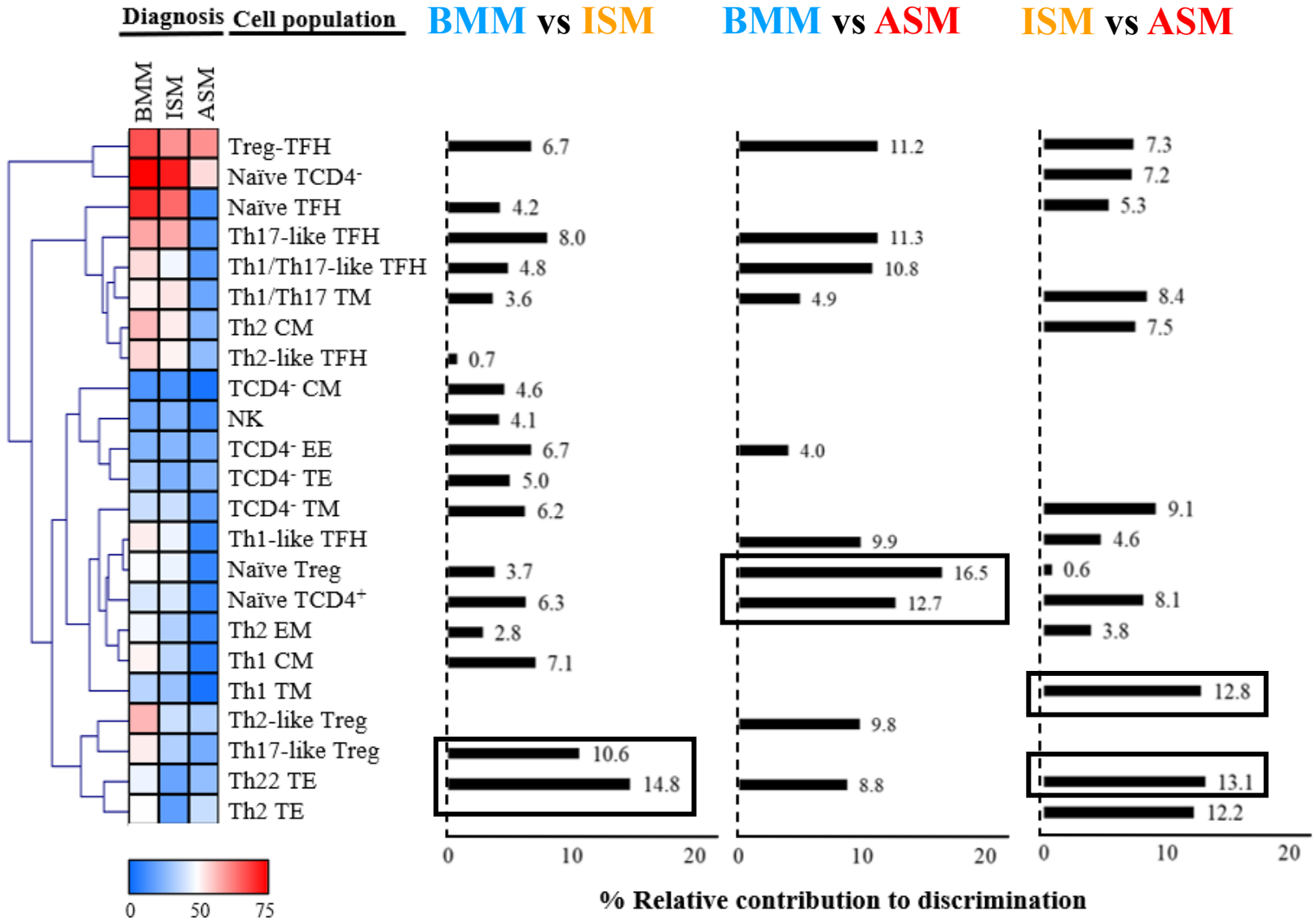
BMM vs ASM



ISM vs ASM



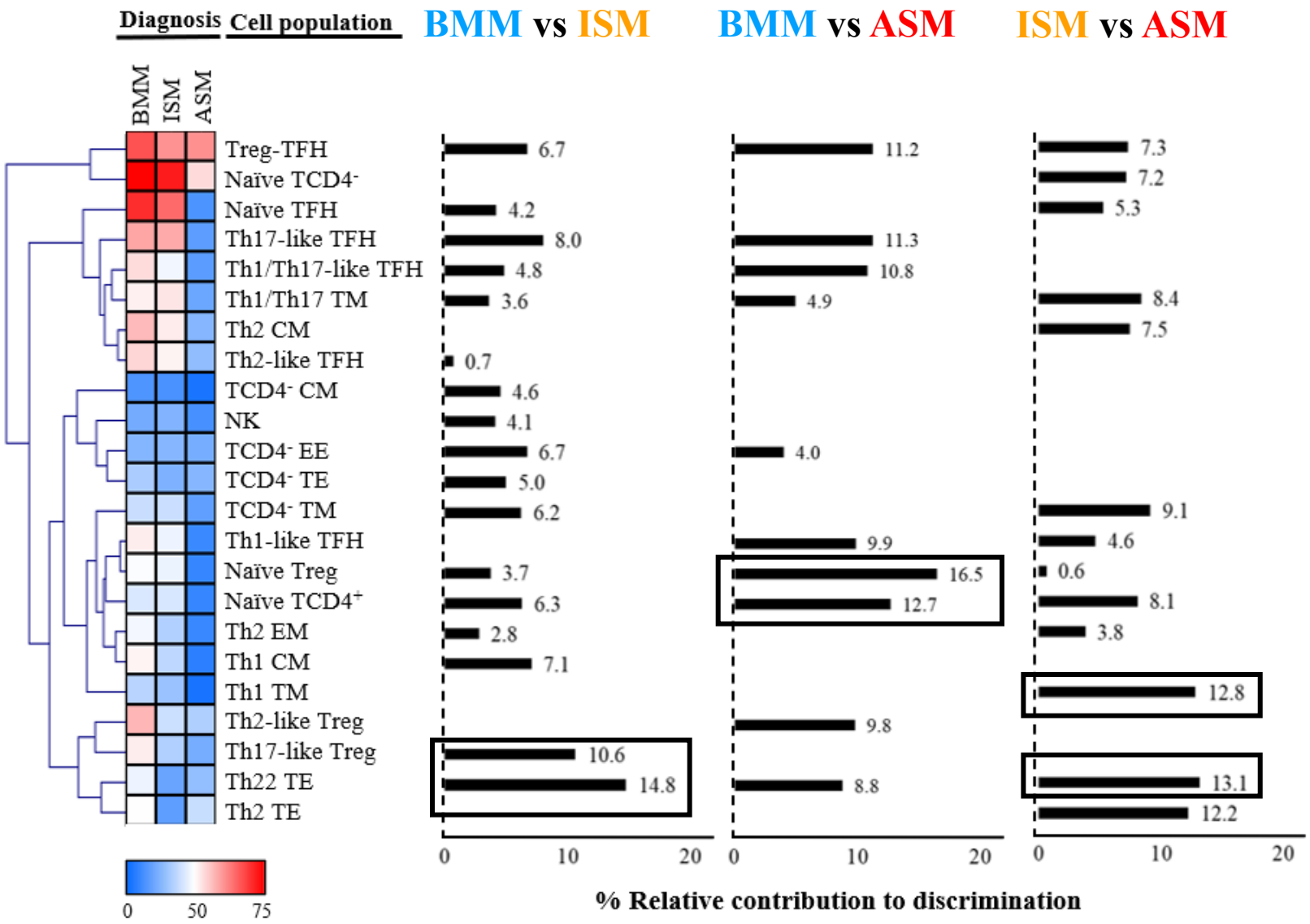
Different immune profiles among the distinct diagnostic subtypes



Pérez-Pons et al, Allergy, 2023

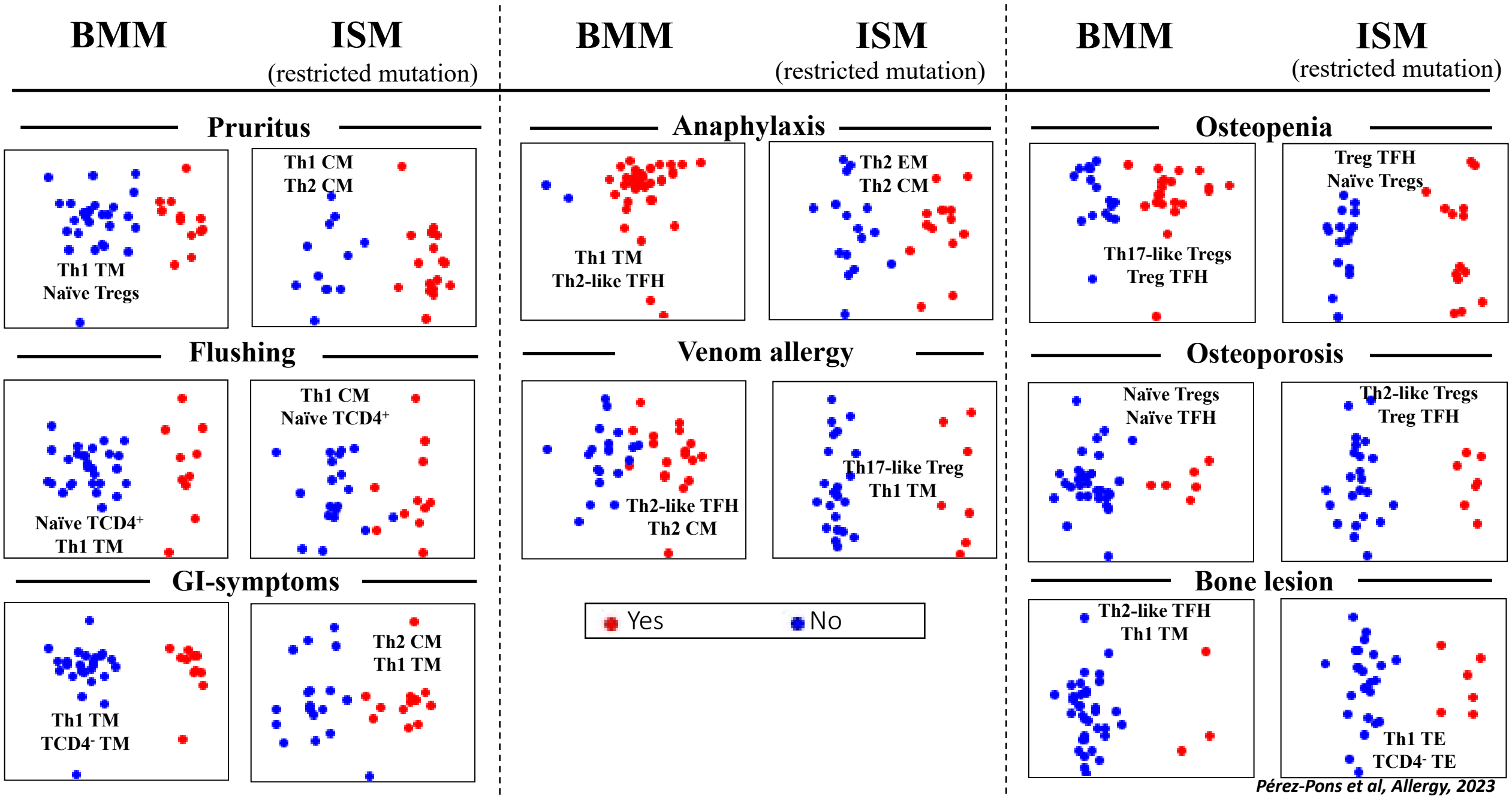
The blood immune profile of distinct diagnostic subtypes of SM

- Overall **decrease of all functional and maturation-associated subsets of T cells in ASM**.
- **Increased TCD4⁻ naïve, TFH, Th1/Th17, Th17 and Th2 subset counts** together with a **decrease of most TCD4⁻ cell subsets** in **BMM and ISM**.
- **Th17-like Tregs and Th22 TE** contribute the most to the discrimination between **BMM and ISM.**
- **Naïve TCD4⁺ and naïve Treg** contributed to discriminate between **BMM and ASM**.
- **Th1 TM and Th22 TE** were those T cell subsets which contributed the most to separate **ISM from ASM.**



Pérez-Pons et al, Allergy, 2023

Potential association between the T cell blood immune profile and clinical features of SM



Pérez-Pons et al, Allergy, 2023

Summary remarks

- SM patients display an ***in vivo*** **activation of blood circulating monocytes and dendritic cells**, with an altered distribution of their different subsets, which might reflect an **enhanced tissue migration** of more mature (intermediate and non-classical) monocyte cell compartments and myeloid dendritic cells.

- The above chronic inflammatory/innate cell profile is present **across distinct diagnostic subtypes of systemic mastocytosis** (BMM, ISM and ASM) with **some distinctive features among them**, particularly as regards dendritic cells with progressively decreased myDC from BMM to ISM and ASM, and increased Axl+ DC in BMM but not ISM and ASM patients.

- Altogether these findings point out the potential **downstream involvement of adaptative T cells** (and potentially also B cells), and particularly different **functional compartments of TCD4+ lymphocytes**.

- **NK-cells, T cytotoxic cells** and both **Th1 and Th2 TCD4^{+} cells are decreased** in blood of SM patients, in parallel to an **increased** number (production) of **naïve TCD4^{-} and TCD4^{+} TFH cells**.

- However, markedly different T cell subset immune profiles were found in distinct diagnostic subtypes of SM, with a general **decrease of the majority of T cell subsets in ASM, and increased counts of several TFH subsets in both BMM and ISM** patients.

- **Based on the overall immune profile in blood a potential association emerged between specific T cell subsets and the presence/absence of distinct clinical features** of the disease among BMM and ISM with MC-restricted *KIT*^{D816V}, including anaphylaxyis, HVA, bone lesions and specific MC-release associated symptoms.

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www.euroflow.org

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