Leiden University Medical Center

BEAT COVID1 STUDY

Immunemonitoring by Flow Cytometry

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Mihaela Zlei

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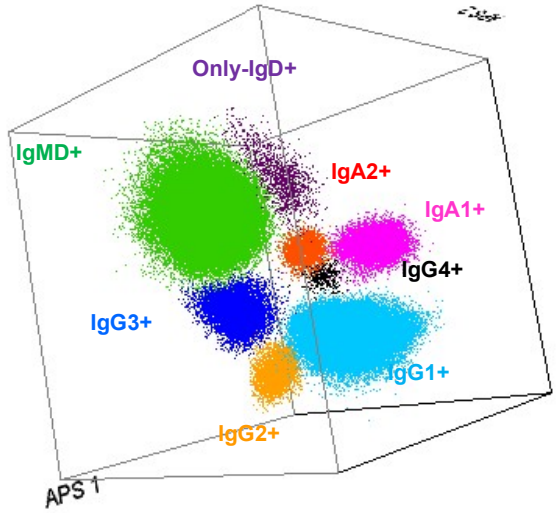
Immunemonitoring Group

NVC ZWOLLE, 25 NOVEMBER 2020



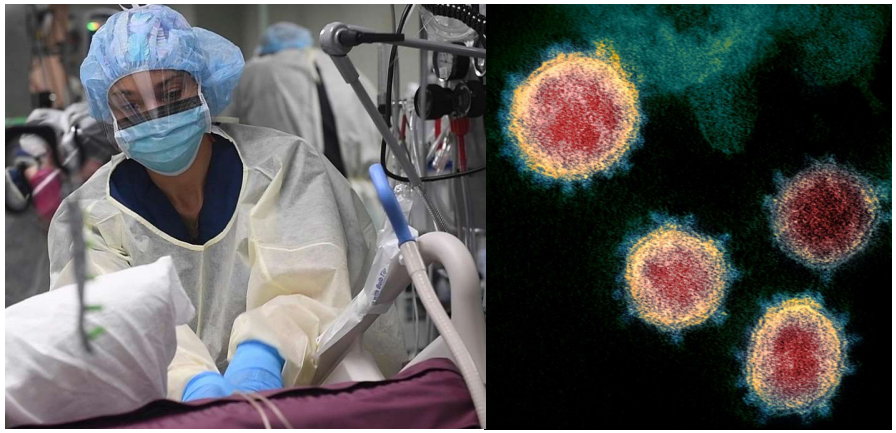
The novel SARS-CoV-2 and COVID-19 outbreak

Outbreak

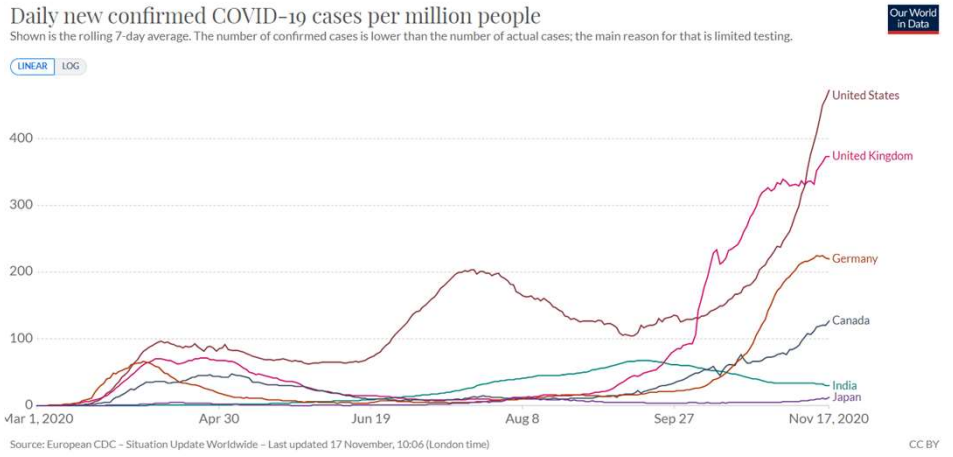
- 30 January 2020: Public Health Emergency of International Concern
- 11 March 2020: WHO declared a **pandemic**

20th Nov. 2020:

- total cases: >57,000,000 (500,000 daily)
- total active cases: >16,000,000
- total deaths: > 1,366,000 (>10,000 daily)



- spread primarily via small droplets from coughing
- estimated R0 of 5.7, before preventive measures implemented
- incubation period: 1 - 14 days (mainly 5)
- up to 80% of exposed people are asymptomatic
- 2-3% need to be hospitalized due to complications
- 20% out of those with complications can dye



- **Vaccine candidates have not yet completed clinical trials**
- **No known specific antiviral medication**
- **No known correlates of anti-SARS-CoV-2 immunity**

Coronavirus disease (COVID-2019) situation reports, WHO, 16 August 2020: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports

OBJECTIVES

Premises

- Current vaccine/ other specific treatment not existing
- Gaps in knowledge concerning immunity to SARS-CoV-2

Objective

Increase the understanding of the COVID-19 disease

- What are the **immune profiles** of different types of groups?
- What are the criteria that define different **disease trajectories**?

Means

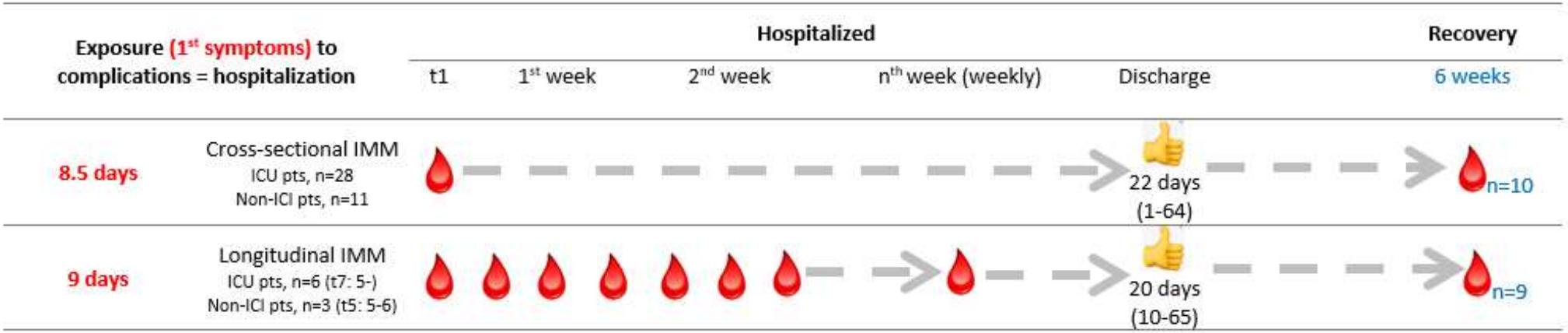
- Investigate cellular immune response in LUMC- hospitalized severe COVID-19 patients
- Integrate the data with serology, molecular biology and functional assays.

BEAT-COVID1 - Biomarker-based Early Anti-inflammatory Therapy for severe COVID-19 (ABR NL73740.058.20)

Identification of biomarkers for prediction of inflammatory processes, in support of BEAT-COVID2 clinical therapy trial

Sub-group coordinators	Study
Ton Rabelink	Functional glycocalyx assays & ECIS test
Tom Ottenhoff, Simone Joosten	Circulating cytokines by Luminex
Annemieke Geluk	Circulating cytokines by UCP LFA (rapid test)
Jutte de Vries, Mariet Feltkamp	SARS-CoV2 specific serology
Eric Snijder, Marjolein Kikkert	SARS-CoV-2 specific neutralization
Ron Hokke, Manfred Wuhrer	Ab glycosylation, serum glycome, α-glycan Abs
Menno Huisman	Coagulation activation parameters
Ton Rabelink	Glycocalyx destruction and heparanase activity
Hermelijn Smits	Stimulation assays
Jacques van Dongen, Frank Staal, Christa Cobbaert	Circulating cellular immune responses, with special attention for the blood B-cell system
Jutte de Vries	SARS-CoV2 viral load in upper airway

EXPERIMENTAL SETUP



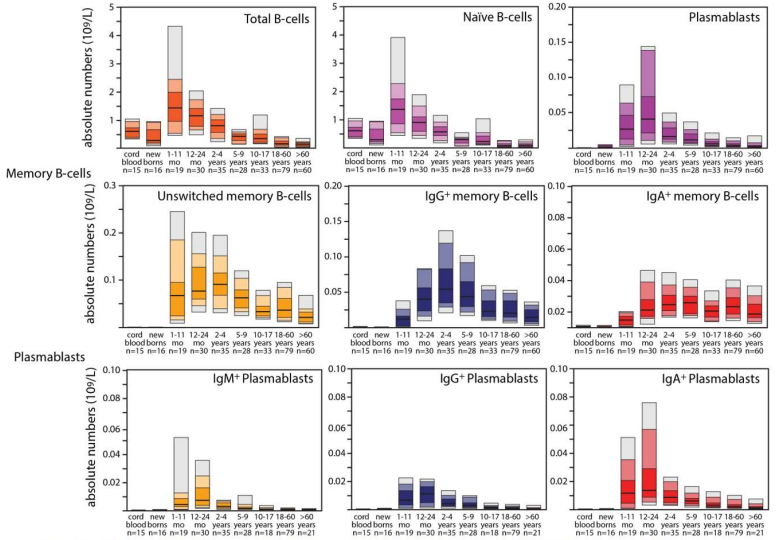
Study group

- **48 patients** included for cellular immune status evaluation (average 62 y)
- 34 ICU patients
- 14 patients from the COVID wards (non-ICU)
- 9 patients - enrolled for a longitudinal immunmonitoring
- 12 healthy controls (>60 y)

Readouts:

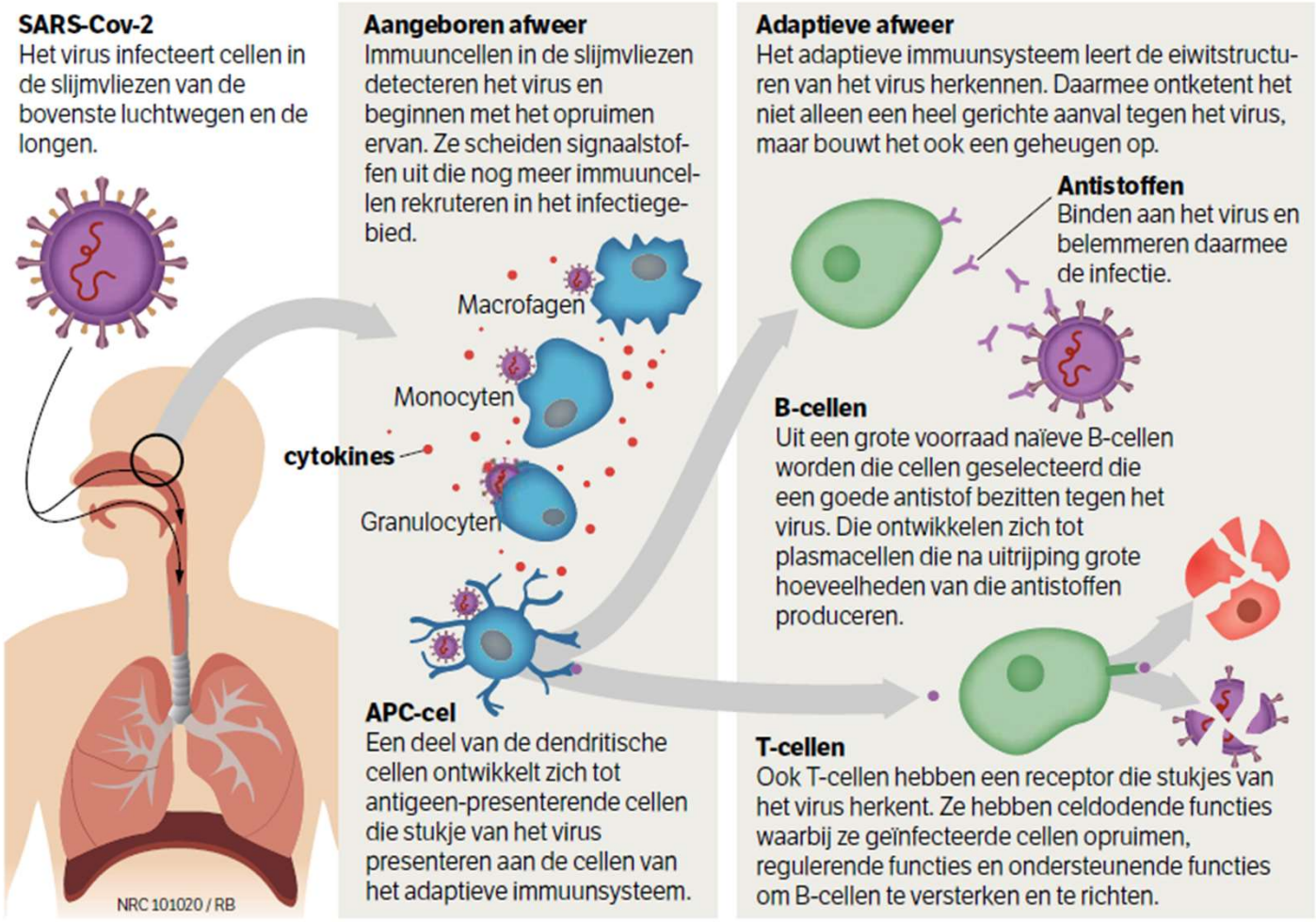
- Flow cytometry of > 250 circulating immune cell subsets
- Stored serum, plasma, PBMCs for further research

Age-related reference values of blood B-cell subsets



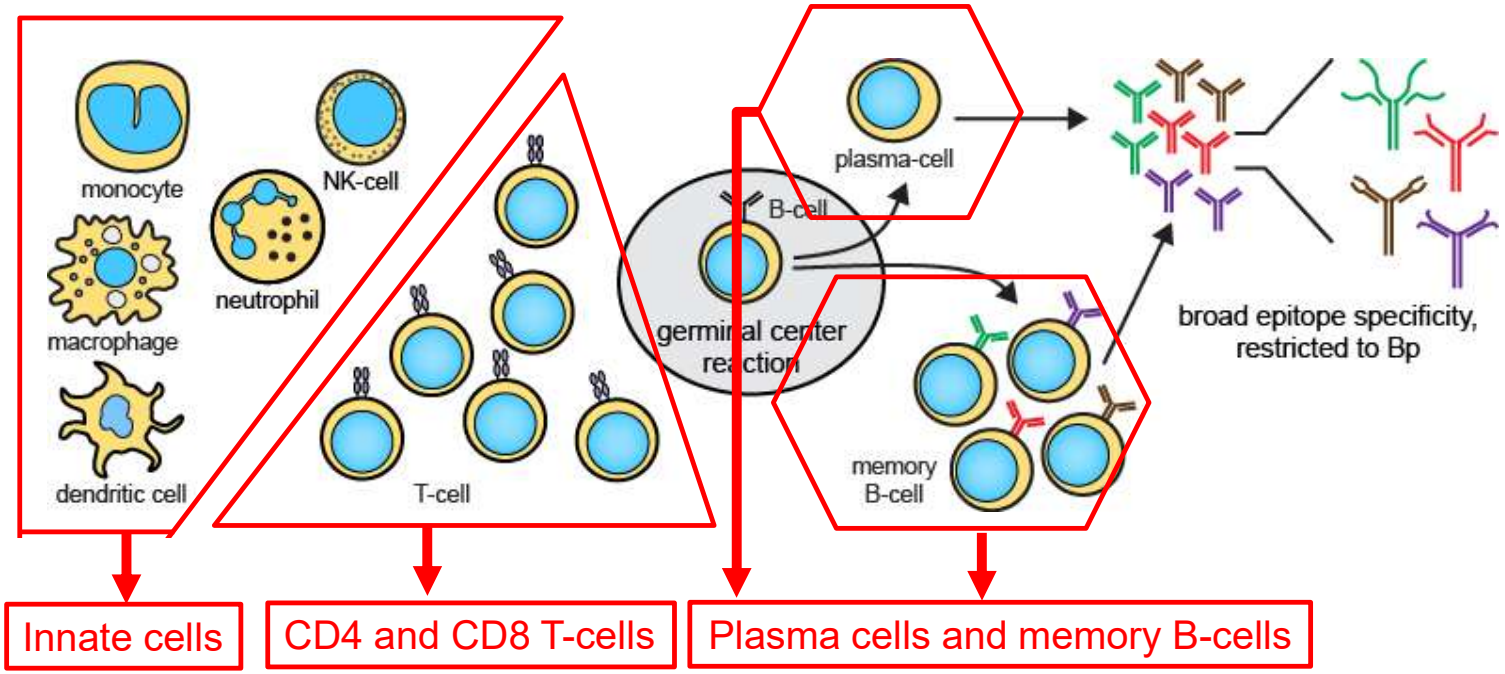
EuroFlow PID report, J.J.M. van Dongen et al. Frontiers Immunol 2019;10, article 1271

De immunologische afweer tegen virussen in een notendop



NRC 10-10-2020

Immune response monitoring



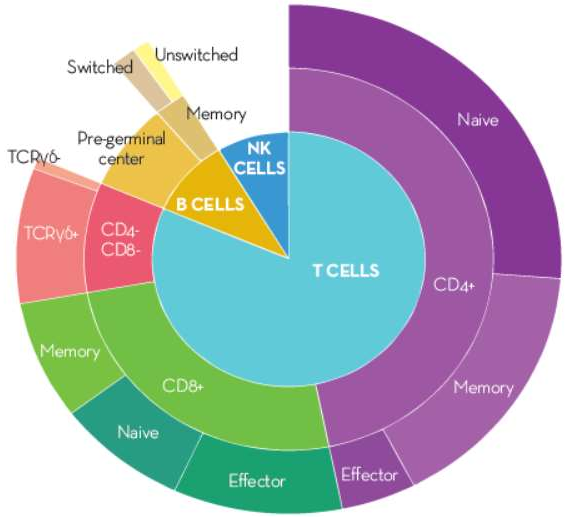
EuroFlow-based next generation flowcytometry in four 14-color tubes:

- 14-color monocyte-macrophage and dendritic cells (DC): **completed: ~22 subsets**
- 14-color CD4+ T-cell populations: **completed: ~85 subsets**
- 14-color CD8+ T-cell subsets and NK-cells: **completed: ~50 subsets**
- 14-color Immature, Memory B-cells, plasma cells, IGH isotypes: **ready: ~115 subsets**

PIDOT Tube – 8 colors, 12 markers

BV421	BV510	FITC	PE	PerCPCy5.5	PECy7	APC	APCH7
CD27	CD45RA	CD8 and SmIgD	CD16 and CD56	CD4 and SmIgM	CD19 and TCRγδ	CD3	CD45

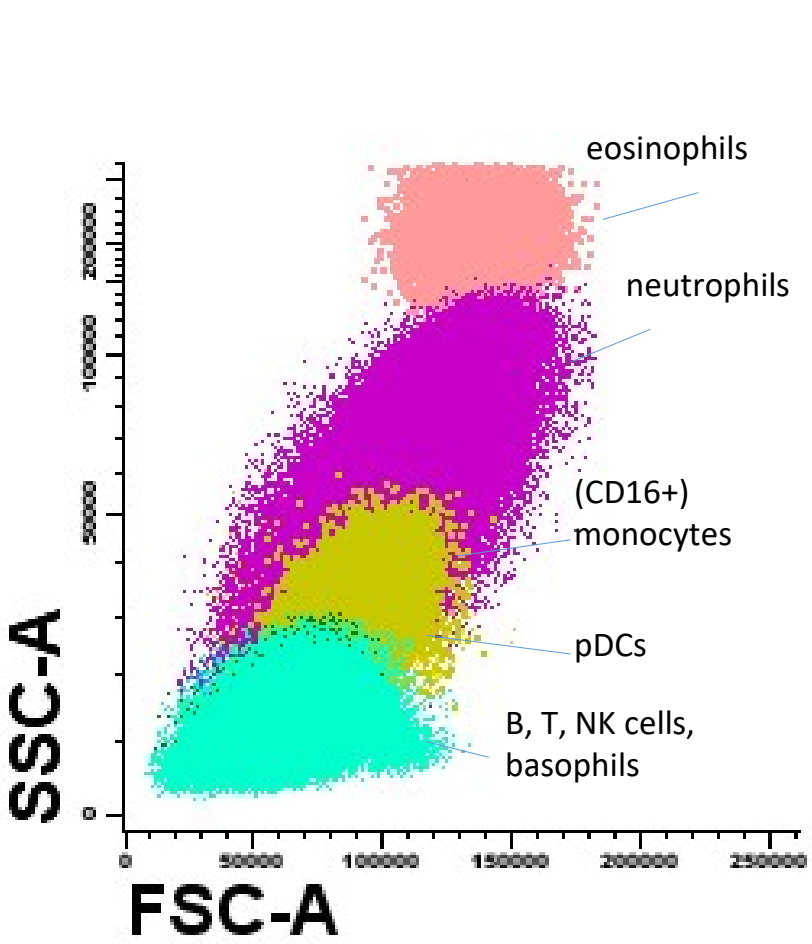
BV605
CD38

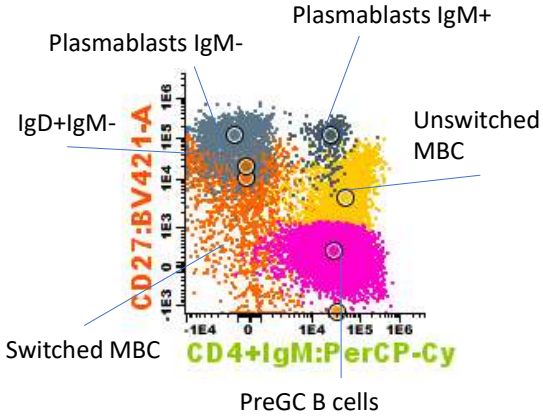


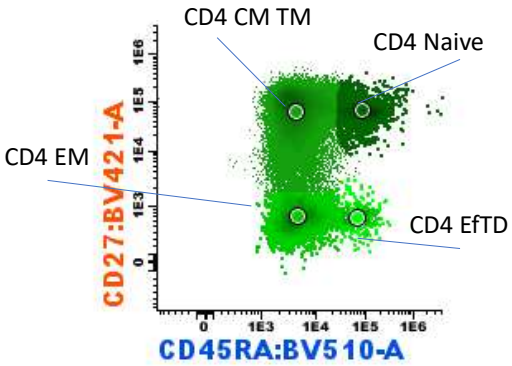
The EuroFlow PID Orientation Tube for Flow Cytometric Diagnostic Screening of Primary Immunodeficiencies of the Lymphoid System

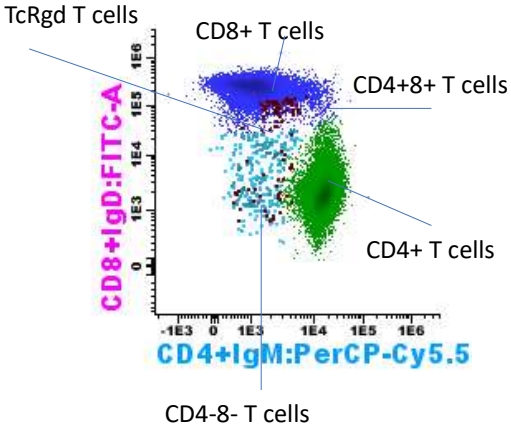
Mirjam van der Burg,[1*] Tomas Kalina,[2*] Martin Perez-Andres,[3*] Marcela Vlkova,[4] Eduardo Lopez-Granados,[5] Elena Blanco, Carolien Bonroy, Ana E. Sousa, Anne-Kathrin Kienzler,[6] Marjolein Wentink,[1] Ester Mejstříková,[2] Vendula Šinkorova[2], Jan Stcuhly,[2] Menno van Zelm,[7] Alberto Orfao,[3] Jacques J.M. van Dongen[8] on behalf of the EuroFlow PID consortium

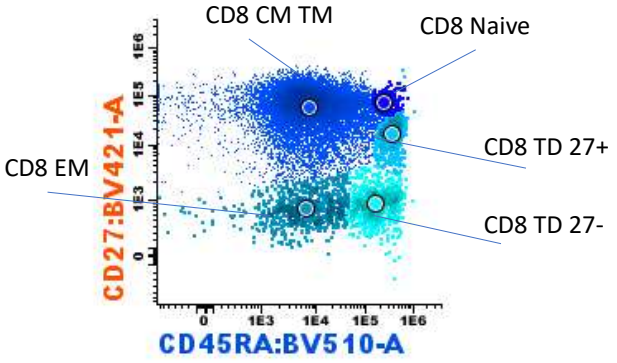
Frontiers in Immunology, March 2019


eosinophils
neutrophils
(CD16+)
monocytes
pDCs
B, T, NK cells,
basophils
SSC-A
FSC-A
0
50000
100000
150000
200000
250000

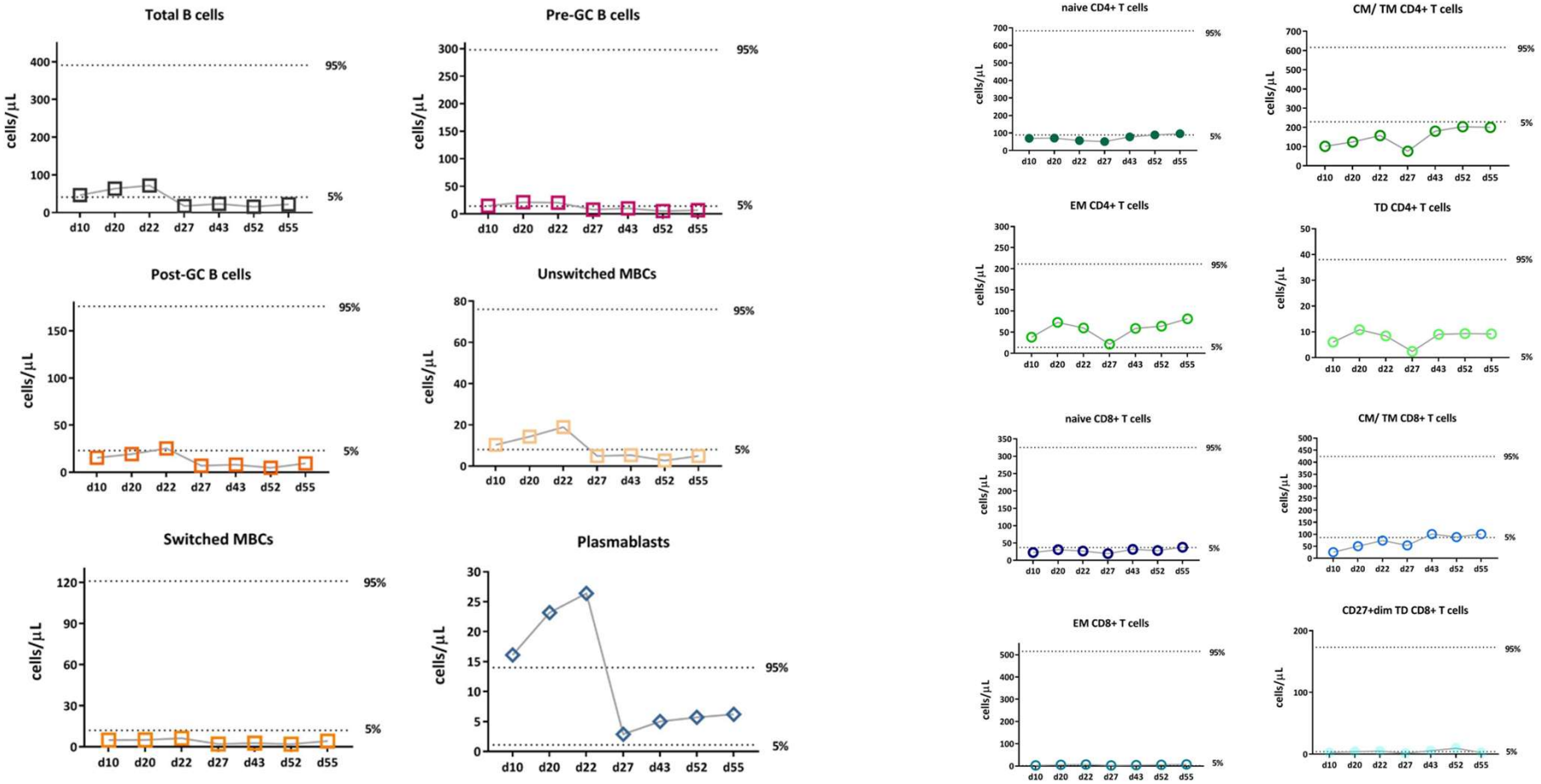

Plasmablasts IgM+
Plasmablasts IgM-
IgD+IgM-
Unswitched
MBC
Switched MBC
PreGC B cells
CD27:BV421-A
CD4+IgM:PerCP-Cy


CD4 CM TM
CD4 Naive
CD4 EM
CD4 EfTD
CD27:BV421-A
CD45RA:BV510-A


TcRgd T cells
CD8+ T cells
CD4+8+ T cells
CD4+ T cells
CD4-8- T cells
CD8+IgD:FITC-A
CD4+IgM:PerCP-Cy5.5


CD8 CM TM
CD8 Naive
CD8 EM
CD8 TD 27+
CD8 TD 27-
CD27:BV421-A
CD45RA:BV510-A

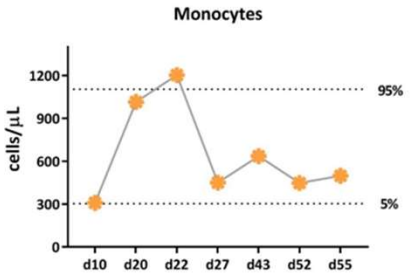
Longitudinal data: B and T cell compartment – Patient example

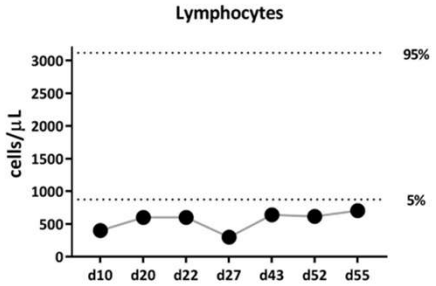


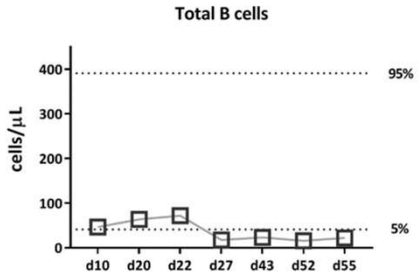
Detailed immune monitoring of a pregnant woman with critical Covid-19

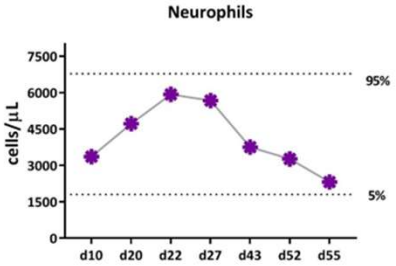
NAdu Fossé[a]KBronsgeest[a]MSArbous[b]MZlei[c]SKMyeni[d]MKikkert[b]JJMvan Dongen[c]FJTStaal[c]MLPvan der Hoorn[a]Tvan den Akker[a]

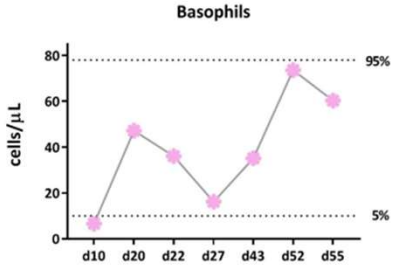
Longitudinal data: B and T cell compartment – Patient example

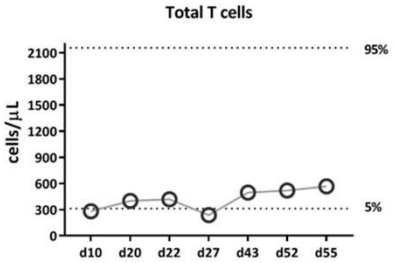


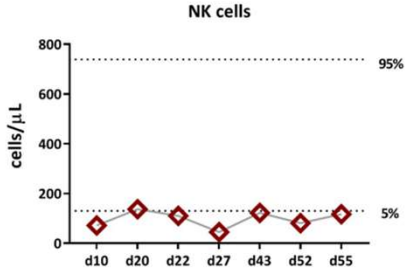


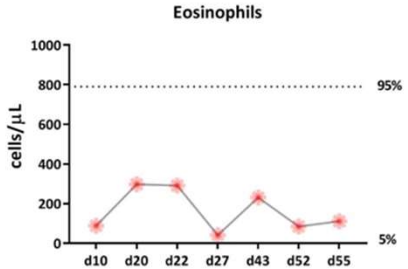












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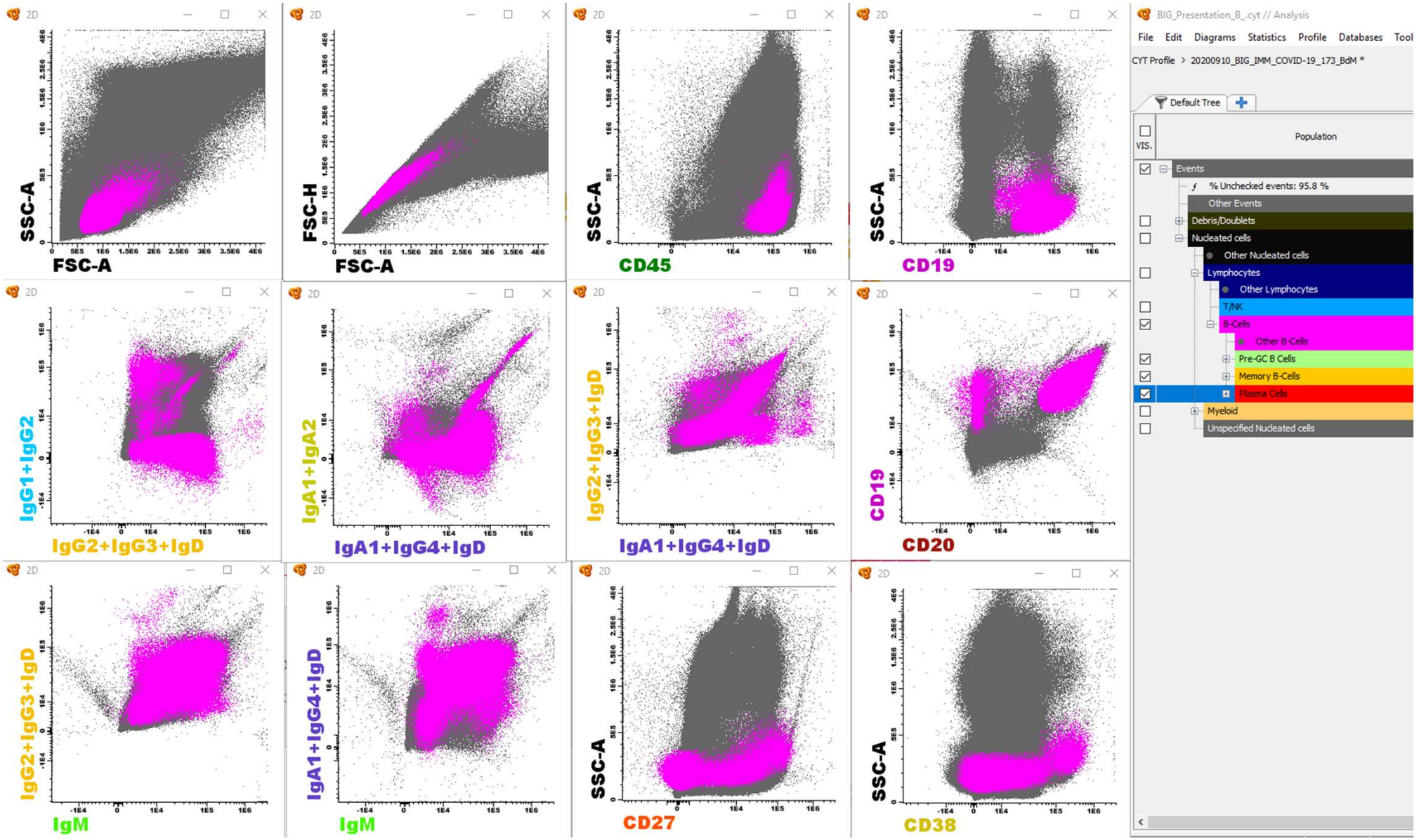
2D
SSC-A
FSC-A
2D
FSC-H
FSC-A
2D
SSC-A
CD45
2D
SSC-A
CD19
BIG_Presentation_B_.cyt // Analysis
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CYT Profile > 20200910_BIG_IMM_COVID-19_173_BdM *
Default Tree
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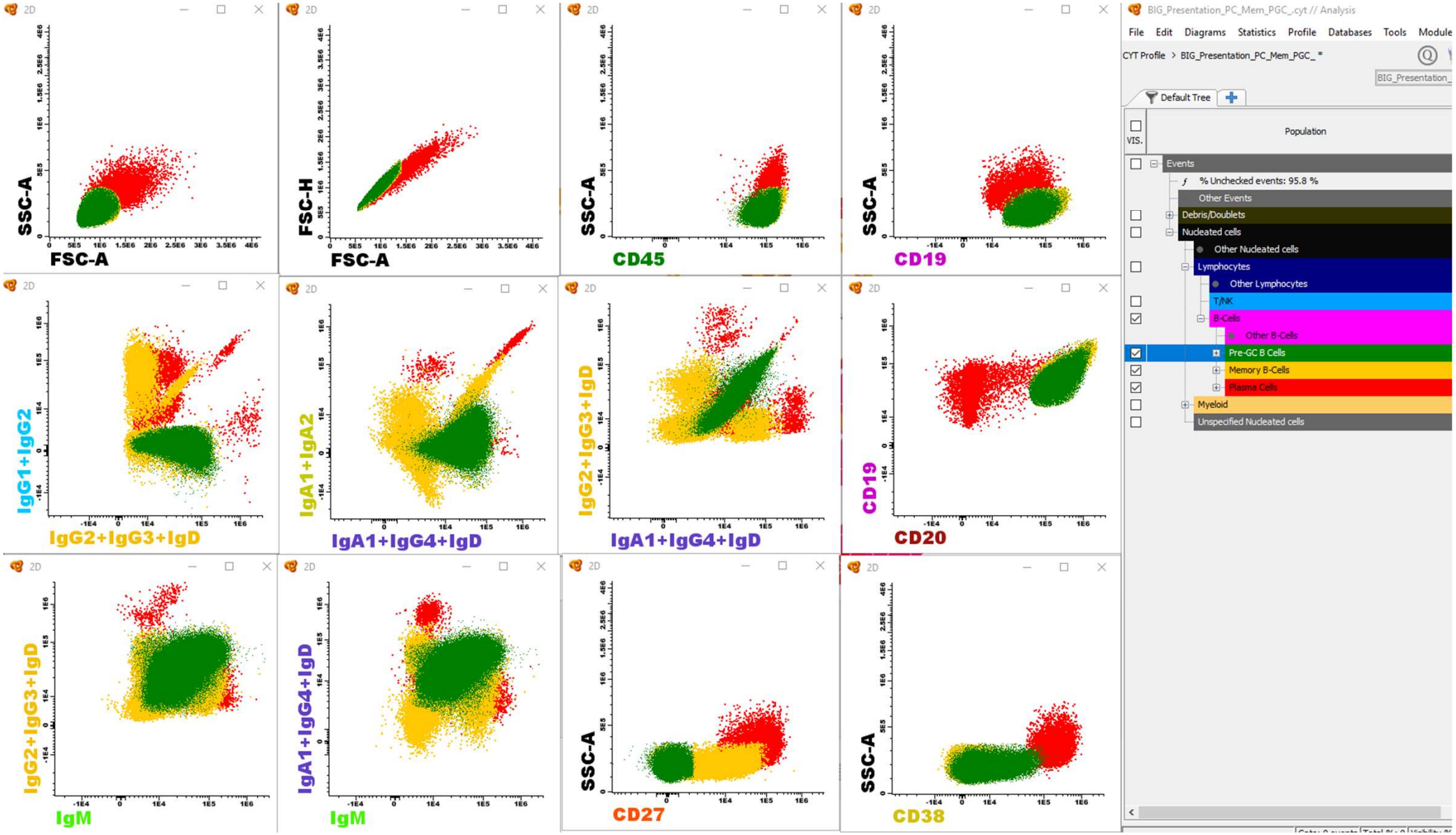
Population
Events
% Unchecked events: 95.8 %
Other Events
Debris/Doublets
Nucleated cells
Other Nucleated cells
Lymphocytes
Other Lymphocytes
T/NK
B-Cells
Other B-Cells
Pre-GC B Cells
Memory B-Cells
Plasma Cells
Myeloid
Unspecified Nucleated cells
2D
IgG1+IgG2
IgG2+IgG3+IgD
2D
IgA1+IgA2
IgA1+IgG4+IgD
2D
IgG2+IgG3+IgD
IgA1+IgG4+IgD
2D
CD19
CD20
2D
IgG2+IgG3+IgD
IgM
2D
IgA1+IgG4+IgD
IgM
2D
SSC-A
CD27
2D
SSC-A
CD38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CD19
CD20
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IgM
2D
IgA1+IgG4+IgD
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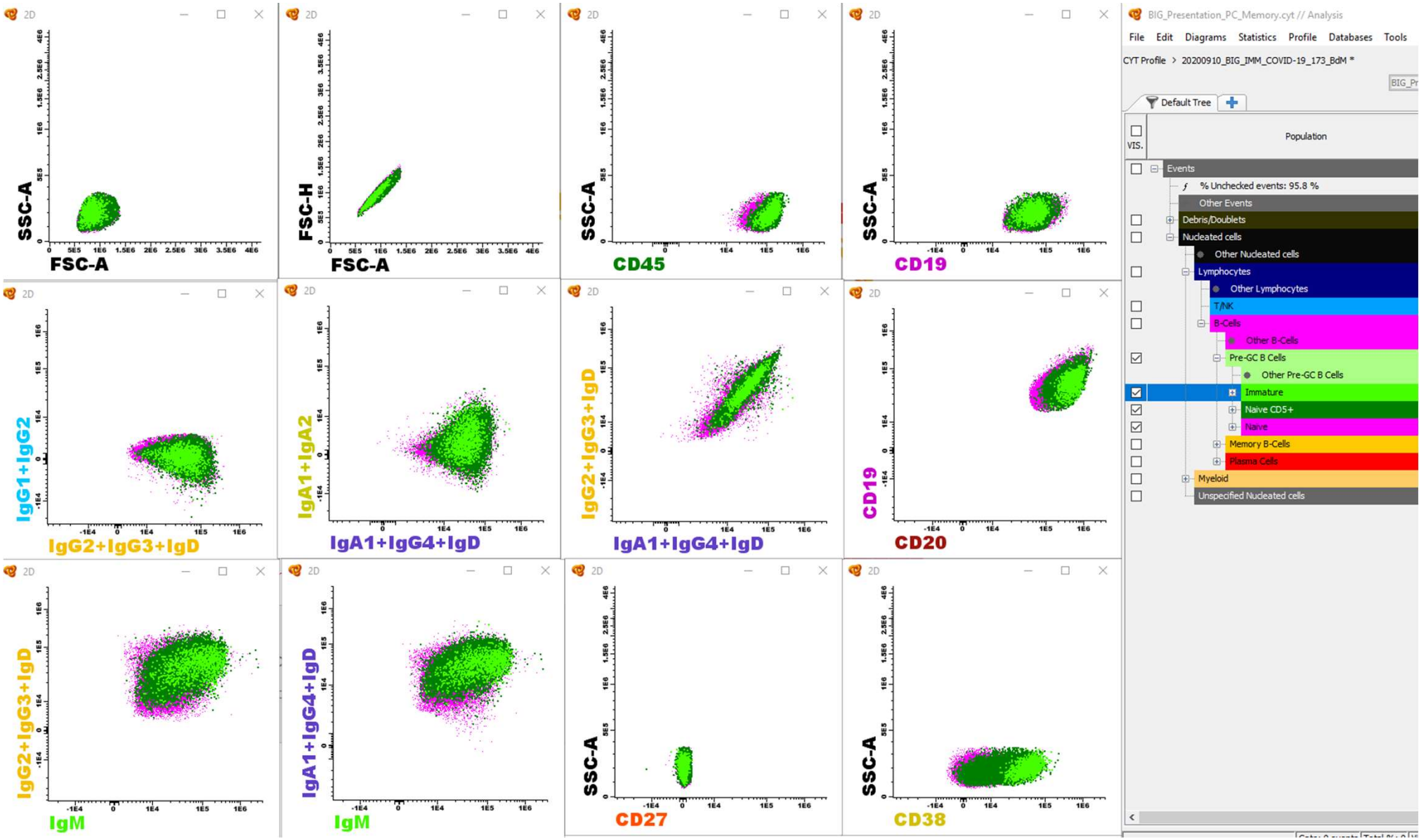
2D
SSC-A
CD27
2D
SSC-A
CD38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BIG_Presentation_PC_Memory.cyt // Analysis
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Other Pre-GC B Cells
Immature
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Naive
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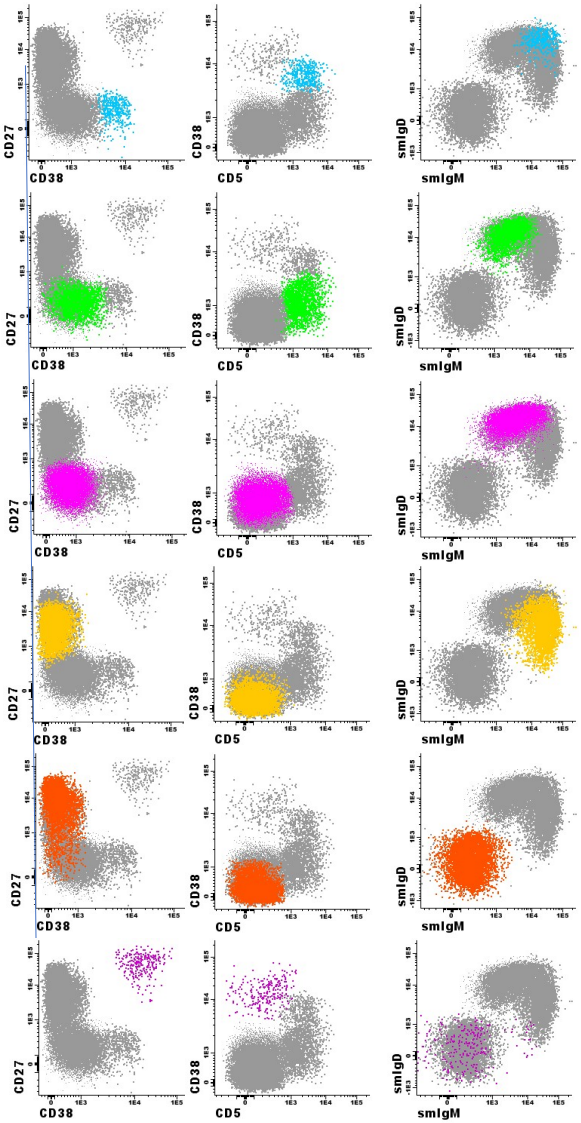
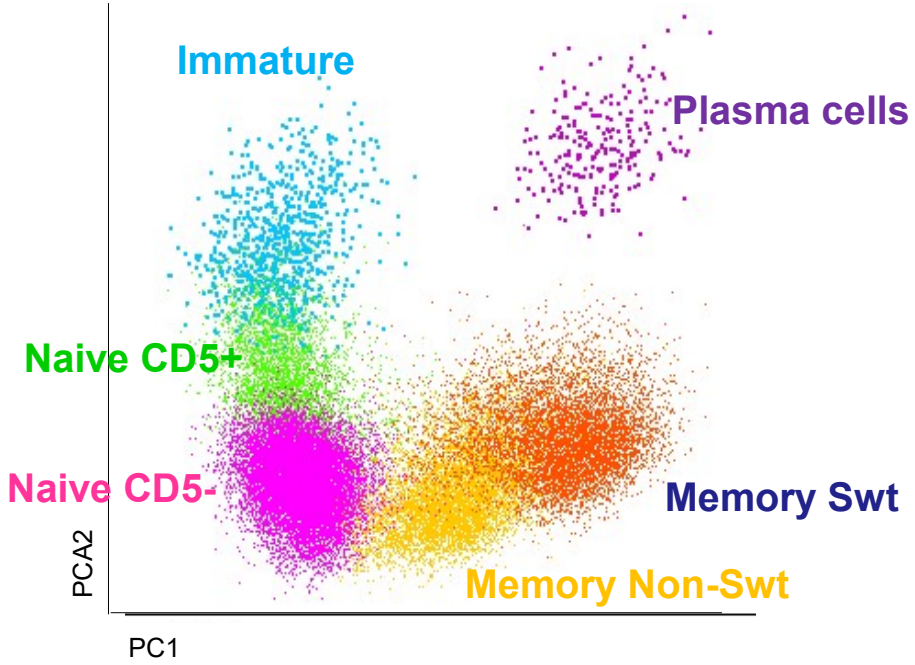
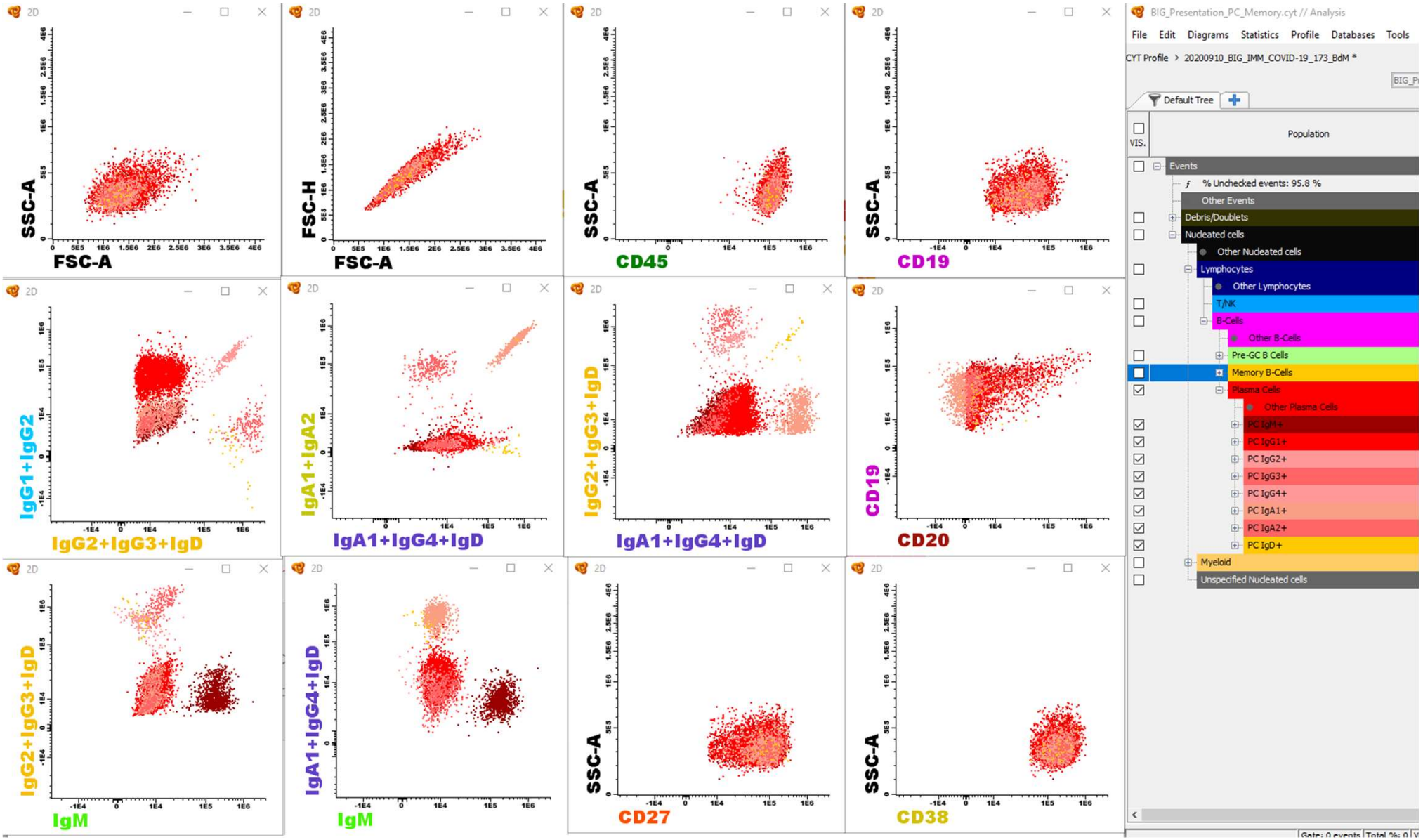


Identification of blood B-cell subsets in APS view

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PC IgG3+
PC IgG4+
PC IgA1+
PC IgA2+
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CD19
CD20
2D
IgG2+IgG3+IgD
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CD27
2D
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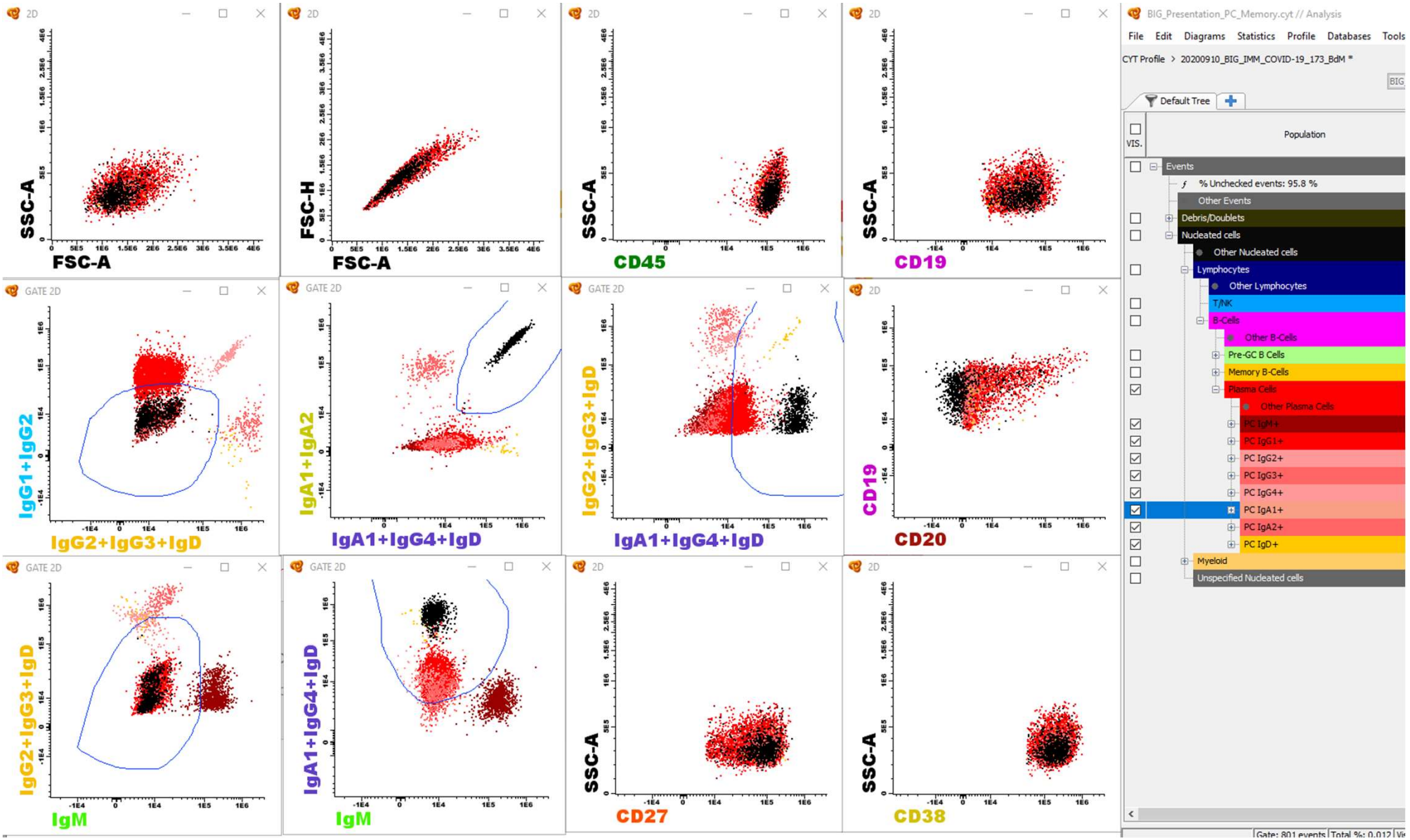
CD38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PC IgG2+
PC IgG3+
PC IgG4+
PC IgA1+
PC IgA2+
PC IgD+
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GATE 2D
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IgA1+IgG4+IgD
GATE 2D
IgG2+IgG3+IgD
IgA1+IgG4+IgD
2D
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CD20
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IgG2+IgG3+IgD
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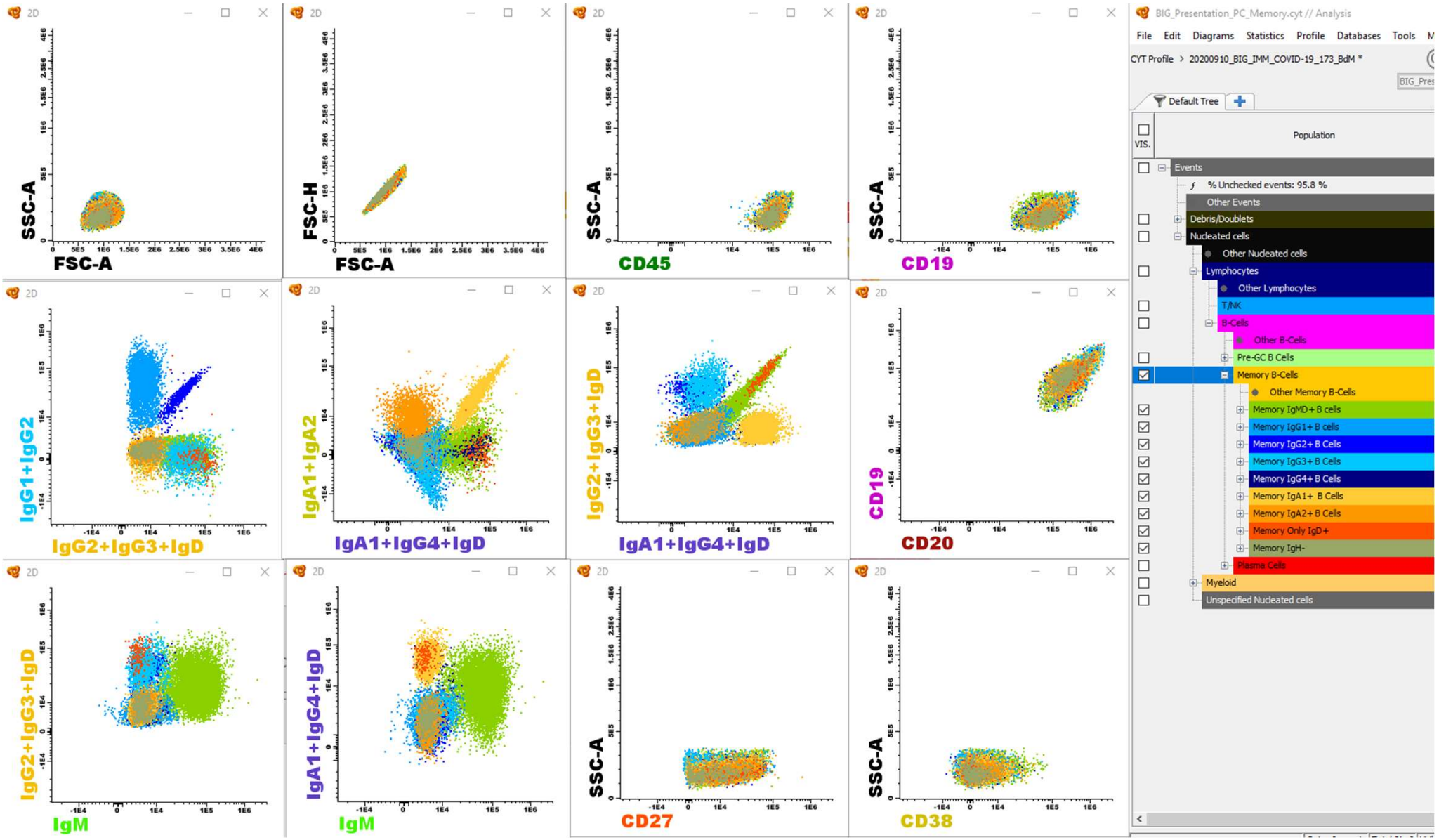
SSC-A
CD38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FSC-A
FSC-H
FSC-A
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CD45
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IgG2+IgG3+IgD
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IgA1+IgG4+IgD
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Memory B-Cells
Other Memory B-Cells
Memory IgMD+ B cells
Memory IgG1+ B cells
Memory IgG2+ B Cells
Memory IgG3+ B Cells
Memory IgG4+ B Cells
Memory IgA1+ B Cells
Memory IgA2+ B Cells
Memory Only IgD+
Memory IgH-
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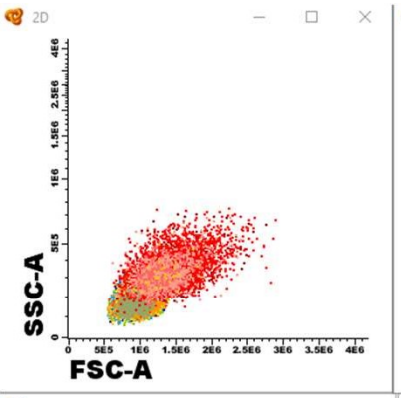
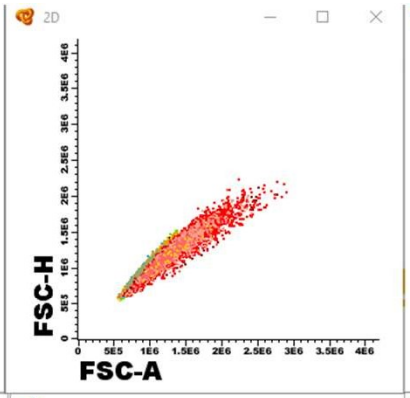
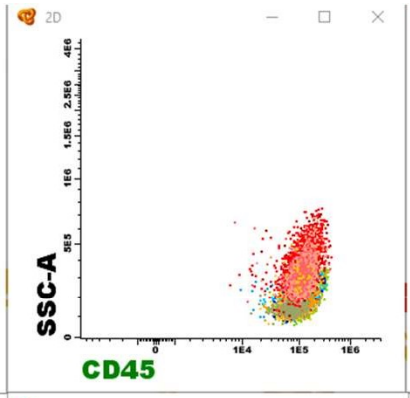
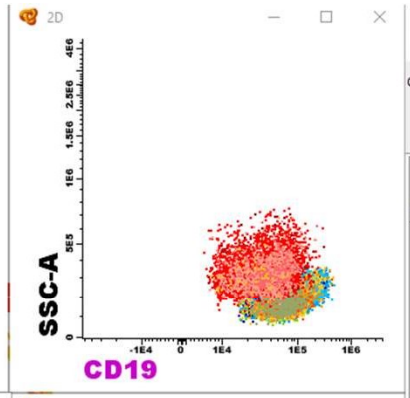
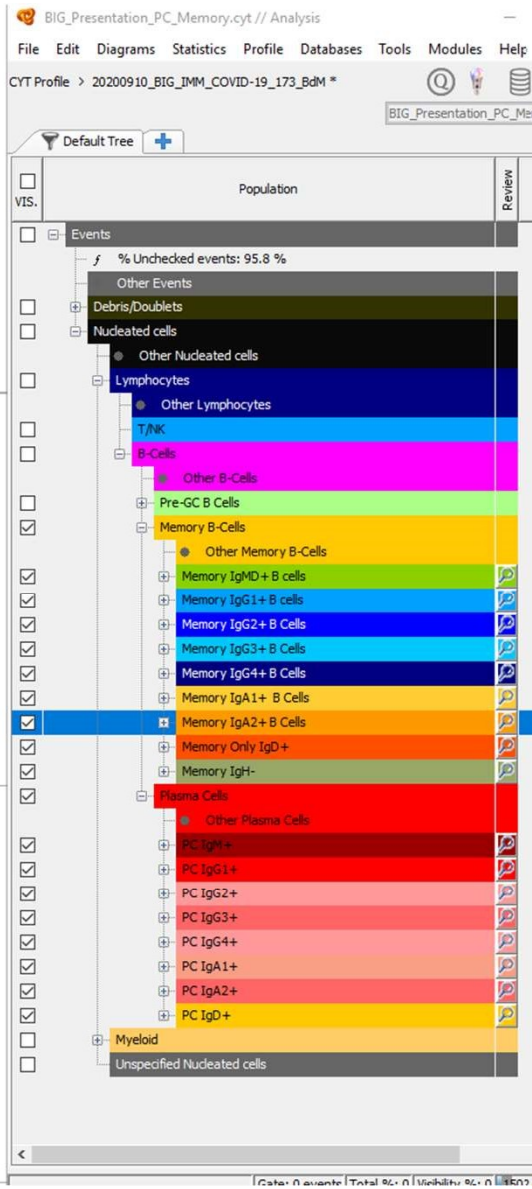
Myeloid
Unspecified Nucleated cells

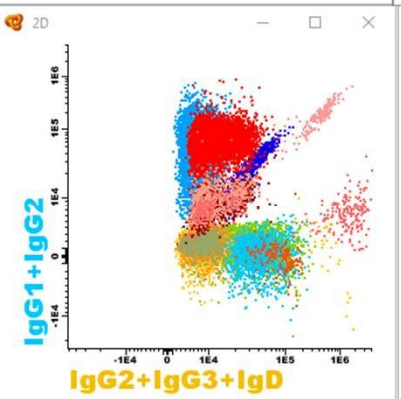

SSC-A
FSC-A

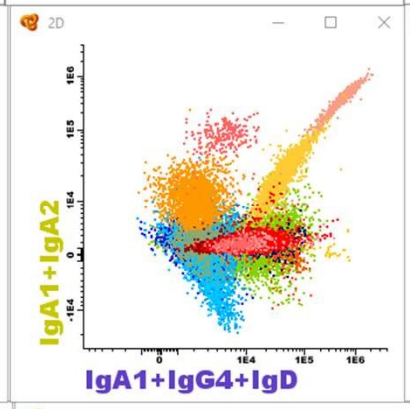

FSC-H
FSC-A

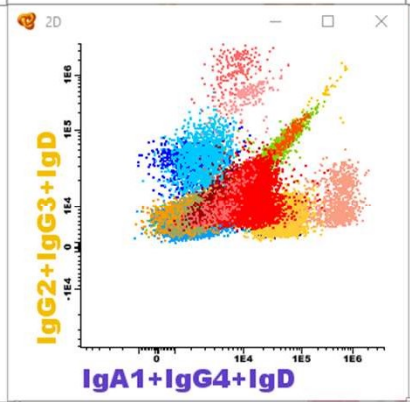

SSC-A
CD45

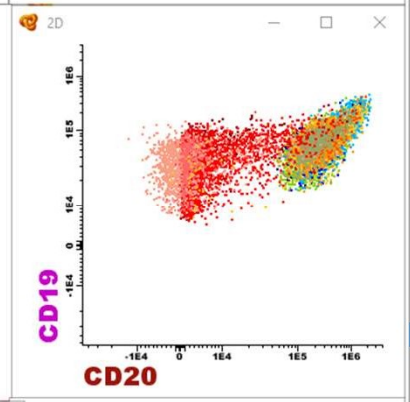

SSC-A
CD19

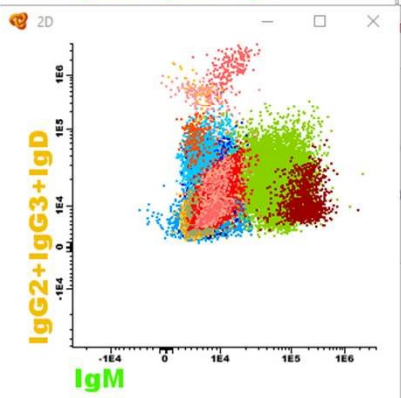

BIG_Presentation_PC_Memory.cyt // Analysis
File Edit Diagrams Statistics Profile Databases Tools Modules Help
CYT Profile > 20200910_BIG_IMM_COVID-19_173_BdM *
Default Tree
VIS.
Population
Review
Events
% Unchecked events: 95.8 %
Other Events
Debris/Doublets
Nucleated cells
Other Nucleated cells
Lymphocytes
Other Lymphocytes
T/NK
B-Cells
Other B-Cells
Pre-GC B Cells
Memory B-Cells
Other Memory B-Cells
Memory IgMD+ B cells
Memory IgG1+ B cells
Memory IgG2+ B Cells
Memory IgG3+ B Cells
Memory IgG4+ B Cells
Memory IgA1+ B Cells
Memory IgA2+ B Cells
Memory Only IgD+
Memory IgH-
Plasma Cells
Other Plasma Cells
PC IgM+
PC IgG1+
PC IgG2+
PC IgG3+
PC IgG4+
PC IgA1+
PC IgA2+
PC IgD+
Myeloid
Unspecified Nucleated cells

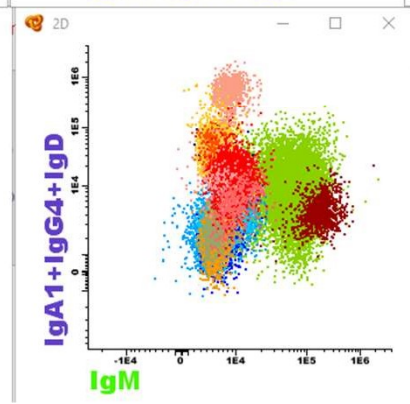

IgG1+IgG2
IgG2+IgG3+IgD

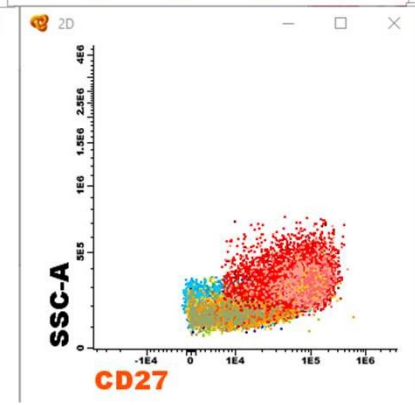

IgA1+IgA2
IgA1+IgG4+IgD

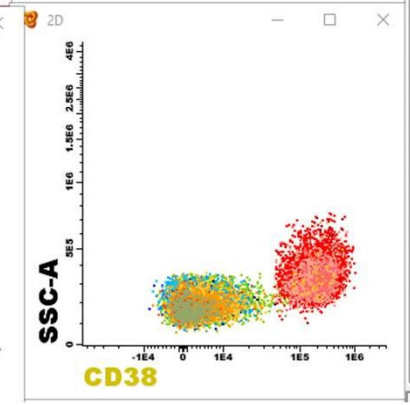

IgG2+IgG3+IgD
IgA1+IgG4+IgD


CD19
CD20

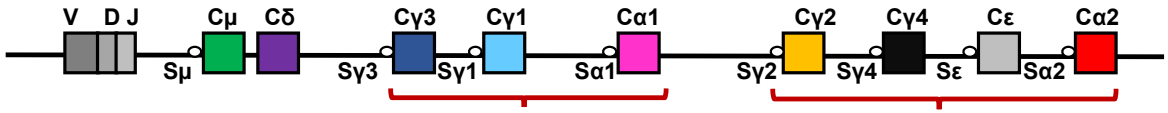

IgG2+IgG3+IgD
IgM


IgA1+IgG4+IgD
IgM

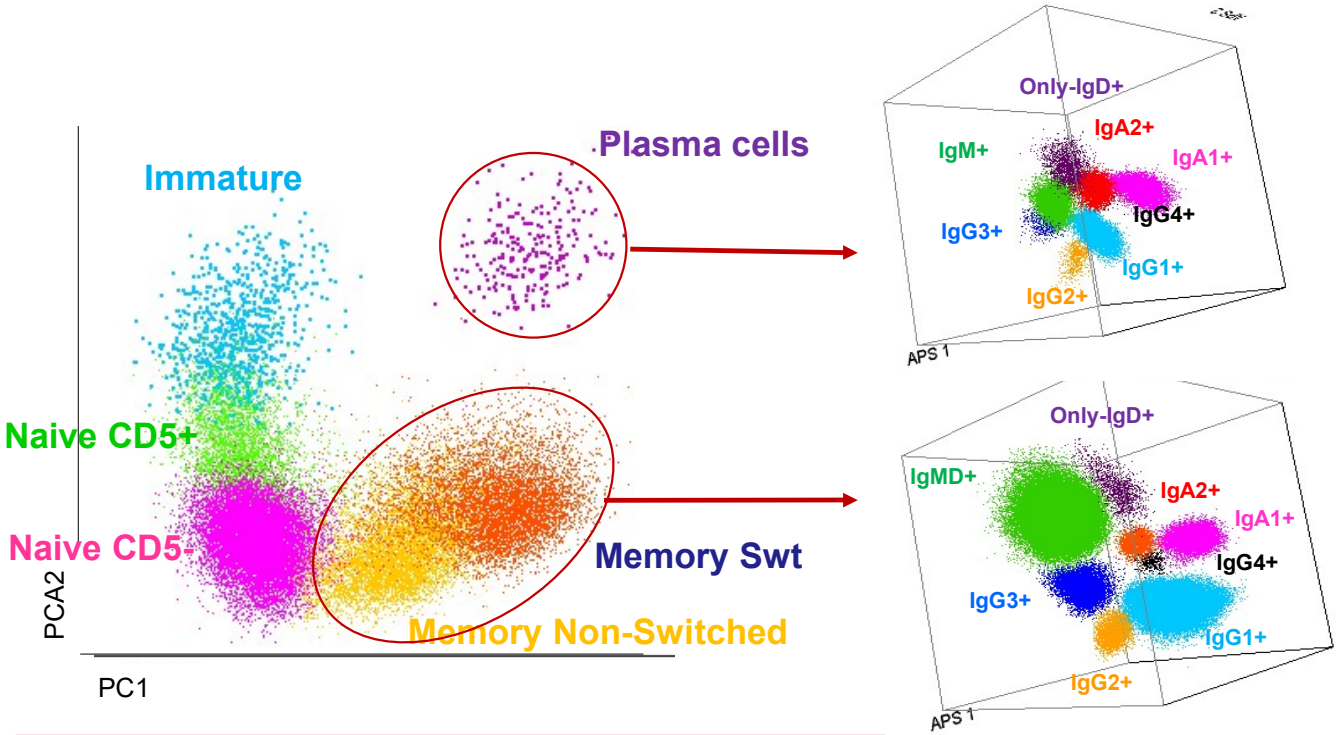

SSC-A
CD27


SSC-A
CD38

IGH isotypes



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

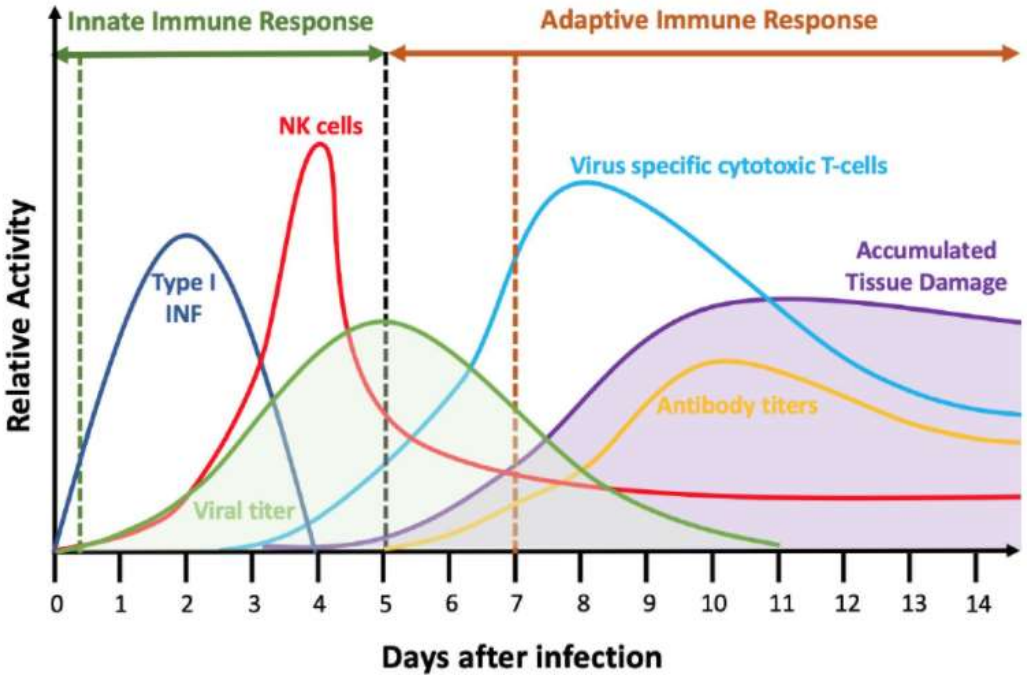


14 colour- combination → ~115 populations

Elena Blanco, Martin Perez, Alberto Orfao, et al. JACI, 2018

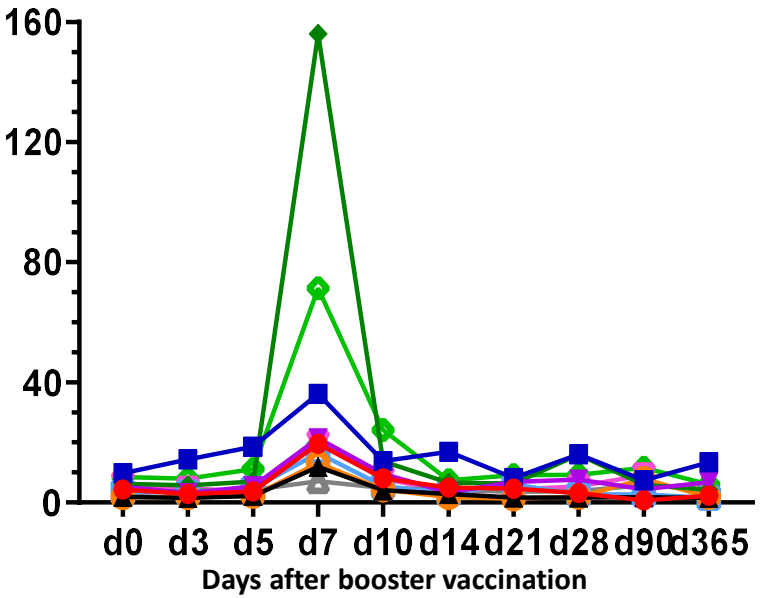
EuroFlow

Model of viral infection in epithelial tissues



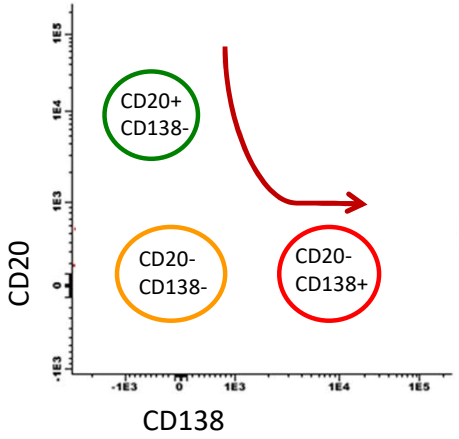
Sego TJ, et al, 2020 May 7. doi:10.1101/2020.04.27.064139

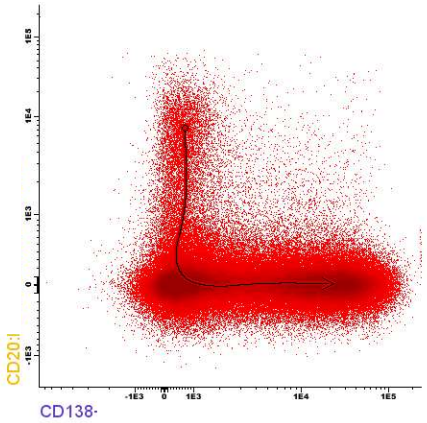
Plasma cell kinetics after booster vaccination with Bordetella pertussis

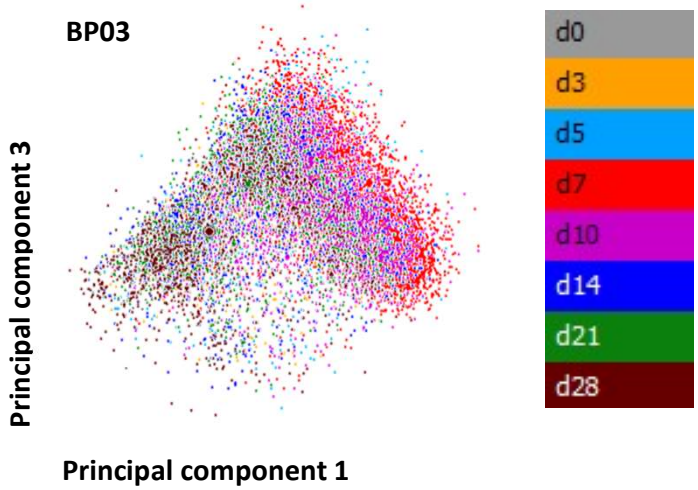


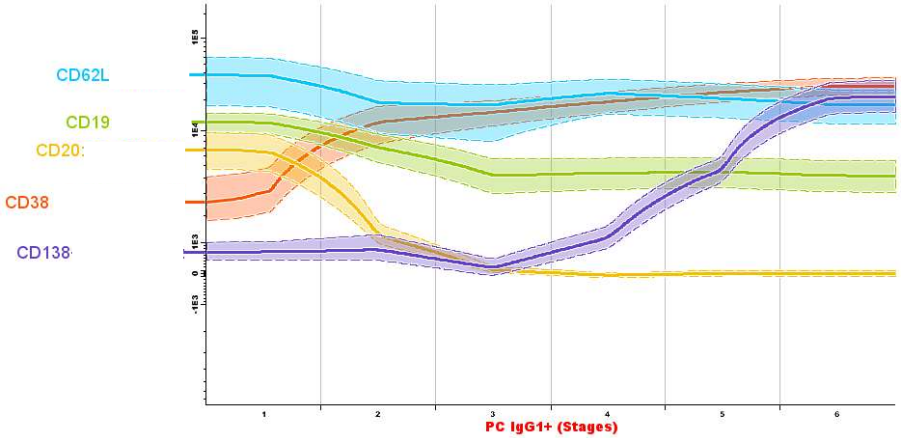
Diks et al, manuscript in preparation

PC MATURATION AFTER BOOSTER AND CHALLENGE



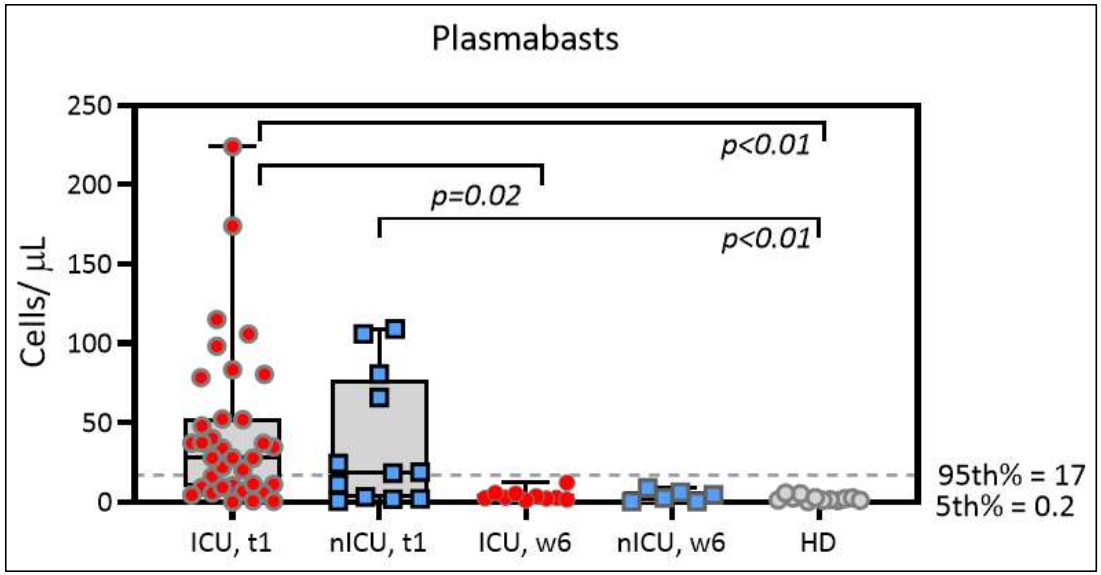




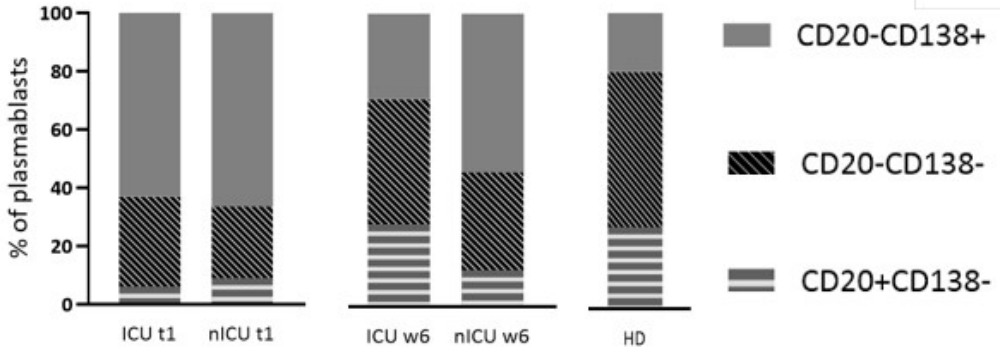


Diks et al, manuscript in preparation

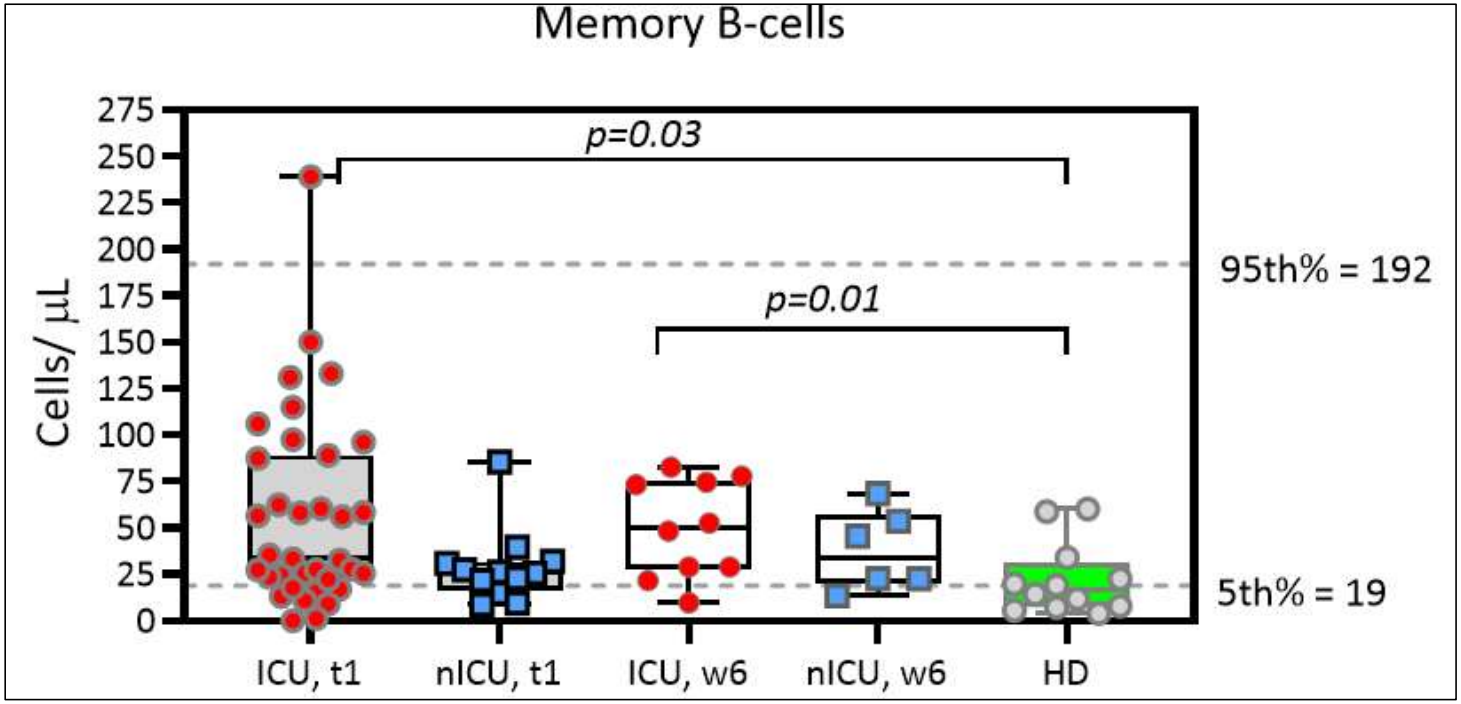
Changes in B-cell compartment, especially kinetics of the plasma cells



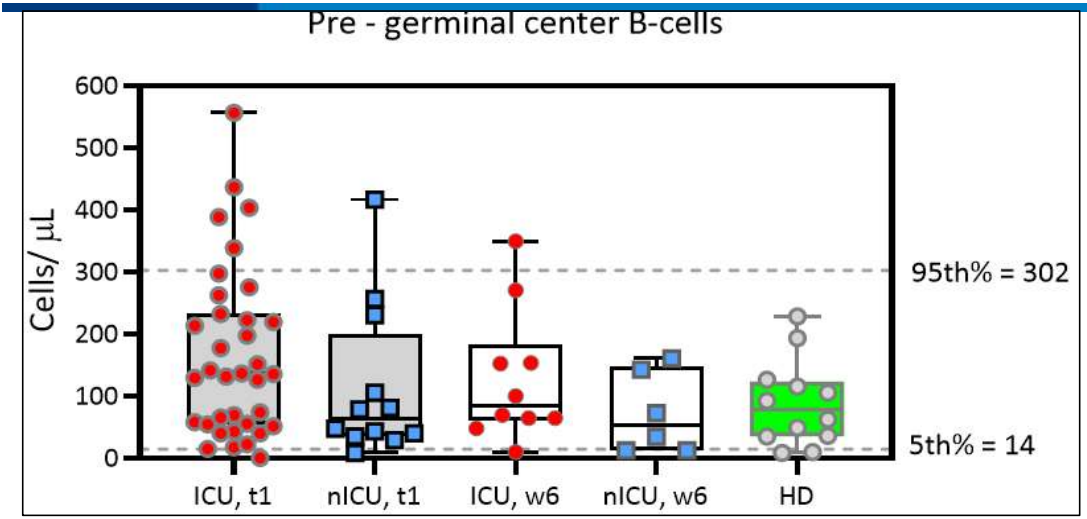
Expansion of the most mature plasma cells in the acute phase of the infection



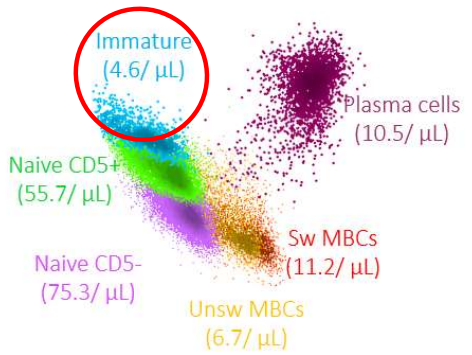
Higher levels of memory B cells in ICU patients 6 weeks after discharge than in healthy donors



Lower level of immature B cells in patients with a late recovery

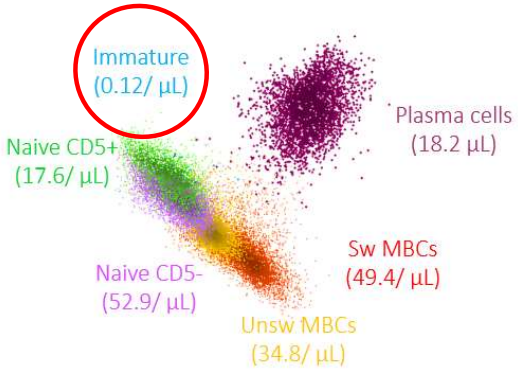


- Pt. 41 (non-ICU, 12 days in hospital)
  - 74 year old
  - day 18 from the first symptoms



B cell compartment: 164 B cells/ µL

Pt. 17 (20 days in ICU)
- 71 year old
- day 17 from the first symptoms



B cell compartment: 173 B cells/ µL

OVERVIEW

- Changes in B-cell compartment, especially kinetics of the plasma cells
- Expansion of the most mature plasma cells in the acute phase of the infection
- Higher levels of memory B cells in ICU patients 6 weeks after discharge than in healthy donors
- Lower level of immature B cells in patients with a late recovery

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EuroFlow