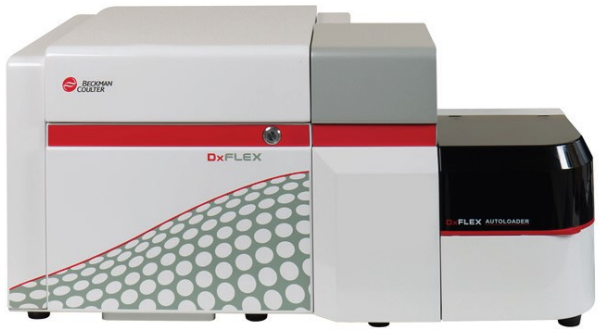
DuraClone,

CytoFLEX LS, DxFLEX, NAVIOS

ΚΑΙ

COVID 19



Μαρία Γεωργίου

LERIVA DIAGNOSTICS

PERSPECTIVE

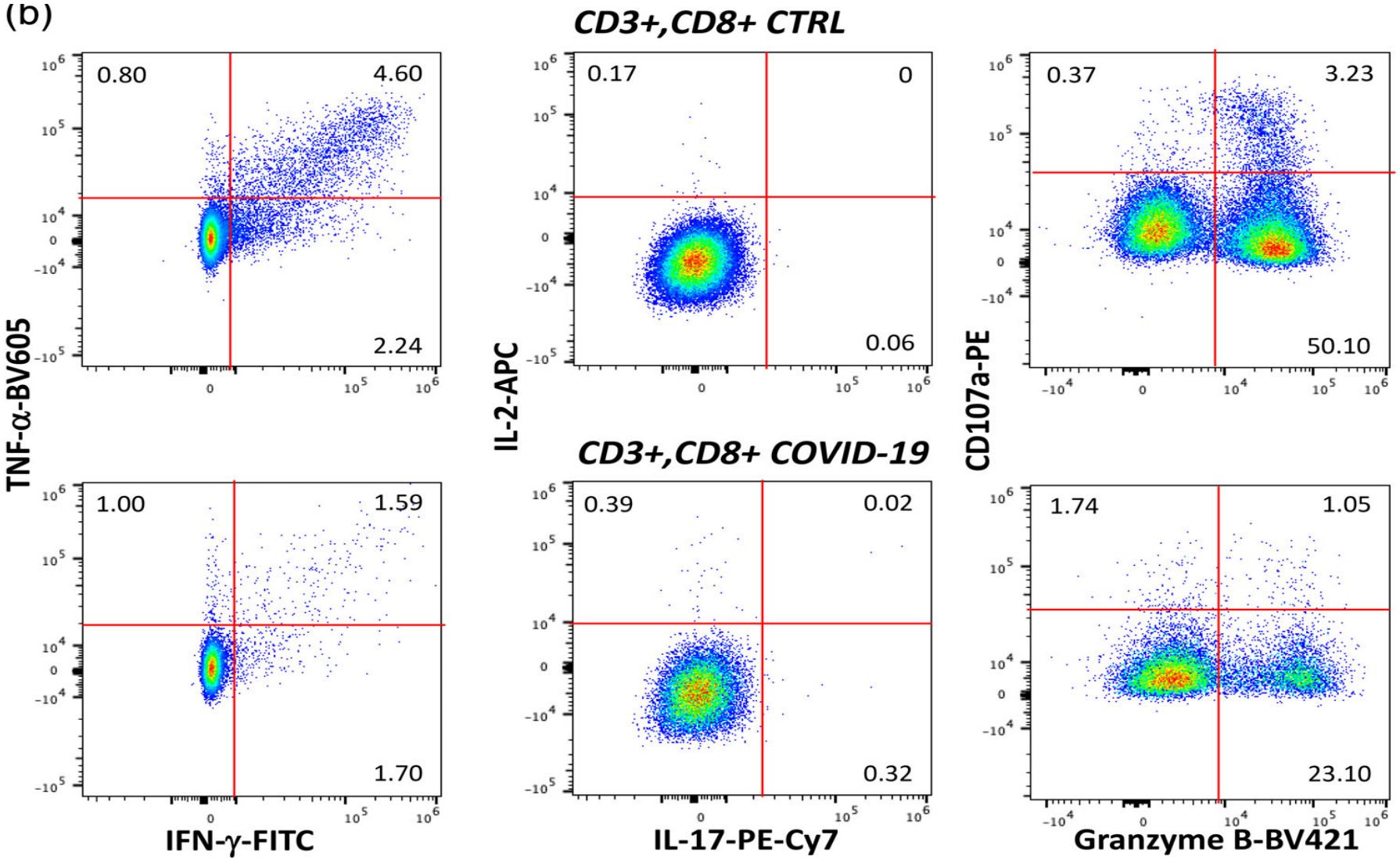
ISAC CYTOMETRY
Journal of Quantitative Cell Science PART A

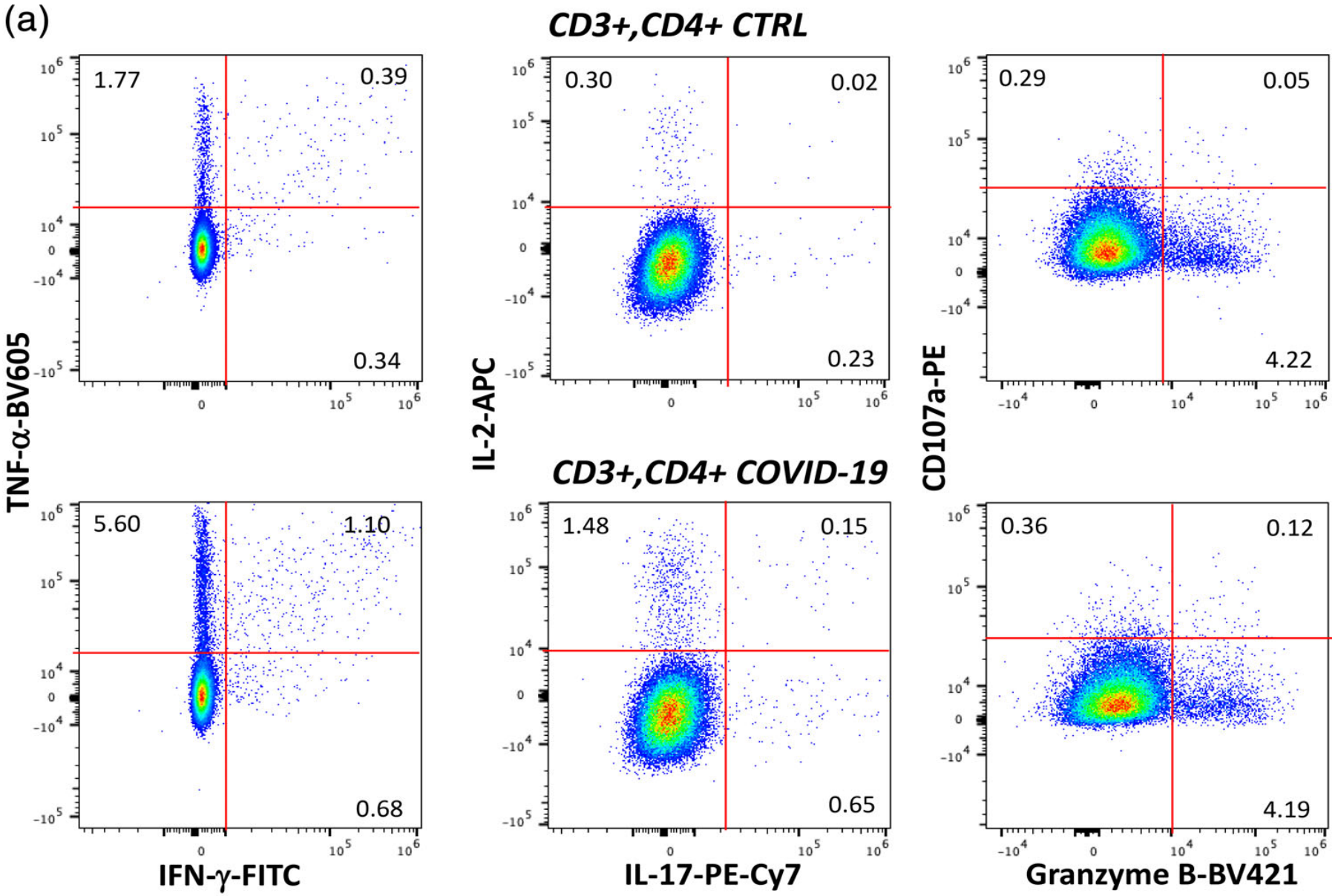
ISAC INTERNATIONAL SOCIETY FOR ADVANCEMENT OF CYTOMETRY

SARS-CoV-2, the Virus that Causes COVID-19: Cytometry and the New Challenge for Global Health

Andrea Cossarizza,[1*] Sara De Biasi,[1†] Giovanni Guaraldi,[2] Massimo Girardis,[3] Cristina Mussini,[2] for the Modena Covid-19 Working Group (MoCo19)[#]

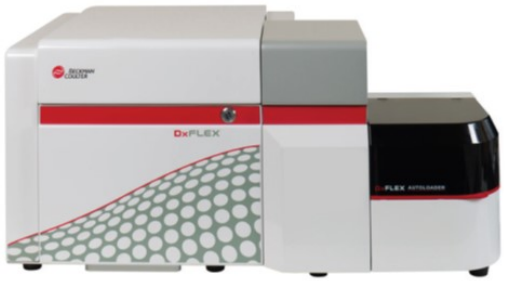
• **Key terms**
SARS-CoV-2; Covid-19; coronavirus; cytometry; CD4; CD8; T cells


(b)
CD3+,CD8+ CTRL
CD3+,CD8+ COVID-19
TNF-α-BV605
IFN-γ-FITC
IL-2-APC
IL-17-PE-Cy7
CD107a-PE
Granzyme B-BV421
0.80
4.60
2.24
0.17
0
0.06
0.37
3.23
50.10
1.00
1.59
1.70
0.39
0.02
0.32
1.74
1.05
23.10


(a)
CD3+,CD4+ CTRL
CD3+,CD4+ COVID-19
1.77
0.39
0.34
0.30
0.02
0.23
0.29
0.05
4.22
5.60
1.10
0.68
1.48
0.15
0.65
0.36
0.12
4.19
TNF-α-BV605
IL-2-APC
CD107a-PE
IFN-γ-FITC
IL-17-PE-Cy7
Granzyme B-BV421

DURAClone* & DURActive*
Product Families

	405 excitation		480 excitation					633 excitation		
	Pacific Blue	Krome Orange	FITC	PE	ECD	PC5.5	PC7	APC	APC-A700	APC-A750
DURAClone IF T Helper Cell	IL-17		IFN-γ				IL-4	CD4		CD3
DURAClone IF T Activation Tube	CD4		IFN-γ	TNFα			IL-2		CD8	CD3
DURAClone IM Treg Tube	Helios	CD45	CD45RA	CD25		CD39	CD4	FoxP3		CD3
DURAClone IM T cell subsets	CD57	CD45	CD45RA	CD197	CD28	CD279	CD27	CD4	CD8	CD3

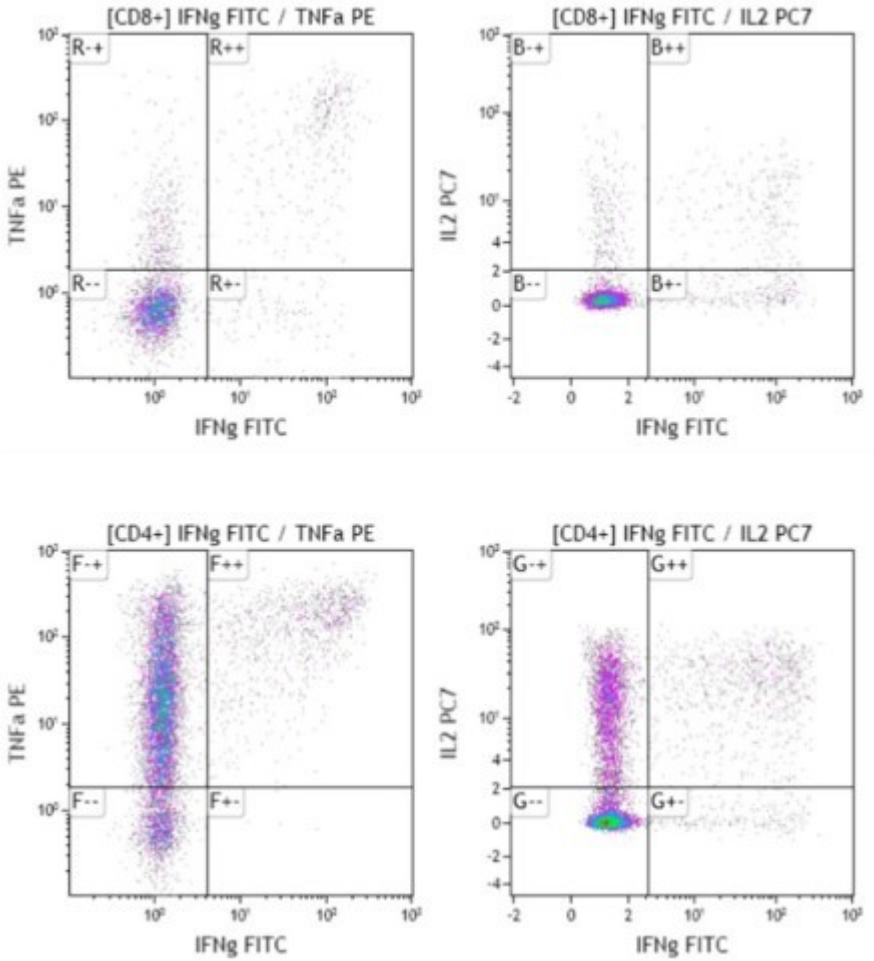


DURAClone* & DURActive* Product Families

DURActive 1 25 tests RUO C11101
Dry Stimulation Mixes for Immune Functional Assays
PMA, Ionomycin, Brefeldin A

DURActive 2 25 tests RUO C11102
Dry Stimulation Mixes for Immune Functional Assays
PMA, Ionomycin

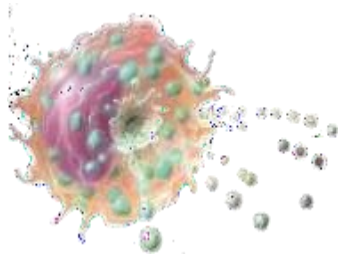
DURActive 3 25 tests RUO C21857
Dry Stimulation Mixes for Immune Functional Assays
LPS, Brefeldin A



REVIEW ARTICLE

ISAC INTERNATIONAL SOCIETY FOR ADVANCEMENT OF CYTOMETRY

CYTOMETRY
Journal of Quantitative Cells Science P A R T A



Relevance of Antibody Validation for Flow Cytometry

Tomas Kalina,1* Kelly Lundsten,2 Pablo Engel3

Thus, at present**, multiparametric cytometry assays require antibody conjugates with known performance criteria under several conditions;** for several cell types, validation data shall be presented for monoclonal antibody reactivity and for antibody conjugate performance. **Consensus on benchmarking methods**, aggregation of comparable data sets across manufacturers and users and public availability of the performance data on clones as well as on antibody conjugates will lead to a qualitatively higher level of information generated by flow cytometry and to a further spread of its applications to basic, translational, and clinical research.

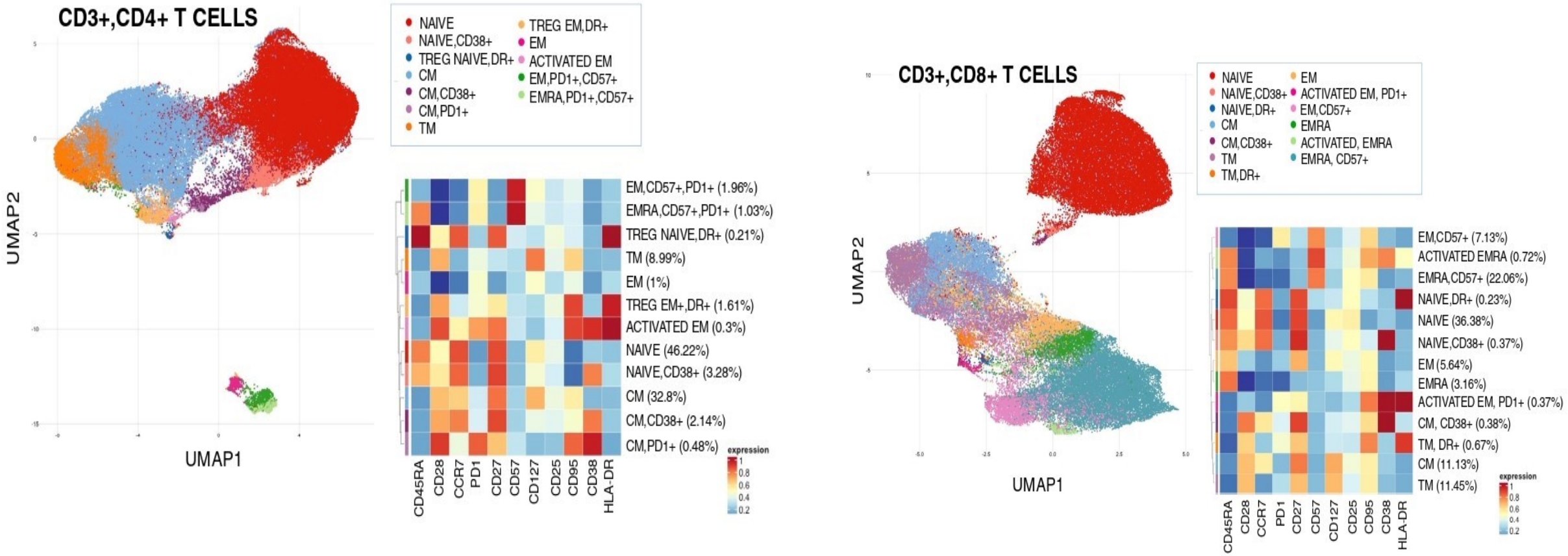


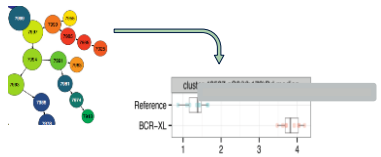
Figure 1. Differentiation, activation and exhaustion of CD4+ or CD8+ T-cell subsets in patients with Covid-19. Representation of an 18-parameter analysis of peripheral blood cells. Cells were stained with the Duraclone IM T cell panel (from Beckman Coulter, FL, USA) added with another five fluorescent mAbs and a marker of cell viability and analyzed on a CytoFLEX LX flow cytometer (Beckman Coulter). Beside side and forward scatters, markers were CD45 conjugated with Krome Orange, CD3 APC-A750, CD4 APC, CD8 AF700, CD27 PC7, CD57 Pacific Blu, CD279 (PD-1) PC5.5, CD28 ECD, CCR7 PE, CD45RA FITC, HLA-DR BUV661, CD127 BV650, CD25 BV785, CD95 BUV395, CD38 BUV496, and PromoFluor-840 (Promokine). Unsupervised analysis of electronically gated CD45+, CD3+, CD4+ or CD45+, CD3+, CD8+ T-cells was first performed by using the Catalyst package (Bioconductor) (16). Second, for both CD4+ and CD8+ T-cell analysis, 10,000 cells per sample were concatenated and transformed. FlowSOM was used to perform the metaclustering ($K = 20$); then, data were represented by the dimensionality reduction method named Uniform Manifold Approximation and Projection (UMAP). It is possible to observe the presence of 12 clusters among CD4+ and 13 among CD8+ T-cells and to see the high amount of naive cells in both T cell populations, and how the distribution of naive, memory, activated and exhausted lymphocytes is different among different types of CD4+ or CD8+ T lymphocytes.

Unsupervised and Supervised Machine Learning Algorithms in Cytobank

SPADE

Clusters and compares relative expression levels

CITRUS



Clusters and identifies differences between data sets

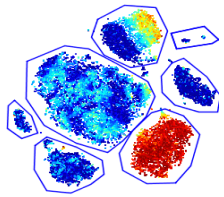
2012

2014

2016

2018

viSNE



Reduces dimensionality and visualization

FlowSOM

Clusters and maps to a self-organizing map

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