ANÁLISIS AUTOMATIZADO DE LAS NEOPLASIAS DE CÉLULAS B MADURAS

CIC Centro de Investigación del Cáncer

VNiVERSiDAD D SALAMANCA
CAMPUS DE EXCELENCIA INTERNACIONAL

USal 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

6° Curso Práctico de Citometría de Flujo
Valencia, 29 de septiembre de 2023

IS THERE A NEED FOR AUTOMATED FCM DATA ANALYSIS?

Why Data Bases are useful?

FCM: from research laboratories to clinical diagnostics

⇩

Digital instruments

>4 to >25 color flow cytometers

Higher analytical speed

⇩

Exponential growth of complex data with hundreds populations in a data file

⇩

(BIG data approaches needed)

EuroFlow

EuroFlow strategy in the diagnostic work-up of CLPD

ORIGINAL ARTICLE

EuroFlow antibody panels for standardized *n*-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes

JJM van Dongen[1], L Lhermitte[2], S Böttcher[3], J Almeida[4], VHJ van der Velden[1], J Flores-Montero[4], A Rawstron[5], V Asnafi[2], Q Lécrevisse[4], P Lucio[6], E
E Macintyre
018708)

SECTION 2. LYMPHOID SCREENING TUBE (LST)

J Flores-Montero[1], J Almeida[1], JJ Pérez[2], V Asnafi[3], L Lhermitte[3],
MB
AW
A Ra
L Se
JJM
[1]USA
[4]UNIK
The N
Centr
[9]SUM
Salamanca, S

SECTION 8. ANTIBODY PANEL FOR B-CELL CHRONIC LYMPHOPROLIFERATIVE DISEASES (B-CLPD)

S Böttcher[1], A Rawstron[2], P Lucio[3], R de Tute[2], J Flores-Montero[4],
Q Léc
M Brü
[1]UNIKI
[4]USAL,
[7]Erasm

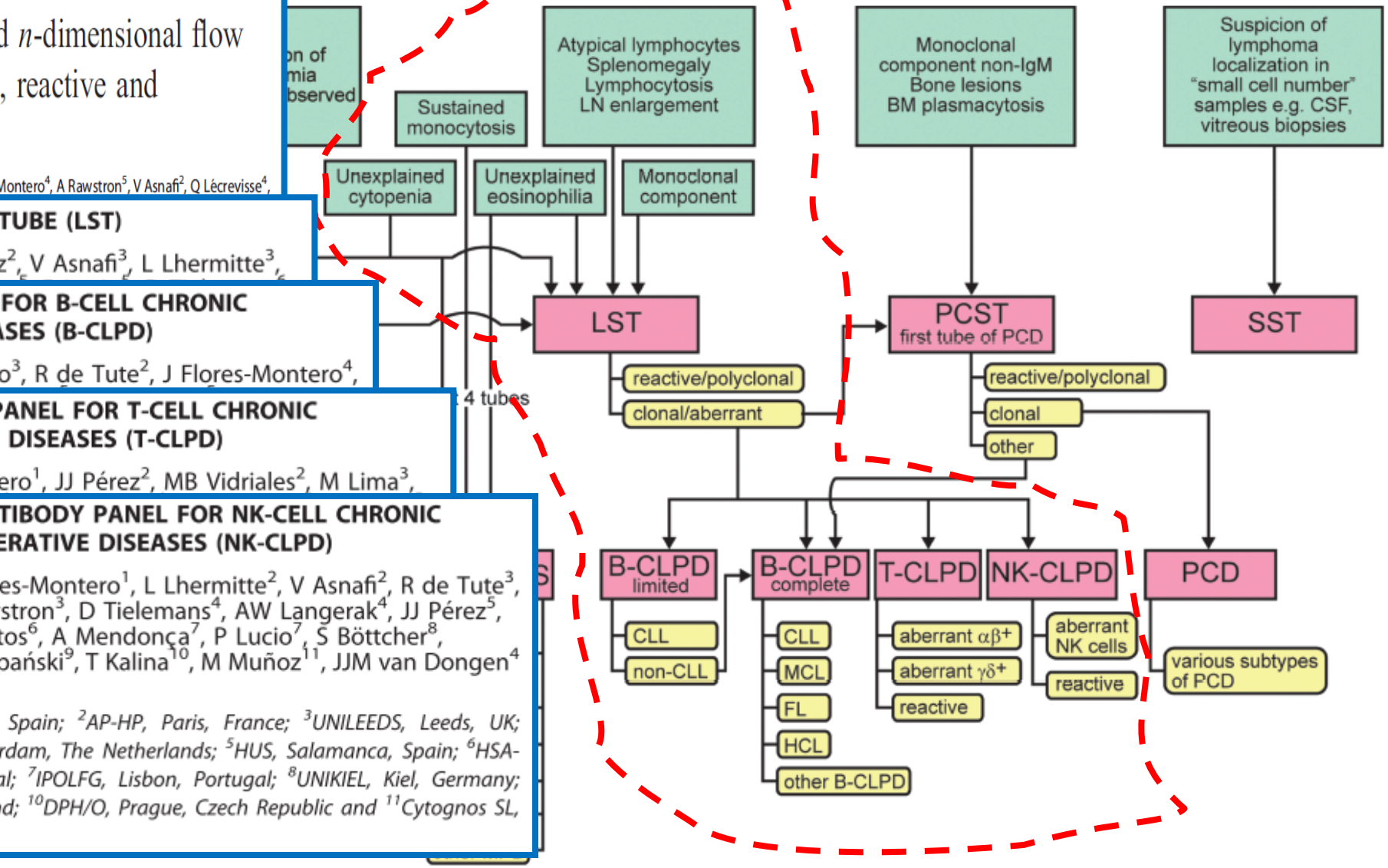
SECTION 9. ANTIBODY PANEL FOR T-CELL CHRONIC LYMPHOPROLIFERATIVE DISEASES (T-CLPD)

J Almeida[1], J Flores-Montero[1], JJ Pérez[2], MB Vidriales[2], M Lima[3],
AH San
E Maci
M Culle
M Mart
[1]USAL,
Portuga
[6]UNIKIE
[9]SUM, Z
Salaman

SECTION 10. ANTIBODY PANEL FOR NK-CELL CHRONIC LYMPHOPROLIFERATIVE DISEASES (NK-CLPD)

J Almeida[1], J Flores-Montero[1], L Lhermitte[2], V Asnafi[2], R de Tute[3], M Cullen[3], A Rawstron[3], D Tielemans[4], AW Langerak[4], JJ Pérez[5], M Lima[6], AH Santos[6], A Mendonça[7], P Lucio[7], S Böttcher[8], L Sedek[9], T Szczepański[9], T Kalina[10], M Muñoz[11], JJM van Dongen[4] and A Orfao[1]

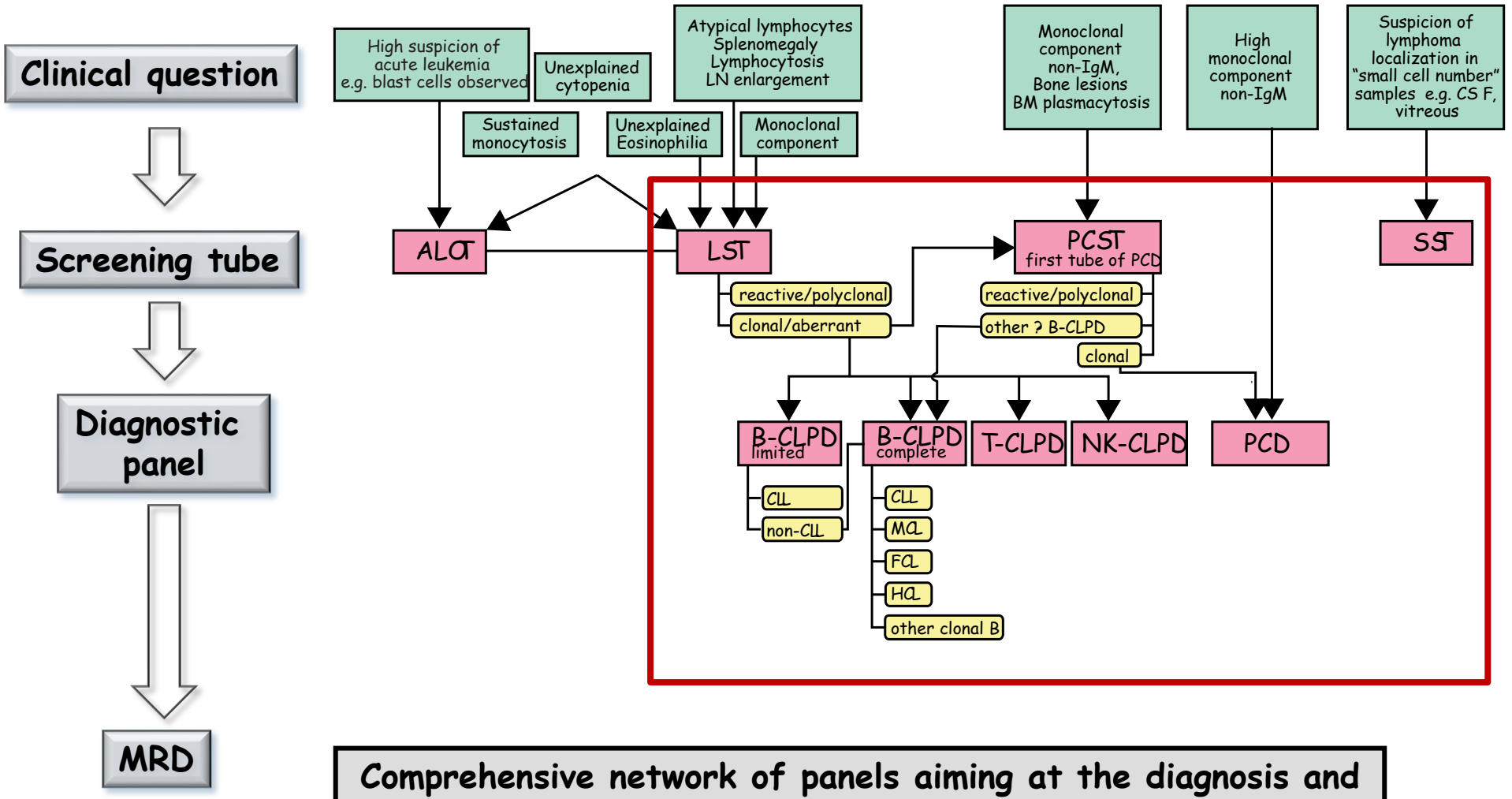
[1]USAL, Salamanca, Spain; [2]AP-HP, Paris, France; [3]UNILEEDS, Leeds, UK; [4]Erasmus MC, Rotterdam, The Netherlands; [5]HUS, Salamanca, Spain; [6]HSA-CHP, Porto, Portugal; [7]IPOLFG, Lisbon, Portugal; [8]UNIKIEL, Kiel, Germany; [9]SUM, Zabrze, Poland; [10]DPH/O, Prague, Czech Republic and [11]Cytognos SL, Salamanca, Spain



EuroFlow

van Dongen et al, Leukemia 2012

The EuroFlow comprehensive approach



Comprehensive network of panels aiming at the diagnosis and characterization of the major WHO entities

LST – Lymphocytosis screening tube

EuroFlow

	PacB	PacO	FITC	PE	PerCp Cy5.5	PE Cy7	APC	APC H7
LST	CD4 CD20	CD45	CD8 sIgλ	CD56 sIgκ	CD5	CD19 TCRγδ	CD3	CD38

Able to identify all the sample major populations of normal vs (expanded and/or aberrant) tumor cells:

Non-hematopoietic cells

T lymphocytes (T-cell subpopulations)

B lymphocytes (B-cell light chain restriction)

NK cells

Plasma cells

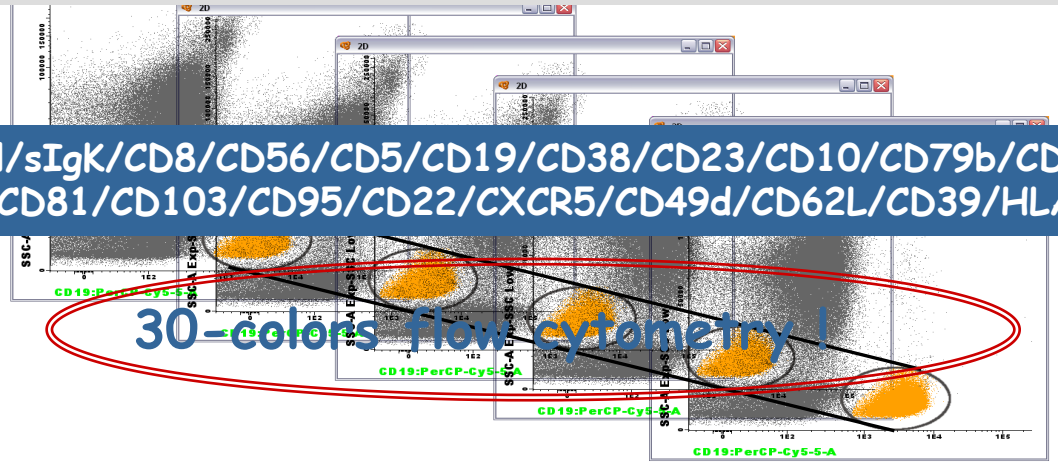
B-NHL panel backbone

van Dongen et al, Leukemia 2012

Responsible scientist: J. Flores Montero

LST + BCLPD classification panel

	Pac Blue	Pac Orange	FITC	PE	PerCP-Cy5.5	PECy7	APC	APC-H7
1= LST	CD20 /CD4	CD45	sIgλ /CD8	sIgK /CD56	CD5	CD19 /TCRγδ	CD3	CD38
2	CD20	CD45	CD23	CD10	CD79b	CD19	CD200	CD43
3	CD20	CD45	CD31	LAIR	CD11c	CD19	sIgM	CD81
4	CD20	CD45	CD103	CD95	CD22	CD19	CXCR5	CD49d
5R	CD20	CD45	CD62L	CD39	HLA-DR	CD19	CD27	



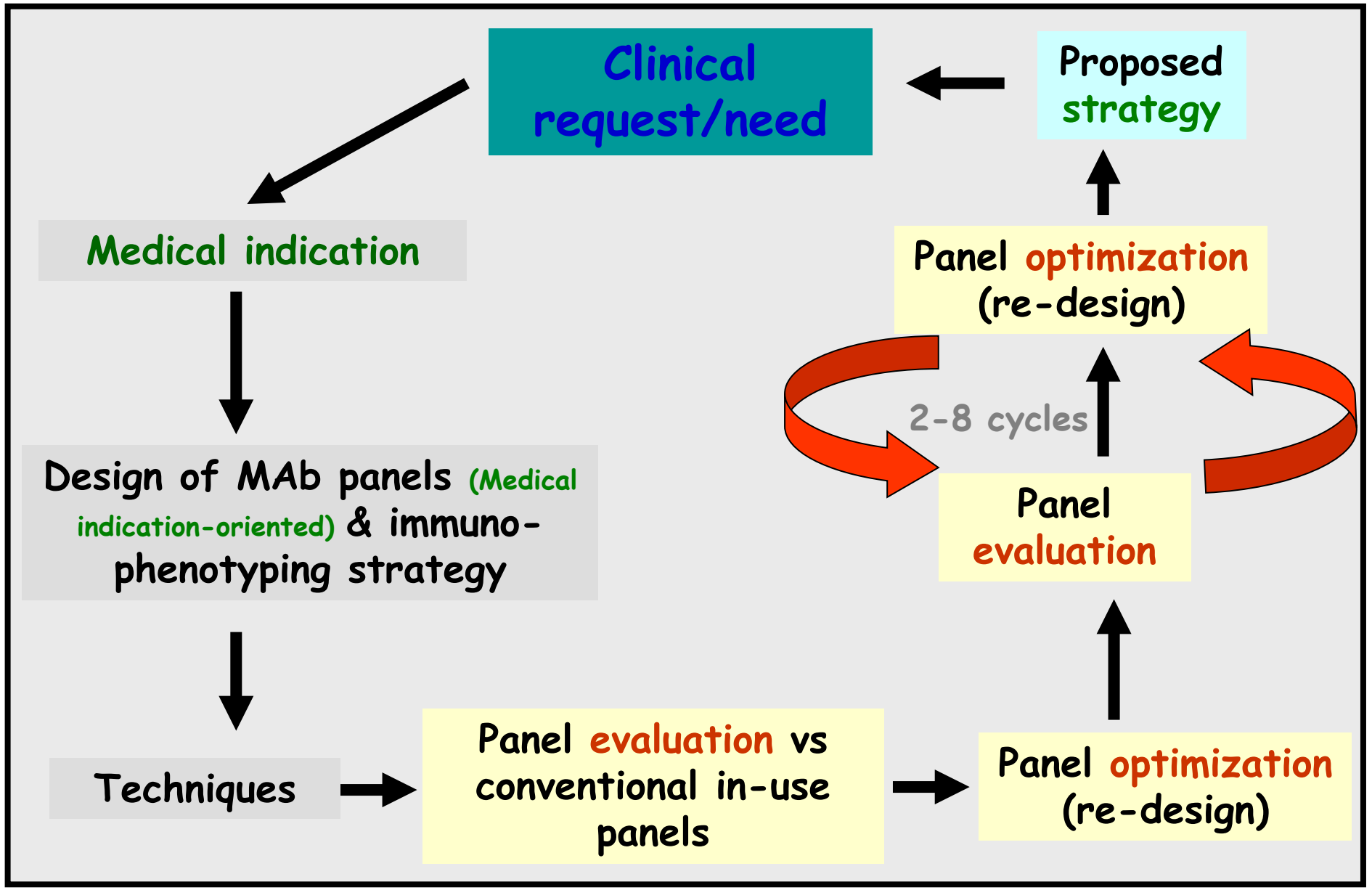
CD20/CD4/CD45/sIgl/sIgK/CD8/CD56/CD5/CD19/CD38/CD23/CD10/CD79b/CD200/CD43/CD31/LAIR1/CD11c/sIgM/CD81/CD103/CD95/CD22/CXCR5/CD49d/CD62L/CD39/HLA-DR/CD19/CD27

30-colors flow cytometry !

Responsible scientist: Sebastian Bottcher

EuroFlow

CONSTRUCTION OF EUROFLOW LEUKEMIA/ LYMPHOMA IMMUNOPHENOTYPING ANTIBODY PANEL



FCM DATA OVERLAYED ON REFERENCE DATA BASES PLUS INTERPRETATION

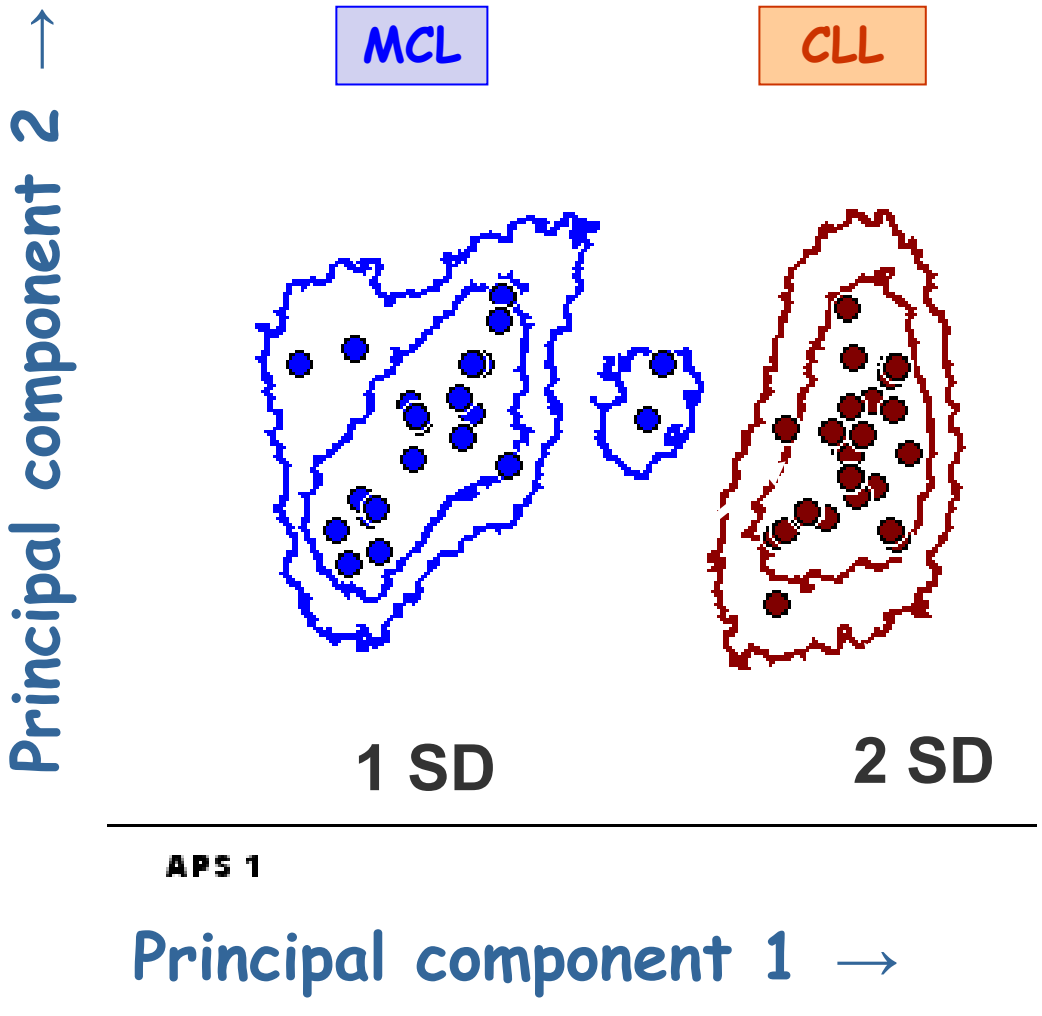
- To evaluate an antibody panel and identify the most informative markers

- To (automatically) gate cell populations in a data file

- To classify a disease into a given lineage, maturation stage and diagnostic category

EuroFlow

MCL vs CLL: PCA of total immunophenotype

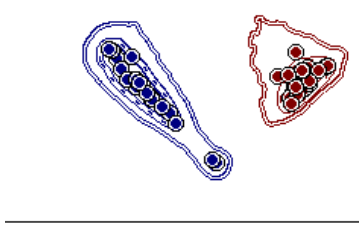


	PC1	
1	IgM	14.09
2	CD200	14.06
3	CD79b	13.39
4	CD23	8.60
5	CD20	6.43
	…	

Responsible scientist: Sebastian Bottcher

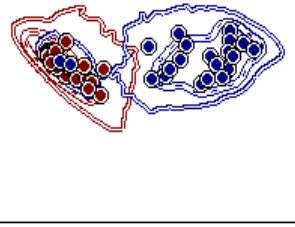
MCL vs CLL: PCA of total immunophenotype

CLL MCL



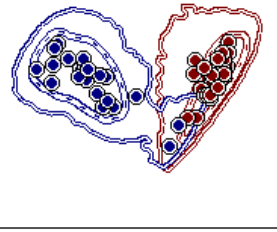
CD200 + IgM

MCL CLL



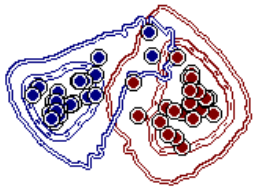
IgM + CD79b

MCL CLL



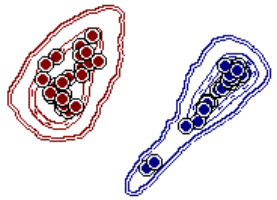
CD23 + IgM

CLL MCL



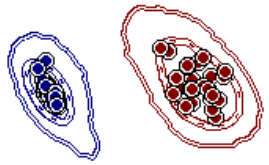
CD23 + CD79b

MCL CLL



CD200 + CD79b

MCL CLL



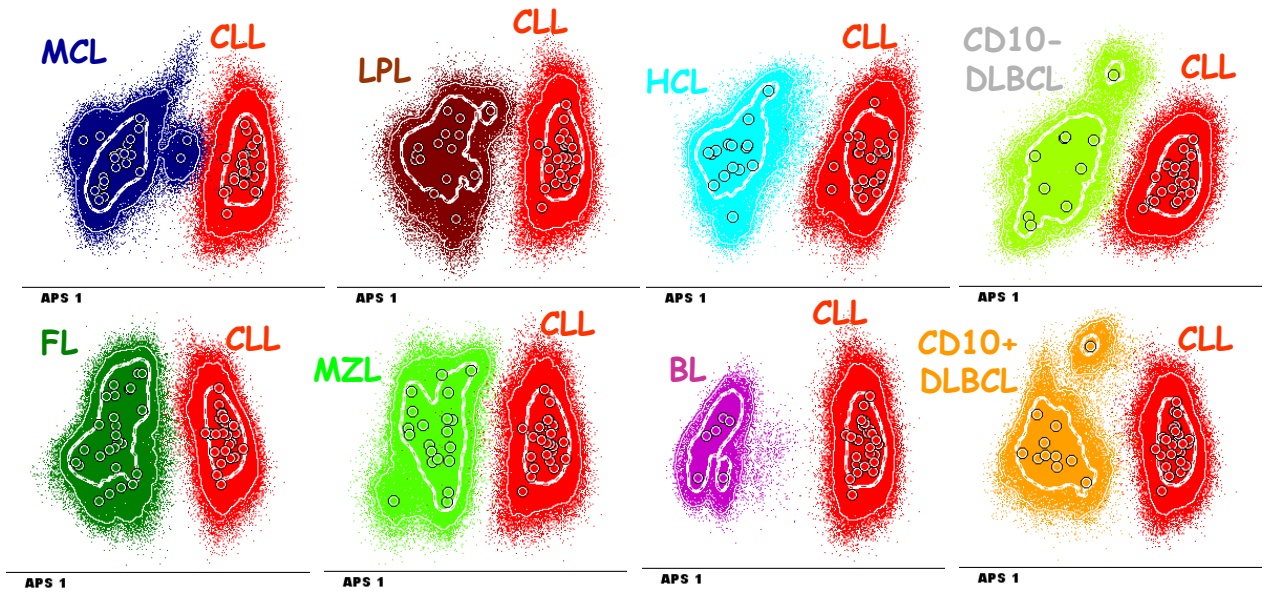
CD200 + CD23

	PC1	
1	IgM	14.09
2	CD200	14.06
3	CD79b	13.39
4	CD23	8.60
5	CD20	6.43
	...	

Responsible scientist:
Sebastian Bottcher

BCLPD classification panel: modular design

Full panel

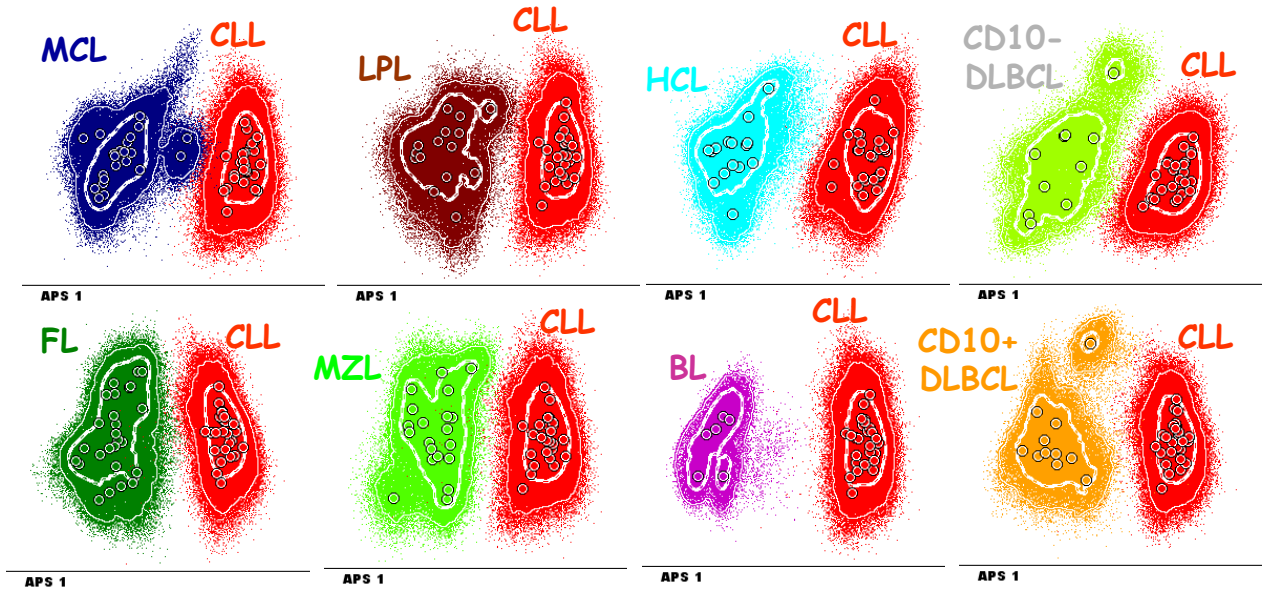


Responsible scientist: Sebastian Bottcher

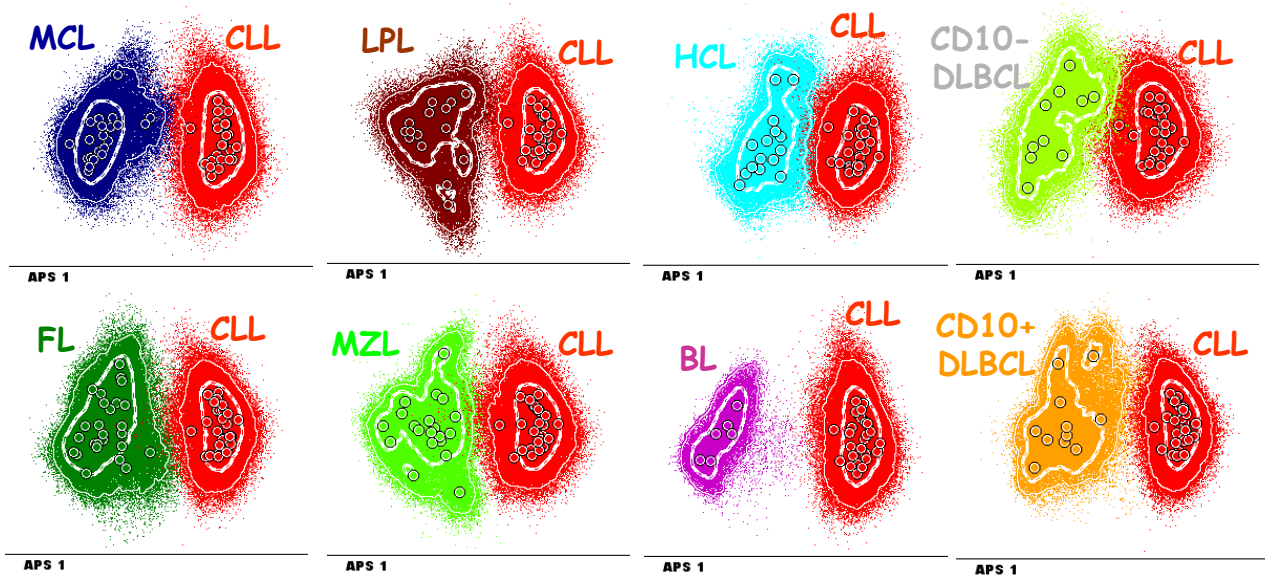
EuroFlow

BCLPD classification panel: modular design

Full panel



Tubes 1 & 2 only

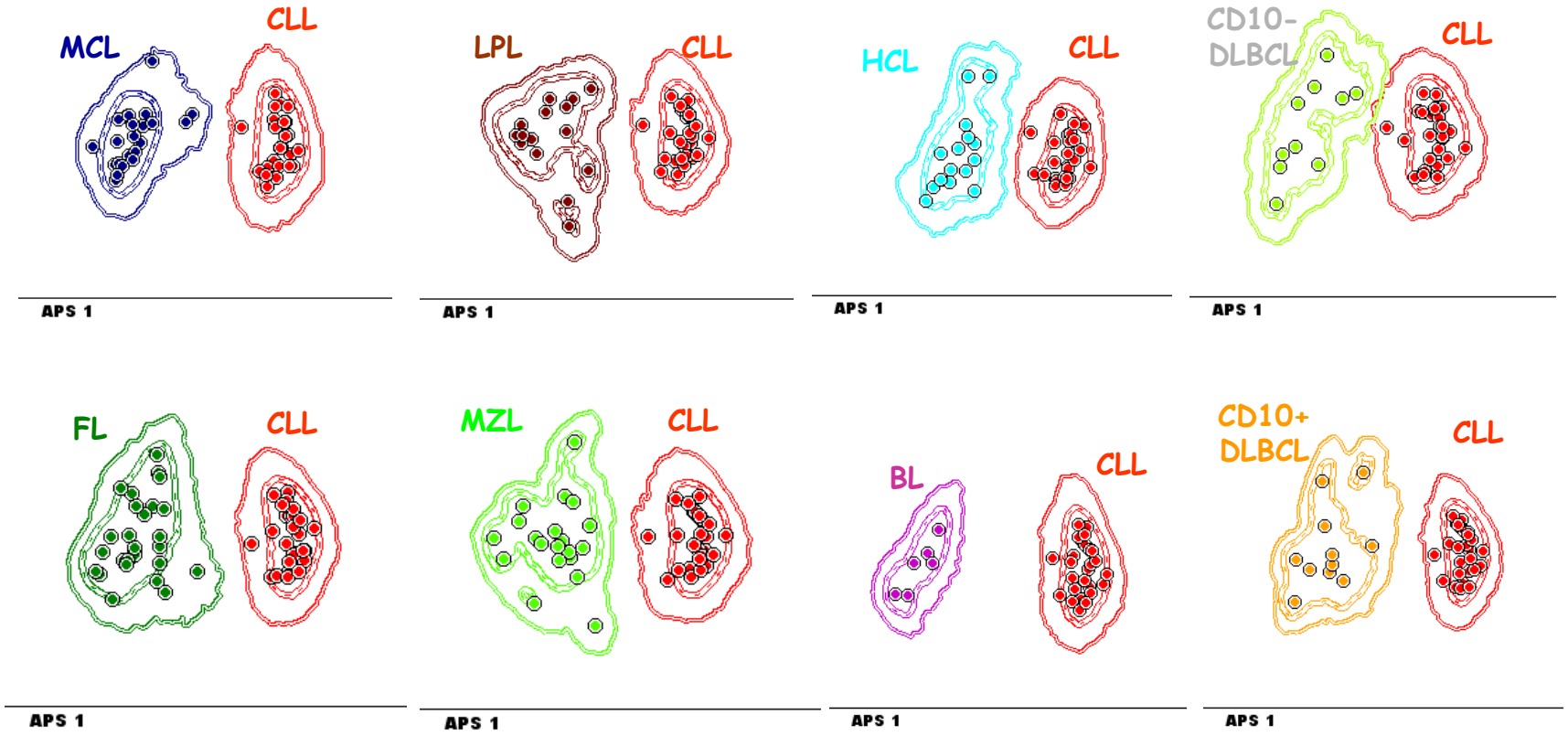


Responsible scientist: Sebastian Bottcher

EuroFlow

BCLPD classification panel: modular design

Tubes 1 & 2 only



Tubes 1 (LST) and 2 only: resolve 100% of CLL and 85% MCL cases

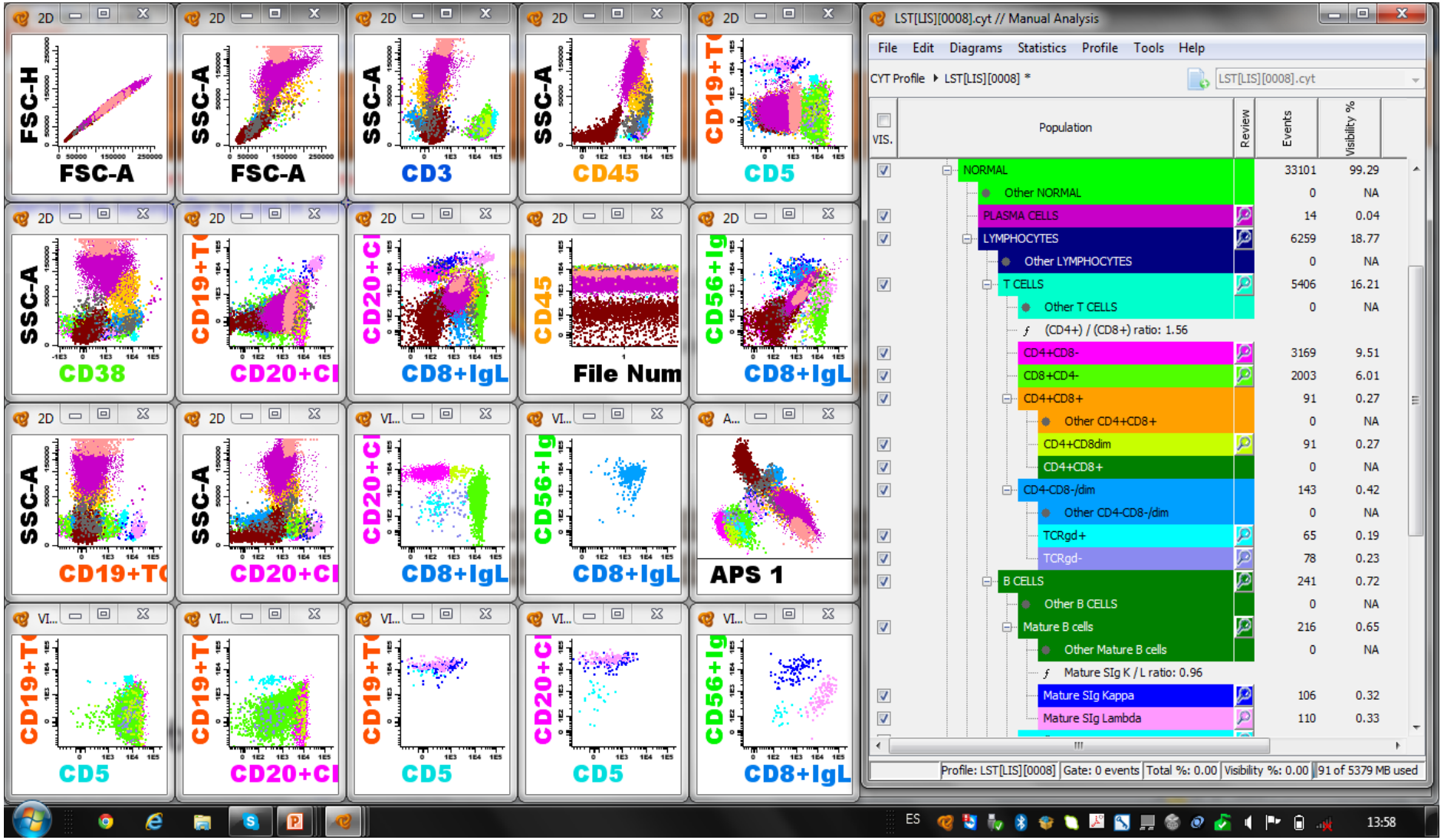
Tubes 1 (LST) only: resolves 48% of CLL and 21% MCL cases

Responsible scientist: Sebastian Bottcher

EuroFlow

GATING IN THE LST TUBE:

35 different cell subsets x mean of 3 gates (n=105 gates)



FCM DATA OVERLAYED ON REFERENCE DATA BASES PLUS INTERPRETATION

EuroFlow

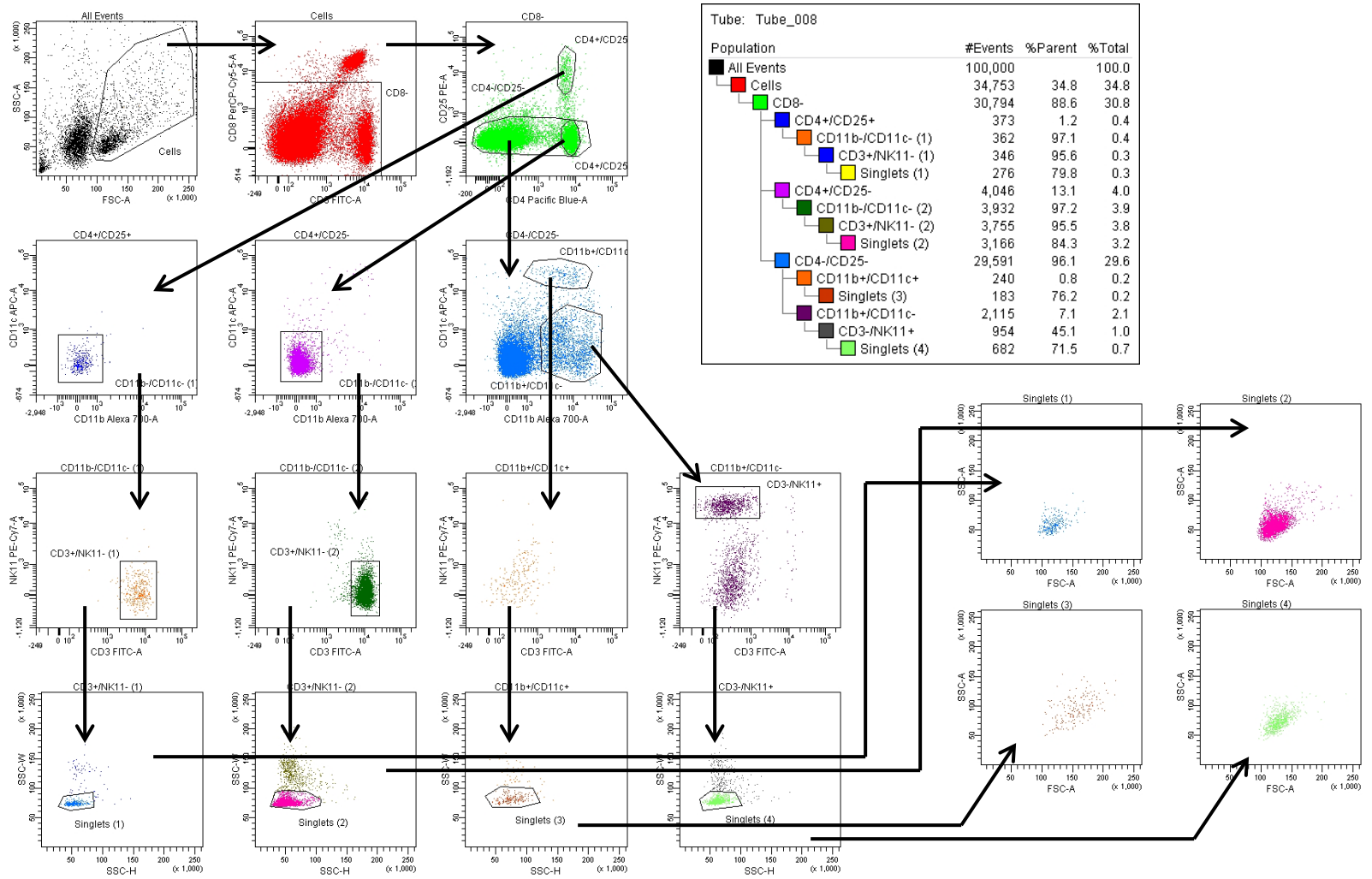
- To evaluate an antibody panel and identify the most informative markers

- To (automatically) gate cell populations in a data file

- To classify a disease into a given lineage, maturation stage and diagnostic category

- Gating groups of events for identification (labeling) of cell populations
  -Automated gating algorithms.

BOOLEAN GATING STRATEGY



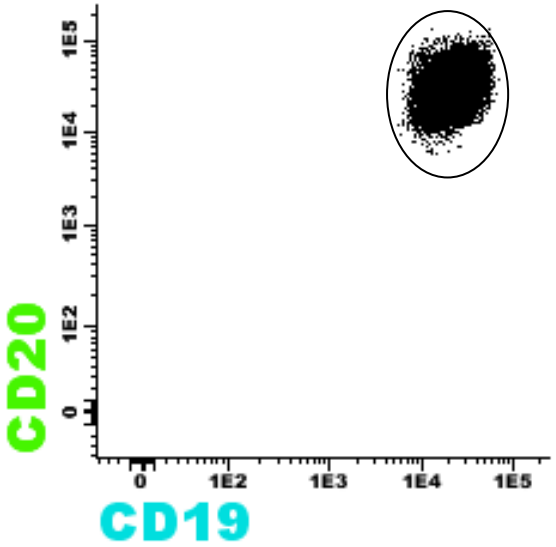
Tube: Tube_008

Population	#Events	%Parent	%Total
All Events	100,000		100.0
Cells	34,753	34.8	34.8
CD8-	30,794	88.6	30.8
CD4+/CD25+	373	1.2	0.4
CD11b-/CD11c- (1)	362	97.1	0.4
CD3+/NK11- (1)	346	95.6	0.3
Singlets (1)	276	79.8	0.3
CD4+/CD25-	4,046	13.1	4.0
CD11b-/CD11c- (2)	3,932	97.2	3.9
CD3+/NK11- (2)	3,755	95.5	3.8
Singlets (2)	3,166	84.3	3.2
CD4-/CD25-	29,591	96.1	29.6
CD11b+/CD11c+	240	0.8	0.2
Singlets (3)	183	76.2	0.2
CD11b+/CD11c-	2,115	7.1	2.1
CD3-/NK11+	954	45.1	1.0
Singlets (4)	682	71.5	0.7

Prepared by A.Salvador

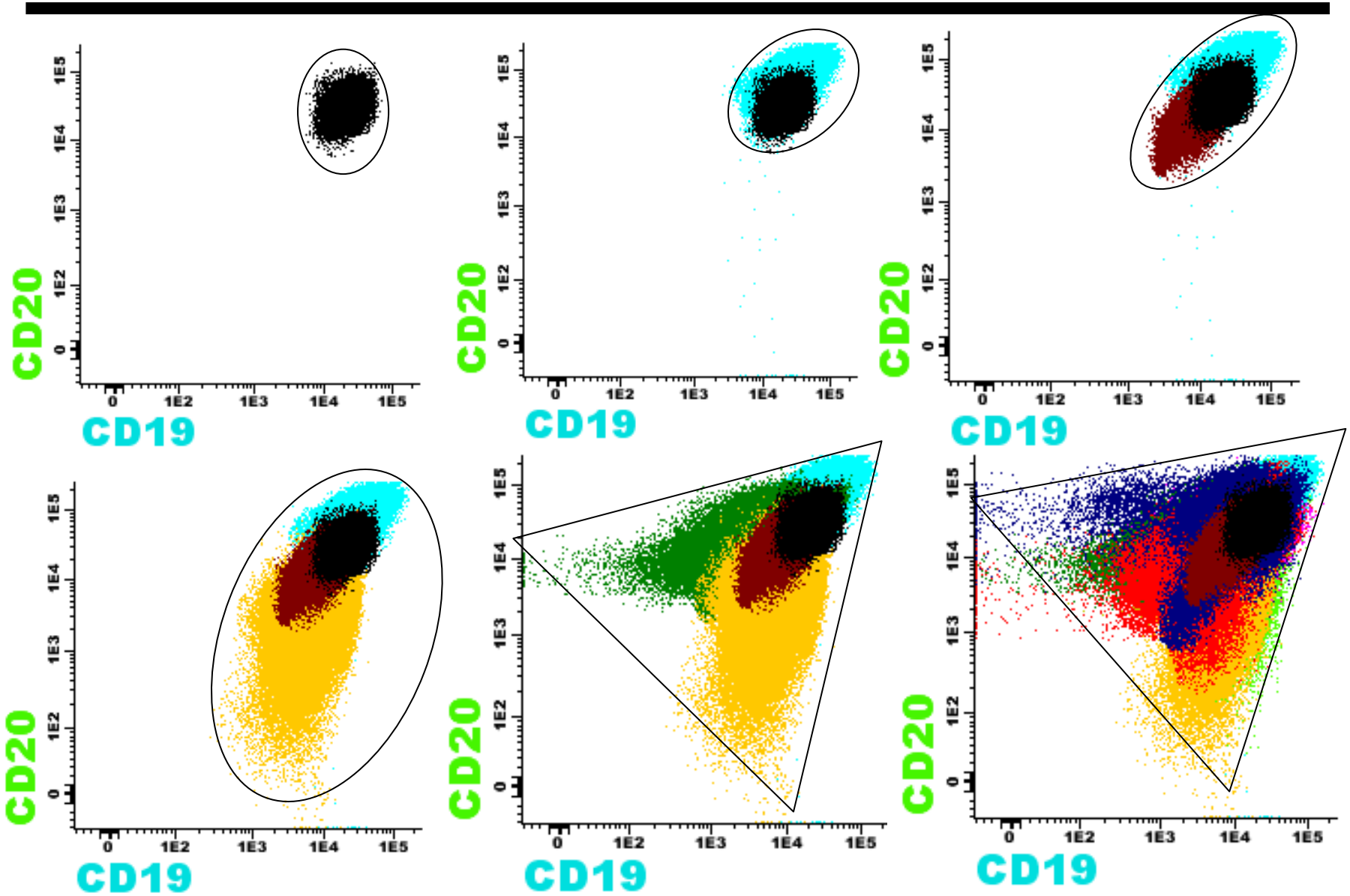
GATING IN THE LST TUBE:

35 different cell populations x mean of 3 gates (105 gates)



GATING IN THE LST TUBE:

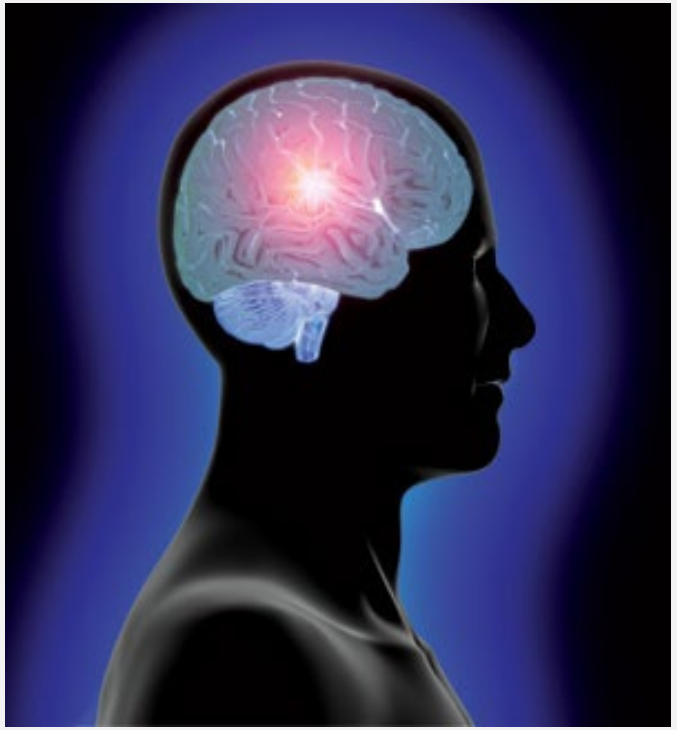
35 different cell populations x mean of 3 gates (105 gates)



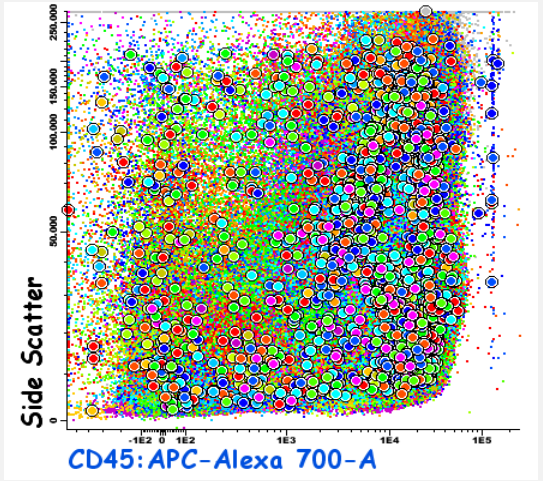
Automated identification of cell populations

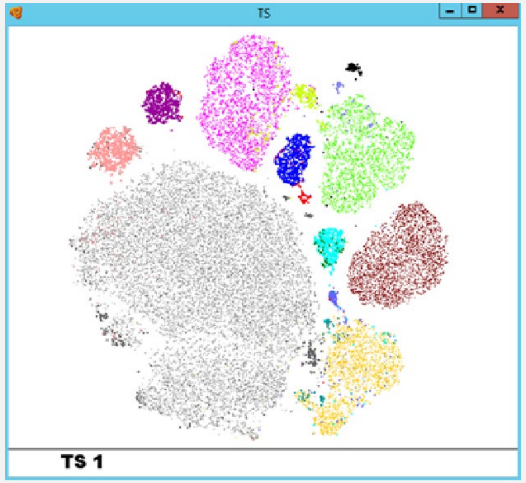
Basic principles

Identifying the pathways that link individual events in an (N)-dimensional space



Groups of cells





Responsible scientists: Rafael Fluxa, Juan Hernandez, Quentin Lecrevisse

EuroFlow

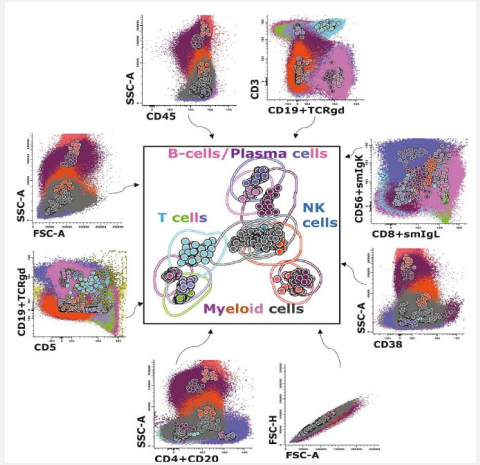
Automated identification of cell populations

Basic principles

Identifying the pathways that link individual events in an (N)-dimensional space

Algorithm for fast comparison of groups of events vs. a **Reference Database**

Clustering phase → **Groups of events** → **Classification phase** → **Cell populations**



Responsible scientists: Rafael Fluxa, Juan Hernandez, Quentin Lecrevisse

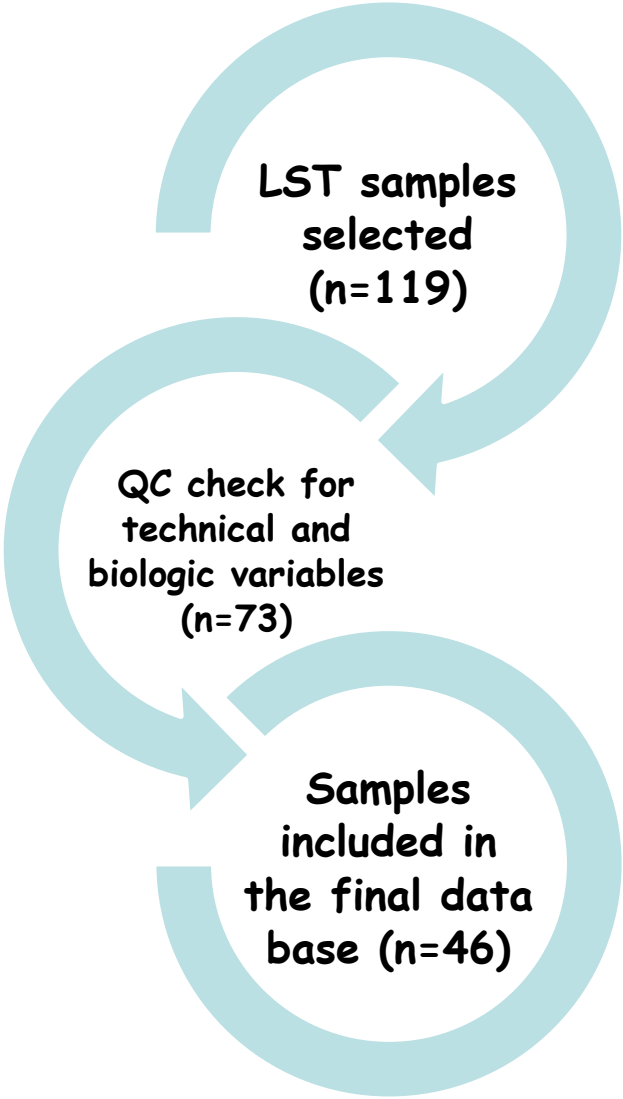
EuroFlow

EUROFLOW LST DATABASE CONSTRUCTION STEPS

EuroFlow

Key steps

1. Selection / Staining and acquisition of normal-reactive bone marrow samples with LST
2. Inspection of technical quality
3. Analysis and identification of all cell populations in the sample
4. Samples incorporation to the data base
5. Exclusion of biological and/or technical outliers
6. Prospective validation



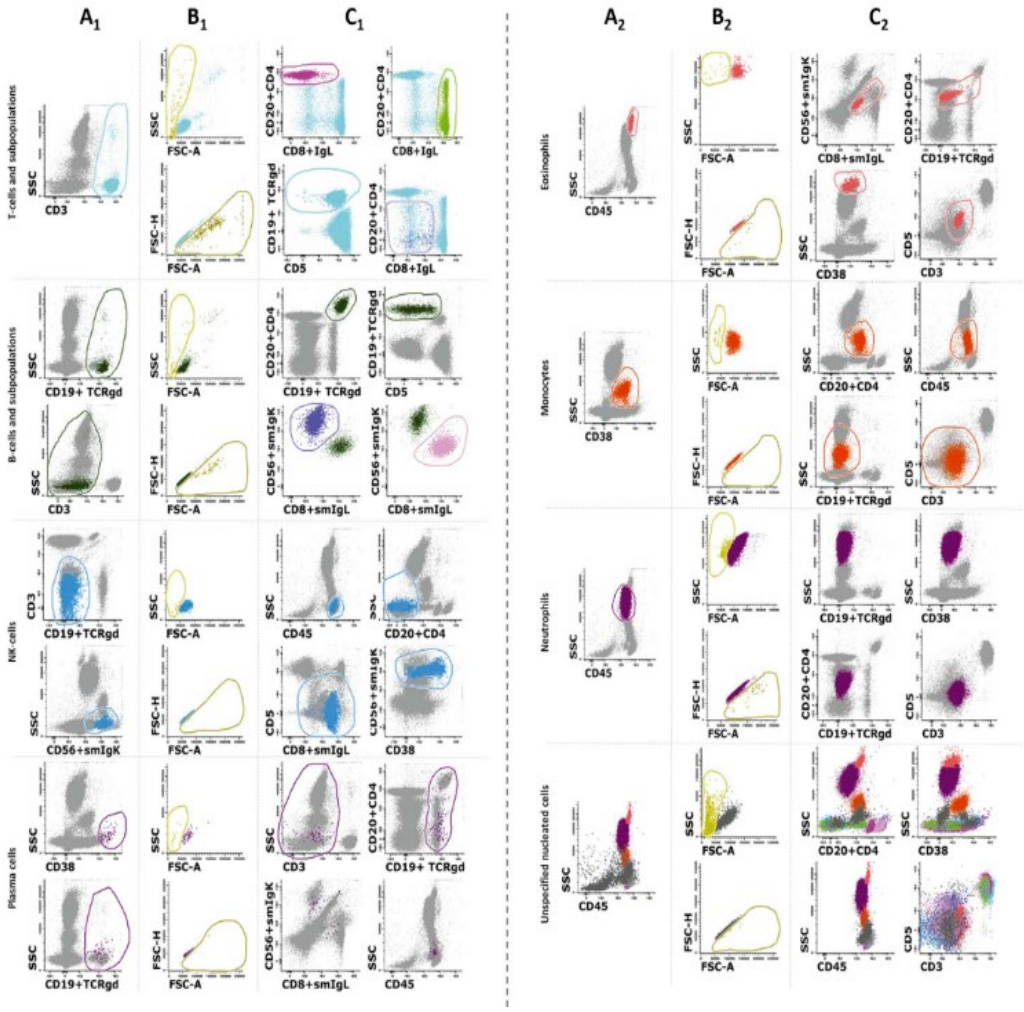
Flores-Montero, Journal of Immunological Methods 2019

EUROFLOW LST DATABASE CONSTRUCTION STEPS

Key steps

1. Selection / Staining and acquisition of normal-reactive blood samples with LST
2. Inspection of technical quality
3. Manual gating and identification of all cell populations in the sample.
4. Samples incorporation to the data base
5. Exclusion of biological and/or technical outliers
6. Prospective validation

Gating strategy for normal PB populations



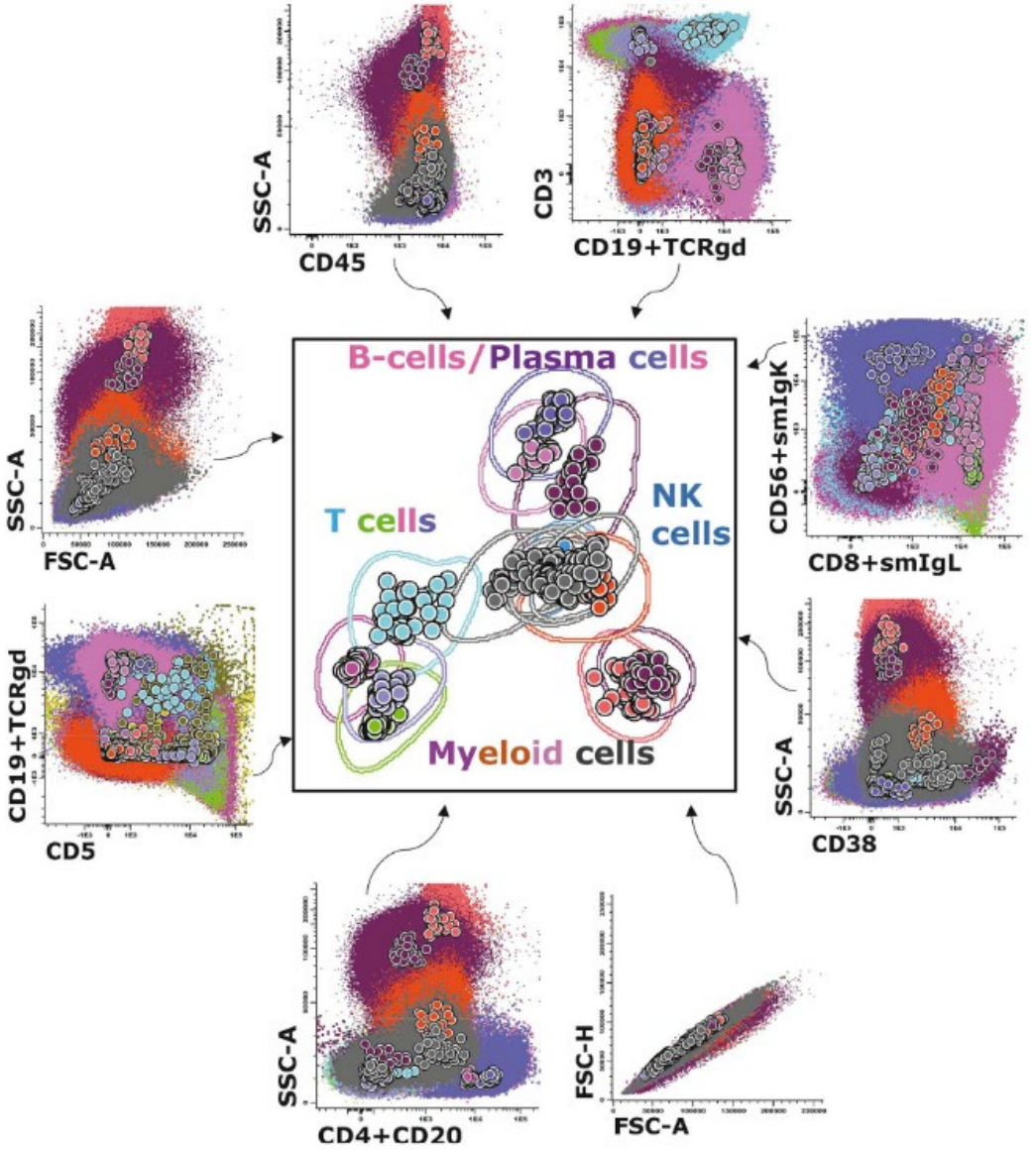
Flores-Montero, Journal of Immunological Methods 2019

EUROFLOW LST DATABASE CONSTRUCTION STEPS

EuroFlow

Key steps

1. Selection / Staining and acquisition of normal-reactive blood samples with LST
2. Inspection of technical quality
3. Manual gating and identification of all cell populations in the sample.
4. Samples merged & incorporated to the database
5. Exclusion of biological and/or technical outliers
6. Prospective validation



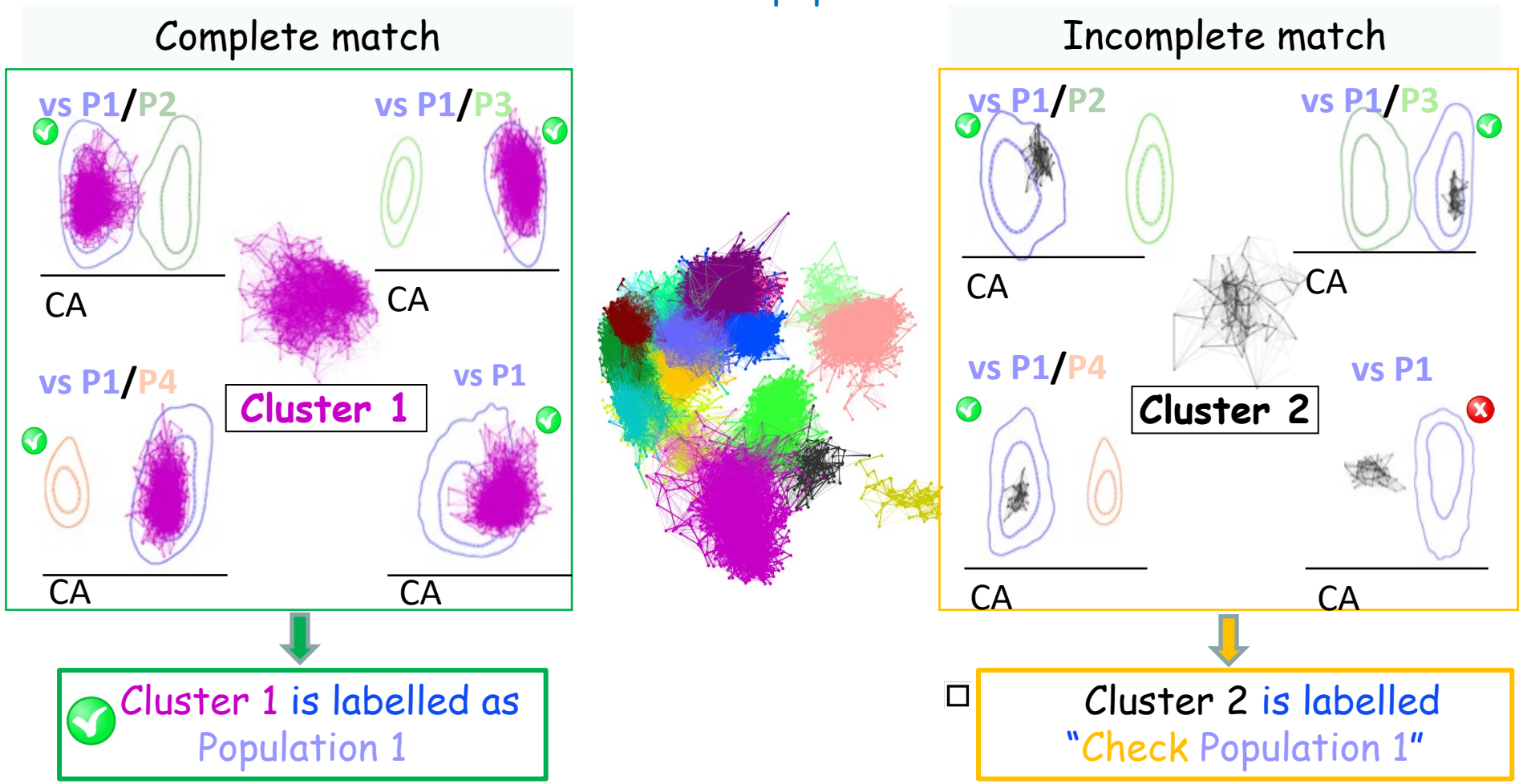
Flores-Montero, Journal of Immunological Methods 2019

Automated gating: classification phase

Classification algorithm

EuroFlow

Cluster vs reference database populations

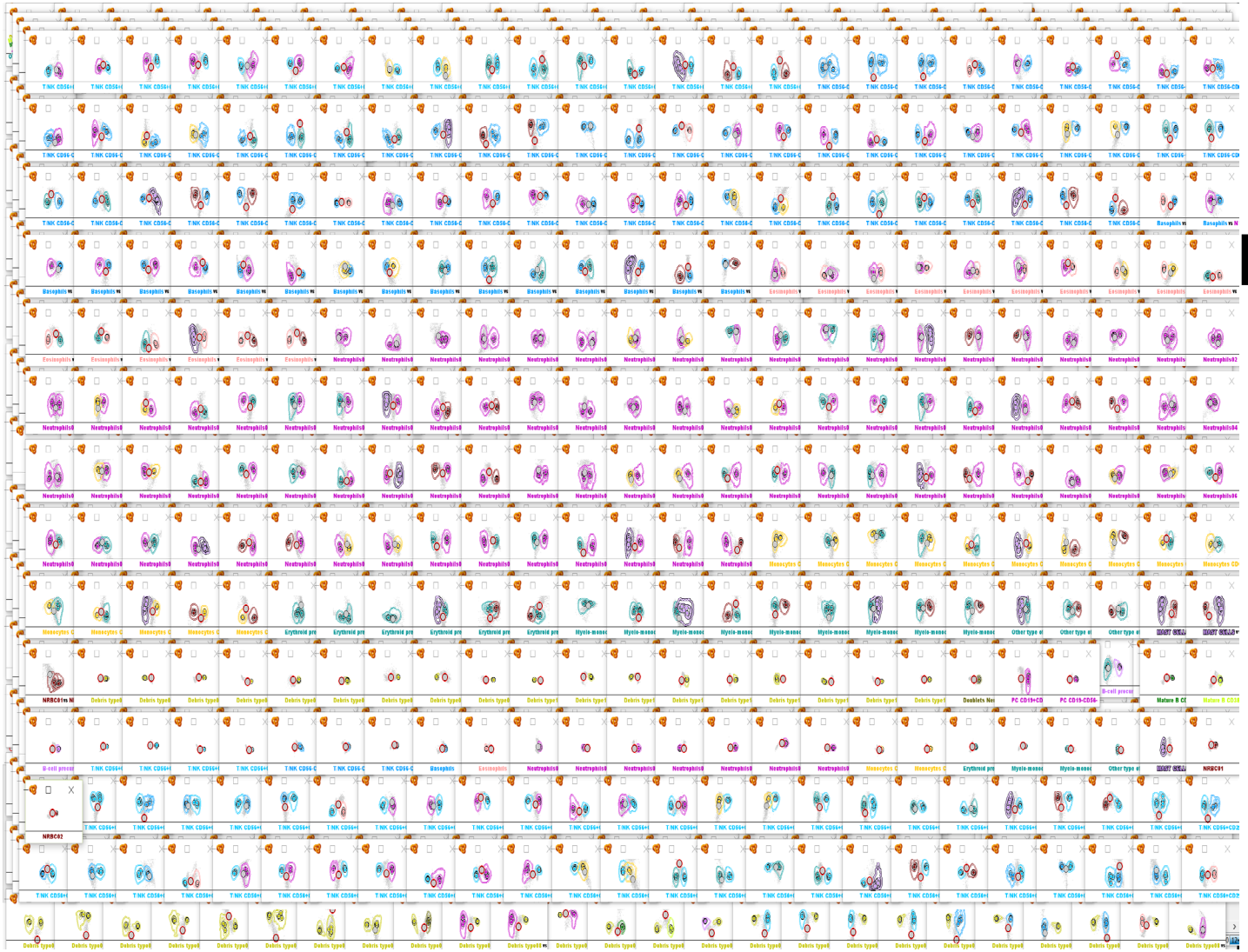


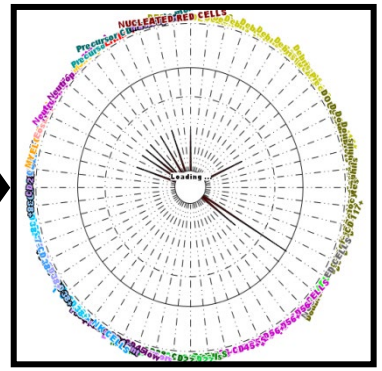
Cluster 1 is labelled as Population 1

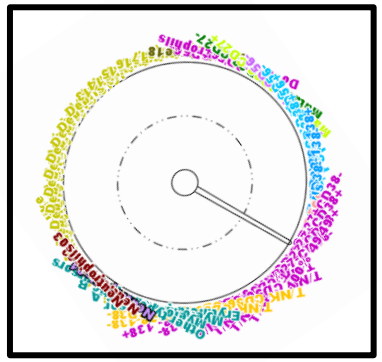
Cluster 2 is labelled "Check Population 1"

AUTOMATED IDENTIFICATION OF CELL POPULATIONS AGAINST THE LST DATABASE

Library containing all 2815 CA possible comparisons





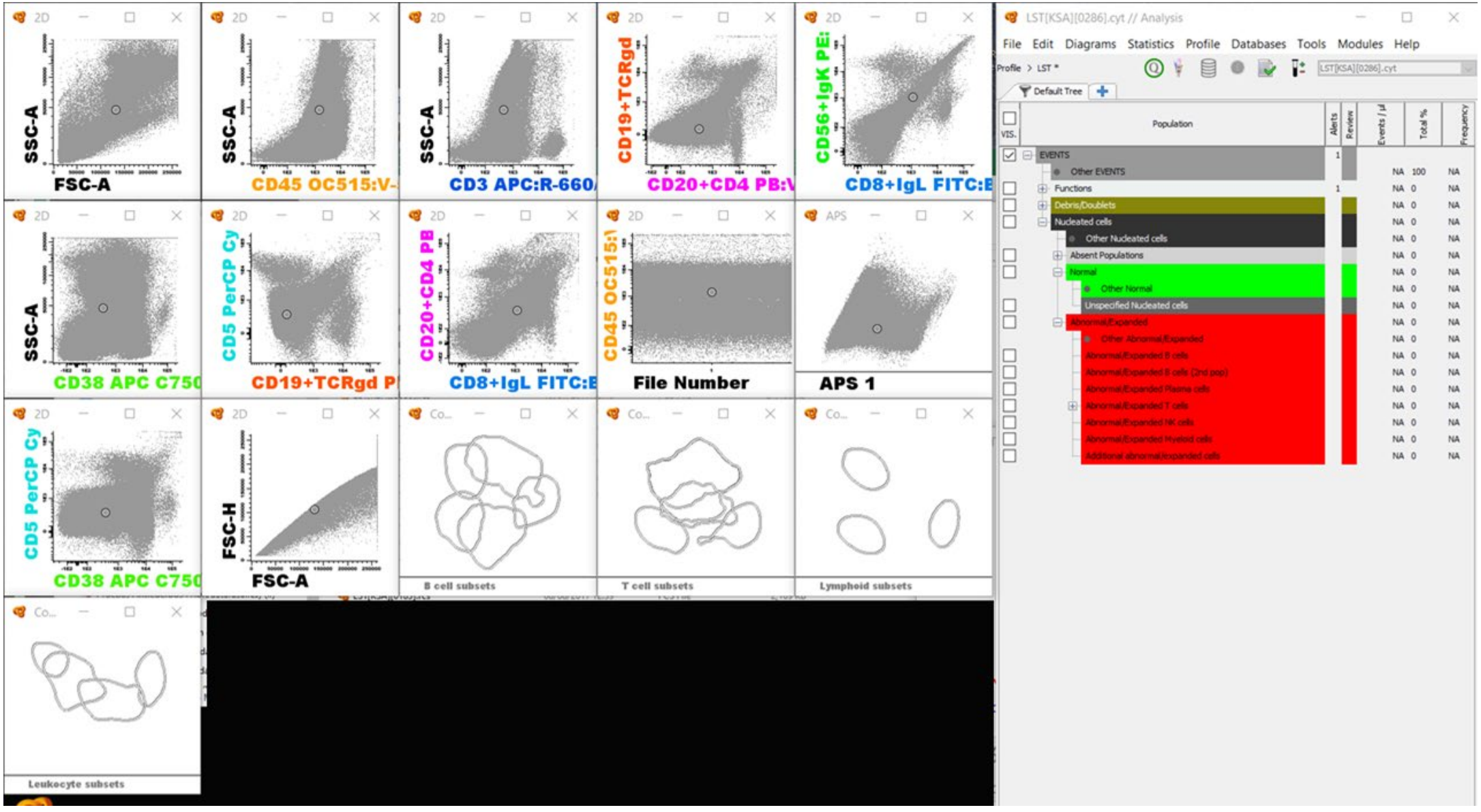


EuroFlow

EuroFlow automated gating (AG&I) with LST data bases

EuroFlow

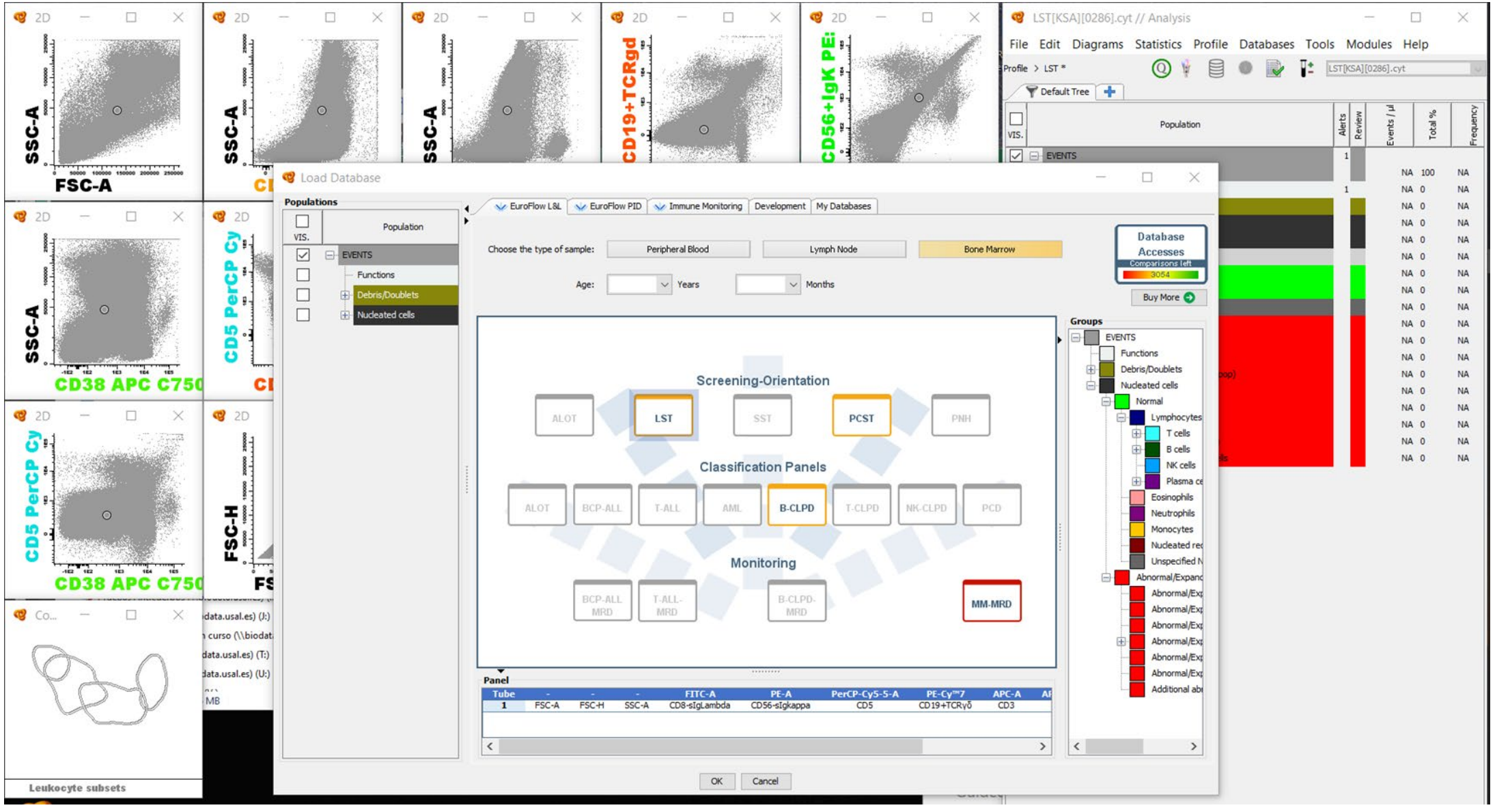
Read file(s)



EuroFlow automated gating (AG&I) with LST BM database

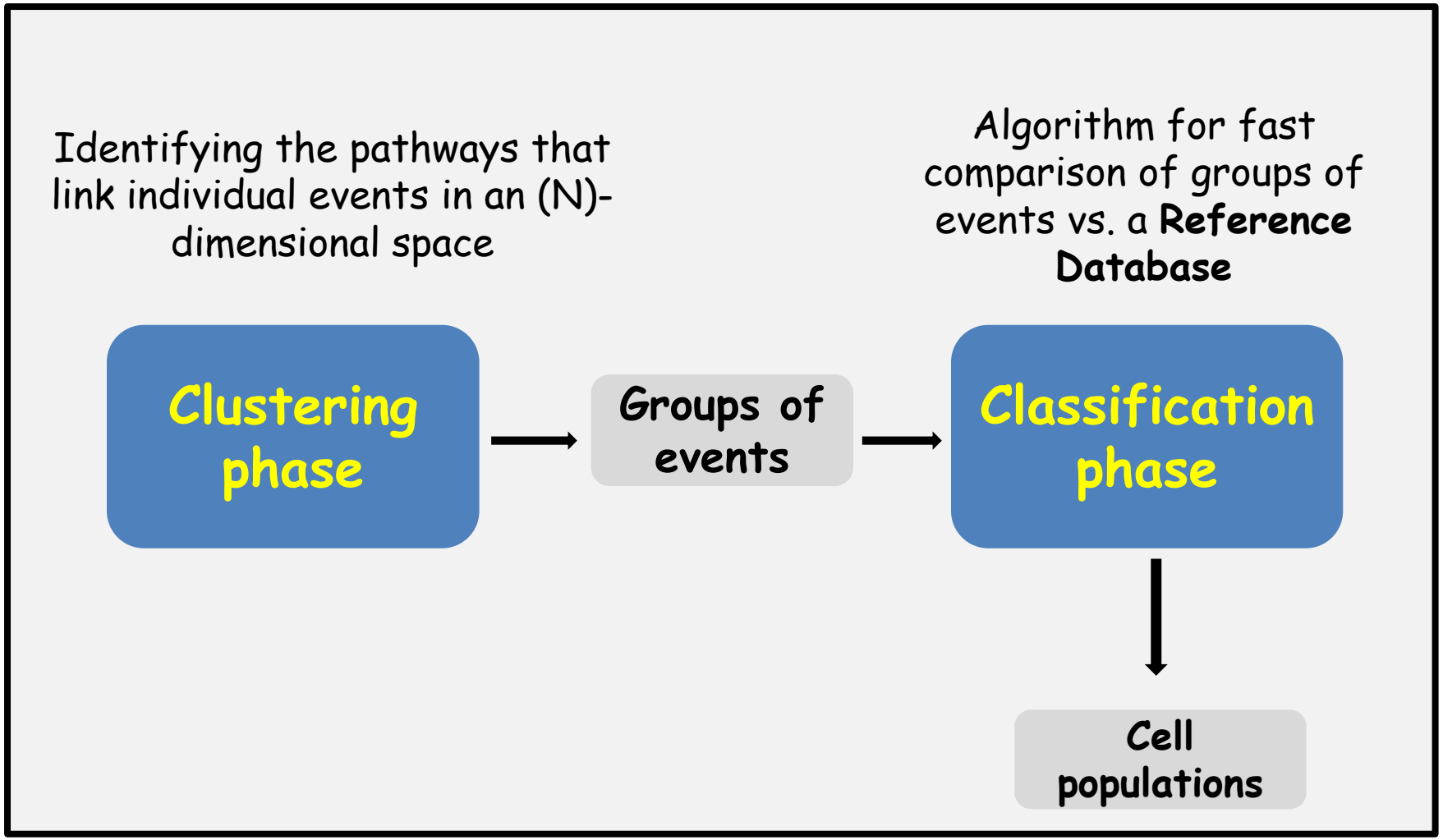
EuroFlow

Data base selection



Automated identification of cell populations

Basic principles



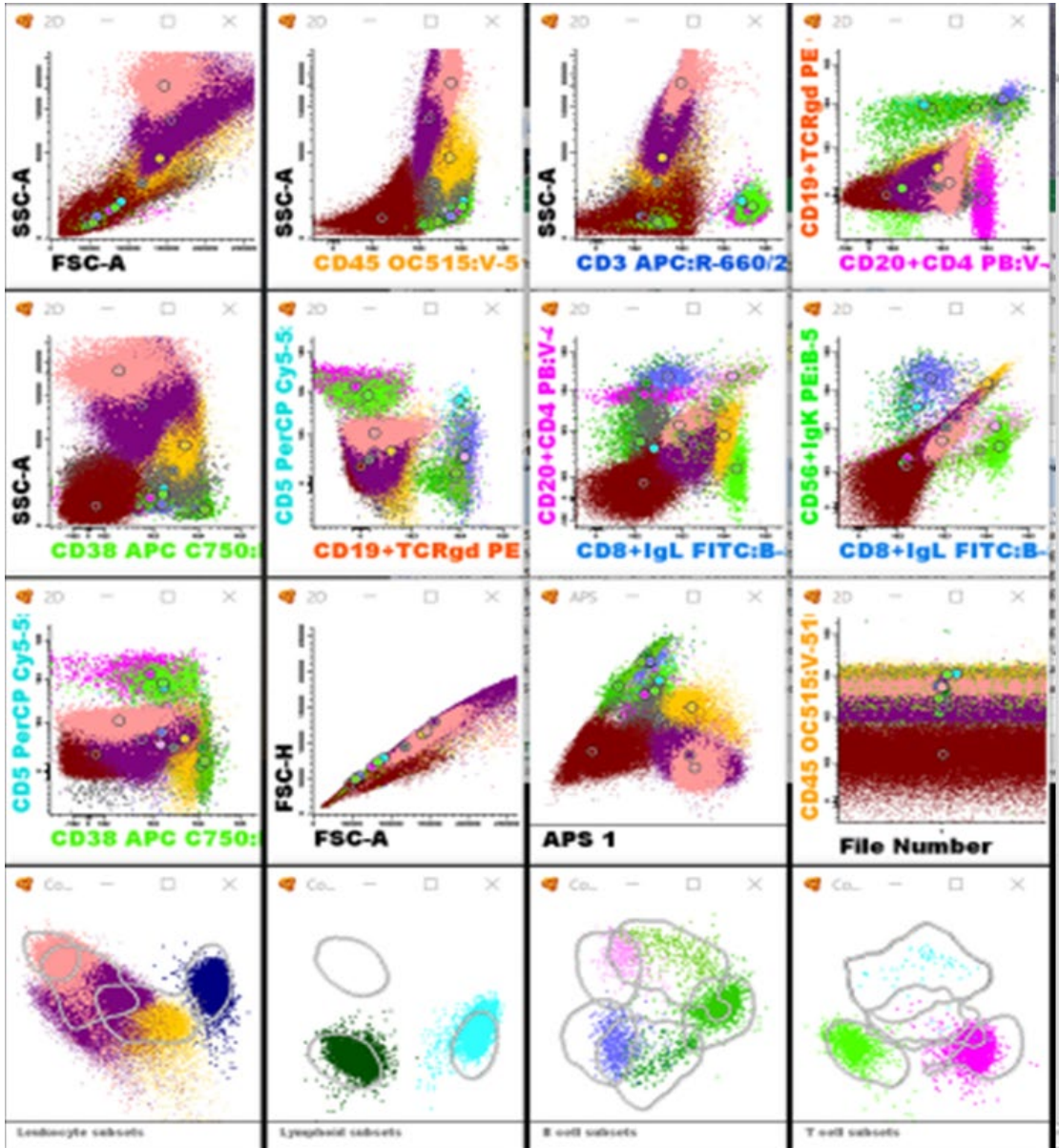
Responsible scientists: Rafael Fluxa, Juan Hernandez, Quentin Lecrevisse

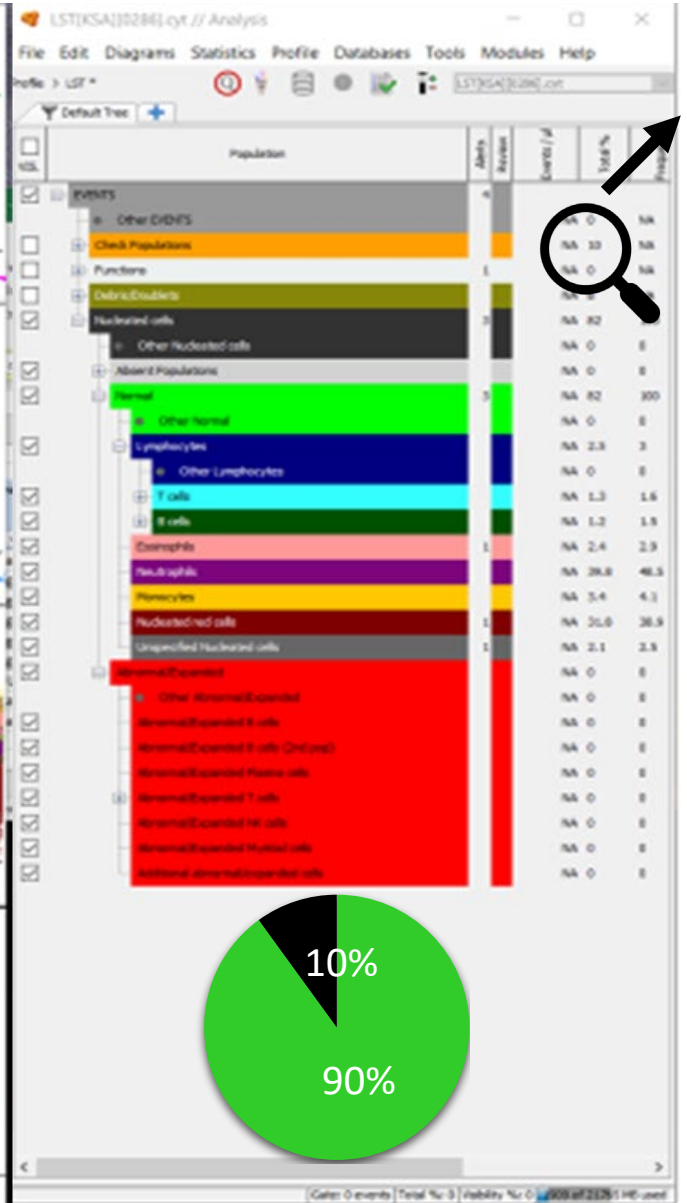
EuroFlow

EuroFlow automated gating (AG&I) with LST BM database

EuroFlow

Automated gating output with events to "check"



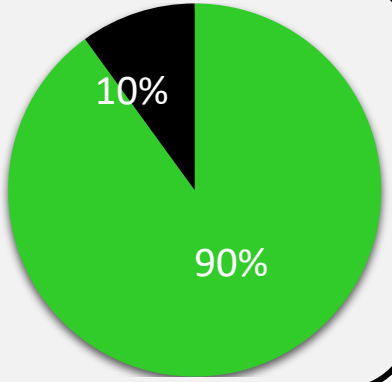


10%

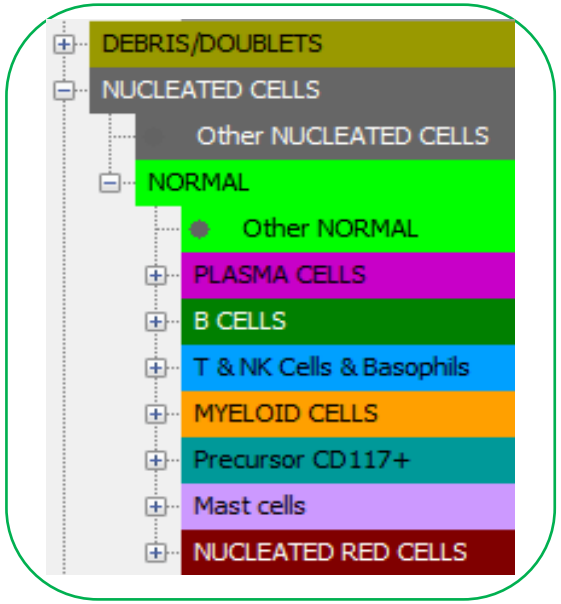
- Normal cell populations unequivocally identified
- To be Checked:
  - Clusters that deviate >2SD from cell populations in the database

CLASSIFICATION OF EVENTS INTO CELL POPULATIONS

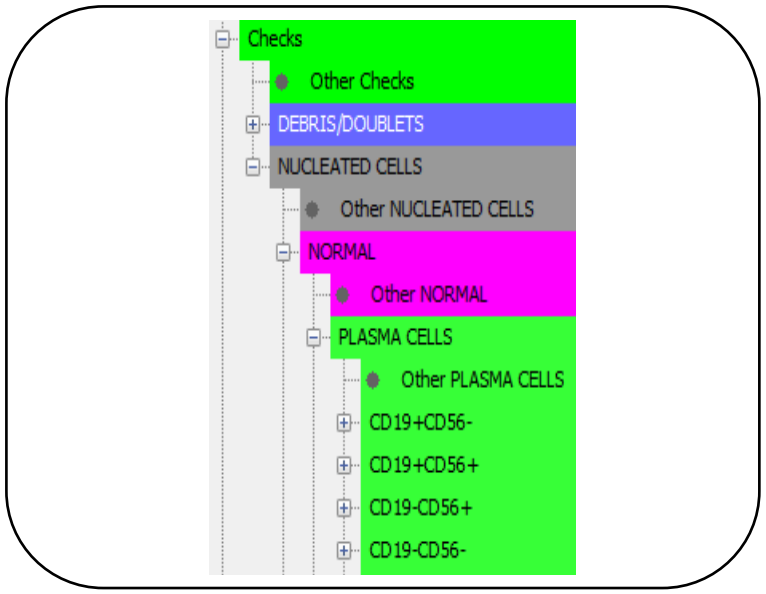
- Normal cell populations unequivocally identified
- To be Checked:
  - Clusters that deviate >2SD from the cell populations in the data base



No need to be checked



Need to be checked:



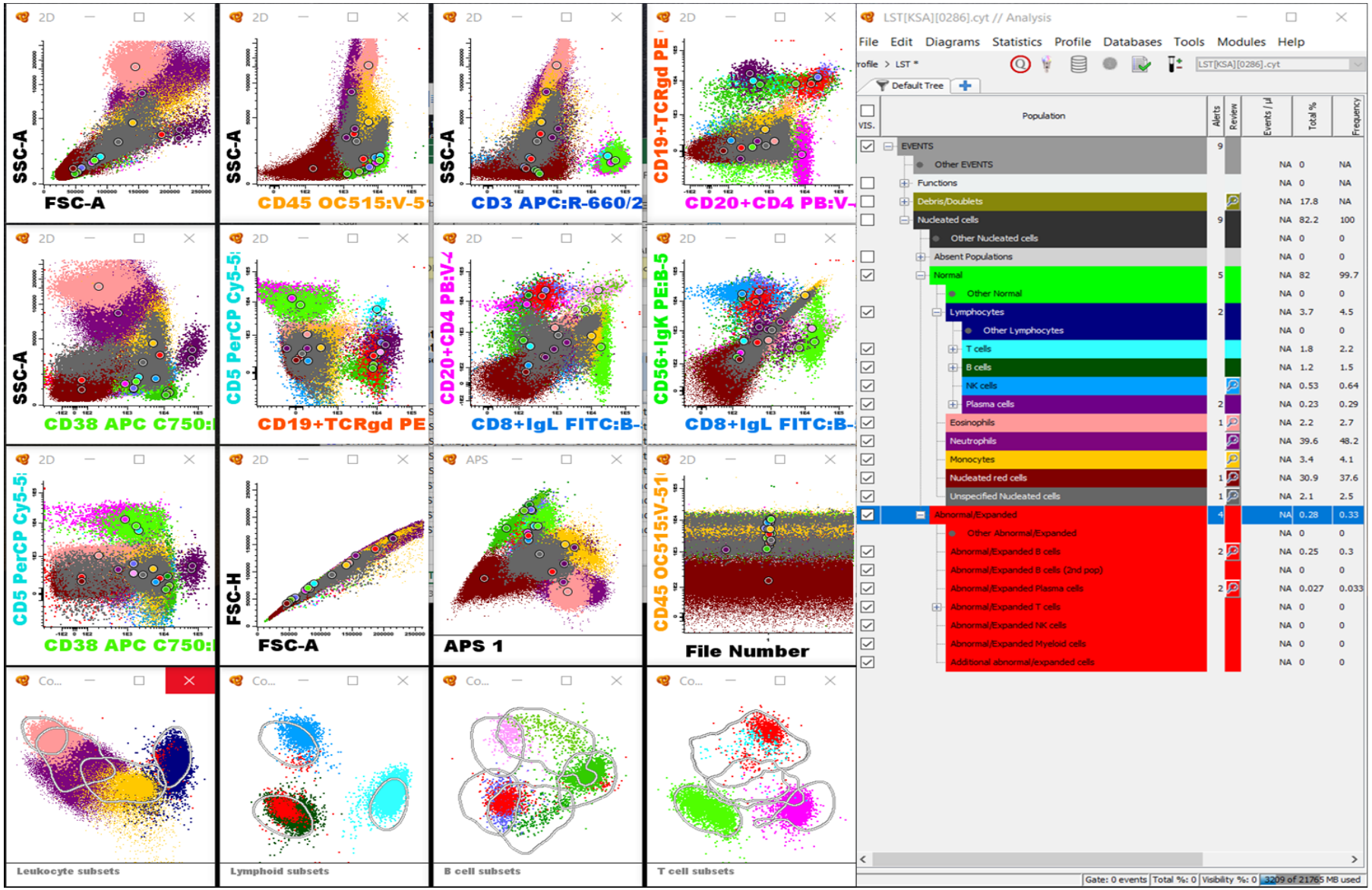
**Limit of detection established at 40 cellular events*

EuroFlow

EuroFlow automated gating (AG&I) with LST BM database

EuroFlow

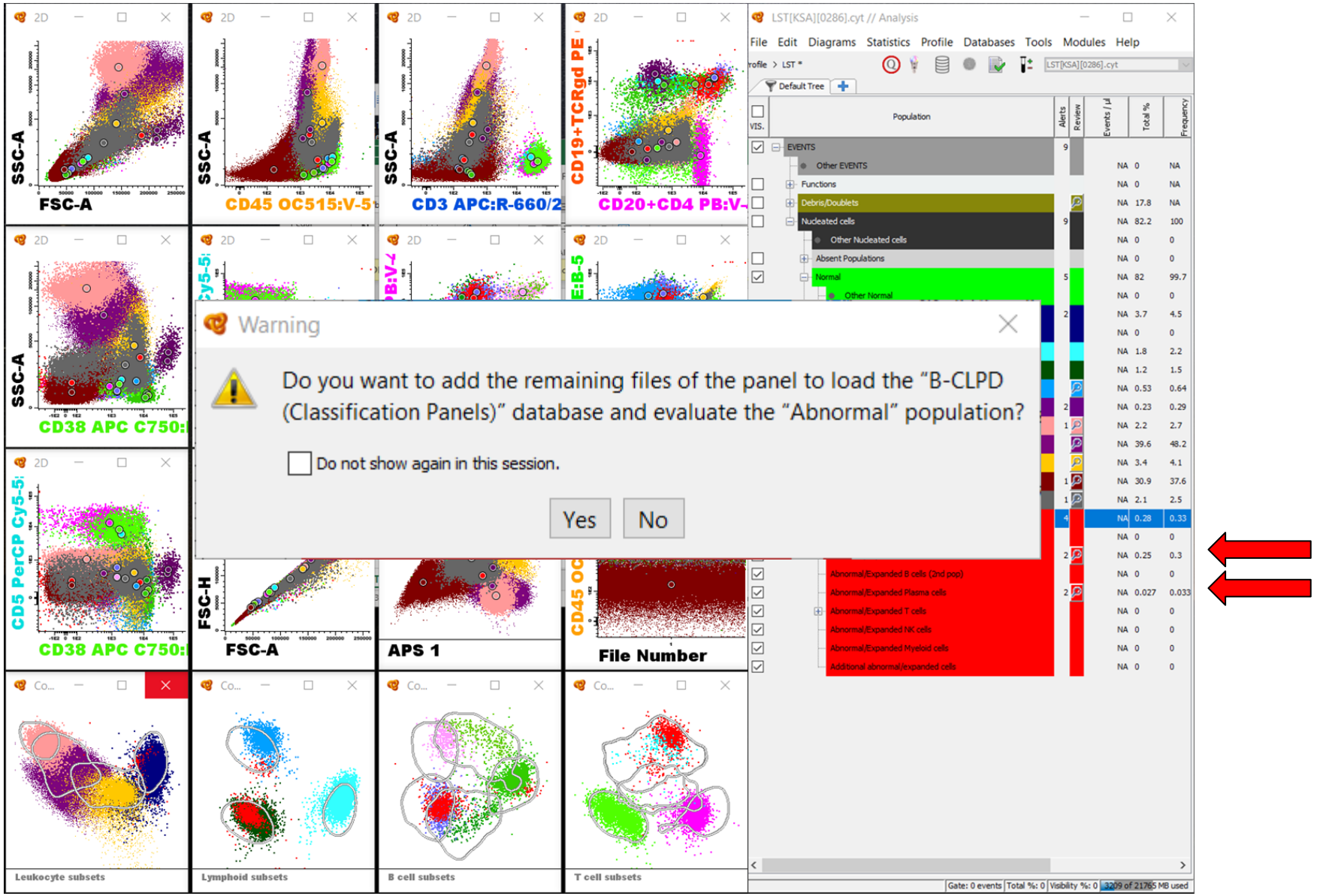
All events gated after labelling events in "check"



EuroFlow automated gating (AG&I) with LST BM database

EuroFlow

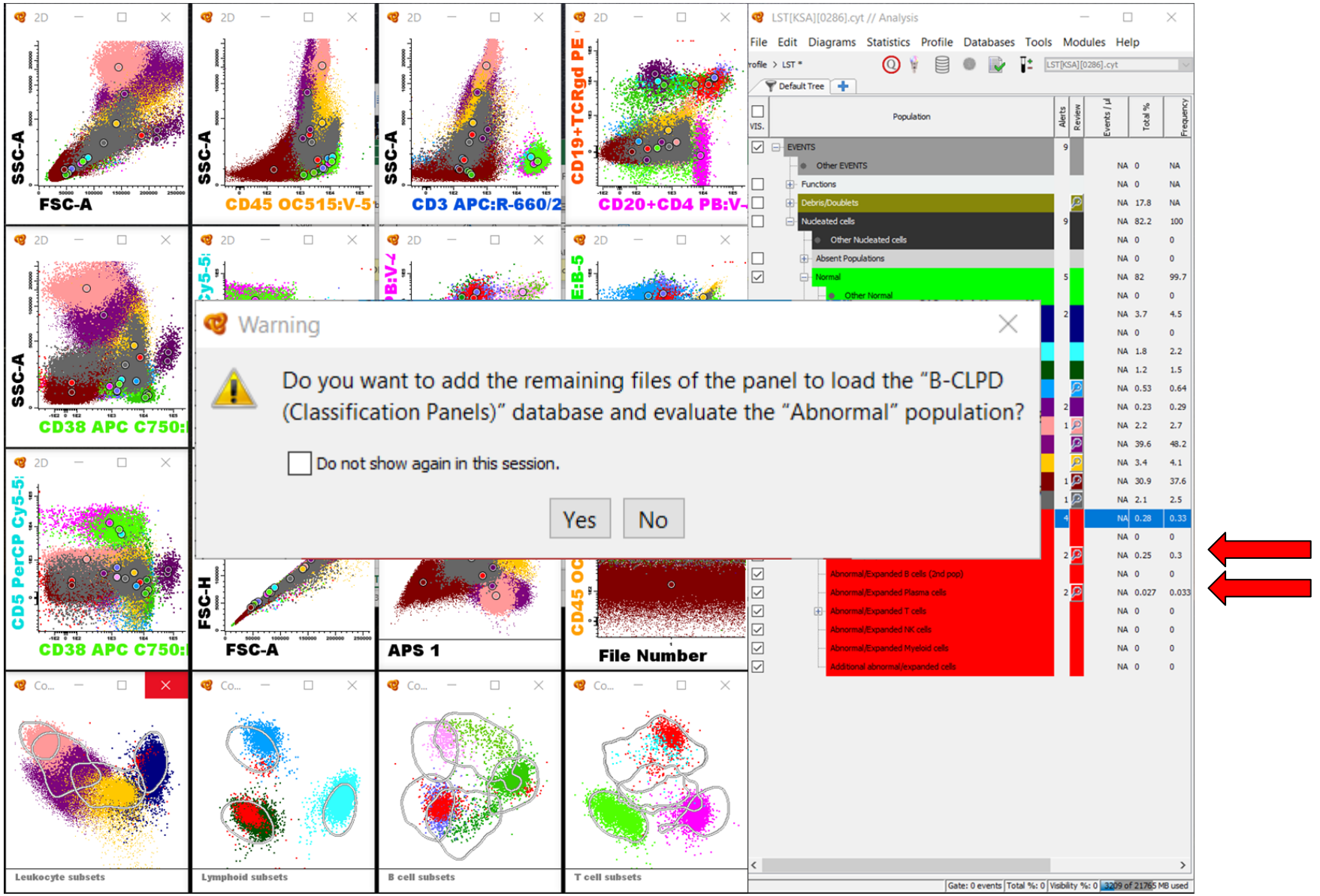
Final classification into a disease category



EuroFlow automated gating (AG&I) with LST BM database

EuroFlow

Final classification into a disease category



FCM DATA ANALYSIS IN CLINICAL LABS

Targets per data file

-Number of each cell population

- Normal
- Increased
- Decreased
- Imbalanced

- Immunophenotype of cell populations

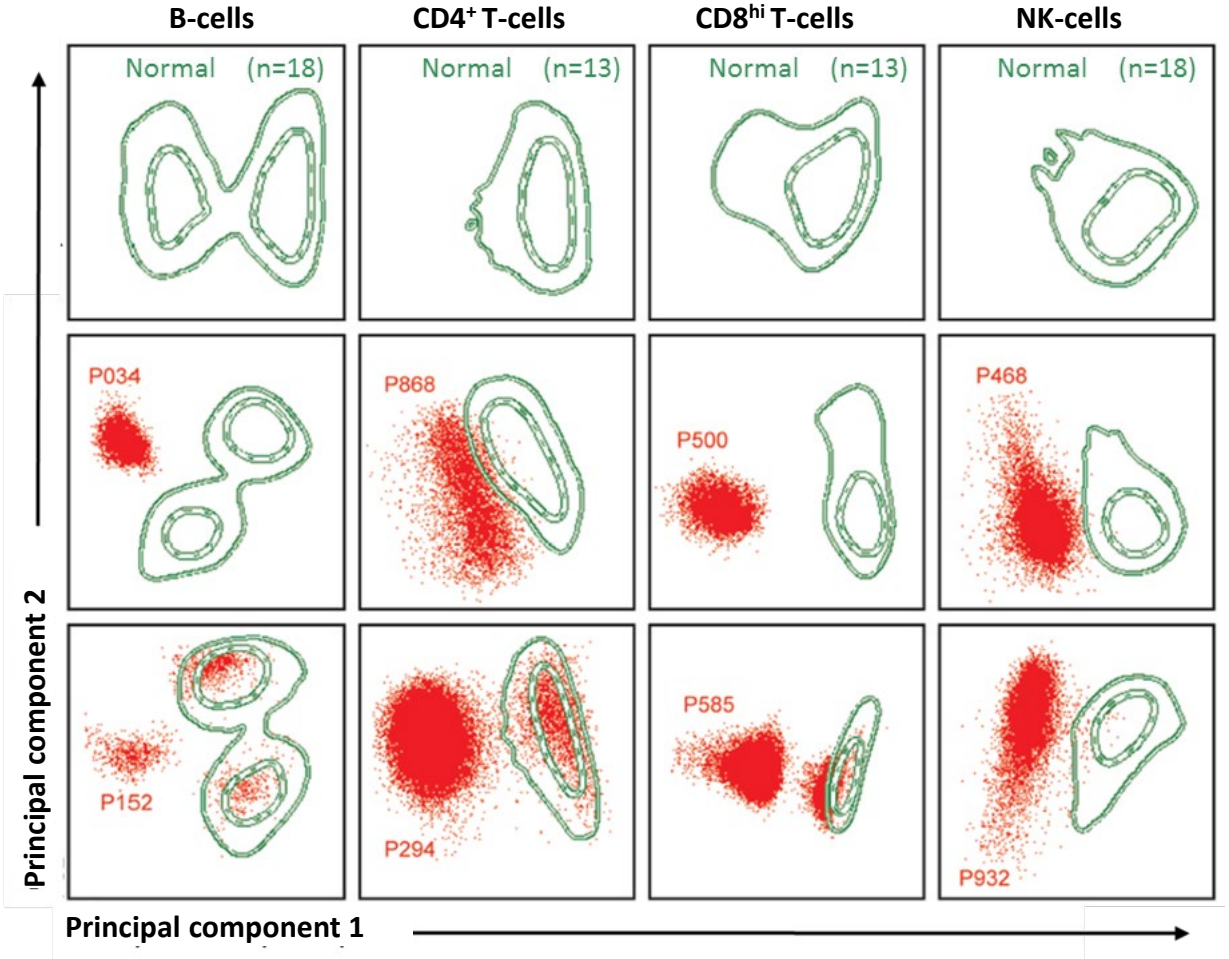
- Normal
- Reactive vs clonal
- Aberrant (what tumor type)

EuroFlow

Validation of the EuroFlow LST tube for detection of mature lymphoid tumor cells

EuroFlow

Principal component analysis against a database of normal PB

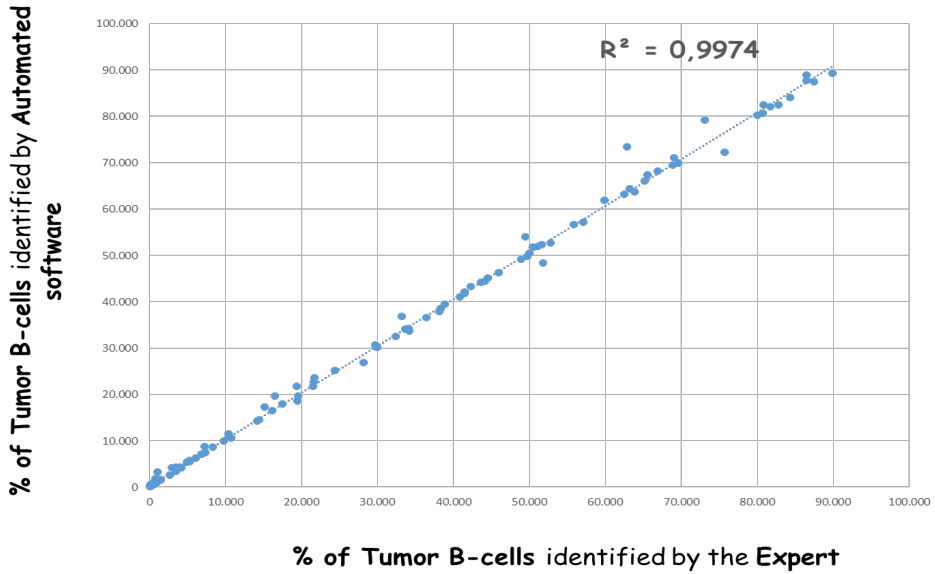


van Dongen et al, Leukemia 2012; Flores-Montero et al J Immunol Meth, 2019

Reference data base interpretation vs final WHO diagnosis

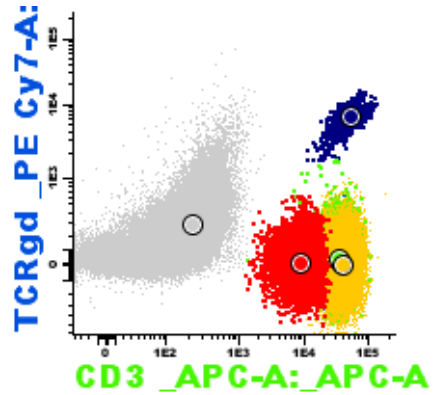
Criteria for abnormal lymphoid cells	N cases (%)
Aberrant immunophenotypic profile (n=227)	227/233 (97.4%)
Altered number /distribution (n=172)	172/233 (73.8%)
Total (n=233)	**233/233 (100%)**

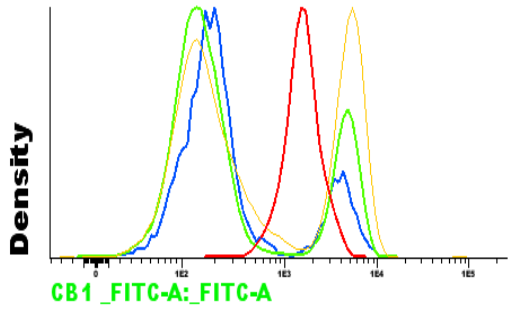
PERCENT TUMOR B-CELLS BY MANUAL vs AUTOMATED GATING (n=113)



Upgraded (8C / 13-markers) LST vs Classic LST + TRBC1 staining tube (n=18)

	Classic LST	TRBC1 tube % cells (min - max)	Upgraded LST
CD4+	**50.1%** (11.6 - 74.5)	**51.3%** (10.9 - 74.7)	**50.3%** (11.3 - 74.9)
TRBC1+		**40.3%** (35.5 - 52.5)	**40.4%** (34.6 - 51.6)
TRBC1-		**59.8%** (47.5 - 64.5)	**59.7%** (48.4 - 65.4)
CD8+	**43.2%** (20.9 - 88.3)	**41.5%** (20.4 - 88.9)	**43%** (20.8 - 88.5)
TRBC1+		**38.9%** (1 - 69.1)	**39.4%** (1 - 70.8)
TRBC1-		**61.2%** (30.9 - 99)	**60.7%** (29.2 - 99)
CD4-/CD8-	**4.3%** (0.2 - 10.6)	**4.5%** (0 - 11)	**4.2%** (0.2 - 10.1)





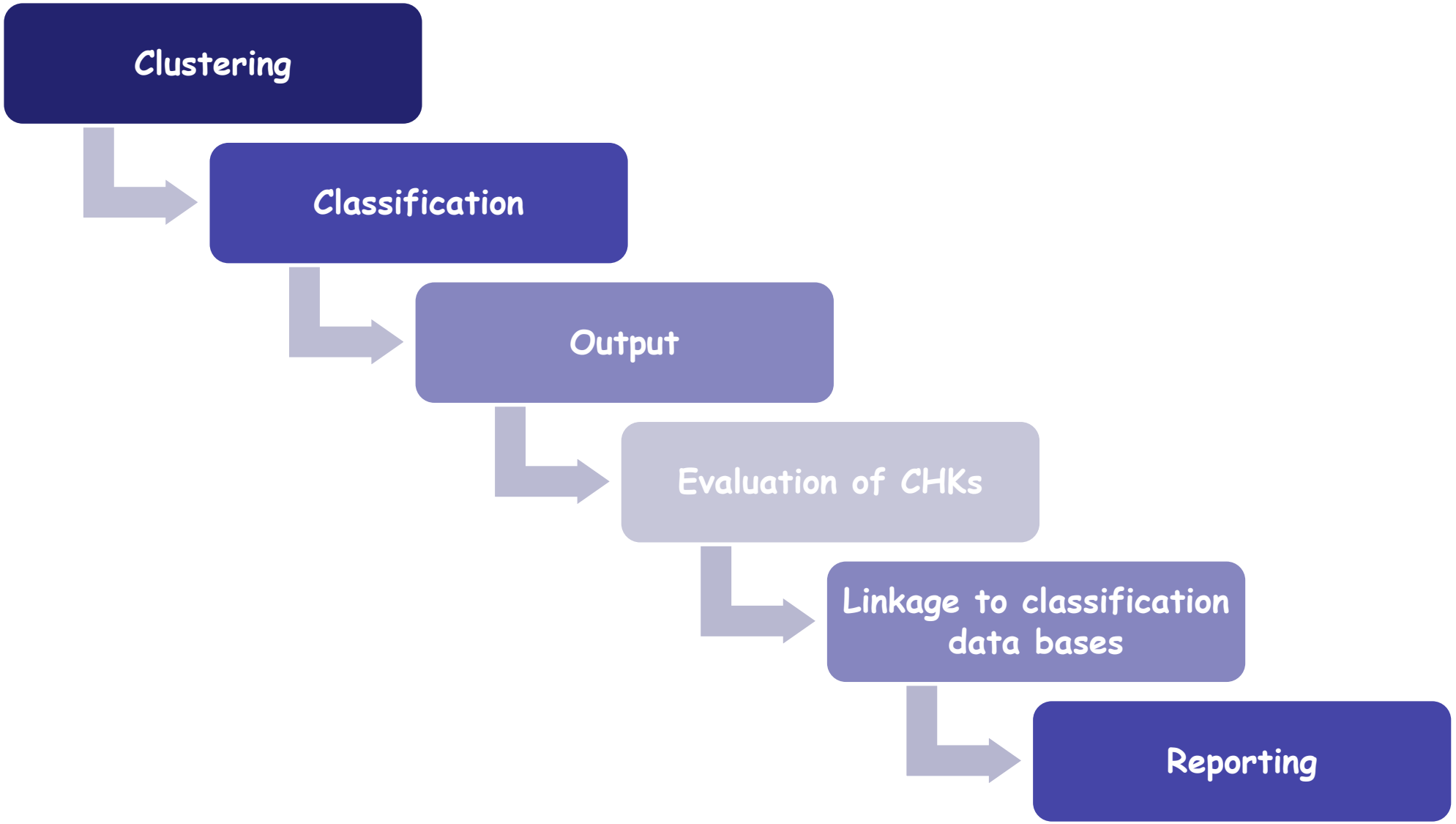
T CD4+
T CD8+
T TCRγδ+
T CD4-/CD8-/TCRγδ-
Clonal T-cells

p = n.s.

Responsible scientist: Juan Flores-Montero and Julia Almeida

Automated gaiting & identification flow chart

EuroFlow



FCM DATA OVERLAYED ON REFERENCE DATA BASES PLUS INTERPRETATION

- To evaluate an antibody panel and identify the most informative markers

- To (automatically) gate cell populations in a data file

- To classify cell populations in a sample into a potential diagnostic category

EuroFlow

FCM DATA ANALYSIS IN CLINICAL LABS

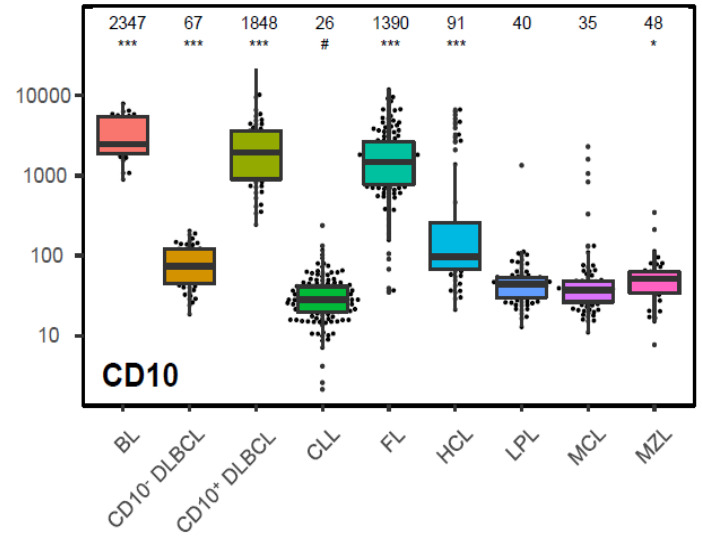
Targets per data file

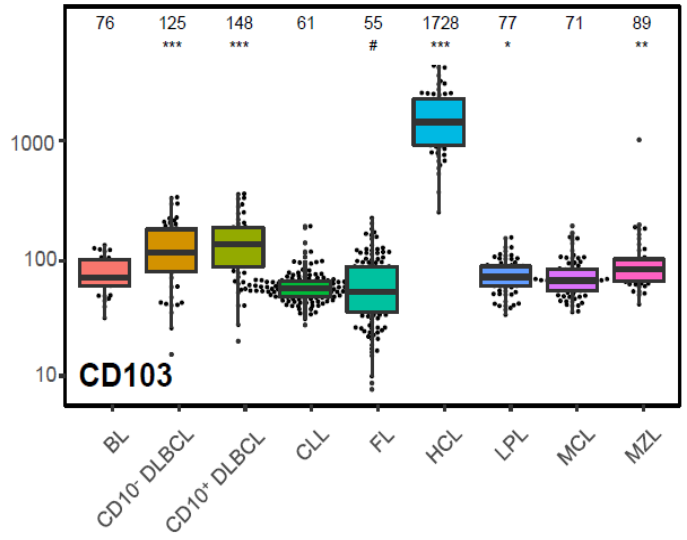
- Number of each cell population
    - Normal
    - Increased
    - Decreased
    - Imbalanced
- Immunophenotype of cell populations
    - Normal
    - Reactive vs clonal
    - Aberrant (what tumor type)

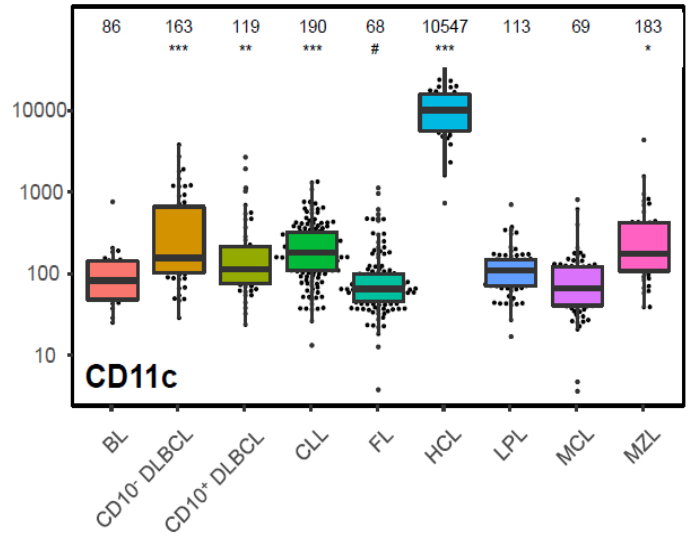
BIOLOGICAL (+CLINICAL) INTERPRETATION

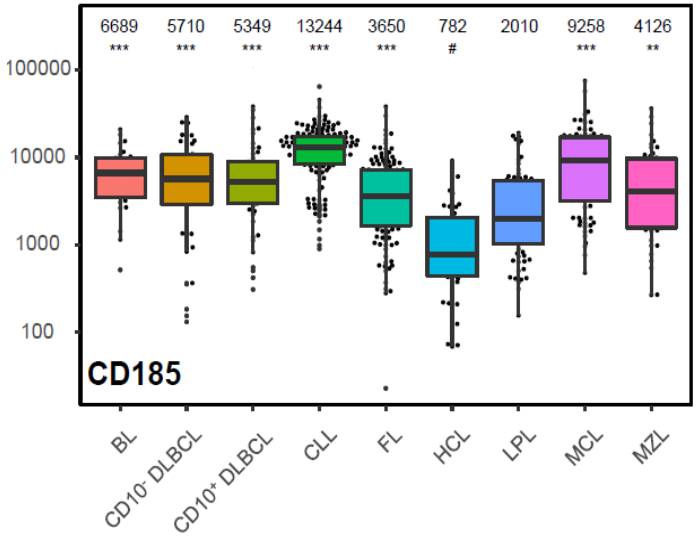
EuroFlow

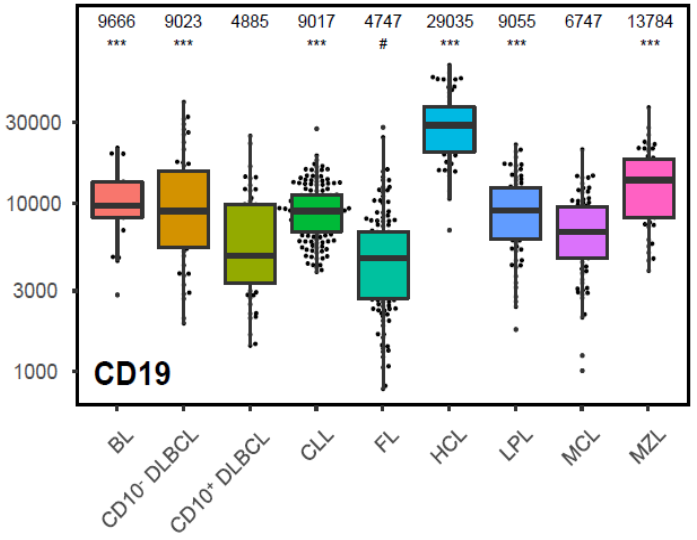
EuroFlow LST+ BCLPD panel database marker map per WHO 2016 diagnostic category

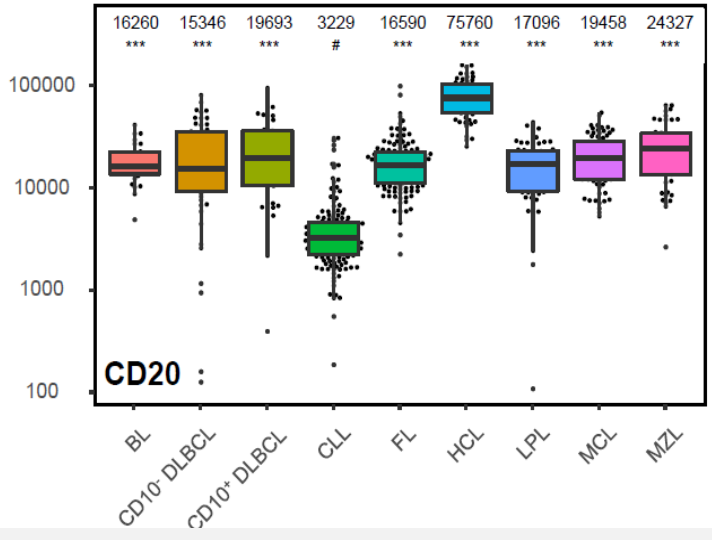






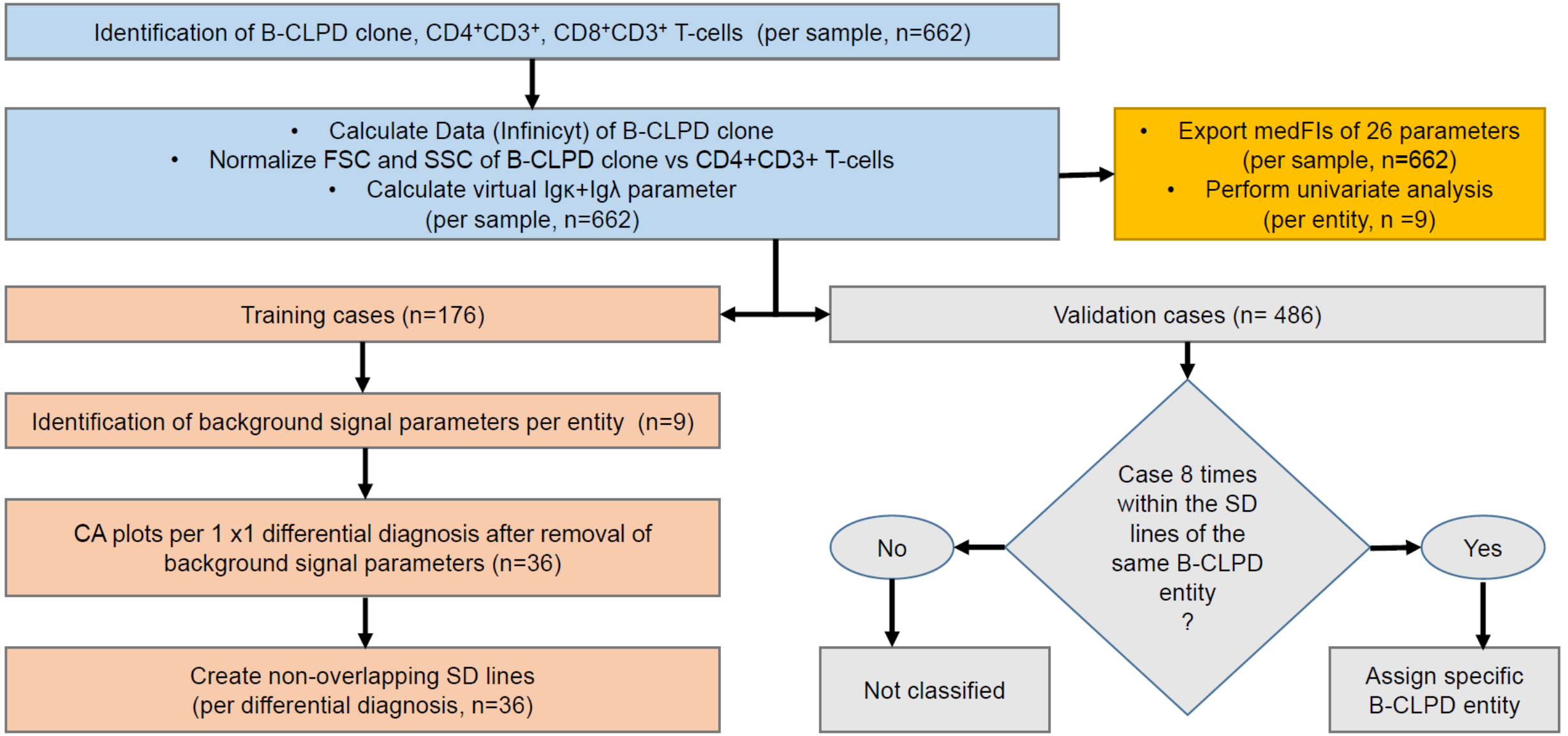






Bottcher et al, Leukemia 2020 (under revision)

EuroFlow LST + BCLPD database construction and validation



Bottcher et al, Leukemia 2021 (under revision)

Classification Algorithms (2006-2023)

CA	***C**anonical **A**nalysis*, based on Canonical Variate Analysis (CVA)
SVM	Based on the ***S**upport **V**ector **M**achine*
APS	***A**utomated **P**opulation **S**eparator*, based on the classical Principal Component Analysis (PCA) algorithm
CA-vSD	***CA** with **v**ariable **S**tandard **D**eviation Delimitation*
NAPS	***N**eighborhood **A**utomated **P**opulation **S**eparator* (*NAPS*), based on Neighbourhood Component Analysis (NCA)

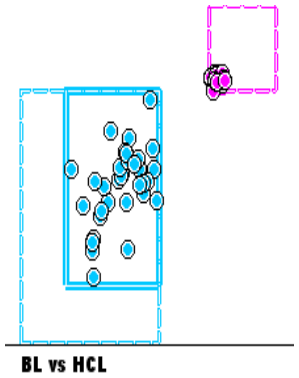
Although based on classical algorithms, all these approaches have been specifically developed to our applications

Overall, each method could in principle provide 4 classification outcomes vs the gold standard (WHO classification) compatible with:

1) A single correct diagnostic entity;
2) Multiple possible diagnoses including the correct one;
3) A misclassification and
4) Unclassifiable

HCL: 1 X 1 DIFFERENTIAL DIAGNOSIS

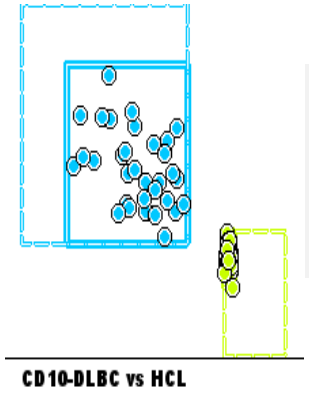
HCL vs BL



CD23 72%
CD31 26%
CD22 2%

CD103 65% CD38 15%
CD305 13% CD19 5%
CD20 2%

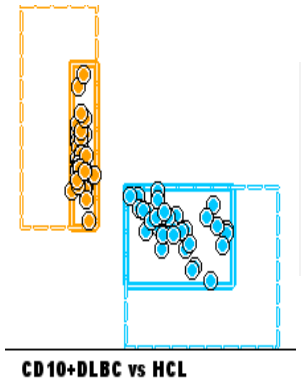
HCL vs CD10⁻DLBCL



CD103 75%
CD305 16%
CD31 9%
CD22 1%

CD95 46% CD11c 40%
CD19 8% CD20 7%

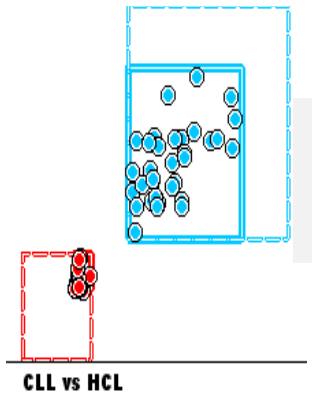
HCL vs CD10⁺ DLBCL



CD95 57%
CD45 19%
CD81 14%
CD19 10%

CD103 44% CD31 39%
CD305 12% CD22 3%
CD19 2%

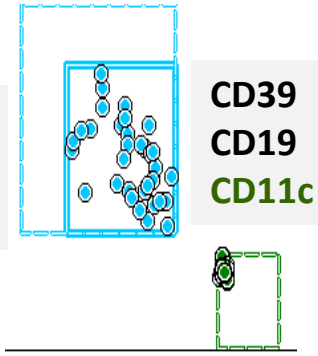
HCL vs CLL



CD95 96%
CD20 3%
CD19 1%

CD103 71% CD31 15%
CD305 11% CD22 3%

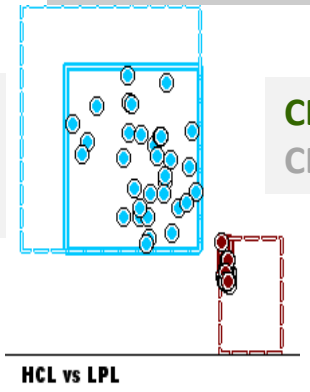
HCL vs FL



CD39 70%
CD19 20%
CD11c 10%

CD103 58% CD31 31%
CD305 11% CD22 1%
CD20 <1%

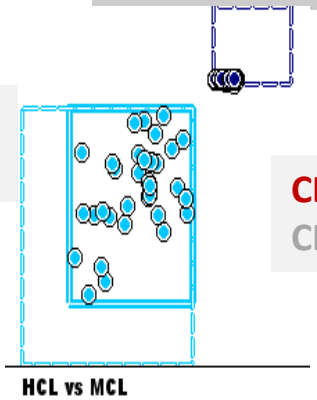
HCL vs MZL/LPL



CD305 94%
CD19 6%

CD103 92% CD11c 6%
CD22 1% CD20 <1%

HCL vs MCL

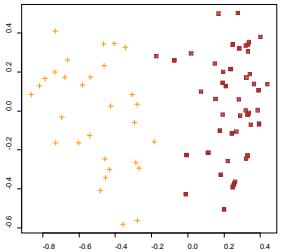


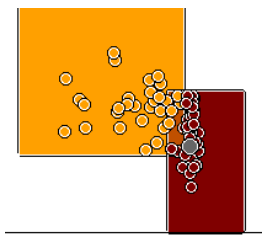
CD103 93%
CD11c 7%

CD305 74% CD22 15%
CD19 8% CD20 4%

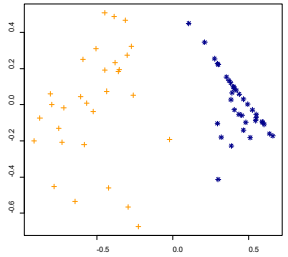
New algorithms for the diagnostic classification of B-cell chronic lymphoproliferative disorders

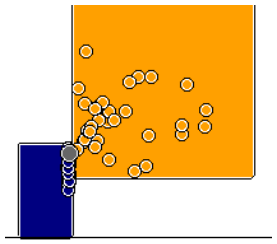
CD10+DLBCL vs LPL/MZL



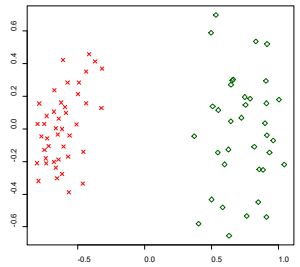


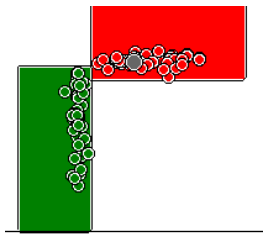
CD10+DLBCL vs MCL



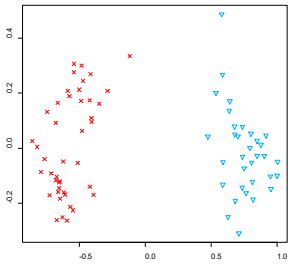


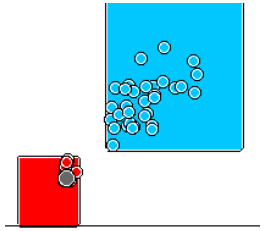
CLL vs FL



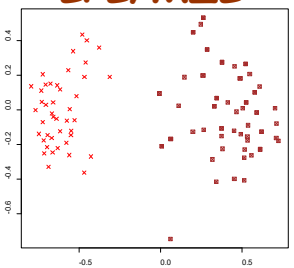


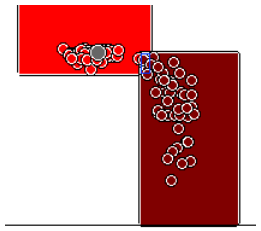
CLL vs HCL



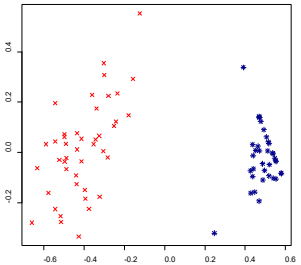


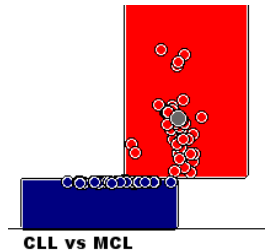
CLL vs LPL/MZL

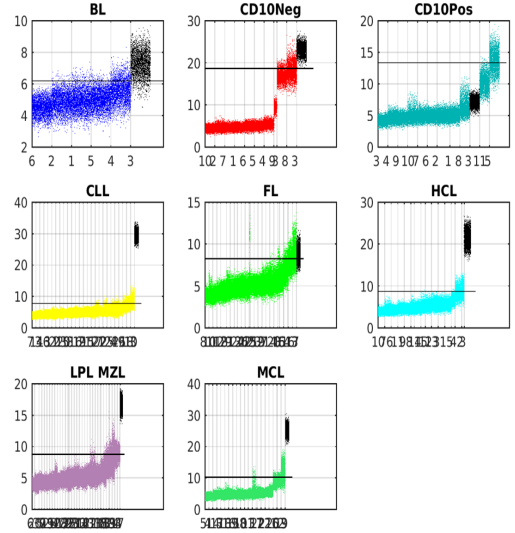




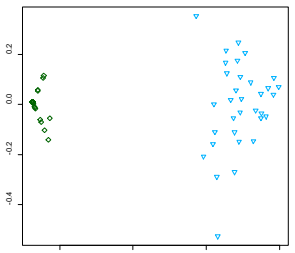
CLL vs MCL

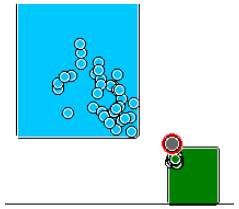




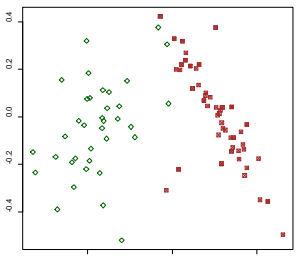


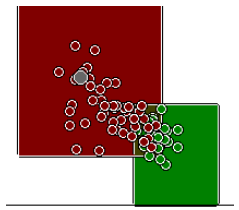
FL vs HCL



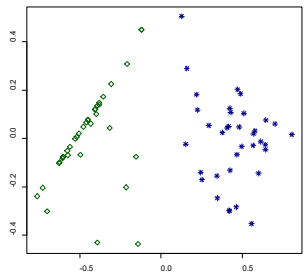


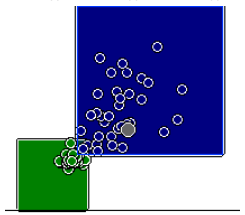
FL vs LPL/MZL



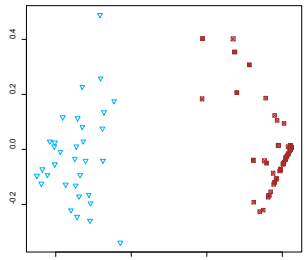


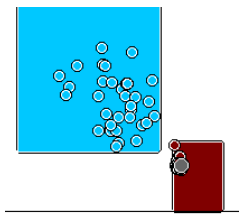
FL vs MCL



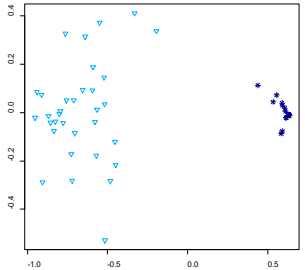


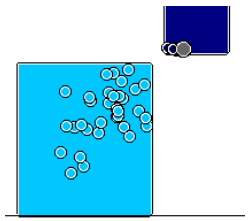
HCL vs LPL/MZL



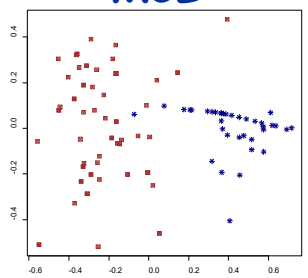


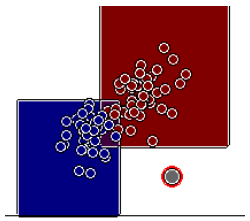
HCL vs MCL

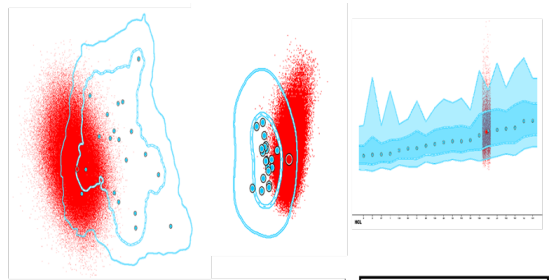




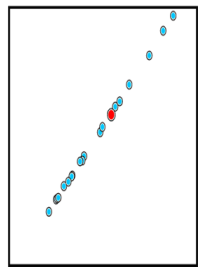
LPL/MZL vs MCL





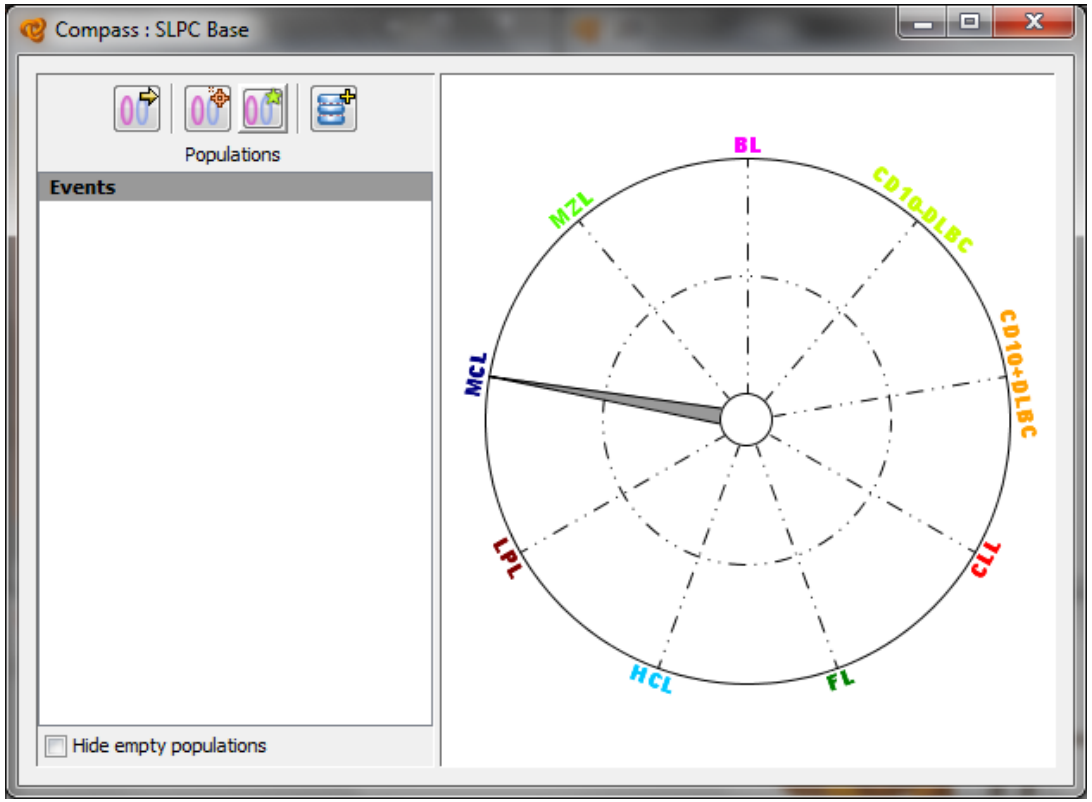


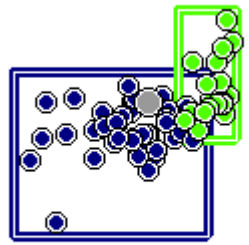
Group	Score	BL	CD10+DLBC	CD10+DLBC	CLL	FL	HCL	LPL	MCL	MZL
BL	15.80%	NA	5.15	10.45	27.06	12.81	4.81	24.99	30.79	10.38
CD10+DLBC	72.67%	94.85	NA	59.66	85.81	82.59	5.35	88.65	90.59	75.84
CD10+DLBC	59.41%	89.55	40.34	NA	71.59	63.92	3.20	72.87	76.81	57.02
CLL	36.81%	72.94	14.19	28.41	NA	42.75	3.02	44.85	60.58	27.78
FL	43.00%	87.19	17.41	36.08	57.25	NA	4.70	51.62	65.46	24.29
HCL	96.29%	95.19	96.65	96.80	96.98	95.30	NA	94.93	97.16	97.29
LPL	38.67%	75.01	11.35	27.13	55.15	48.38	5.07	NA	65.88	21.44
MCL	28.11%	69.21	9.41	23.19	39.42	34.54	2.84	34.12	NA	12.11
MZL	59.24%	89.64	24.16	42.98	72.22	75.71	2.71	78.56	87.89	NA



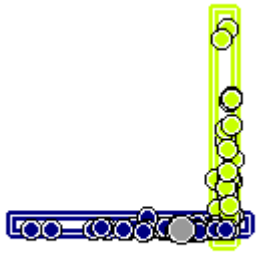
BCLPD: Diagnostic classification of individual cases vs a reference data base

BCLPD: Diagnostic classification of individual cases

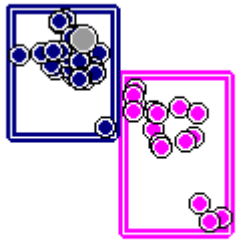




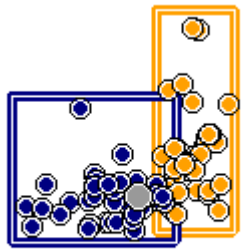
MCL vs MZL [Events]



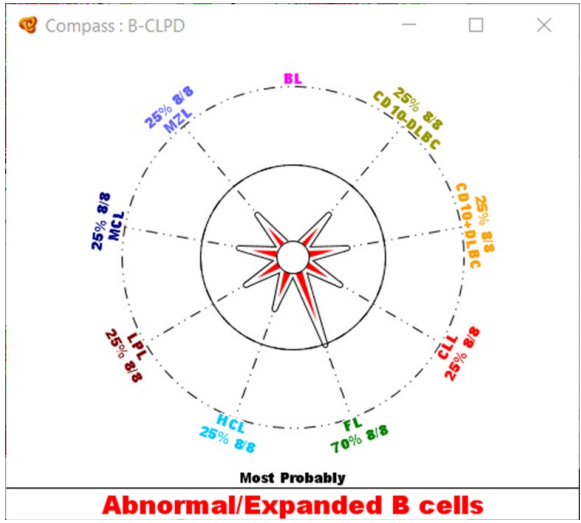
CD10-DLBC vs MCL [Events]



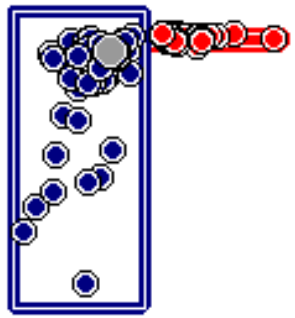
BL vs MCL [Events]



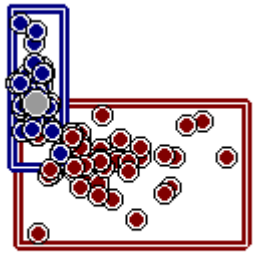
CD10+DLBC vs MCL [Events



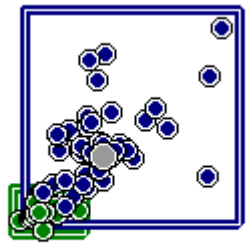
Abnormal/Expanded B cells



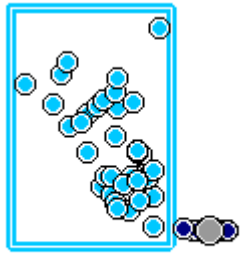
CLL vs MCL [Events]



LPL vs MCL [Events]



FL vs MCL [Events]



HCL vs MCL [Events]

BCLPD: Performance of different algorithms for the diagnostic classification of individual cases

	CA	SVM	APS	CA-vSD	NAPS
Correct diagnosis	90.6%	86.8%	86.0%	53.2%	80.4%
Misclassified	9.1%	11.9%	11.4%	7.3%	16.8%
Not classified	0.3%	1.3%	2.6%	39.5%	2.8%
% of single diagnoses	44.5%	42.3%	49.0%	58.7%	76.5%
Summarizing Score (CorrInd)	76.5	76.6	75.2	75.6	81.5

---- Best

---- Worst

BCLPD: Performance of different algorithms for the diagnostic classification of individual cases

Confusion matrix for NAPS and CA-vSD *(*659 BCLPD test cases)

	Correctness	Not-classified	Misclassified	NAPS ↓
Correctness	381	162	6	549 (83.3%)
Not classified	2	15	1	18 (2.7%)
Misclassified	5	67	20	92 (14%)
CA-vSD →	388 (58.9%)	244 (37%)	27 (4.1%)	659

		Classification algorithm				
WHO Diagnosis	**Predicted diagnosis**	**CA**	**SVM**	**APS**	**CA-vSD**	**NAPS**
BL (n=28)	Correct diagnosis	83.2%	80.6%	77.7%	50.3%	73.5%
	Misclassified	16.8%	15.8%	15.2%	14.0%	23.0%
	Not classified		3.6%	7.1%	35.7%	3.6%
CD10- DLBCL (n=52)	Correct diagnosis	83.3%	72.1%	76.4%	23.8%	72.1%
	Misclassified	16.7%	25.9%	19.8%	8.9%	24.0%
	Not classified		1.9%	3.8%	67.3%	3.8%
CD10+ DLBCL (n=52)	Correct diagnosis	87.4%	90.5%	79.9%	35.0%	69.8%
	Misclassified	12.6%	9.5%	18.2%	9.2%	28.3%
	Not classified			1.9%	55.8%	1.9%
CLL (n=145)	Correct diagnosis	98.6%	98.6%	97.9%	90.4%	97.9%
	Misclassified	0.7%	0.7%	0.8%	0.7%	0.7%
	Not classified	0.7%	0.7%	1.4%	9.0%	1.4%
FL (n=128)	Correct diagnosis	96.6%	93.1%	92.2%	38.2%	88.6%
	Misclassified	3.4%	6.1%	6.3%	0.9%	9.0%
	Not classified		0.8%	1.6%	60.9%	2.3%
HCL (n=58)	Correct diagnosis	94.1%	95.7%	96.6%	91.4%	96.6%
	Misclassified	4.1%	2.6%	1.7%	1.7%	1.7%
	Not classified	1.7%	1.7%	1.7%	6.9%	1.7%
LPL (n=74)	Correct diagnosis	96.1%	86.5%	91.8%	47.1%	77.6%
	Misclassified	3.9%	12.1%	6.8%	4.3%	12.9%
	Not clasified		1.4%	1.4%	48.6%	9.5%
MCL (n=75)	Correct diagnosis	98.6%	97.2%	97.2%	78.7%	95.2%
	Misclassified	1.4%	1.5%	1.4%	1.3%	3.4%
	Not classified		1.3%	1.3%	20.0%	1.3%
MZL (n=47)	Correct diagnosis	78.2%	66.8%	67.9%	37.5%	51.4%
	Misclassified	21.8%	33.2%	32.1%	11.5%	48.6%
	Not classified				51.1%	
Total (n=659)	Correct diagnosis	90.6%	86.8%	86.0%	53.2%	80.4%
	Misclassified	9.1%	11.9%	11.4%	7.3%	16.8%
	Not classified	0.3%	1.3%	2.6%	39.5%	2.8%

Efficiency of EuroFlow LST+BCLPD panel and databases for classification of BCLPD vs WHO 2016

	WHO diagnosis	n	Algorithm-based flow cytometric diagnosis													
			BL	CD10⁻ DLBCL	CD10⁺ DLBCL	CLL	FL	HCL	LPL	MCL	MZL	Not classi-fied	Sensi-tivity	Speci-ficity	PPV	NPV
T1 to T5	BL	13	***10***	0	0	0	0	0	0	0	0	3	77%	99%	67%	99%
	CD10⁻ DLBCL	31	0	***3***	0	0	0	0	0	0	4	24	10%	99%	33%	94%
	CD10⁺ DLBCL	33	5	0	***6***	0	2	0	0	0	0	20	18%	99%	55%	94%
	CLL	125	0	0	0	***116***	0	0	0	0	0	9	93%	100%	99%	98%
	FL	109	0	1	5	0	***35***	0	2	0	0	66	32%	99%	95%	84%
	HCL	38	0	0	0	0	0	***34***	0	0	0	4	89%	100%	97%	99%
	LPL	54	0	2	0	0	0	0	***22***	2	1	27	41%	99%	88%	93%
	MCL	56	0	0	0	0	0	0	0	***42***	0	14	75%	99%	93%	97%
	MZL	27	0	3	0	1	0	1	1	1	***10***	10	37%	99%	67%	96%
T1 + T2	BL	13	***8***	0	0	0	0	0	0	0	0	5	62%	99%	62%	99%
	CD10⁻ DLBCL	31	0	***2***	0	0	0	1	0	0	1	27	6%	100%	50%	94%
	CD10⁺ DLBCL	33	3	0	***7***	0	1	1	0	0	0	21	21%	99%	54%	95%
	CLL	125	0	0	0	***111***	0	0	0	0	1	13	89%	100%	99%	96%
	FL	109	2	0	5	0	***37***	0	0	0	0	65	34%	100%	97%	84%
	HCL	38	0	0	0	0	0	***25***	0	0	0	13	66%	100%	93%	97%
	LPL	54	0	0	0	0	0	0	***6***	1	3	44	11%	100%	75%	90%
	MCL	56	0	0	1	0	0	0	1	***26***	0	28	46%	100%	93%	93%
	MZL	27	0	2	0	1	0	0	1	1	***4***	18	15%	99%	44%	95%

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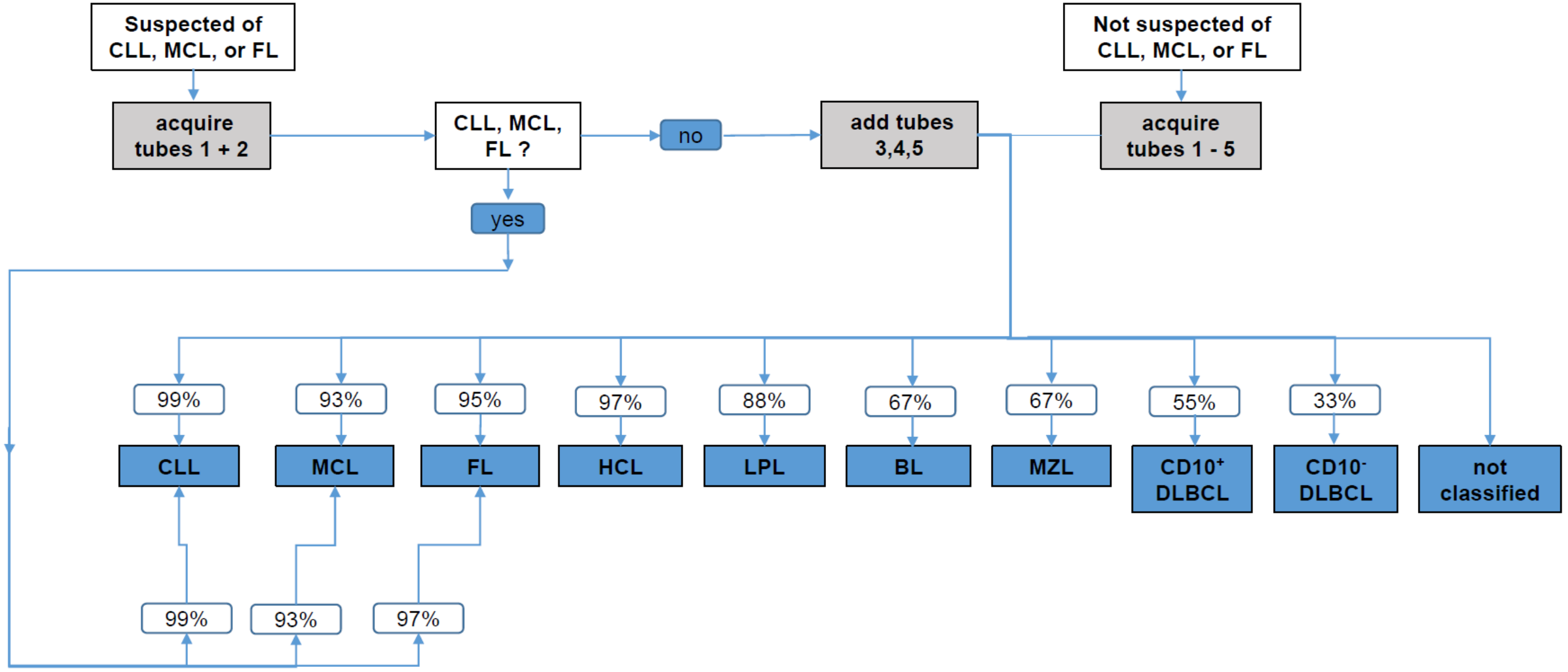
EuroFlow LST+BCLPD panel and databases for classification of BCLPD vs WHO 2016: diagnostic algorithm



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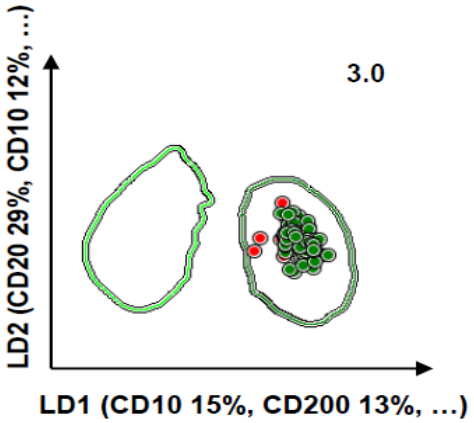
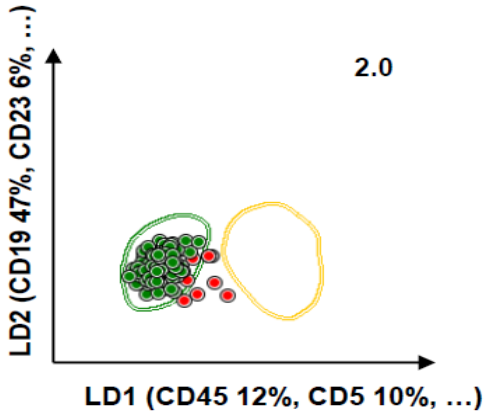
Efficiency of EuroFlow LST+BCLPD panel and databases for classification of BCLPD vs WHO 2016: CLL as an example

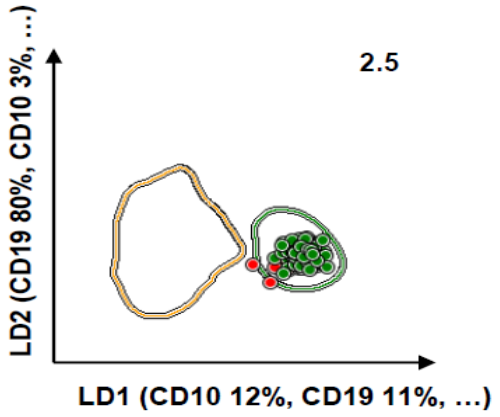
CLL vs. BL



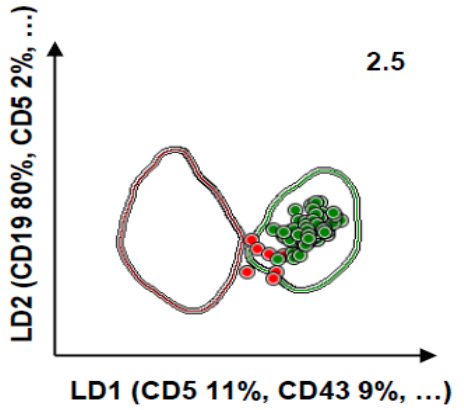
CLL vs. CD10⁻ DLBCL



CLL vs. CD10⁺ DLBCL

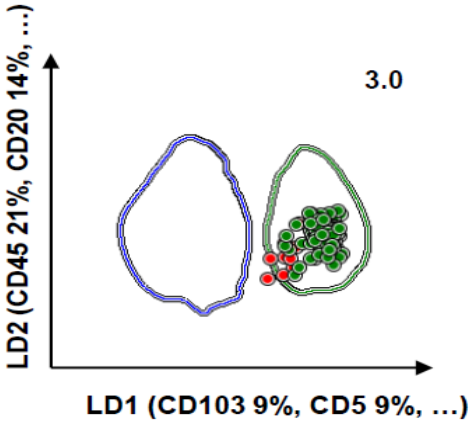


CLL vs. FL

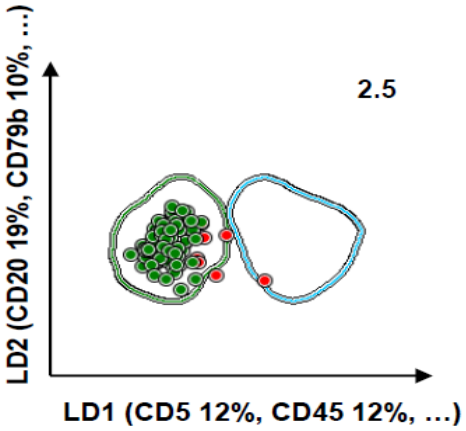


Highly accurate classification of typical and atypical CLL

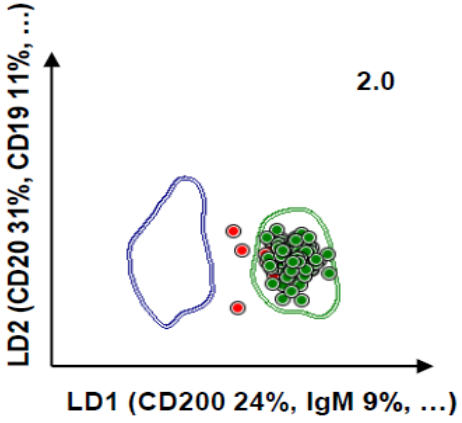
CLL vs. HCL



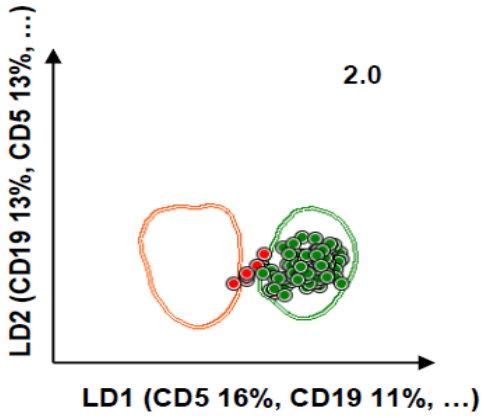
CLL vs. LPL



CLL vs. MCL



CLL vs. MZL



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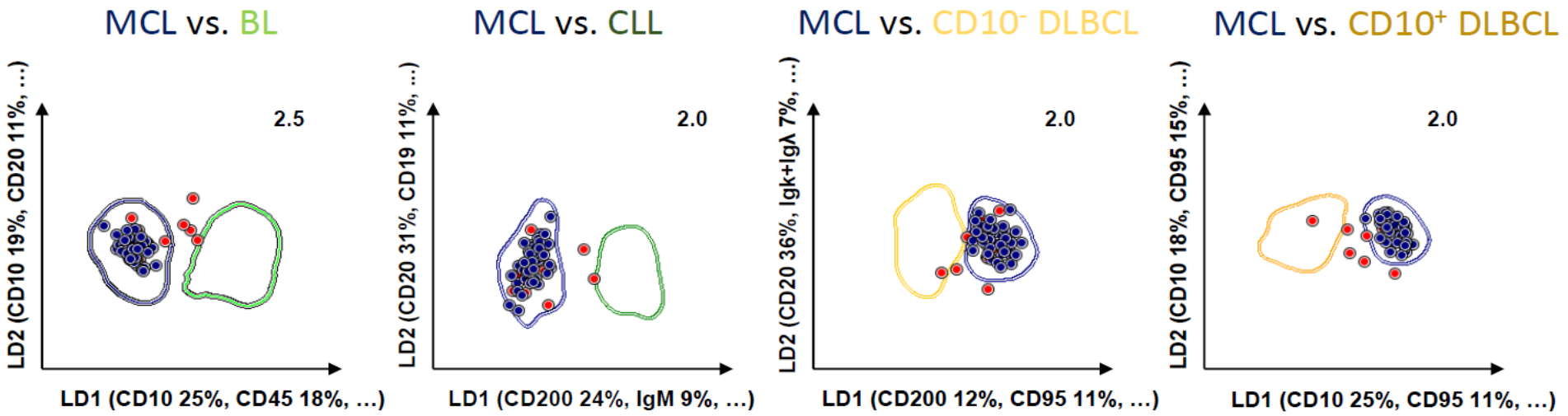
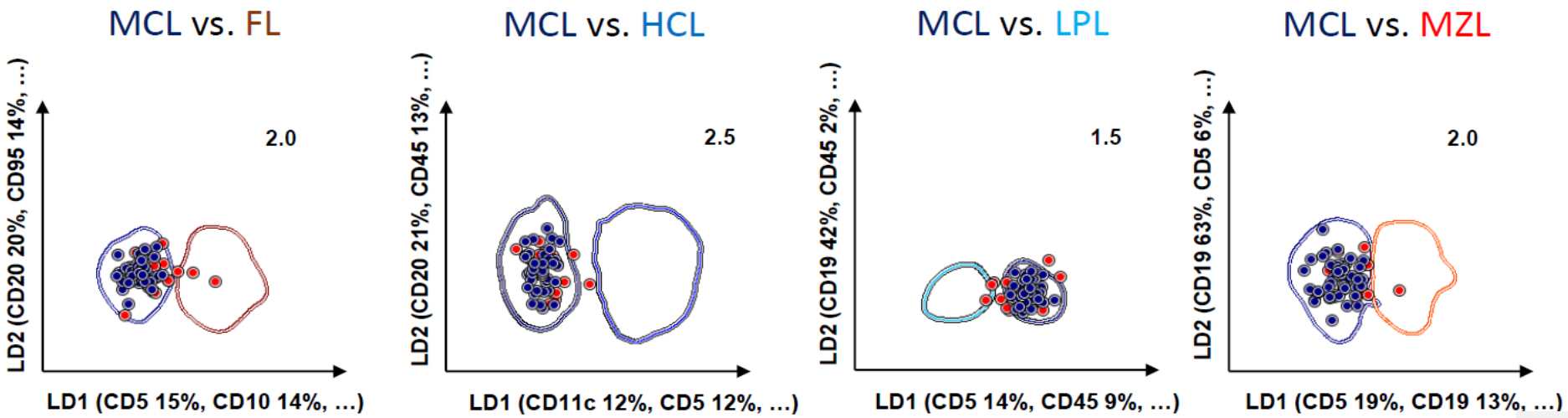
Efficiency of EuroFlow LST+BCLPD panel and databases for classification of BCLPD vs WHO 2016: MCL as an example





Highly accurate classification of typical and atypical MCL

Bottcher et al, Blood Adv 2022

FROM THE LAB TO THE CLINICAL SIDE

- Graphics are useful for expert visualization of complex data but …

- Graphics have to be translated into common (medical) language (numbers and words)

REPORT

- Numbers:
  - Counts per cell population
  - (age-matched) reference ranges
  - Quality of sample (hemodilution) and analysis (LOD, LOQ)

-Words / Text:
  - Marker expression levels 2SD (normal) vs 3SD (low-high) vs 4SD (very low-very high)
  - Compatible with diagnosis A (probability >95%)

EuroFlow

EuroFlow automated gating (AG&I) with LST PB database

Automated reporting

CELLULARITY (estimated based on total nucleated cells analyzed)

Cell concentration in the sample: 15600 cells/µl.

Reference age range: ≥ 50 years

Population	Frequency (%)	Reference (%)	Cells/µl	Reference (cells/µl)
Lymphocytes	**9.4**	(27.4 - 47.8)	**1465**	(1784 - 3902)
T cells	**7.1**	(17.7 - 40.4)	**1108**	(1249 - 3019)
CD4+CD8-	**4.5**	(10.4 - 25.6)	**694**	(721 - 1753)
CD8+CD4-	**2.1**	(2.9 - 19.8)	322	(202 - 1564)
CD4-CD8-/dim	0.59	(0.032 - 2.7)	91.3	(2.2 - 166)
TCRgd+	0.41	(0.099 - 2.7)	64.5	(6 - 166)
TCRgd-	0.17	(0.032 - 0.29)	**26.8**	(2.2 - 20.8)
NK cells	**1.2**	(1.9 - 8.7)	187	(153 - 740)
Plasma cells	**1.1**	(0.002 - 0.19)	**170**	(0.14 - 16)
Eosinophils	0.12	(0.03 - 4.3)	18.9	(1.6 - 297)
Neutrophils	**79.1**	(37.9 - 60.5)	**12339**	(1990 - 4881)
Monocytes	6.9	(5.7 - 12.9)	**1084**	(287 - 896)
Total Abnormal/Expanded cells	**4.4**	-	**694**	-
Abnormal/Expanded B cells	**4.4**	-	**694**	-

Absent populations: Mature B cells, Mature Sig Kappa, Mature Sig Lambda

Sample with 22.9 % of debris.

The reference values displayed are calculated according to: Percentile (5-95).

IMMUNOPHENOTYPIC DESCRIPTION OF ABNORMAL/EXPANDED CELLS

ABNORMAL/EXPANDED B CELLS IMMUNOPHENOTYPE

$FSC^{lo}SSC^{normal}$ CD4/$CD20^{++}$(98.8%) $CD45^{lo/+}$(96.7%) CD5$^-$ CD19/$TCRgd^{+/++}$(82.3%) CD38$^-$. sIglambda+

lo: low; hi: high.

Database normal cells have been used for the automated immunophenotypic description of the abnormal cells.

COMMENT

Peripheral blood sample with leukocytosis, neutrophilia and monocytosis. The absolute count of eosinophils and lymphocytes is normal.

Decreased number and/or proportion of T cells, CD4+CD8-, CD8+CD4- and NK cells detected. The proportion and/or absolute number of TCRgd- and B cells is increased.

In the analyzed peripheral blood, there are no alterations in the relative or absolute distribution of the lymphoid populations: CD4-CD8-/dim and TCRgd+.

The CD4/CD8 ratio (2.2) is normal.

In the analyzed sample, an abnormal expanded B cell population (4.4%, 694 cells/µl) is detected with aberrant immunophenotype (FSC^{lo} $CD45^{lo/+}$(96.7%)) and with monoclonal expression of immunoglobulin (sIglambda+). This is associated with the expansion of no abnormal circulating plasma cells (1.1%, 170 cells/µl). -

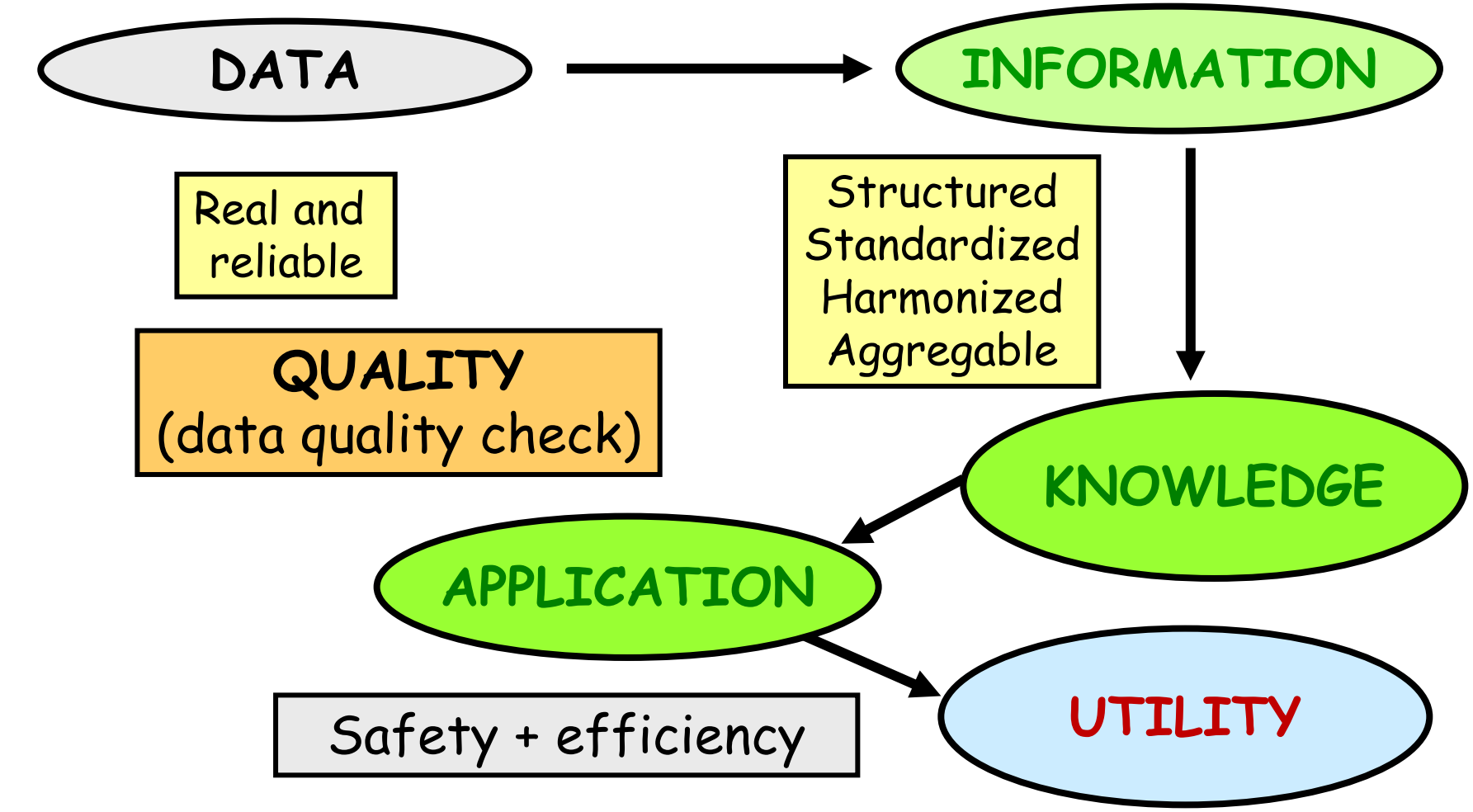
Add additional comment:

CONCLUSION

An abnormal expanded B cell population (4.4%) with aberrant immunophenotype detected. The characterization and classification of these cells are required.

Add additional conclusion:

FROM BIG DATA TO APPLIED KNOWLEDGE

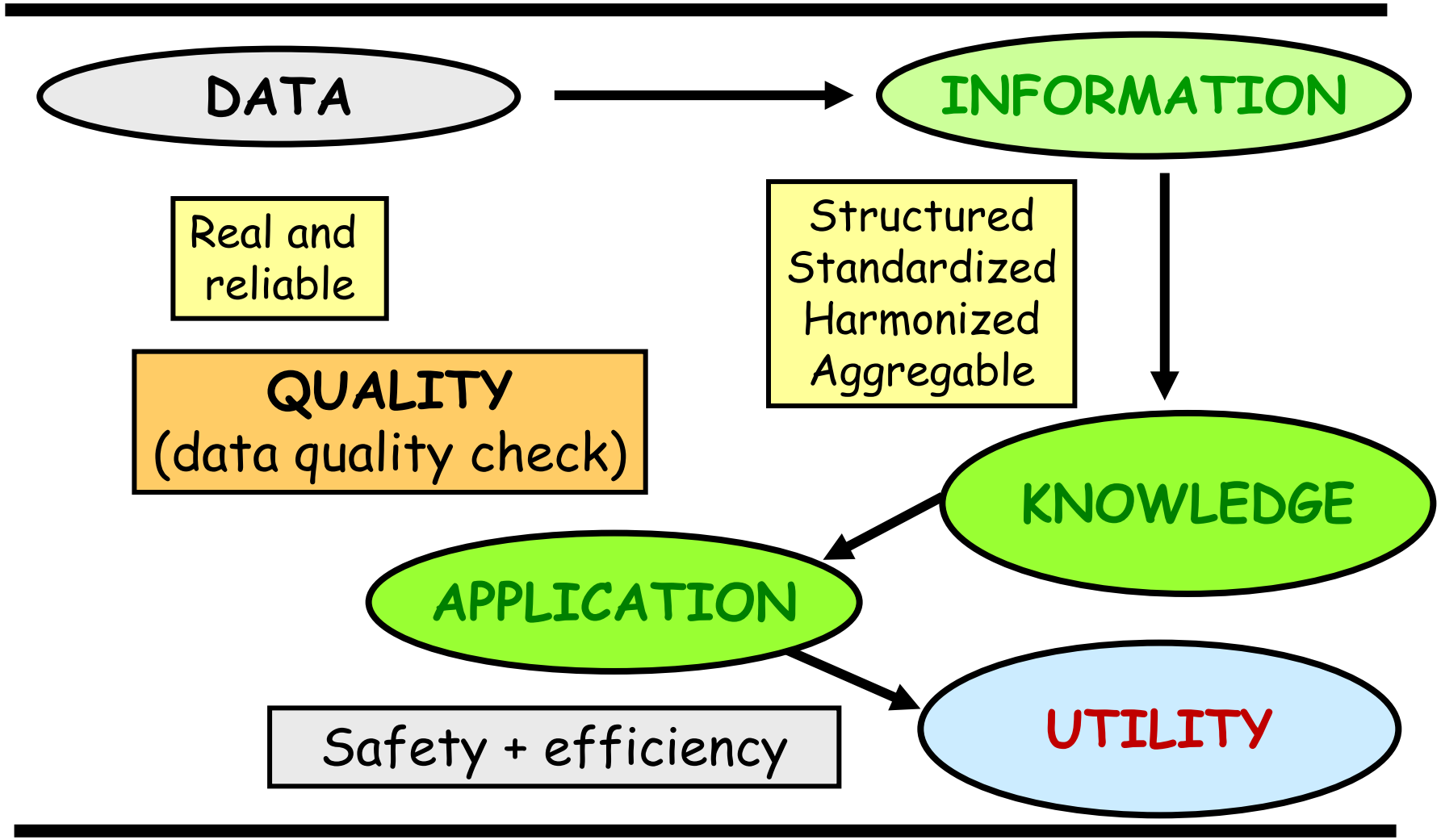


Automate
Cost savings
Quality
Accuracy

Augment (Predict)
Self learning
Better prediction
Human interactive

Amplify (decide)
Autonomous
Boost human activities

FROM BIG DATA TO CLINICALLY USEFUL AUTOMATED ASSAYS



Automation of data analysis and interpretation goes beyond current data analysis procedures and approaches with an increased value and utility

Concluding remarks

- Flow cytometry is much more than data, but its future requires appropriate and maximized extraction and usage of information that data provide
  - Pictures are not enough

- Low quality data will lead to low quality clinical and research information and more limited clinical utility and knowledge
  - Standardization is mandatory

-

- New data analysis tools are needed for maximum benefit from data in the clinical and research settings
  - Automation is the way to go

Tools are required, but most importantly planning is needed

EuroFlow

AKNOWLEDGEMENTS

EuroFlow

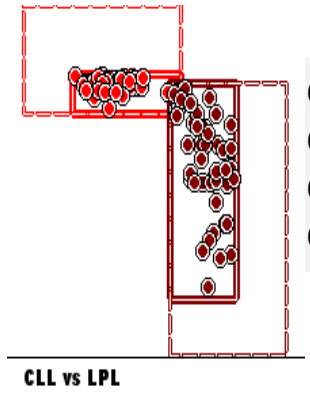
EuroFlow is part of ESLHO a scientific working group of EHA
(European Hematology Association)

Euroflow is an independent scientific consortium, which aims at innovation in flow cytometry for improving diagnostic patient care
www.euroflow.org

MUCHAS GRACIAS

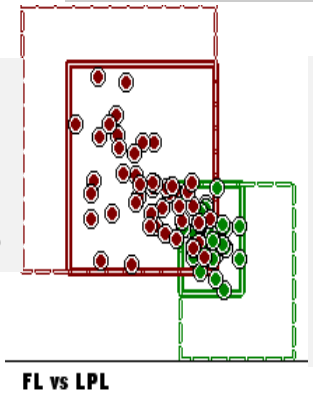
MZL/LPL and FL: 1 X 1 DIFFERENTIAL DIAGNOSIS

MZL/LPL vs CLL



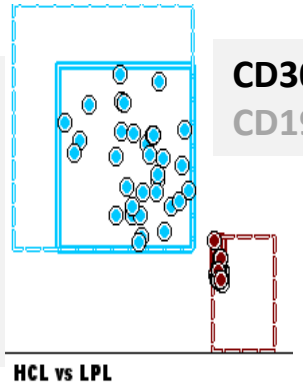
CD95	44%		
CD31	33%		
CD22	13%		
CD79b	10%		
CD45	28%	CD200	20%
CD5	19%	CD23	15%
IgM	8%	CD43	6%
CD20	3%		

MZL/LPL vs FL



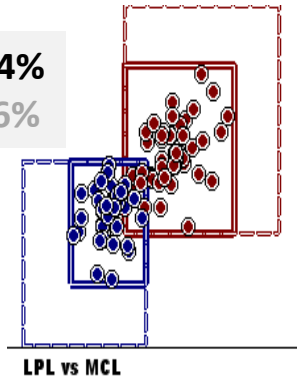
CD49d	44%		
CD38	20%		
CD200	16%		
CD27	14%		
CD81	4%		
CD45	2%		
CD62L	39%	CD43	23%
CD39	17%	CD31	14%
CD19	4%	CD10	3%

MZL/LPL vs HCL



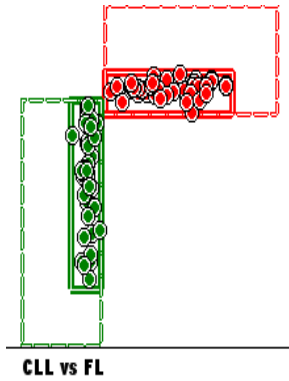
CD305	94%		
CD19	6%		
CD103	92%	CD11c	6%
CD22	1%	CD20	<1%

MZL/LPL vs MCL



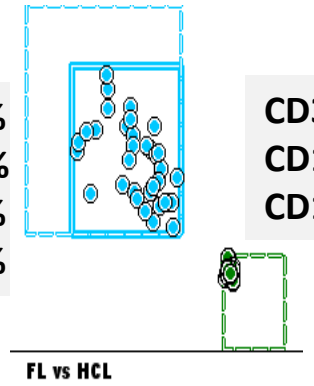
CD31	48%		
CD49d	44%		
HLADR	3%		
CD19	3%		
CXCR5	3%		
CD11c	53%	CD62L	19%
CD200	10%	CD95	6%
CD5	6%	CD45	6%

FL vs CLL



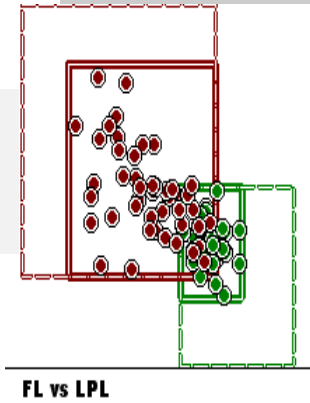
CD10	50%		
CD79b	18%		
CD45	16%		
CD22	16%		
CD31	36%	CD5	22%
CD43	20%	CD200	15%
CD81	5%	CD19	2%

FL vs HCL



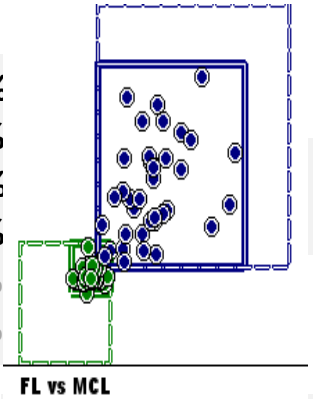
CD39	70%		
CD19	20%		
CD11c	10%		
CD103	58%	CD31	31%
CD305	11%	CD22	1%
CD20	<1%		

FL vs MZL/LPL



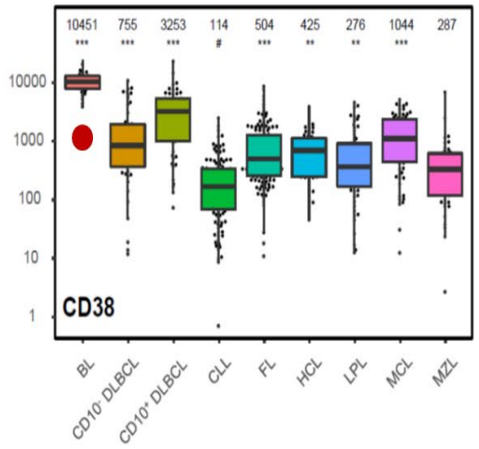
CD49d	44%		
CD38	20%		
CD200	16%		
CD27	14%		
CD81	4%		
CD45	2%		
CD62L	39%	CD43	23%
CD39	17%	CD31	14%
CD19	4%	CD10	3%

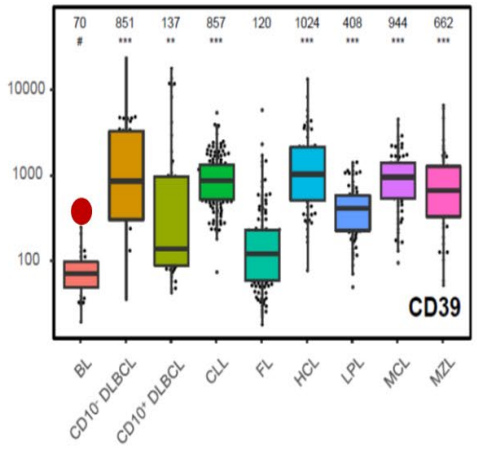
FL vs MCL

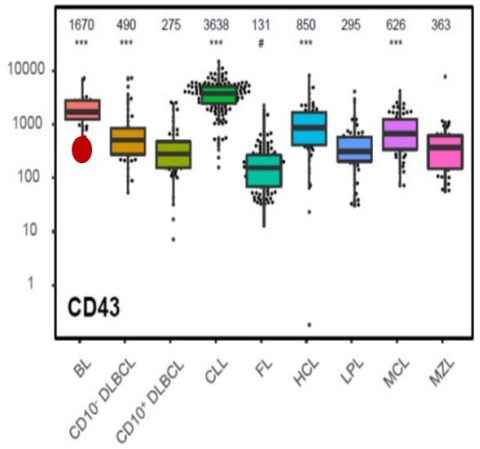


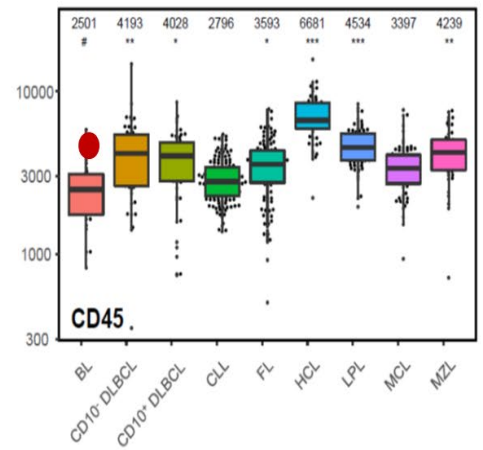
CD39	53%		
CD5	47%		
CD305	44%	CD31	28%
CD43	26%	CD10	2%

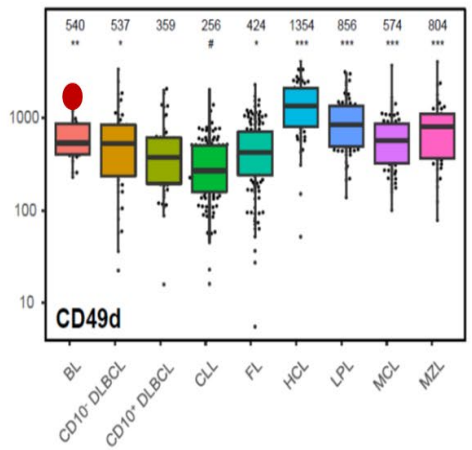
EuroFlow LST+ BCLPD panel database marker map per WHO 2022 diagnostic category

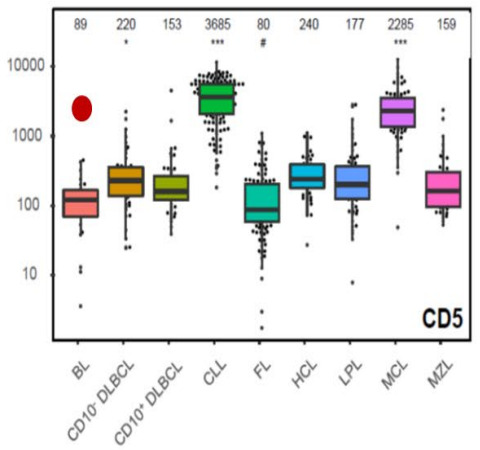


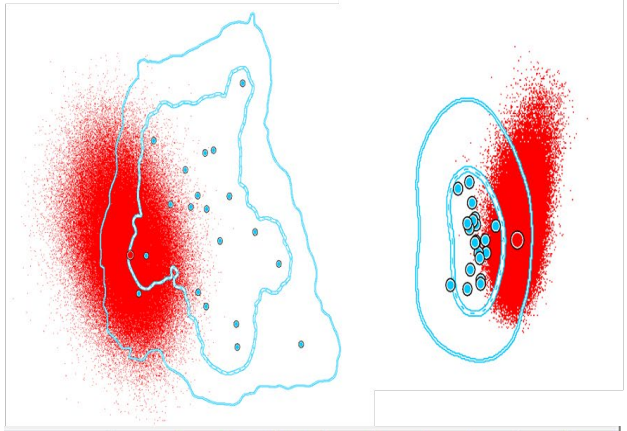


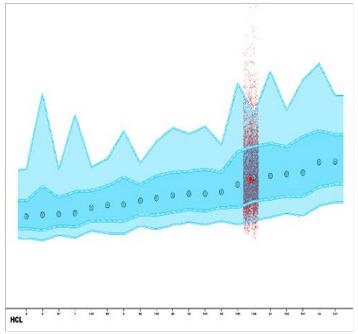




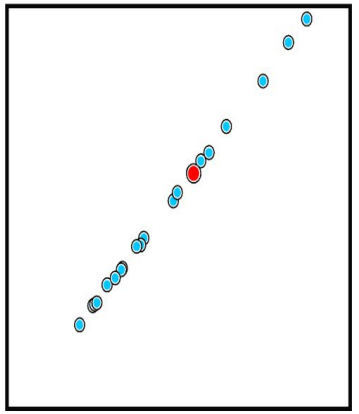








Group	Score	BL	CD10-DLBC	CD10+DLBC	CLL	FL	HCL	LPL	MCL	MZL
BL	15.80%	NA	5.15	10.45	27.06	12.81	4.81	24.99	30.79	10.36
CD10-DLBC	72.67%	94.85	NA	59.66	85.81	82.59	3.35	88.65	90.59	75.84
CD10+DLBC	59.41%	89.55	40.34	NA	71.59	63.92	3.20	72.87	76.81	57.02
CLL	36.81%	72.94	14.19	28.41	NA	42.75	3.02	44.85	60.58	27.78
FL	43.00%	87.19	17.41	36.08	57.25	NA	4.70	51.62	65.46	24.29
HCL	96.29%	95.19	96.65	96.80	96.98	95.30	NA	94.93	97.16	97.29
LPL	38.67%	75.01	11.35	27.13	55.15	48.38	5.07	NA	65.88	21.44
MCL	28.11%	69.21	9.41	23.19	39.42	34.54	2.84	34.12	NA	12.11
MZL	59.24%	89.64	24.16	42.98	72.22	75.71	2.71	78.56	87.89	NA



EuroFlow

Bottcher et al, Blood Adv 2022

AVAILABLE DATA BASES (August 2022)

- L & L diagnostic panels:

- LST in blood
- LST in bone marrow
- ALOT in blood
- ALOT in bone marrow

- L & L MRD panels

- MM-MRD in bone marrow
- MM-CTPC in blood
- BCP-ALL MRD in bone marrow
- BCP-ALL MRD in blood

- L & L classification panels:

- ALOT (acute leukemias)
- LST (B-cell CLPD)
- BCLPD (B-cell CLPD)

- PID panels

- PIDOT in blood (reference ranges)

Internal EuroFlow tests:

- TCD4 cells in blood (ref. ranges)
- Cytotoxic T/NK in blood
- B-cells and PC in blood

- Under construction:

- PID IgH-isotype (ref. ranges)
- IMM-Innate cells/Mo/DC
- PNH

EuroFlow