New applications of FCM in the diagnosis and monitoring of B-cell chronic lymphoproliferative disorders

VNiVERSiDAD D SALAMANCA

Cancer Research Centre & Dpt. Medicine
University Hospital of Salamanca University of Salamanca

XVII CONGRESO CHILENO DE HEMATOLOGÍA & VII CONGRESO DE MEDICINA TRANSFUSIONAL
27 AL 30 DE OCTUBRE 2010, HOTEL DE LA BAHÍA, COQUIMBO , Chile

IMMUNOPHENOTYPING OF HAEMATOLOGICAL MALIGNANCIES

- 1953/1994: From the development of the instruments & techniques to the WHO classifications of haematological malignancies.

- 1994/2006: The ability to specifically identify leukaemic cells: from normal phenotypes to aberrant phenotypic profiles.

- 2006/-: Recent contributions of immuno-phenotyping of haematological malignancies: pointing to the future.

FCM IMMUNOPHENOTYPING IN THE 80`S: PANELS OF REAGENTS AND TECHNIQUES

PANELS OF REAGENTS:

- Panels of relevant markers to support suspected diagnosis of:
  - AML, ALL
  - MM
  - B-CLPD, T-CLPD

TECHNIQUES:

- Isolation of MNC
- Indirect and direct IF
- Single stainings

- Difficult to distinguish normal/leukemic cells
- Few fluorochrome conjugated MAb available
- Few fluorochrome available

MATUTES et al SCORE FOR B-CLL

MARKER	EXPRESSION	SCORE
CD5	positive	1
CD23	positive	1
FMC7	negative	1
sIg	dim	1
CD22/CD79b	dim/dim	1

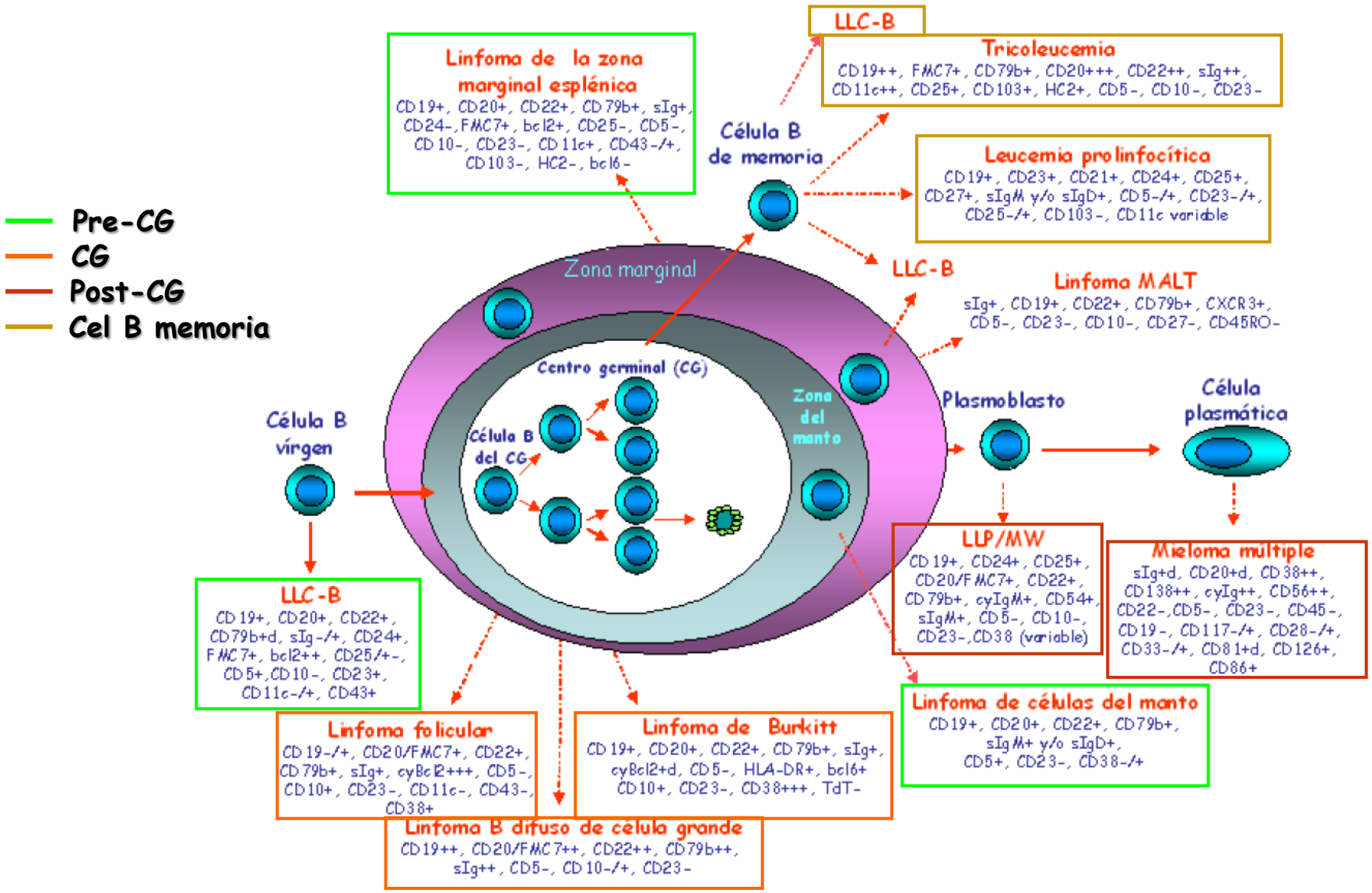
Scores in CLL range from 3 to 5, while in other B-CLPD range between 0 and 2

Matutes et al, Leukemia 1994

IMMUNOPHENOTYPIC PATTERNS OF DIFFERENT TYPES OF B-CLPD (*Orfao et al, In: "B-CLL". Humana Press, 2004*)

	sIg	CD5	CD10	CD20	CD11c	CD23	CD24	CD25	CD38	CD43	CD79b	CD103	FMC7
B-CLL	d	+	-	d	-/+	++	+	+	-/+	+	d	-	-
PLL	+	-/+	-	+	-/+	-/+	+	-/+	-/+	-/+	+	-	+
HCL	+	-	-	++	++	-	-/+	++	-	-	+	+	+
SMZL	+	-/+	-	+	+	-	+	-/+	-	-	+	-/+	+
LPL	+	-	-	+	-	-	+	+	-/+	-	+	-	-/+
MCL	+	+	-	+	-/+	-	+	-/+	-	+	+	-	-/+
FL	+	-	+	+	-/+	-/d	+	-/+	+	-	+	-	+
LDBCL	+	-	-	+	-/+	-	-/+	-	+	-	+	-	+
BL	-/+	-	+	+	-	-	+	-	++	-/+	-/+	-	+

Origen de los SLPC-B según su célula homóloga normal



GC: Centro germinal

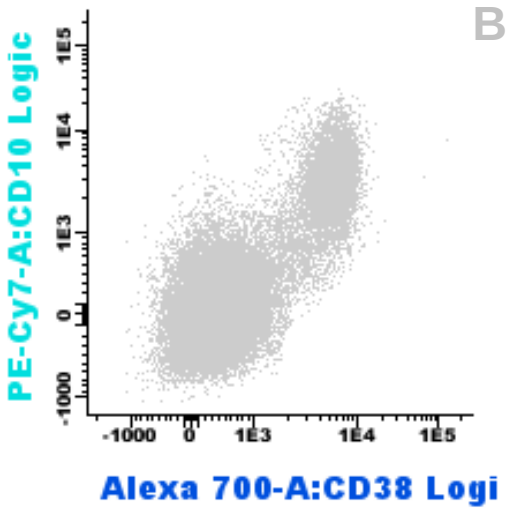
IMMUNOPHENOTYPING OF HAEMATOLOGICAL MALIGNANCIES

- 1953/1994: From the development of the instruments & techniques to the WHO classifications of haematological malignancies.

- 1994/2006: The ability to specifically identify leukaemic cells: from normal phenotypes to aberrant phenotypic profiles.

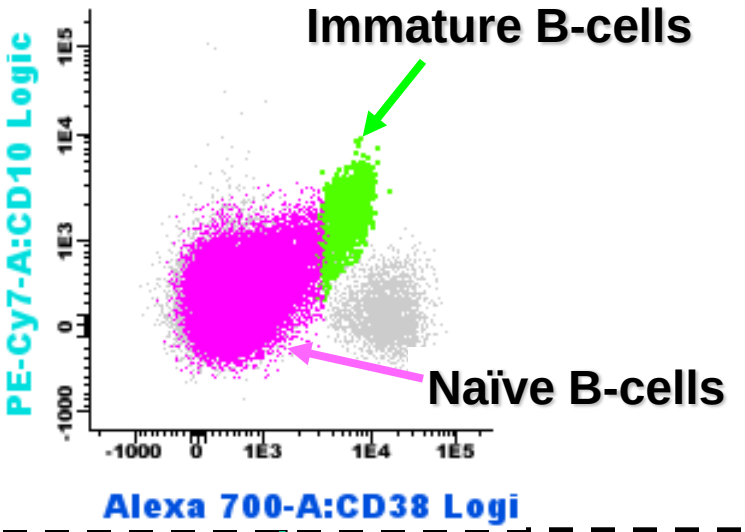
- 2006/-: Recent contributions of immuno-phenotyping of haematological malignancies: pointing to the future.

MATURATION-ASSOCIATED NORMAL PB B-CELL SUBSETS



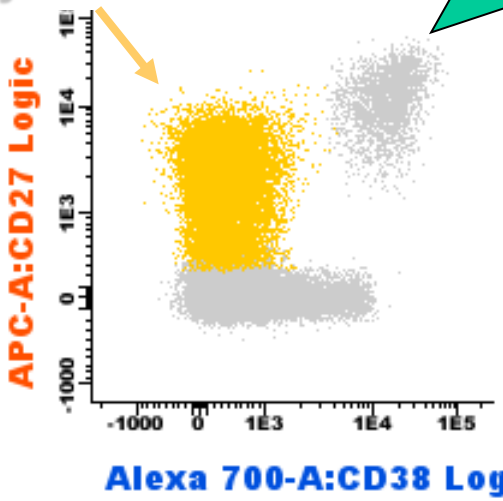
BM

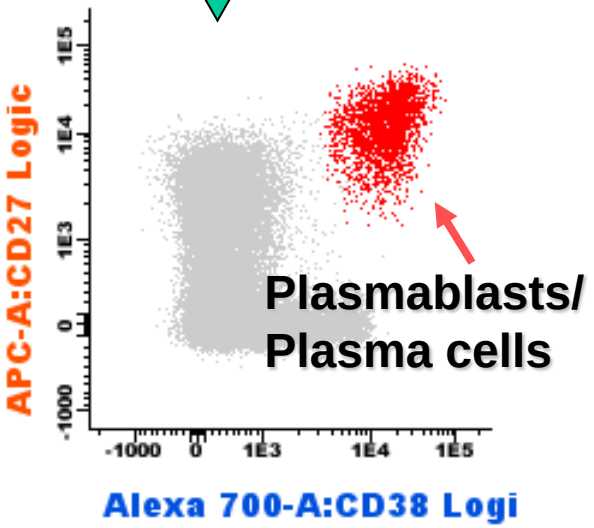
PB



Somatic Hypermutation/IgH Switch

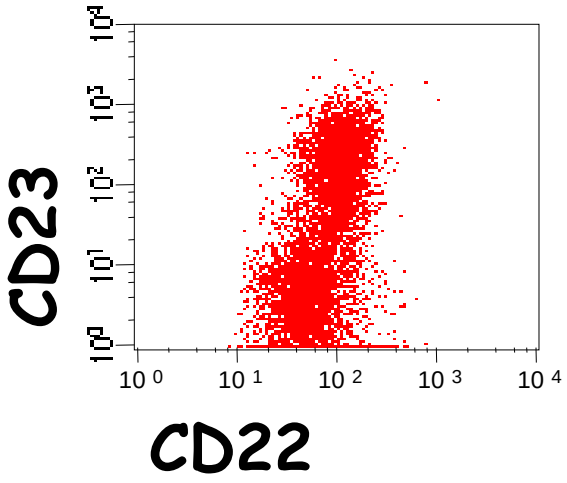
Memory B-cells

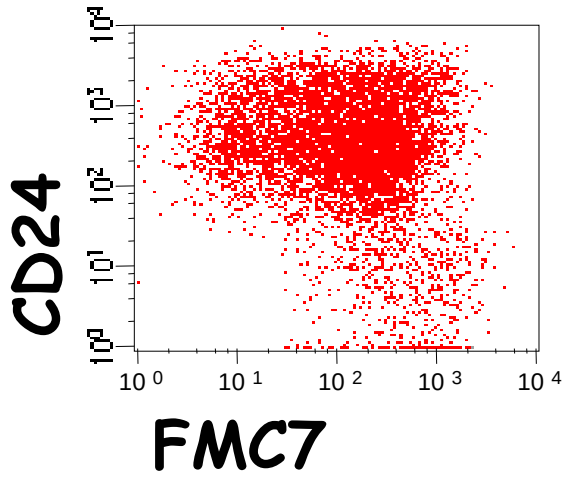




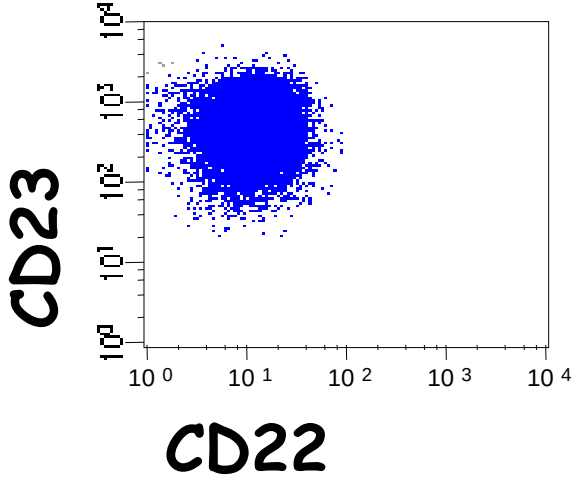
ABERRANT PHENOTYPES

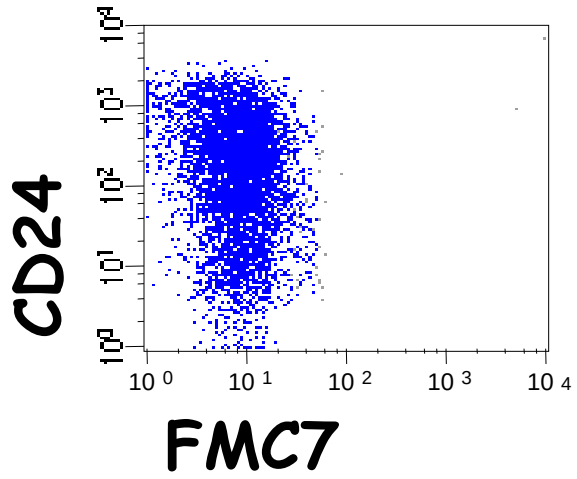
Normal PB B-cells

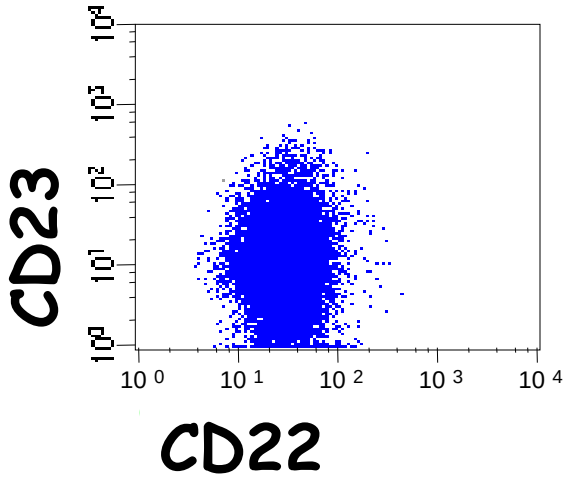


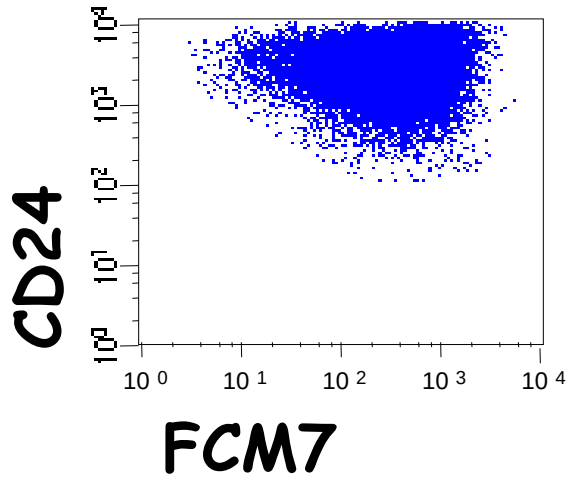


Leukemic B-cells

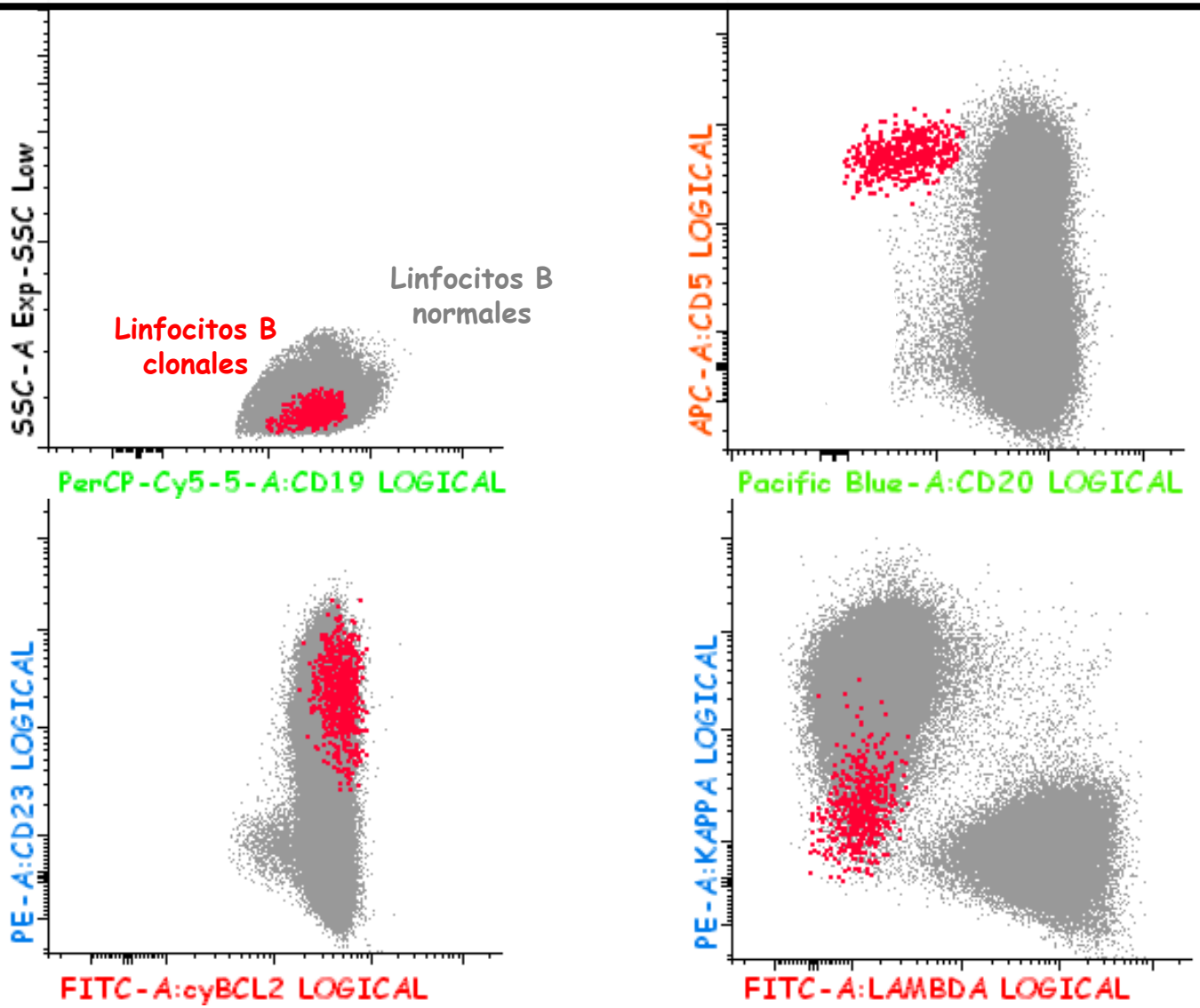








Immunophenotypic identification of PB B-cells with a CLL-like phenotype



*0.35% of all B-cells & 0.03% of all leucocytes

FCM in the study of hematological malignancies

1. **Making the diagnosis**

   Normal ↔ reactive/regenerating ↔ malignant

   Annually > 300,000 new patients with a hematological malignancy in developed countries

2. **Classification of hematopoietic malignancies**

   - **relation with prognosis**
   - **relevance of risk-group definition in treatment protocols**

   Based on differentiation characteristics and particularly on chromosome aberrations, resulting in fusion gene transcripts or aberrantly (over) expressed genes

3. **Evaluation of treatment effectiveness**

   Detection of minimal residual disease (MRD):

   MRD-based risk-group stratification (treatment reduction or treatment intensification)
   Annually > 400,000 follow-up samples in leukemia patients

FCM in the study of BCLPD

1. **Making the diagnosis (of clonality)**
   Normal ↔ reactive/regenerating ↔ clonal / malignant

2. **Classification into WHO categories**
   - relation with prognosis
   - relevance of risk-group definition in treatment protocols

   Based on differentiation characteristics and particularly on chromosome aberrations, resulting in fusion gene transcripts or aberrantly (over) expressed genes

3. **Evaluation of treatment effectiveness**
   Detection of minimal residual disease (MRD):

   MRD-based risk-group stratification (treatment reduction or treatment intensification)

PHENOTYPIC DIAGNOSIS OF CLONALITY: SEQUENTIAL STEPS

- Lineage assessment

-Identification of clonality within a lymphoid lineage

-Definition of maturation stage and disease type

DIAGNOSIS OF CLONALITY: CLINICAL QUESTIONS

Is this lymphocytosis clonal?

-Is this tissue enlarged because of an underlying clonal LPD?

-Are there clonal lymphocytes in this "apparently" normal tissue?/Is there minimal disease?

DIAGNOSIS OF CLONALITY: CLINICAL QUESTIONS

Is this lymphocytosis clonal?

-Is this tissue enlarged because of an underlying clonal LPD?

-Are there clonal lymphocytes in this "apparently" normal tissue?/Is there minimal disease?

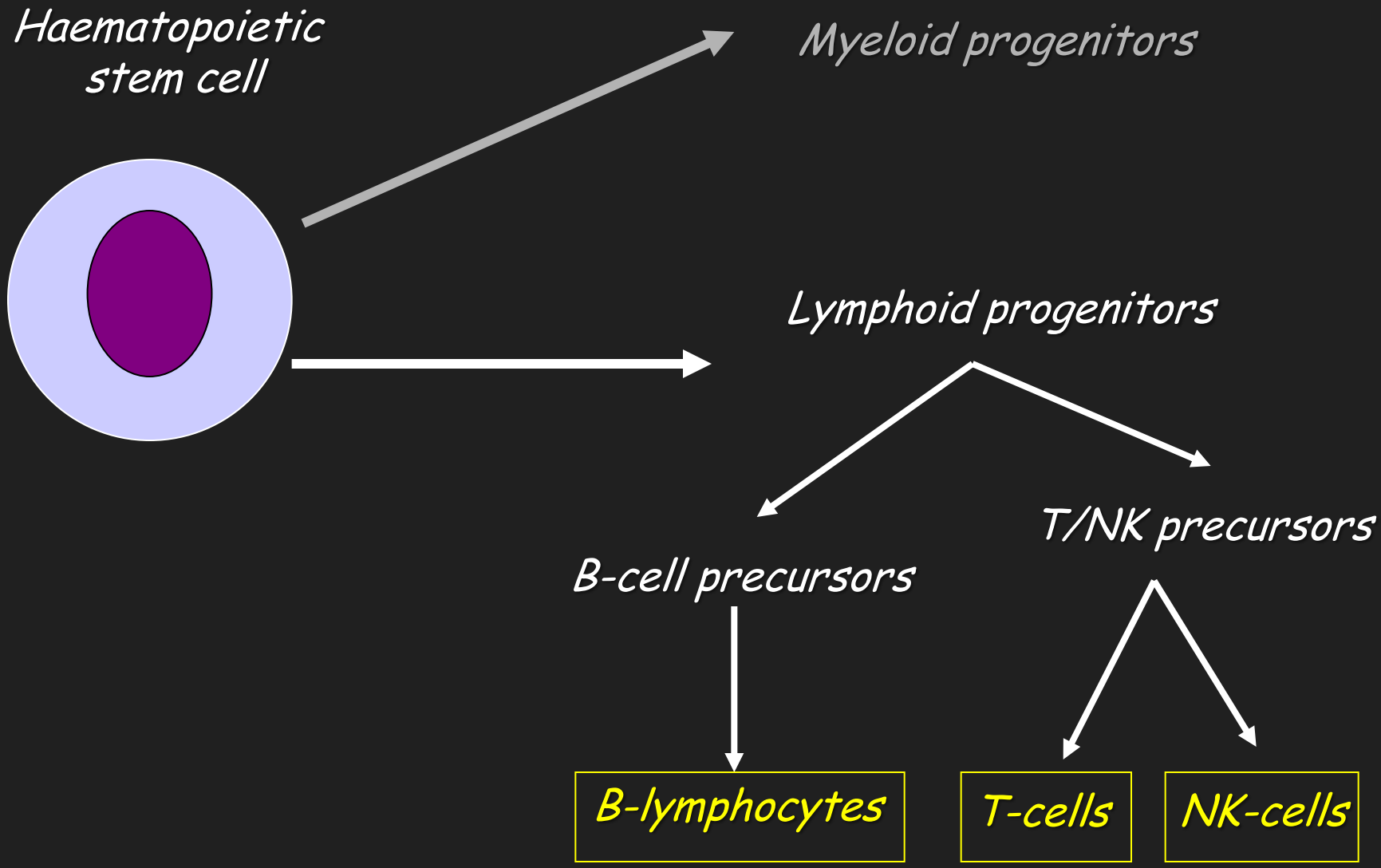
PHENOTYPIC DIAGNOSIS OF CLONALITY: SEQUENTIAL STEPS

- Lineage assessment

-Identification of clonality within a lymphoid lineage

-Definition of maturation stage and disease type

LYMPHOPOIESIS



PHENOTYPIC ASSESSMENT OF CELL LINEAGE IN CLPD

T-cell lineage:

- CD3, TCR

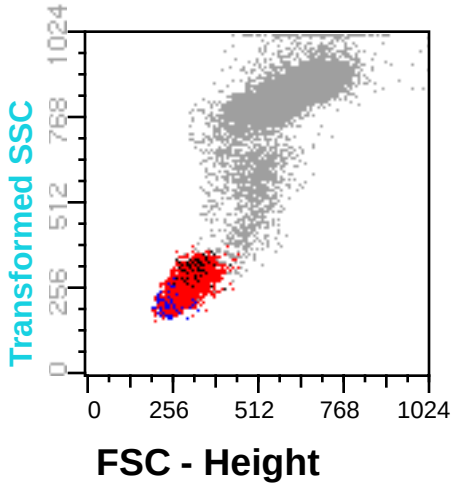
B-cell lineage:

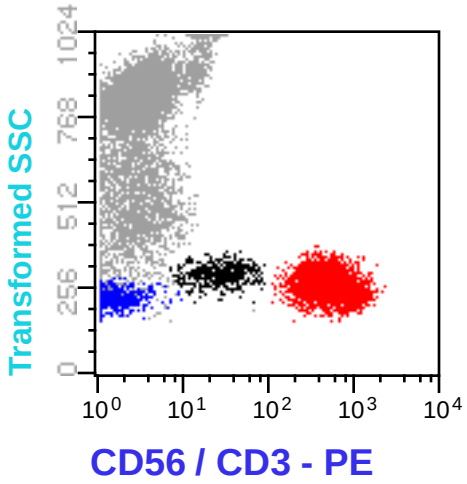
- CD19, CD20, sIg, cIg, CD38+++, CD138

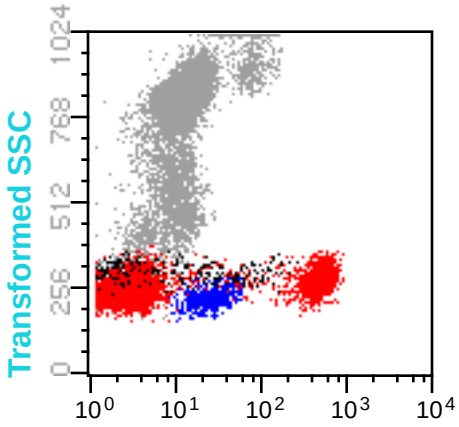
NK-cell lineage:

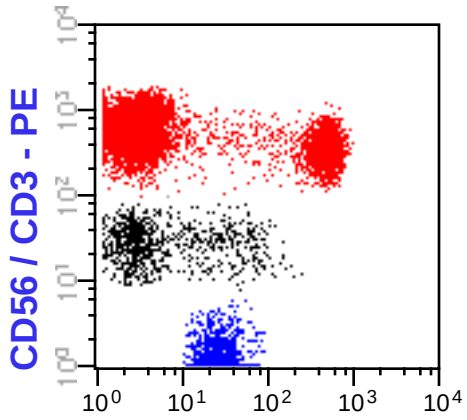
- CD56, CD16 (CD3 negative)

RAPID PHENOTYPIC SCREENING OF PB LYMPHOCYTOSIS









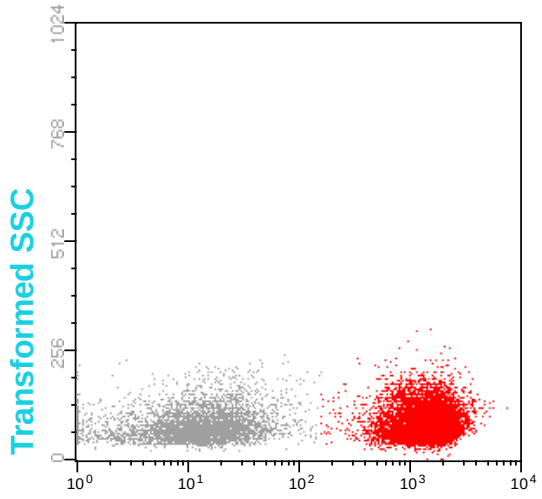
PHENOTYPIC DIAGNOSIS OF CLONALITY: SEQUENTIAL STEPS

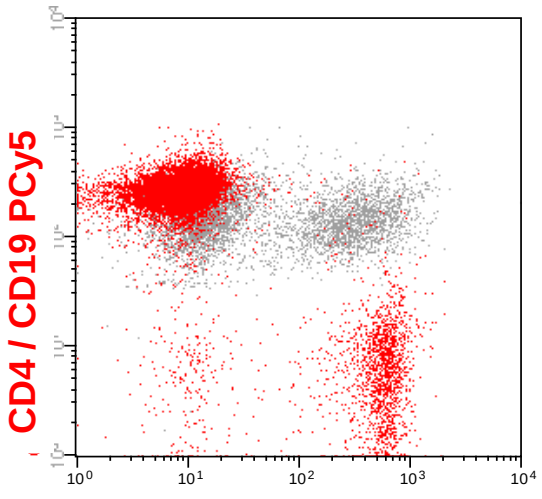
- Lineage assessment

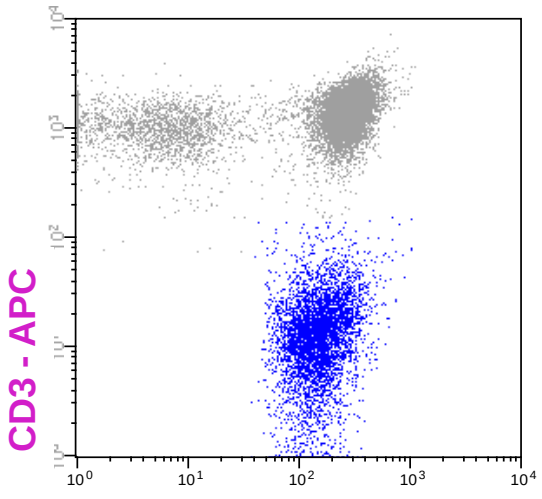
-Identification of clonality within a lymphoid lineage

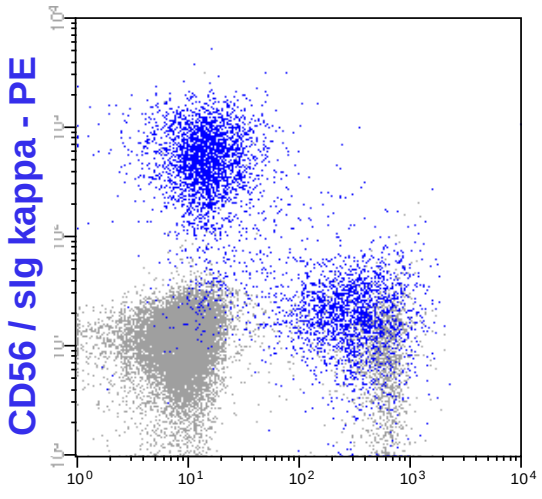
-Definition of maturation stage and disease type

RAPID PHENOTYPIC SCREENING OF PB LYMPHOCYTOSIS









RAPID FCM SCREENING OF CLONAL B-CELLS: ABSOLUTE LYMPHOCYTOSIS (> 3.5 x109/L)

	Conventional Morphology	Flow Cytometry	Final Diagnosis
Reactive	25	32	32
Doubtful	21	0	
Neoplastic	63	77	77

RAPID FCM SCREENING OF CLONAL B-CELLS: ABSOLUTE LYMPHOCYTOSIS (> 3.5 x109/L)

	Conventional Morphology	Flow Cytometry	Final Diagnosis
Reactive	25	32	32
Doubtful	21	0	
Neoplastic	63	77	77

Sensitivity	95%	100%
Specificity	75%/100%*	100%

*Considering only the neoplastic group

Lymphocytosis Screening Tube (LST)

Aim: *DIAGNOSTIC SCREENING OF B, T & NK CELL CLPD to identify the cell lineage involved (in the lymphocytosis), to detect the most common aberrant phenotypes (CD5), and to assess B-cell clonality*

Pacific Blue	Pacific Orange	FITC	PE	PerCP Cy5.5	PECy7	APC	APC-H7
CD20 CD4	CD45	CD8 Anti-Igλ	CD56 Anti-Igκ	CD5	CD19 Anti-TCRγδ	sCD3	CD38

EuroFlow

Lymphocytosis Screening Tube (LST)

Aim: *DIAGNOSTIC SCREENING OF B, T & NK CELL CLPD to identify the cell lineage involved (in the lymphocytosis), to detect the most common aberrant phenotypes (CD5), and to assess B-cell clonality*

Pacific Blue	Pacific Orange	FITC	PE	PerCP Cy5.5	PECy7	APC	APC-H7
CD20 CD4	CD45	CD8 Anti-Igλ	CD56 Anti-Igκ	CD5	CD19 Anti-TCRγδ	sCD3	CD38

- Markers aiming at identification of B-lineage cells, common B-cell aberrancies and to assess B-cell clonality

EuroFlow

Lymphocytosis Screening Tube (LST)

Aim: *DIAGNOSTIC SCREENING OF B, T & NK CELL CLPD to identify the cell lineage involved (in the lymphocytosis), to detect the most common aberrant phenotypes (CD5), and to assess B-cell clonality*

Pacific Blue	Pacific Orange	FITC	PE	PerCP Cy5.5	PECy7	APC	APC-H7
CD20 CD4	CD45	CD8 Anti-Igλ	CD56 Anti-Igκ	CD5	CD19 Anti-TCRγδ	sCD3	CD38

- Markers aiming at identification of B-lineage cells, common B-cell aberrancies and to assess B-cell clonality
- Markers aiming at identification of T cells and T-cell subsets:
  - .- TCRγδ vs TCRαβ (non-TCRγδ)
  - .- CD4+/CD8-; CD4-/CD8+; CD4+/CD8+; CD4-/CD8-

  and T-cell aberrancies

EuroFlow

Lymphocytosis Screening Tube (LST)

Aim: *DIAGNOSTIC SCREENING OF B, T & NK CELL CLPD to identify the cell lineage involved (in the lymphocytosis), to detect the most common aberrant phenotypes (CD5), and to assess B-cell clonality*

Pacific Blue	Pacific Orange	FITC	PE	PerCP Cy5.5	PECy7	APC	APC-H7
CD20 CD4	CD45	CD8 Anti-Igλ	CD56 Anti-Igκ	CD5	CD19 Anti-TCRγδ	sCD3	CD38

- Markers aiming at identification of B-lineage cells, common B-cell aberrancies and to assess B-cell clonality
- Markers aiming at identification of T cells and T-cell subsets:
  - .- TCRγδ vs TCRαβ (non-TCRγδ)
  - .- CD4+/CD8-; CD4-/CD8+; CD4+/CD8+; CD4-/CD8-

  and T-cell aberrancies
- Markers aiming at identification of NK cells, and NK-cell subsets

EuroFlow

DIAGNOSIS OF CLONALITY: CLINICAL QUESTIONS

- Is this lymphocytosis clonal?

-Is this tissue enlarged because of an underlying clonal LPD?

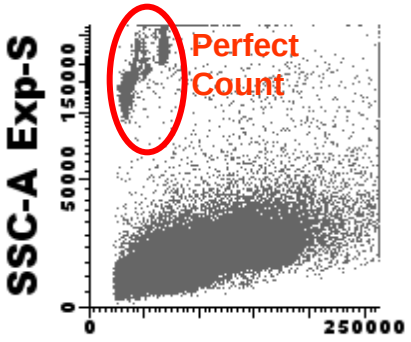
-Are there clonal lymphocytes in this "apparently" normal tissue?/Is there minimal disease?

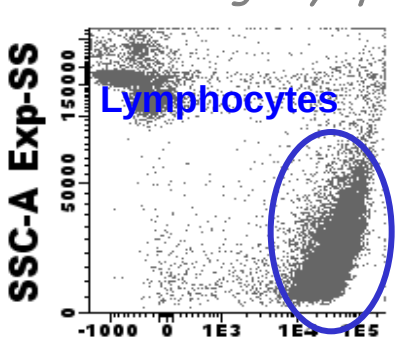
FC on FNA samples as a screening diagnostic tool

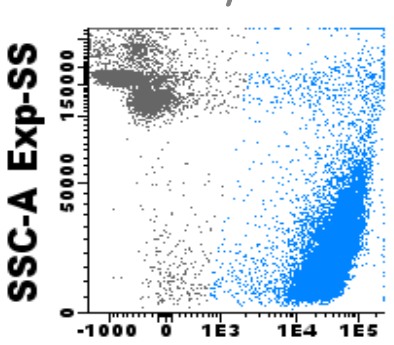
GATING STRATEGY for the immunophenotypic identification of lymphoid cells

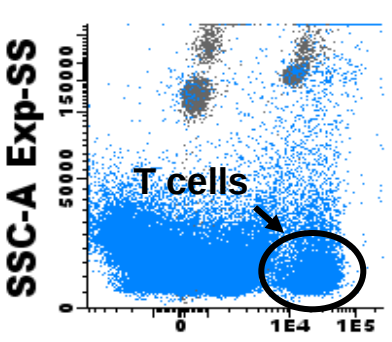
CD8+anti-λ FITC / anti-κ PE / CD4 + CD19 PerCPCy5.5 / CD3 APC / CD45 APCCy7

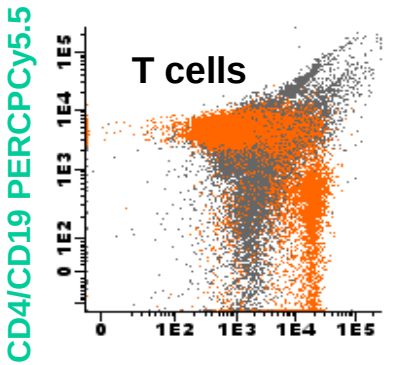
Pathologic lymphoid tissue sample

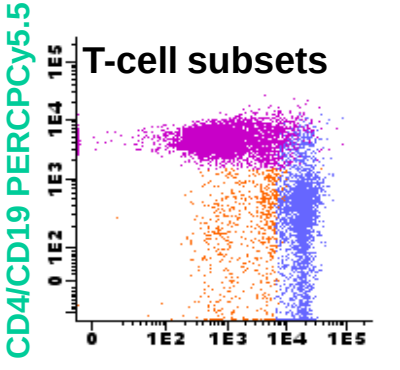


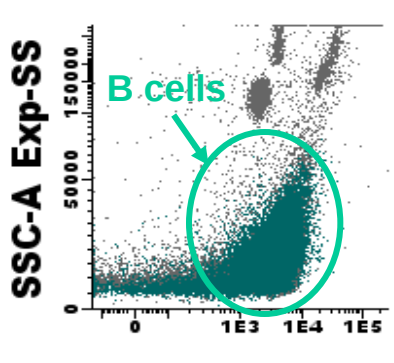


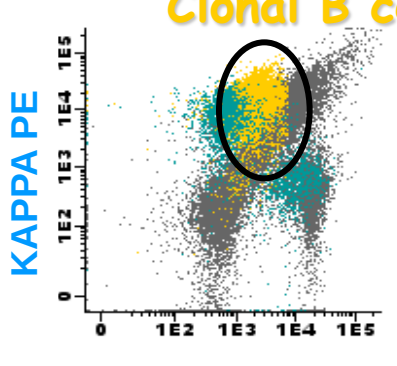








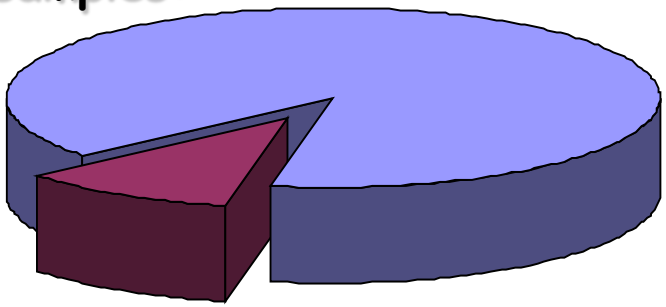




FC on FNA samples as a screening diagnostic tool

Comparison of cytology, FC and histology

Total FNA samples:
n=446
(from 400 cases)



Valuable samples
n=48 (10.5%)
(from 41 patients)

Only for cytology: 35
Only for FCM: 3
For FCM & cytology: 10 | <3%

Usually due to scanty cellularity or PB contamination

Valuable samples
n= 398 (89.5%)
(from 359 patients)

Degree of concordance between FNAC, FC and histology:

Concordant samples:
373/398 (94%)
from 336 cases
84% from the total samples

Discordant samples:
25/398 (6%)
from 23 cases
5.5% from the total samples

Distribution of concordant samples by FCM and cytology by diagnostic subgroups

DIAGNOSIS	FNA (n=373)		GANGLIONAR BIOPSY (n=136)
	FCM	CYTOLOGY	
REACTIVE PROCESS OR HL	182	182	30
TYPE I LYMPHADENITIS		37	
TYPE II LYMPHADENITIS		38	
OTHER REACTIVE PROCESSES		86	
HODGKIN`S LYMPHOMA		21	16
B-NON HODGKIN LYMPHOMA	121	121	82
T-NON HODGKIN LYMPHOMA	6	6	5
SOLID TUMOR	47	47	17
PLASMA CELL DYSCRASIA	11	11	
ACUTE MYELOID LEUKEMIA	1	1	
T-ACUTE LYMPHOBLASTIC LEUKEMIA	5	5	2

N. of concordant samples: 373/398 (94%) from 336 cases

Results expressed as number of concordant samples. HL: Hodgkin's lymphoma. FCM: flow cytometry

Comparison between FCM, cytology and histology: DISCREPANT CASES

CASE N.	*Age/Sex*	*FCM*	*Cytology*	*Histopathologic diagnosis*	*PCR/SB/FISH*	*Final diagnosis*
1	74/M	RP	ST	B-NHL	NV	B-NHL
2	80/M	RP	ST	-	-	**ST**
3	35/M	RP	ST	RP	-	**RP**
4	64/M	RP	PCD	-	-	**PCD**
5	85/F	RP	NHL	B-NHL	NV	**B-NHL**
6	77/M	T-NHL vs RP	NHL	T-NHL	TCRab+	**T-NHL**
7	19/F	RP	RP	B-NHL	-	B-NHL
8	80/M	RP vs ST	RP	B-NHL	NV	B-NHL
9	73/F	B-NHL	NHL	HL	t(14;18)+	HL + **B-NHL**
10	41/F	B-NHL	RP	RP	BCR+	**B-NHL**
11	79/M	B-NHL	RP	B-NHL	BCR+	**B-NHL**
12	88/F	B-NHL	RP	-		**B-NHL**
13	71/M	B-NHL + ST	ST	ST		**ST + B-NHL**
14	73/F	B-NHL + T-NHL	NHL	T-NHL + ATYPICAL B-CELLS	T-NHL	**B-NHL + T-NHL**
15	74/M	B-NHL	NHL	T-NHL + ATYPICAL B-CELLS	T-NHL	**B-NHL** + T-NHL
16	75/F	T-NHL	NHL	B-NHL	TCRb+	**T-NHL**
17	73/F	T-NHL	HL	HL	TCRb+	HL + **T-NHL**
18	36/M	T-NHL	RP	T-NHL	TCRb+	**T-NHL**
19	477M	AML	RP	-	-	**AML**
20	48/M	PCD	RP	-	-	**PCD**
21	58/M	PCD	RP	-	-	**PCD**
22	44/F	DCL	GS	GS	-	**DCL**

F: female; M: male; RP: reactive process; ST: solid tumor; B-NHL: B-Non Hodgkin Lymphoma; T-NHL: T-Non Hodgkin Lymphoma; AML: acute myeloid leukemia; PCD: plasma cell dyscrasia; DCL: dendritic cell lymphoma; NHL: non-Hodgkin lymphoma; GS: Granulocytic sarcoma; HL: Hodgkin`s lymphoma; TCR: T-cell receptor; CLL: Chronic lymphoid leukemia; -: not analyzed; NV: non valuable;

DIAGNOSIS OF CLONALITY: CLINICAL QUESTIONS

Is this lymphocytosis clonal?

-Is this tissue enlarged because of an underlying clonal LPD?

-Are there clonal lymphocytes in this "apparently" normal tissue?/Is there minimal disease?

LEPTOMENINGEAL DISEASE IN HEMATOLOGICAL MALIGNANCIES

- Clinical parameters used to define risk for CNS involvement/relapse in B-NHL, ALL and AML are found to be of limited predictive value

- Although cytologic analysis of CSF has a great specificity, it has a limited sensitivity with a reported false-negative rate of between 20%-60%
- Recent single center studies suggest that FCM can increase the sensitivity of conventional cytology for the identification of CSF involvement, particularly in B-NHL

ANÁLISIS DE MUESTRAS DE LCR MEDIANTE CMF

Muestras de LCR estabilizadas (Transfix, Immunostep SL)

↓

centrifugar (1x) y concentrar (300mL)

↓

Marcaje celular de 1/3 de la muestra

Pacific Blue	Pacific	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
CD20	CD45	CD8 and smIgλ	CD56 and smIgκ	CD4	CD19	smCD3 and CD14	CD38

Adicionar esferas PerfectCOUNT (Cytognos SL) y Medir (Citómetro de flujo FACSCanto II; BDB)

Negativo

↓

Marcar de nuevo las 2/3 partes restantes de muestra

Positivo

↓

Panel adaptado al fenotipo del LNH-B

FLOW CYTOMETRY VS CYTOLOGY FOR DETECTION OF CSF IN AGGRESSIVE B-NHL (n=123)

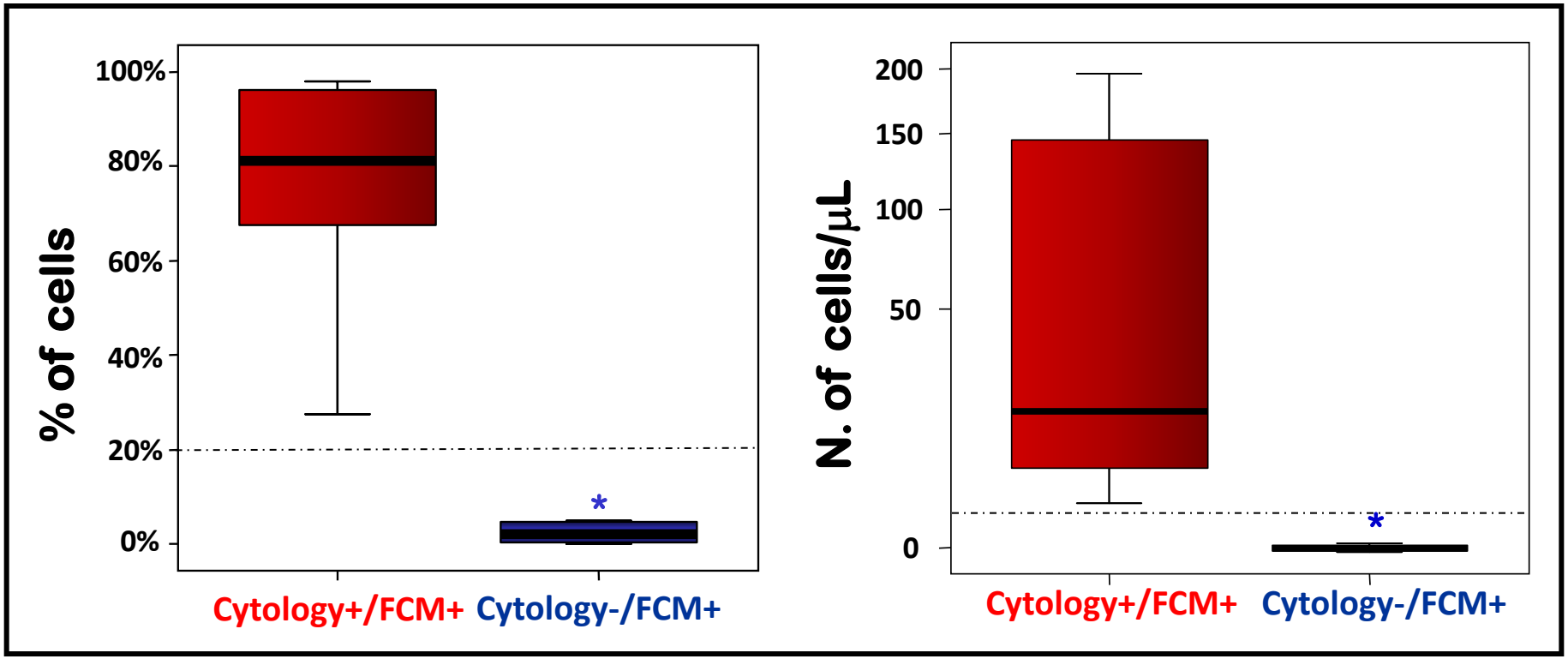
	Flow Cytometry	Cytology	P
Positive CSF	27/123 (22%)	7/123 (6%)	<0.0001

Cytology	Flow Cytometry −	Flow Cytometry +
−	95/123 (77%)	17/123 (14%)
+	1/123 (1%)*	7/123 (6%)
Susp.	−	3/123 (2%)

*The presence of neoplastic cells was ruled out by further immunocytochemical analyses

Quijano et al, J Clin Oncol, 2009

ABSOLUTE AND RELATIVE NUMBERS OF NEOPLASTIC B-CELLS IN INFILTRATED CSF SAMPLES BY FCM

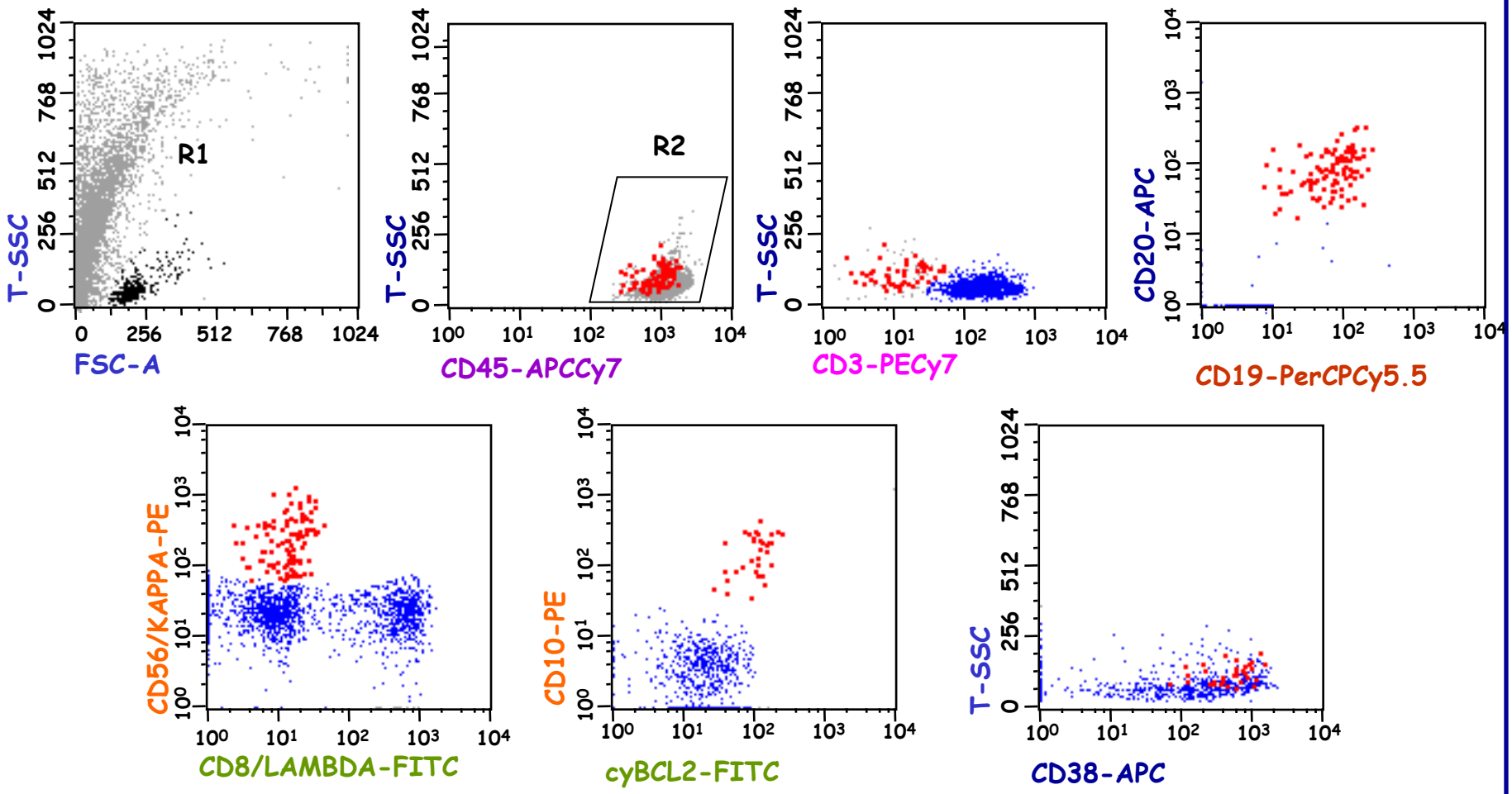


*p<0.001

*Cut-off: <20% and <1 neoplastic B-cell/µL

Quijano et al, J Clin Oncol, 2009

LEPTOMENINGEAL DISEASE IN A DLBCL (tFL): LOW LEVEL INFILTRATION OF CSF

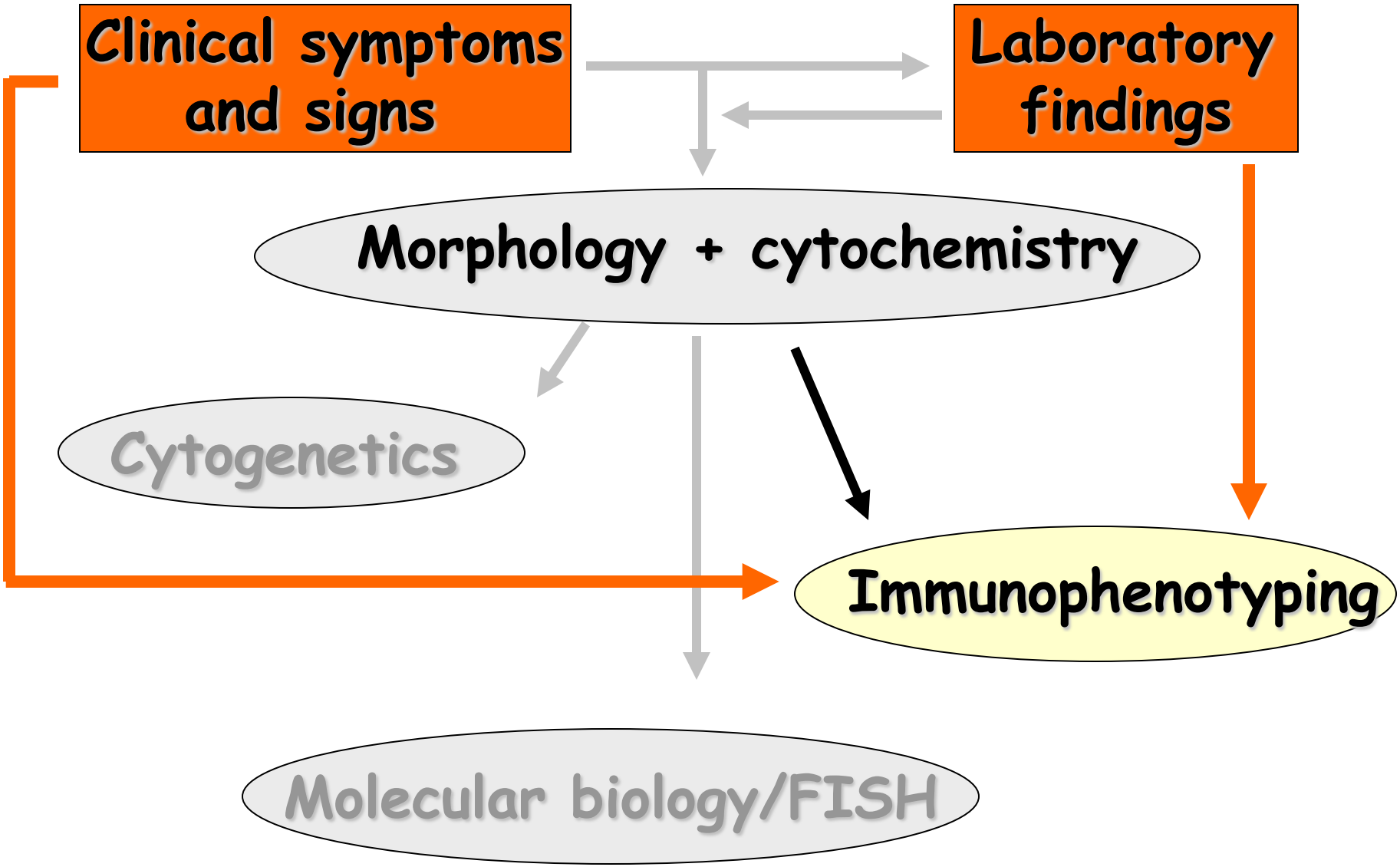


T-cells Neoplastic B-cells (6%)

FREQUENCY OF FCM+ in B-NHL according to diagnosis

B-NHL SUBTYPE	At Diagnosis	%	At Relapse	%
DLBCL	21/129	16%	7/21	30%
Burkitt	2/23	9%	3/3	100%
Follicular	4/15	27%	1/6	17%
Mantle-cell	3/11	27%	1/2	50%
Plasmablastic	0/4	0%	0/0	0%
Splenic marginal zone	1/4	25%	1/1	100%
Richter syndrome	1/1	100%	0/0	0%
Other B-NHL	2/3	67%	0/1	0%
TOTAL	**34/190**	**18%**	**12/33**	**36%**

DIAGNOSIS OF HAEMATOLOGICAL MALIGNANCIES



FCM in the study of BCLPD

1. Making the diagnosis (of clonality)
   Normal ↔ reactive/regenerating ↔ clonal / malignant

   Annually > 300,000 new patients with a hematological malignancy in developed countries

2. Classification into WHO categories
   - relation with prognosis
   - relevance of risk-group definition in treatment protocols

   Based on differentiation characteristics and particularly on chromosome aberrations, resulting in fusion gene transcripts or aberrantly (over) expressed genes

3. **Evaluation of treatment effectiveness**
   Detection of minimal residual disease (MRD):

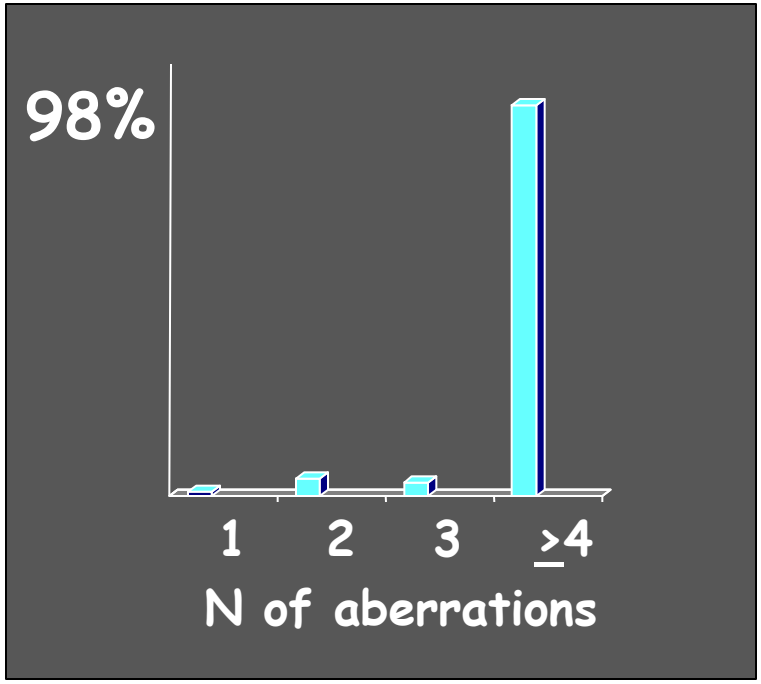
   MRD-based risk-group stratification (treatment reduction or treatment intensification)

FCM in the study of BCLPD

Evaluation of treatment effectiveness

Detection of MRD:

High applicability and sensitivity

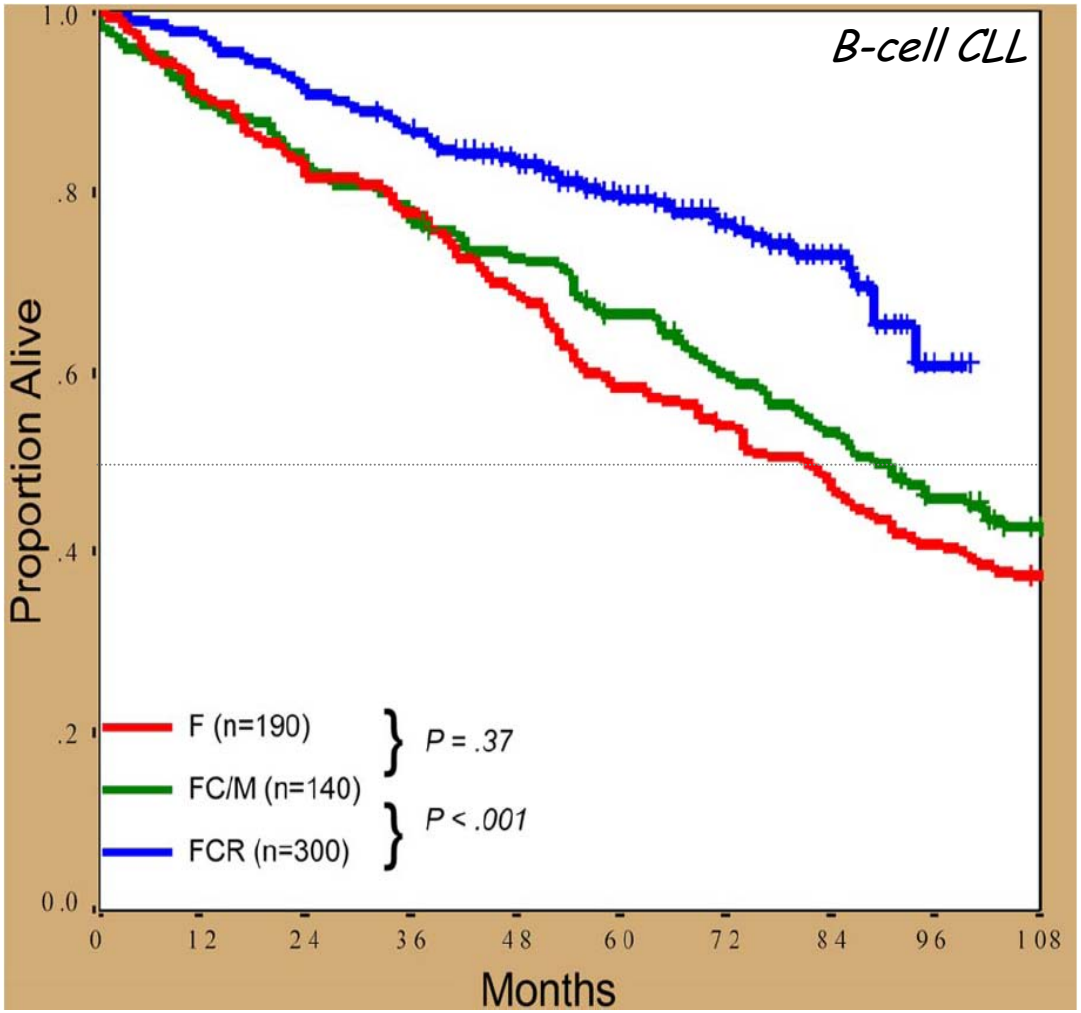


Phenotypic aberrancy	*Case*	*% of B lymphocytes*[a]		*Sensitivity level*
		Aberrant	*Normal*	
FMC7^{-}/CD5$^{+\ or\ ++}$/CD19^{+}	1	0.007	3.44	10^{-5}
	2	0.006	2.56	10^{-5}
	3	0.012	1.64	10^{-4}
FMC7^{+}/CD5^{++}/CD19^{+}	4	0.019	1.45	10^{-4}
FMC7^{++}/CD5^{+}/CD19^{+}	5	0.022	1.10	10^{-4}
CD22^{+d}/CD23^{+}/CD19^{+}	6	0.017	3.08	10^{-4}
	7	0.001	1.28	10^{-5}
	8	0.019	3.93	10^{-4}
	9	0.001	2.05	10^{-5}
	10	0.012	2.53	10^{-4}
CD103^{+}/CD25^{-}/CD19^{+}	11	0.01	2.51	10^{-4}
	12	0.033	0.40	10^{-4}
	13	0.025	1.29	10^{-4}
CD103^{+}/CD25^{++}/CD19^{+}	14	0.003	0.38	10^{-5}
	15	0.003	1.82	10^{-5}
CD10^{-}/CD11c^{++}/CD19^{++}	16	0.05	0.81	10^{-4}
	17	0.03	1.93	10^{-4}
	18	0.02	4	10^{-4}
	19	0.05	3.67	10^{-4}

MRD IN B-CLL

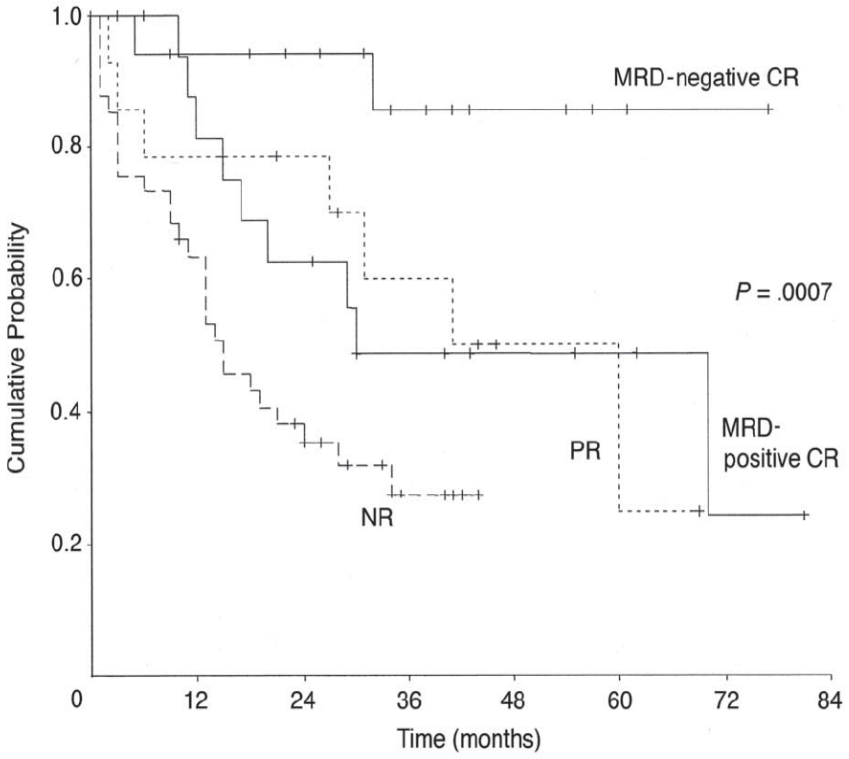
Reference	Aberrant criteria	Sensitivity	Prognostic value
Brugiatelli M et al. (Cancer 1989)	sIgκ+/sIgλ+ ratio	10-2	Yes
Robertson LE et al. (Blood 1992)	sIgκ+/sIgλ+ ratio	10-2	Yes
Leonormand B et al. (Leukemia 1994)	CD19+/CD5+	10-3	Yes
Cabezudo E et al. (Leukemia, 1997)	CD19+/CD5+	10-3	Yes
García-Vela A et al. (Leukemia, 1999)	CD19+/CD79b+d	10-4	Yes
Rawstron AC et al. (Blood 2001)	CD19+/CD20+d/CD5+/CD79b+d	10-4	Yes
Maloum K et al. (Br J Haematol 2002)	CD19+/CD20+d/CD5+/CD79b+d	10-4	Yes
Gupta R et al. (Am J Clin Pathol 2004)	CD19+/CD5+	10-3	Not analyzed
Bottcher S et al. (Leukemia 2004)	CD19+/CD5+/CD43+/CD20+d	10-4	Not analyzed
Moreton P et al. (J Clin Oncol 2005)	CD19+/CD20+d/CD5+/CD79b+d	10-5	Yes
Montillo M et al. (Cancer Invest 2005)	CD19+/CD20+d/CD5+/CD79b+d	10-5	Yes
Moreno et al (Blood 2006)	Autologous transplantation (n=25) Allogeneic transplantation (n=15)		Yes No

Why performing MRD monitoring in B-cell CLPD?

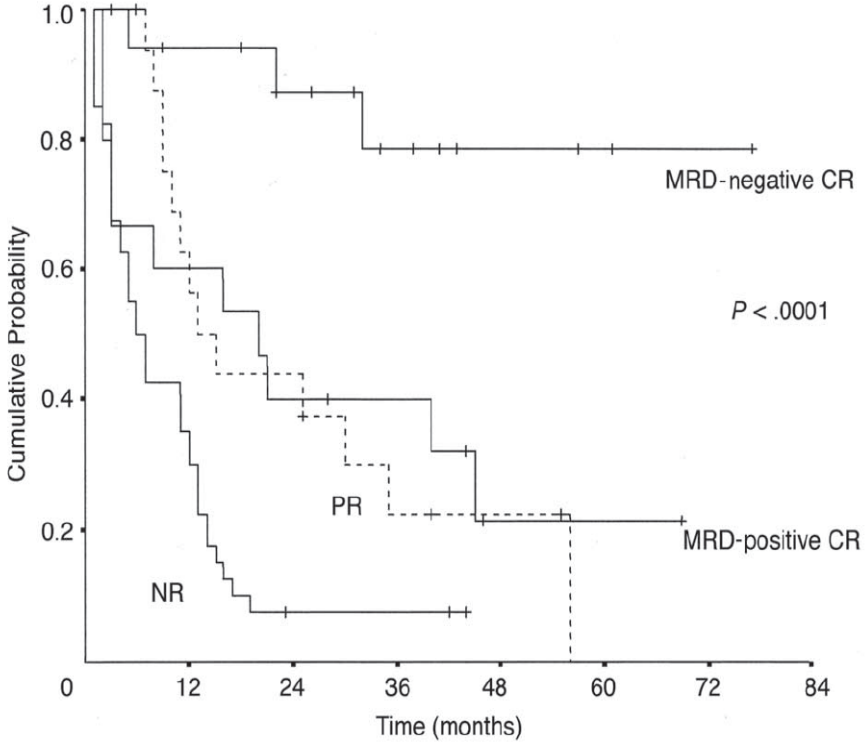


MRD IN B-CLL by flow cytometry
Clinical impact

Overall survival



Treatment free survival



IMMUNOPHENOTYPING OF HAEMATOLOGICAL MALIGNANCIES

- 1953/1994: From the development of the instruments & techniques to the WHO classifications of haematological malignancies.

- 1994/2006: The ability to specifically identify leukaemic cells: from normal phenotypes to aberrant phenotypic profiles.

- 2006/-: **Recent contributions of immuno-phenotyping of haematological malignancies: pointing to the future.**

CLINICAL APPLICATIONS OF IMMUNOPHENOTYPING OF B-CLPD

Diagnosis

Diagnostic classification

Treatment monitoring

FUTURE DEVELOPMENTS

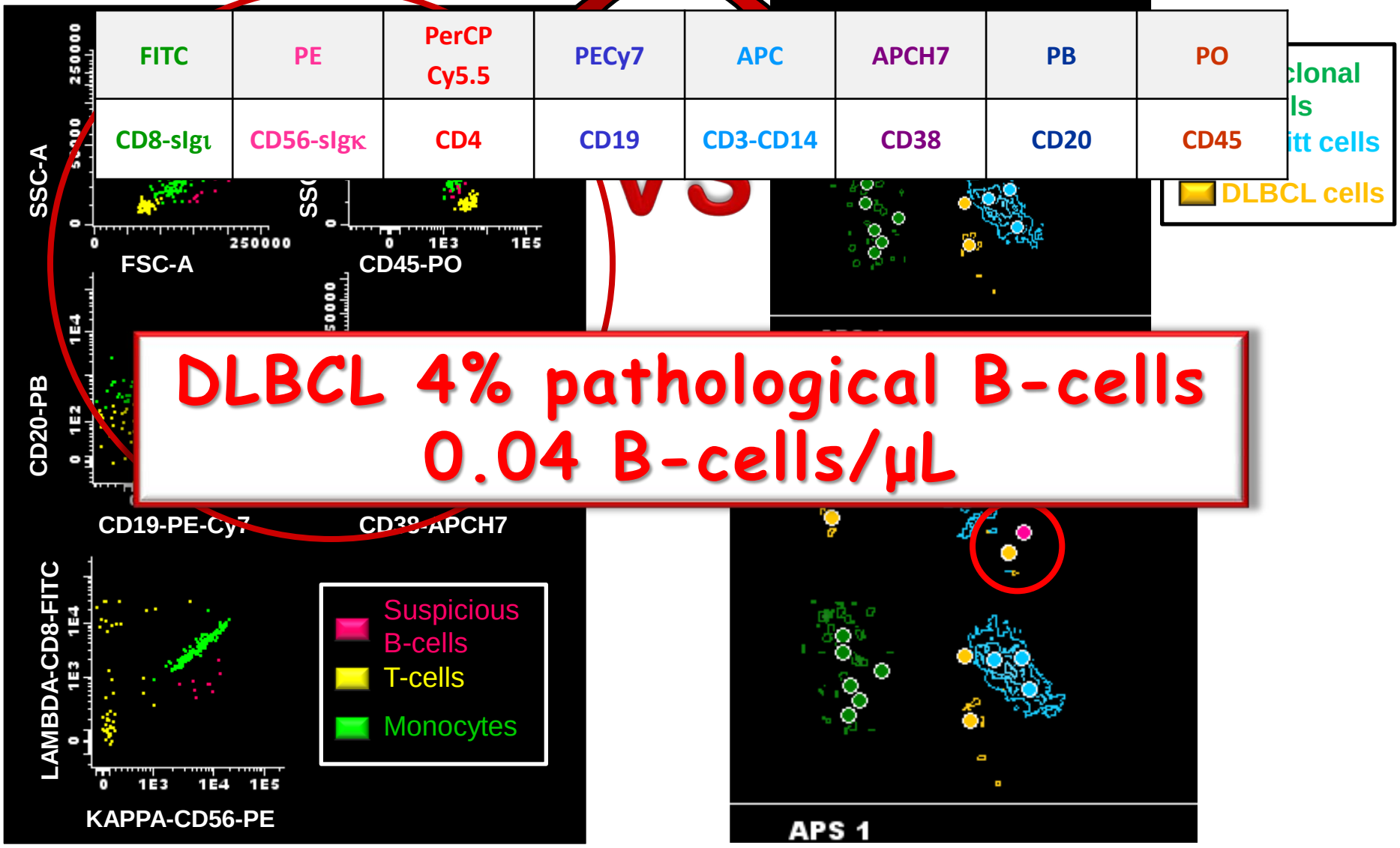
- Can we still improve the sensitivity of flow cytometry for the diagnosis of CSF involvement?

- Can we build an internal control for the detection of (minimal) CSF contamination with blood-derived cells?

- Can we develop new tools to help in distinguishing normal vs pathological B-cells?

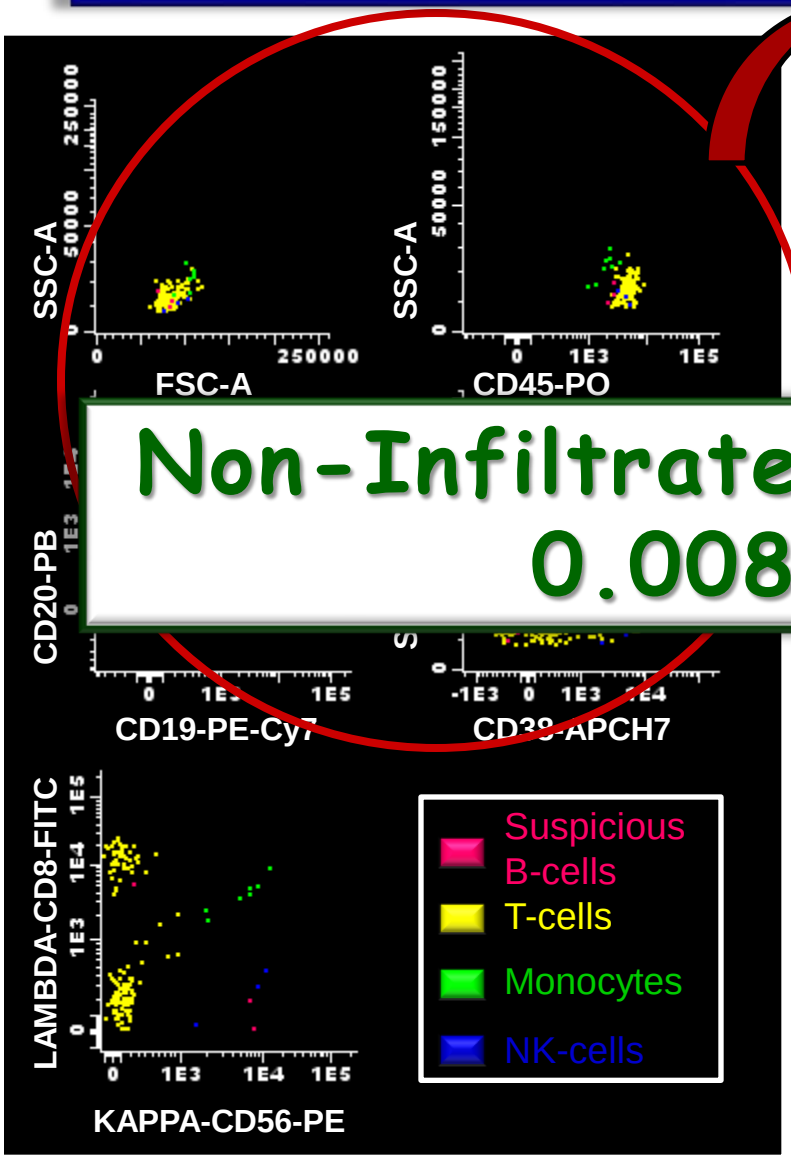
SUSPICIOUS B-CELLS VS REFERENCE LIBRARY: COMPASS ANALYSIS

FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7	PB	PO
CD8-sIgλ	CD56-sIgκ	CD4	CD19	CD3-CD14	CD38	CD20	CD45

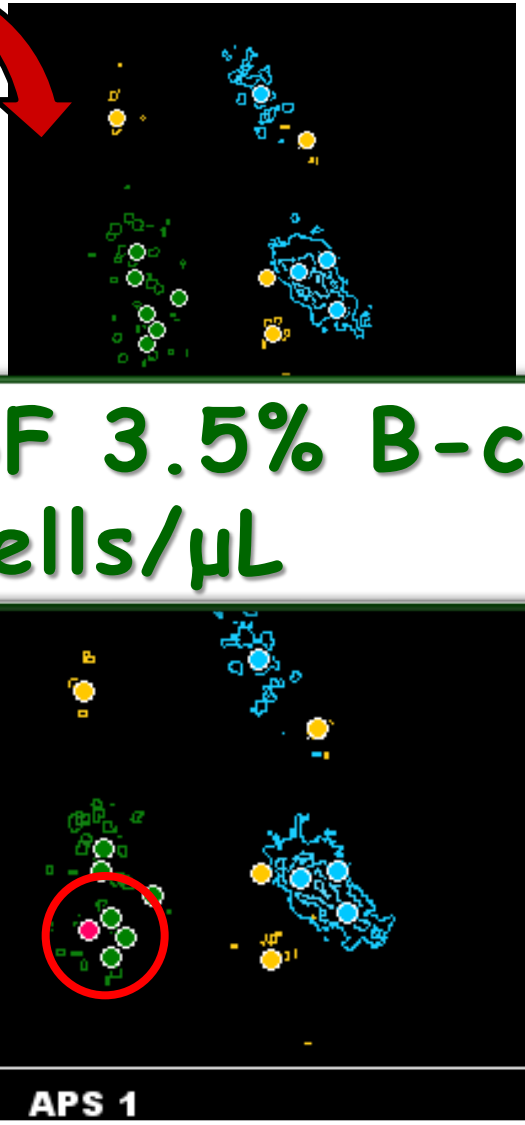


DLBCL 4% pathological B-cells
0.04 B-cells/µL

SUSPICIOUS B-CELLS VS REFERENCE LIBRARY: COMPASS ANALYSIS



VS



Non-Infiltrated CSF 3.5% B-cells
0.008 B-cells/µL

CLINICAL APPLICATIONS OF IMMUNOPHENOTYPING OF B-CLPD

Diagnosis

Diagnostic classification

Treatment monitoring

IMMUNOPHENOTYPIC PATTERNS OF DIFFERENT TYPES OF B-CLPD (*Orfao et al, In: "B-CLL". Humana Press, 2004*)

	sIg	CD5	CD10	CD20	CD11c	CD23	CD24	CD25	CD38	CD43	CD79b	CD103	FMC7
B-CLL	d	+	-	d	-/+	++	+	+	-/+	+	d	-	-
PLL	+	-/+	-	+	-/+	-/+	+	-/+	-/+	-/+	+	-	+
HCL	+	-	-	++	++	-	-/+	++	-	-	+	+	+
SMZL	+	-/+	-	+	+	-	+	-/+	-	-	+	-/+	+
LPL	+	-	-	+	-	-	+	+	-/+	-	+	-	-/+
MCL	+	+	-	+	-/+	-	+	-/+	-	+	+	-	-/+
FL	+	-	+	+	-/+	-/d	+	-/+	+	-	+	-	+
LDBCL	+	-	-	+	-/+	-	-/+	-	+	-	+	-	+
BL	-/+	-	+	+	-	-	+	-	++	-/+	-/+	-	+

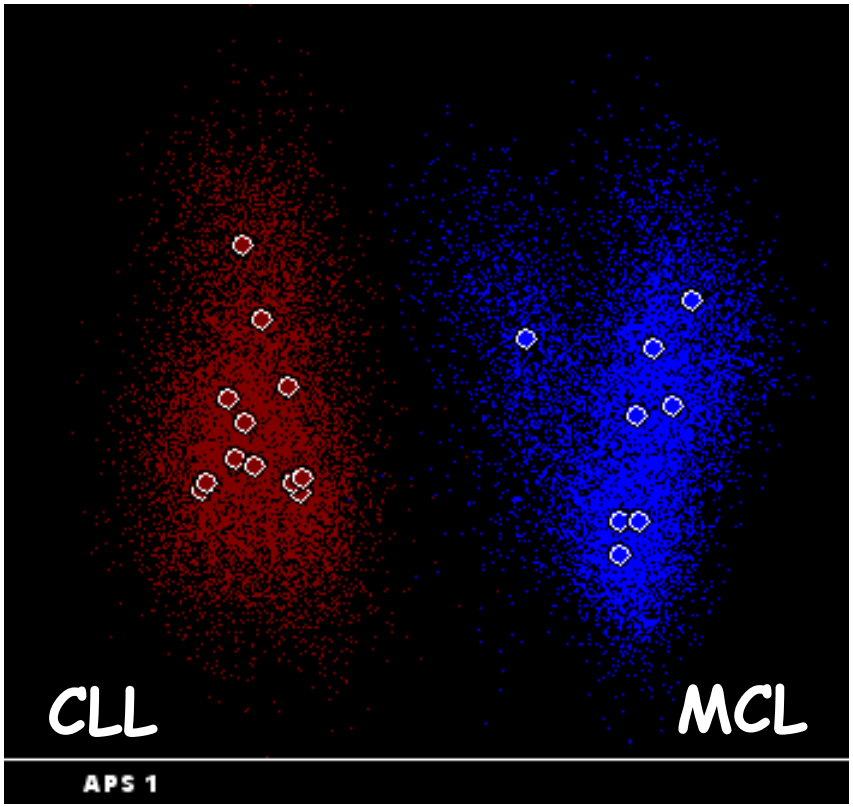
B-CPLD panel

	Pac Blue	Pac Orange	FITC	PE	PerCP-Cy5.5	PECy7	APC	APC-H7
1	CD20	CD45				CD19		
2	CD20	CD45				CD19		
3	CD20	CD45				CD19		
4	CD20	CD45				CD19		
5	CD20	CD45				CD19		

1= LST	CD20 / CD4	CD45	λ/CD8	κ/CD56	CD5	CD19 / TCRγ/δ	CD3	CD38
2	CD20	CD45	CD23	CD10	CD79b	CD19	CD200	CD43
3	CD20	CD45	CD31	LAIR	CD11c	CD19	IgM	CD81
4	CD20	CD45	CD103	CD95	CD22	CD19	CXCR5	CD49d
5	CD20	CD45	CD62L	CD39	HLA-DR	CD19	CD27	

EuroFlow

B-CLPD panel: utility

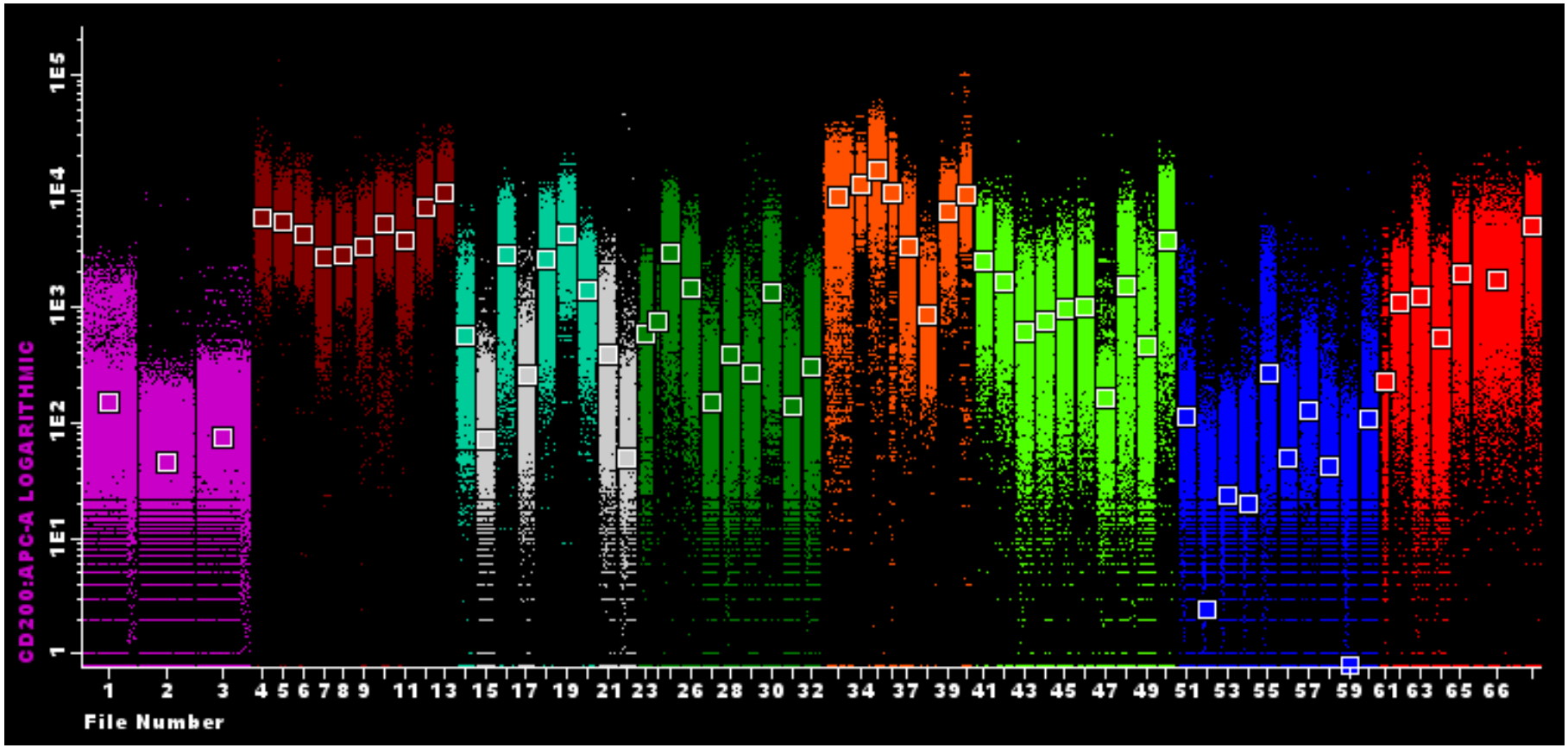


1	IgM	14.02
2	CD200	13.76
3	CD79b	11.94
4	CD23	8.57
5	CD38	7.73

Responsible scientists: Sebastian Bottcher

EuroFlow

Characterization markers:
CD200



BL CLL DLBCL FL HCL LPL MCL MZL

Responsible scientists: Sebastian Bottcher

EuroFlow

EuroFlow B-CLPD panel: summary and perspectives

- B-CLPD panel allows unequivocal classification of most mature B-cell malignancies according to WHO

- All differential diagnoses (n=35) achieved efficiently except for the following 1 vs 1 comparisons:

FL vs DLBC
MZL vs LPL
LPL vs DLBCL
MZL vs DLBCL

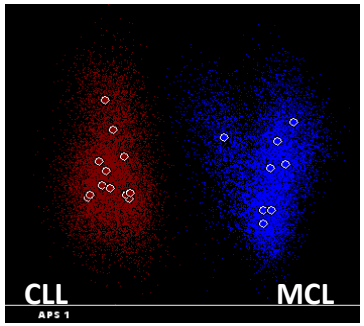
Responsible scientists: Sebastian Bottcher

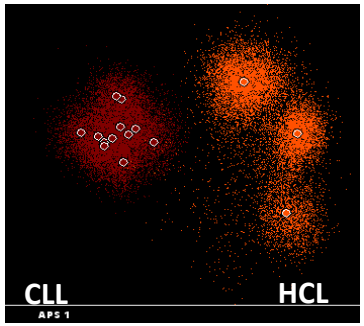
EuroFlow

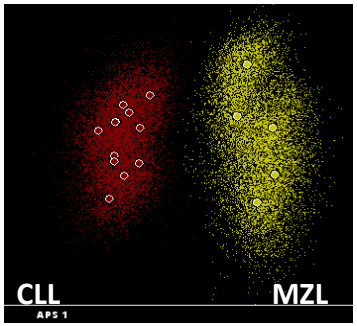
B-NHL classification panel – modular design

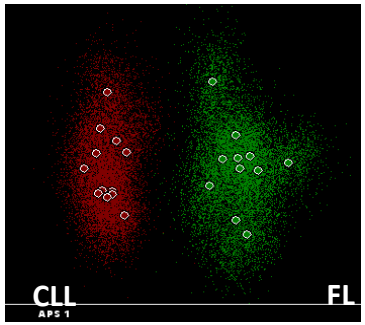
EuroFlow

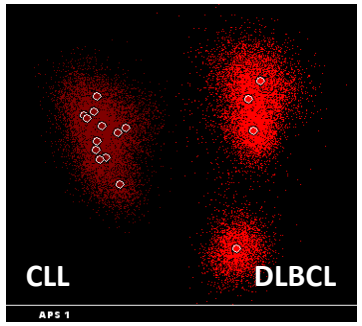
Full panel



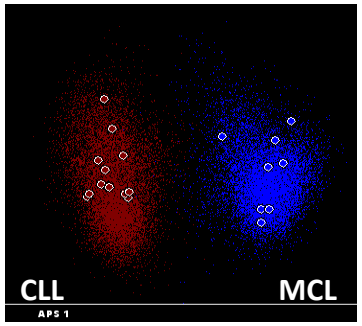


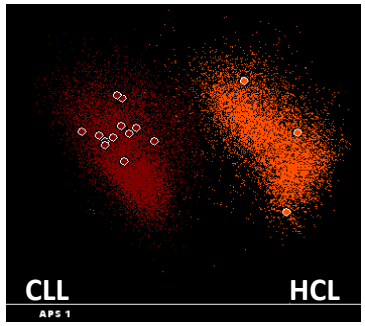


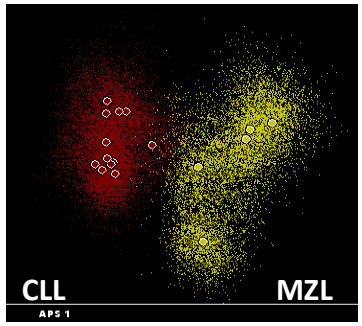


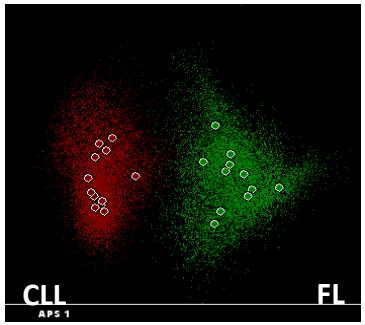


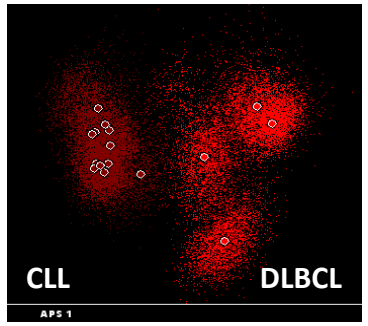
Tubes 1 & 2 only





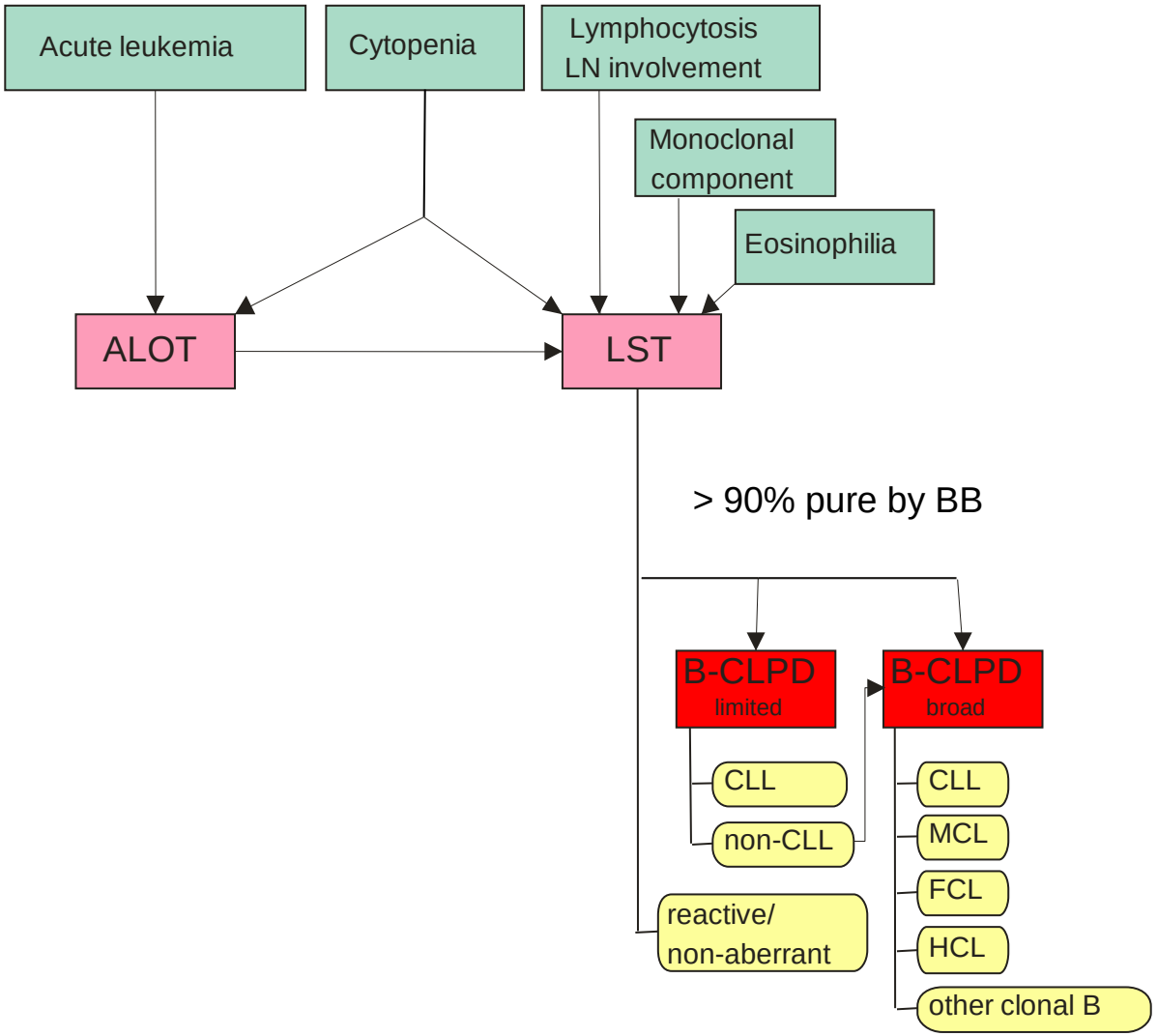






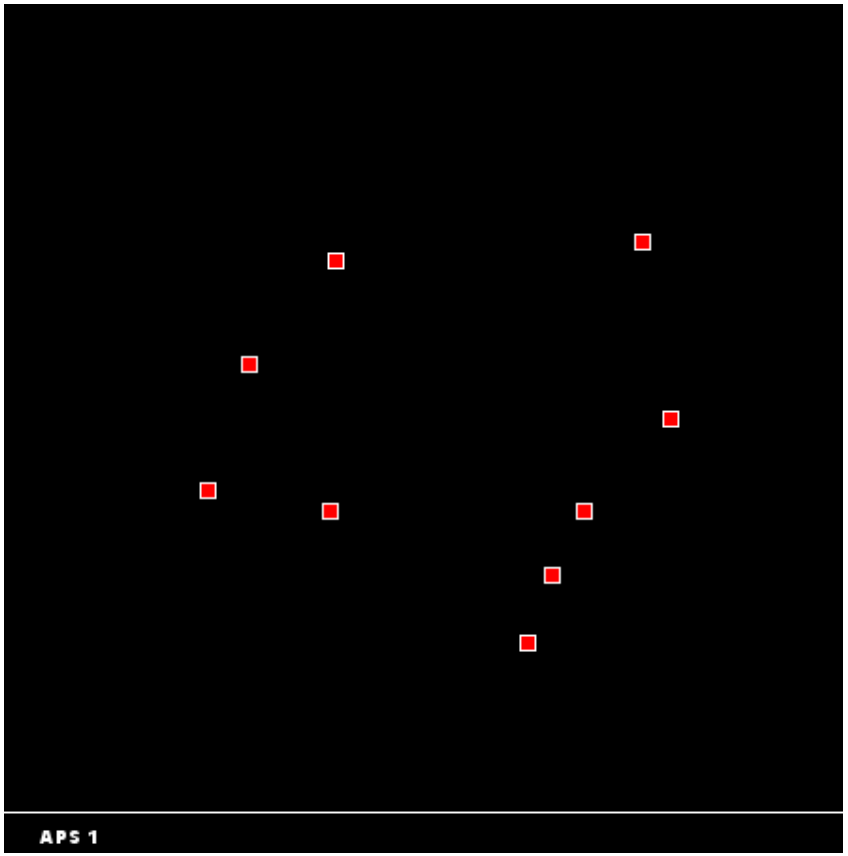
Responsible scientist: Sebastian Bottcher

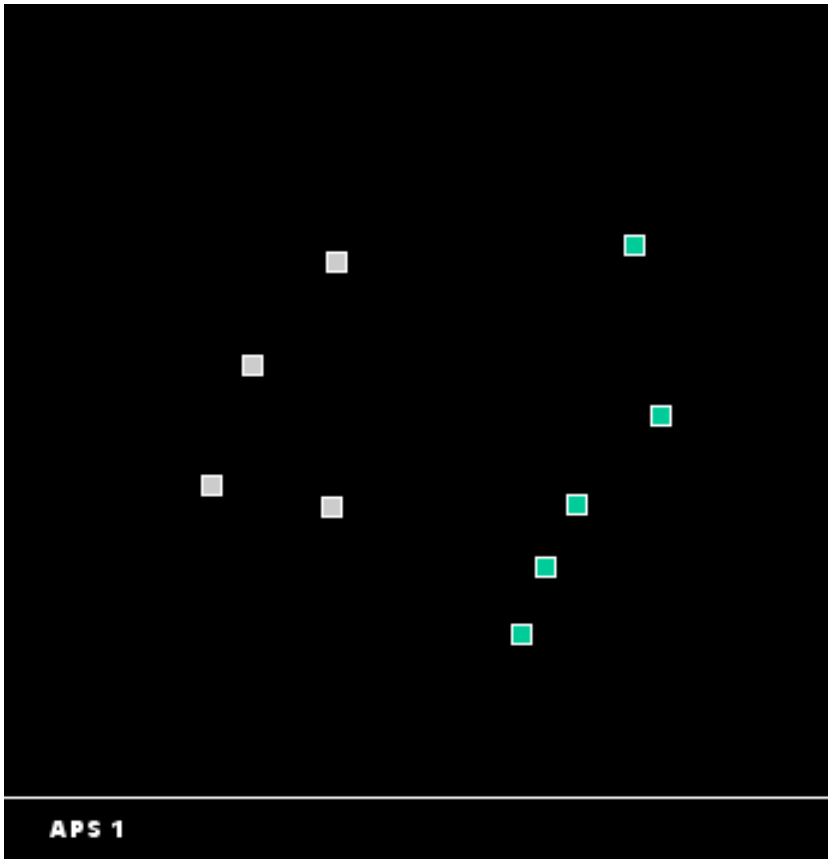
B-CLPD: Diagnostic work-flow



EuroFlow

B-CLPD panel: subclassification of DLBCL

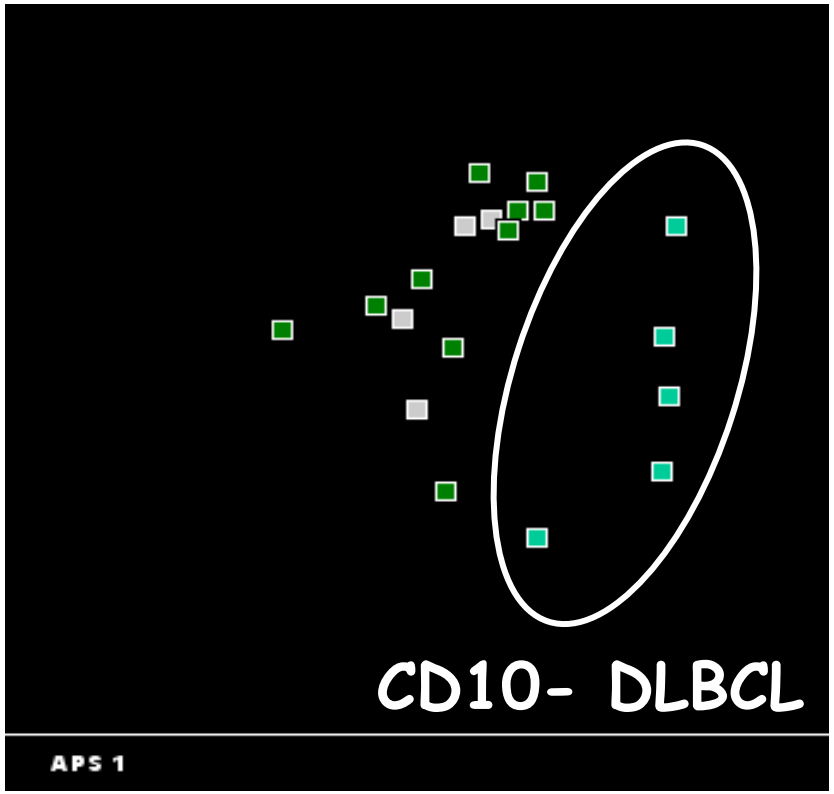




Responsible scientists: Sebastian Bottcher

EuroFlow

B-CLPD panel: subclassification of DLBCL vs FL

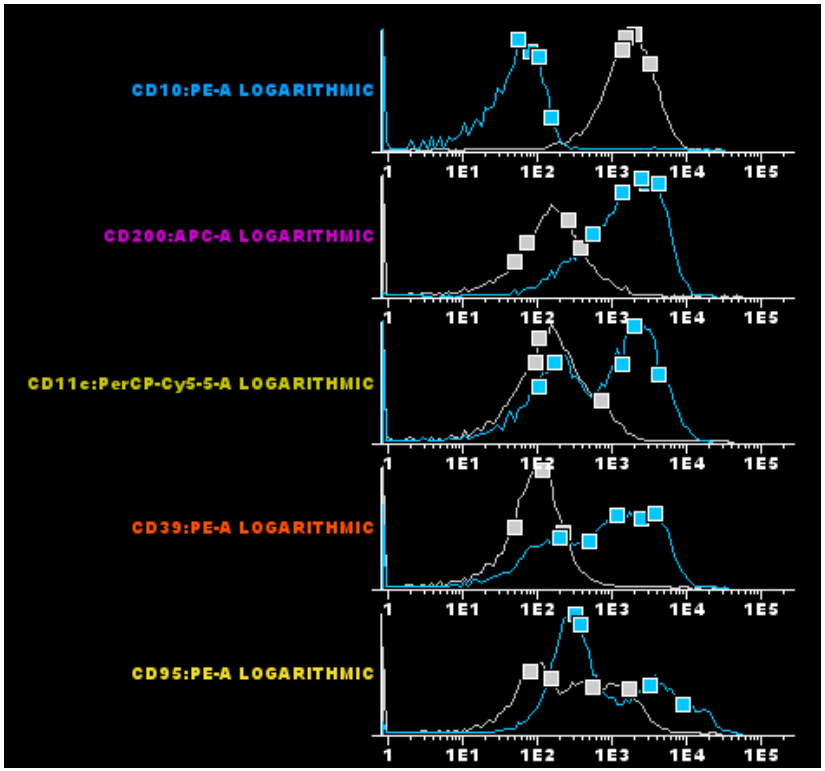


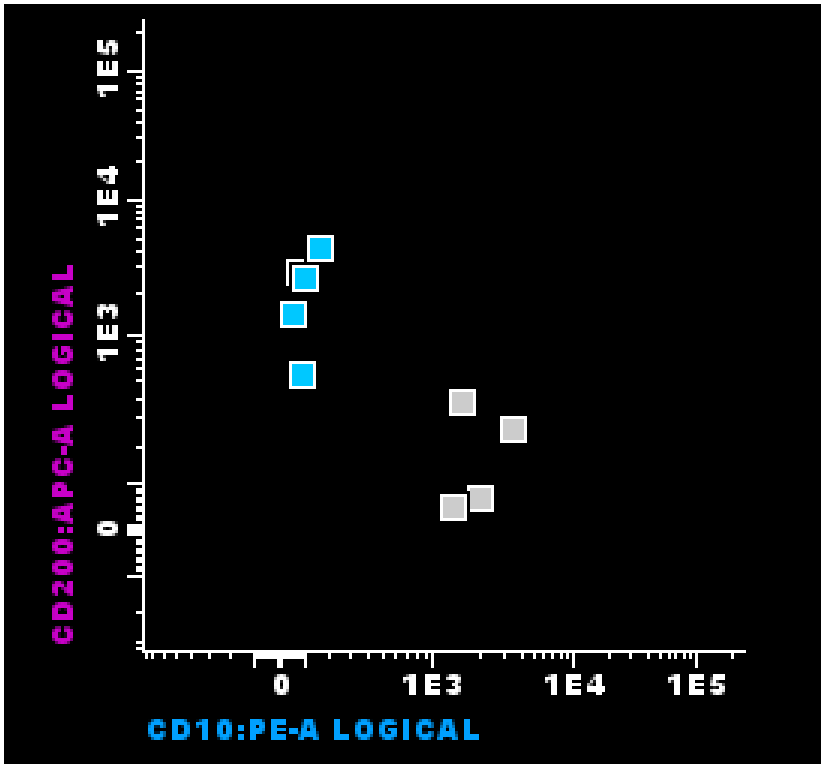
	APS Significance
IgM-APC	11.4
CD10-PE	9.7
CD200-APC	9.1
CD39:PE	8.4

Responsible scientists: Sebastian Bottcher

EuroFlow

B-CLPD panel: subclassification of DLBCL

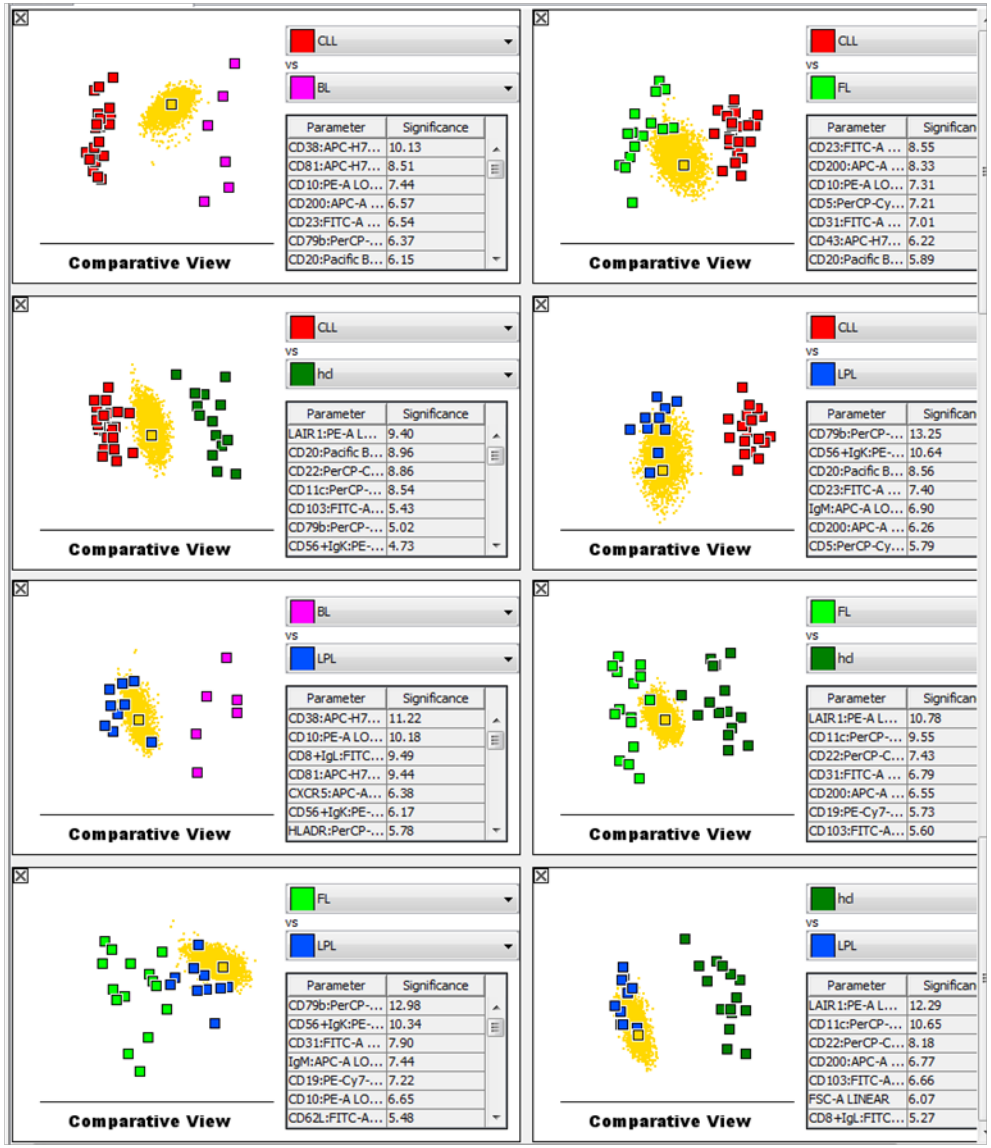




Responsible scientists: Sebastian Bottcher

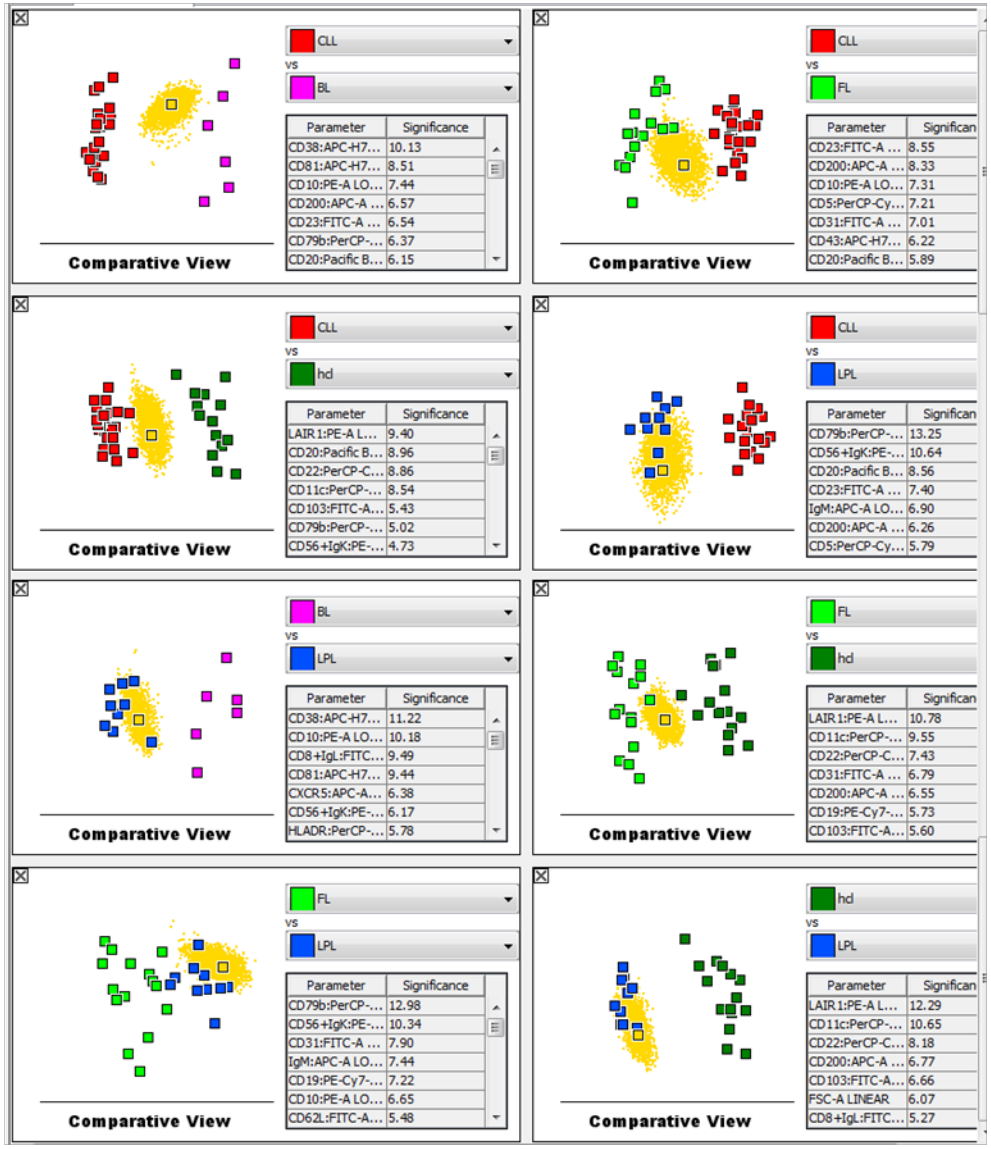
EuroFlow

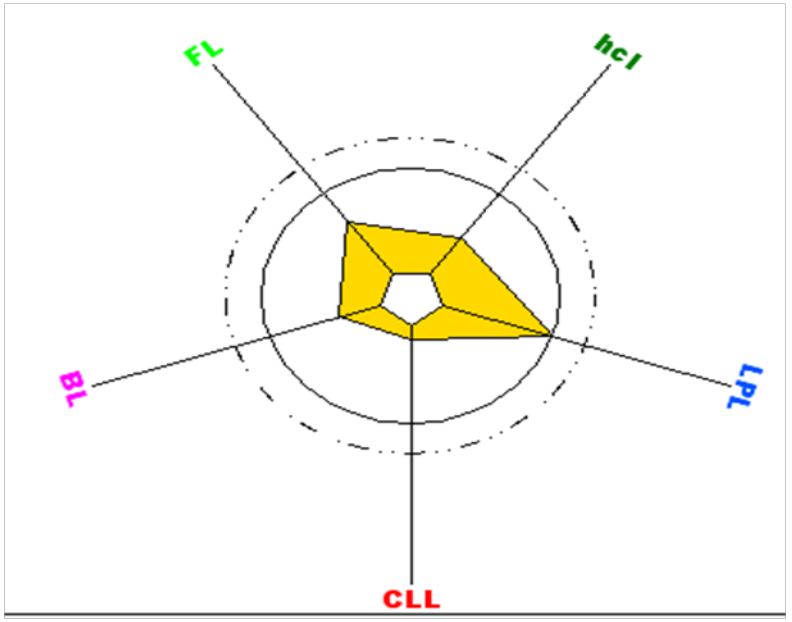
B-CLPD: Comparative analysis of "our case" vs multiple reference groups



Responsible scientists: Sebastian Bottcher

B-CLPD:Comparative analysis of "our case" vs multiple reference groups





Responsible scientists: Sebastian Bottcher

CLINICAL APPLICATIONS OF IMMUNOPHENOTYPING OF B-CLPD

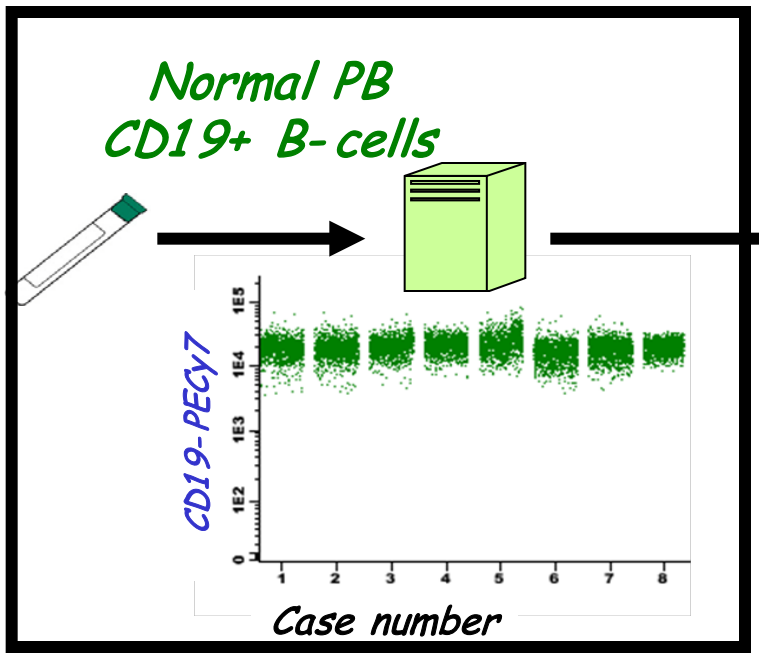
Diagnosis

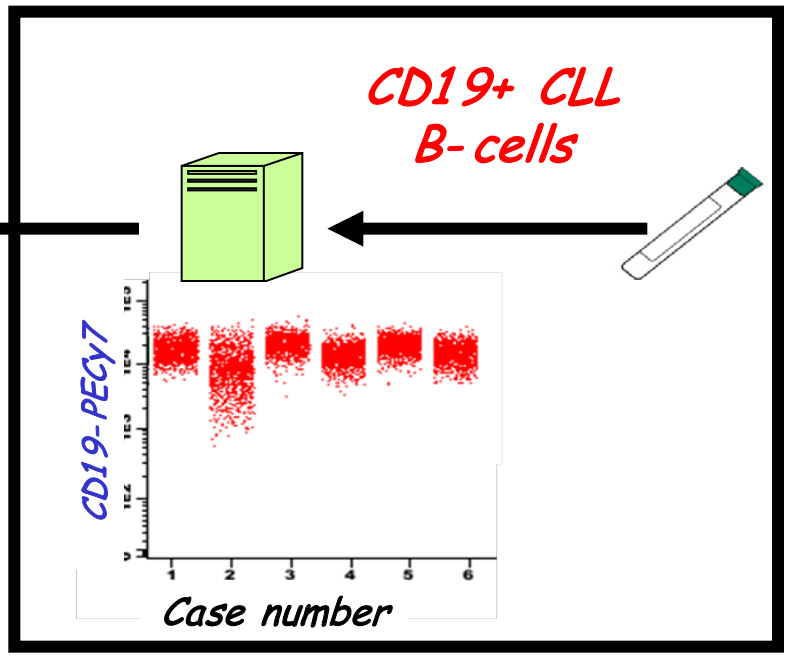
Diagnostic classification

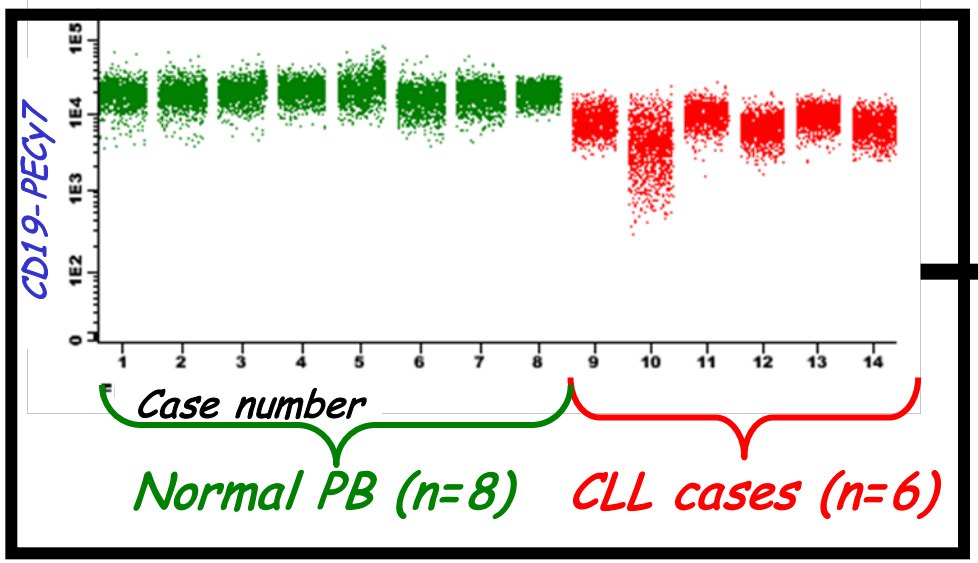
Treatment monitoring

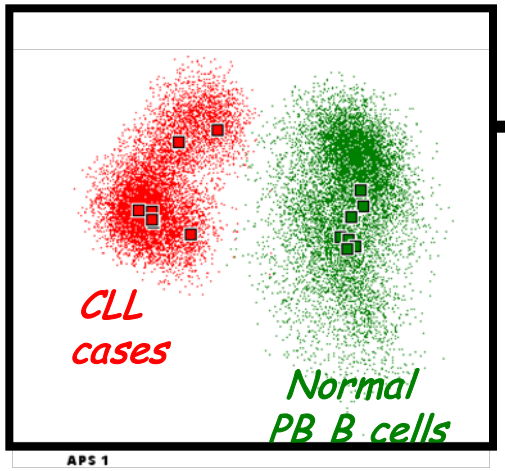
REFERENCE DATAFILES: NORMAL vs. CLL B-CELLS

EuroFlow





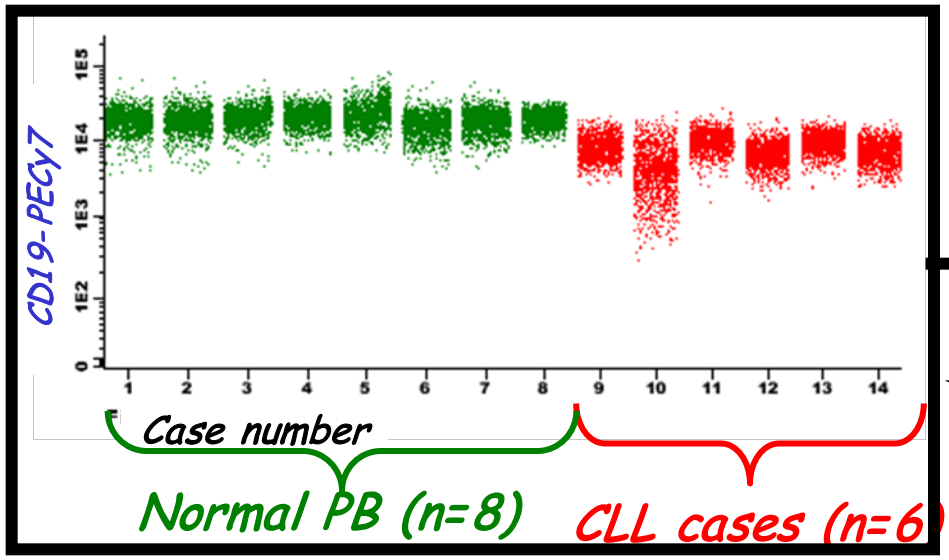


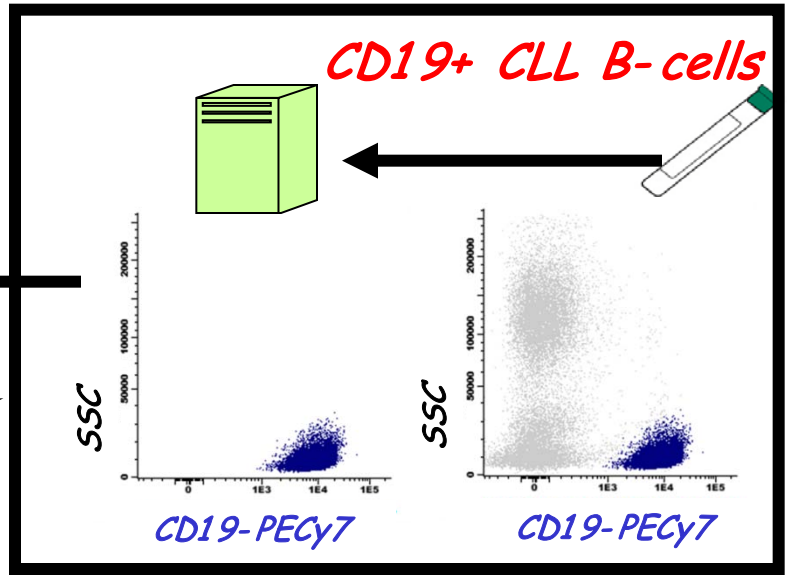


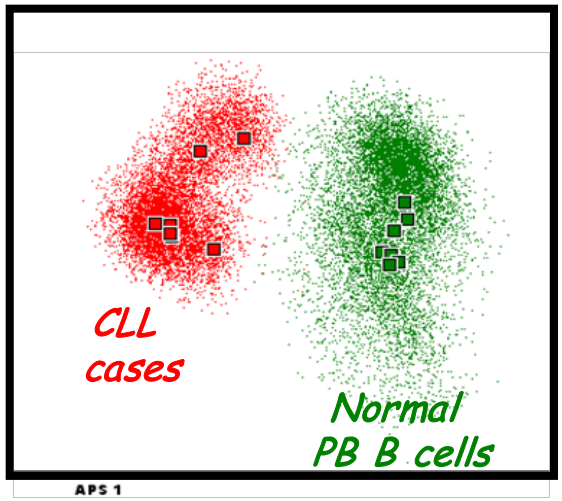
LAIR1	9.3
CD5	8.6
CD79b	8.2
IgM	8.1

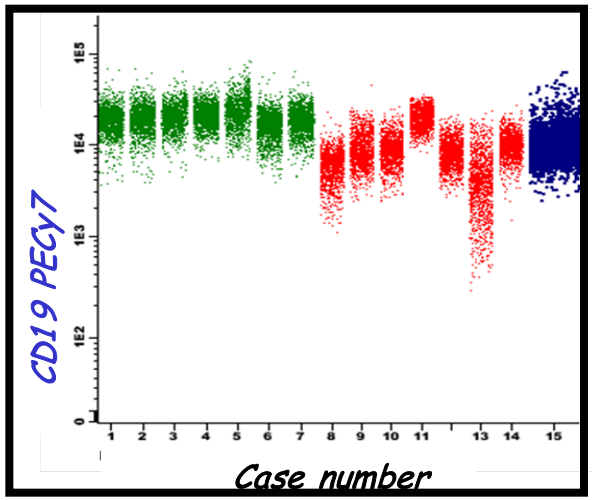
COMPARE A CASE VS NORMAL & CLL B-CELLS

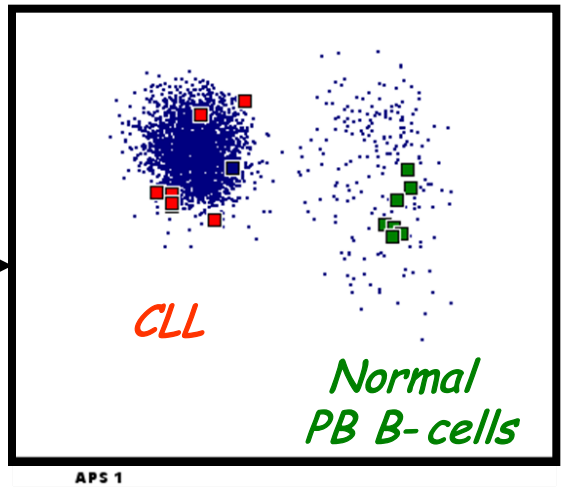
EuroFlow





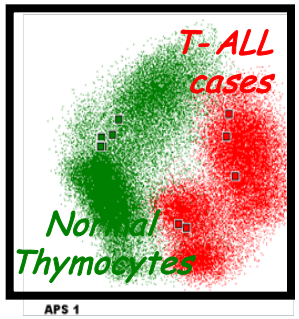
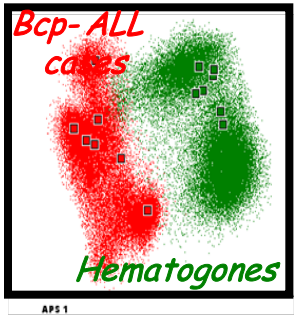
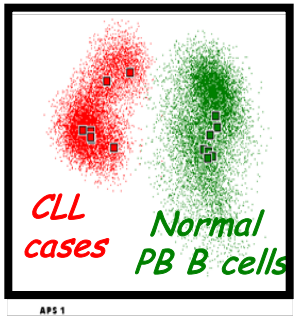
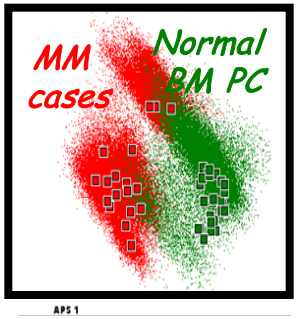
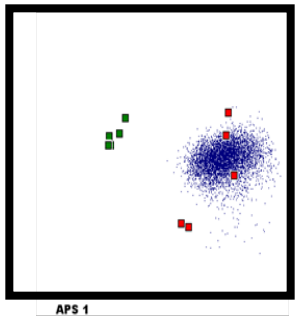
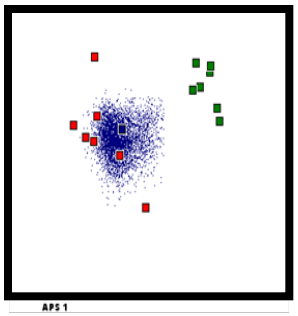
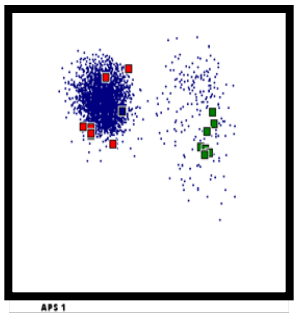
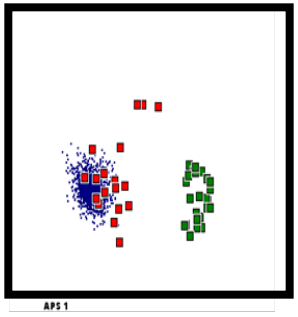
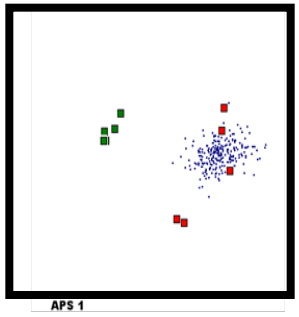
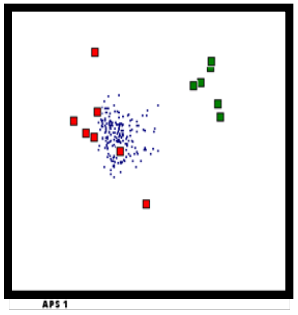
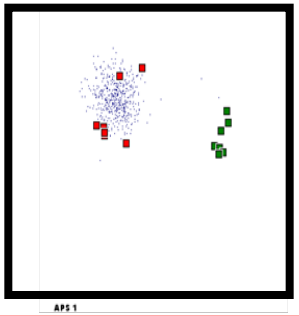
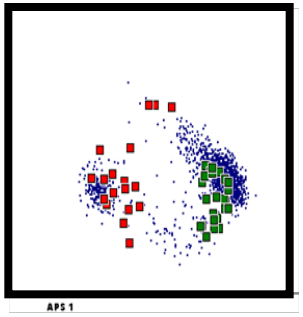






COMPARE A CASE VS NORMAL & NEOPLASTIC B-CELLS

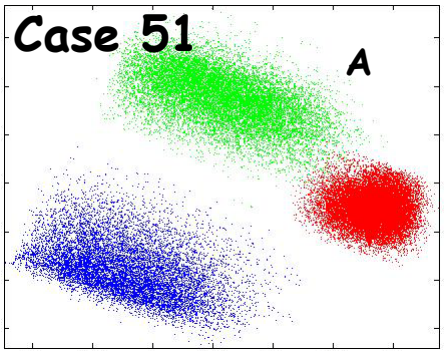
EuroFlow

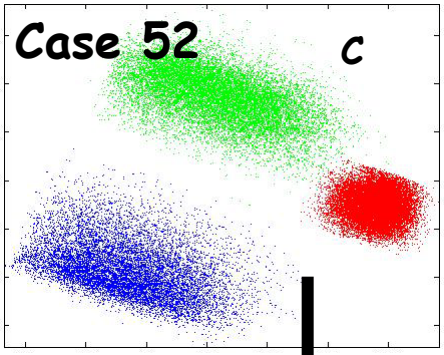
	T-ALL	B-cell precursor ALL	CLL	Multiple myeloma
Reference data files				
Diagnostic samples vs. Reference data files				
MRD samples vs. Reference data files				

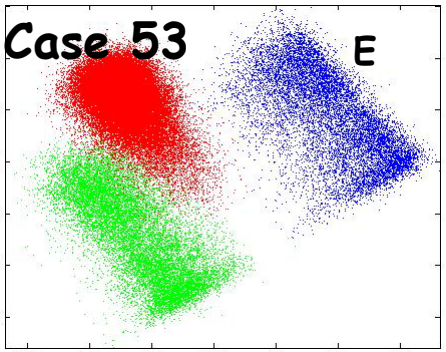
B-CLPD: AUTOMATED IDENTIFICATION OF ABERRANT PHENOTYPES AT DIAGNOSIS

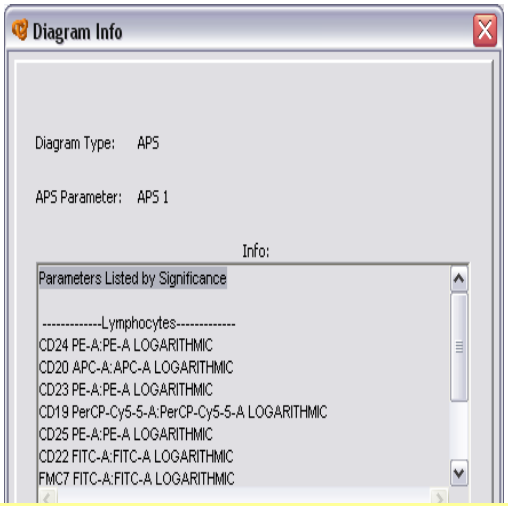
(Only CD19+ B-cells are displayed)

PB SAMPLES AT DIAGNOSIS









Information about the most discriminating parameters: (Most informative panels for the follow-up of MRD)

Pedreira et al, CytometryA 2008

Conclusions

- FCM is useful for the study of B-CLPD for: (i) the diagnosis (of clonality, NHL staging, and CSF infiltration), (ii) classification into WHO groups and (iii) MRD monitoring

- The new strategies developed by the EuroFlow Consortium opens the door for all applications of multiparameter flow cytometry for which a large number of parameters are needed and for the automation of FCM data analysis

EuroFlow

EuroFlow consortium aims at innovation in flow cytometry