

ESTRATEGIAS ACTUALES DE ANALISIS DE DATOS POR CMF: EL PROGRAMA INFINICYT



CANCER RESEARCH CENTER, UNIVERSITY & UNIVERSITY HOSPITAL of SALAMANCA (SPAIN)



Curso Avanzado de Actualización en Oncohematología por citometría de flujo.
Buenos Aires, 30 de mayo de 2011

DIAGNOSTICS IN HEMATO-ONCOLOGY

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 (ALL, AML, CML)

Prepared by J.T.M. van Dongen

APPLICATIONS OF FLOW CYTOMETRY

Flow cytometry: where can it be applied?

70s-90s

Hybridoma technology
Monoclonal antibodies
Fluorochrome-conjugates



From research laboratories
to clinical diagnostics

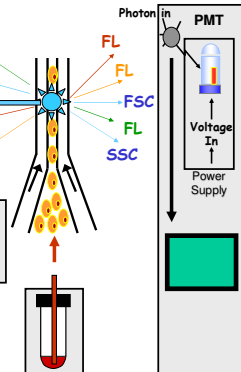
GENERATION OF FLOW CYTOMETRY DATA

LASERS

BLUE LASER
(Aligned and focused)

LASER LIGHT CHANGES:

- Different direction (Scatter)
- Different colour (Fluorescence)



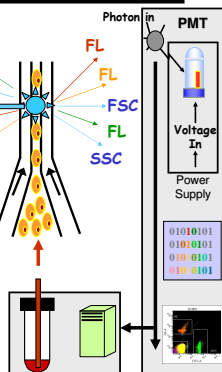
GENERATION OF FLOW CYTOMETRY DATA

LASERS

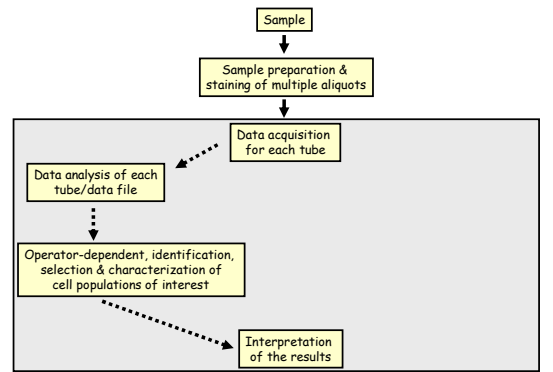
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LASER LIGHT CHANGES:

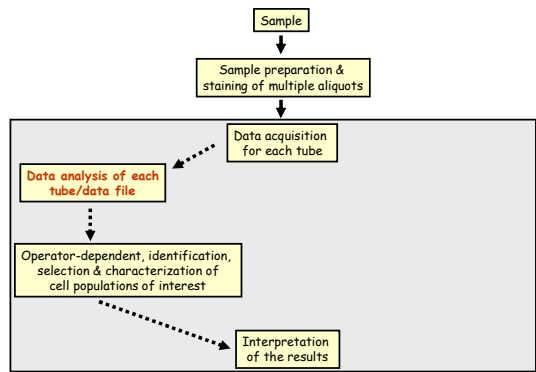
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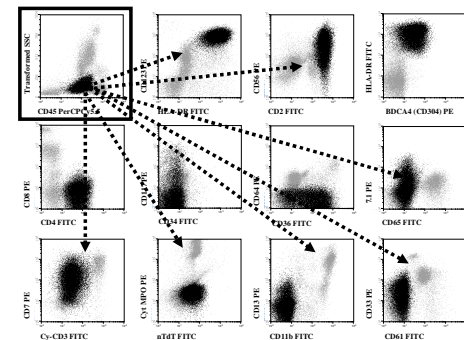
FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH



FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH



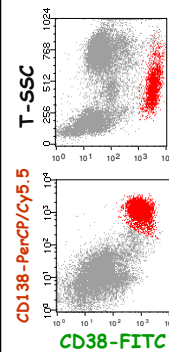
IMMUNOPHENOTYPIC FEATURES OF NEOPLASTIC CELLS



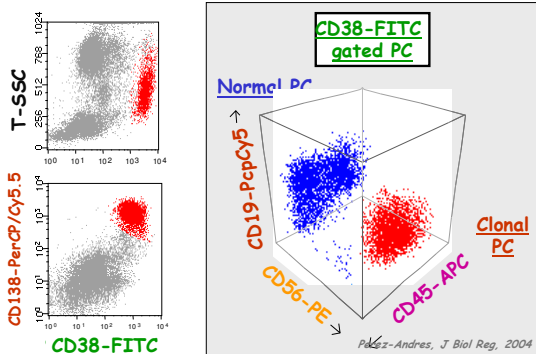
MDS/AML: 3-COLOR STAINING PANEL

- FITC	PE	PerCP/Cy5.5
- cCont.	cCont.	CD45
- nTdt	cMPO	CD45
- cCD3	CD7	CD45
- CD19	cCD79a	CD45
- sCont	sCont	CD45
- HLADR	CD117	CD45
- HLADR	CD123	CD45
- CD11b	CD13	CD45
- CD15	CD16	CD45
- CD36	CD64	CD45
- CD33	CD61	CD45
- CD71	6phA	CD45
- CD65	7.1	CD45
- CD2	CD56	CD45

MONOCLONAL GAMMOPATHIES: IDENTIFICATION OF CLONAL PLASMA CELLS



MONOCLONAL GAMMOPATHIES: IDENTIFICATION OF CLONAL PLASMA CELLS



Do not forget....FCM language is COMPLEX

WIIYGIWYG

What Is In Your Gate Is What You Get

Prepared by A.Salvador

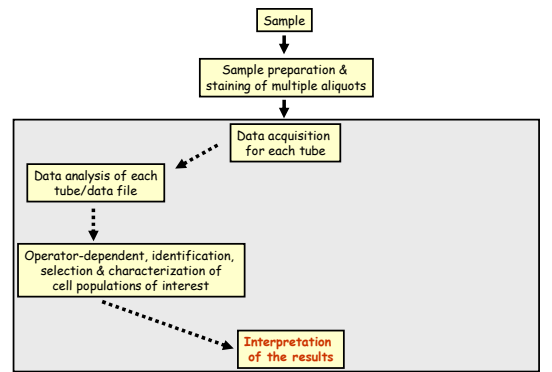
Do not forget....FCM language is COMPLEX

WINIYGIWYF

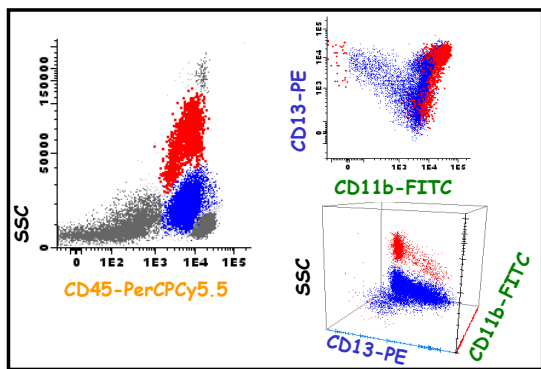
What Is Not In Your Gate Is What You Forget

Prepared by A.Salvador

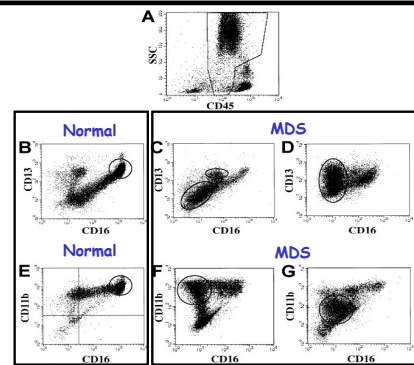
FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH



MDS: COEXISTENCE OF NORMAL & HYPOGRANULAR MATURING NEUTROPHILS

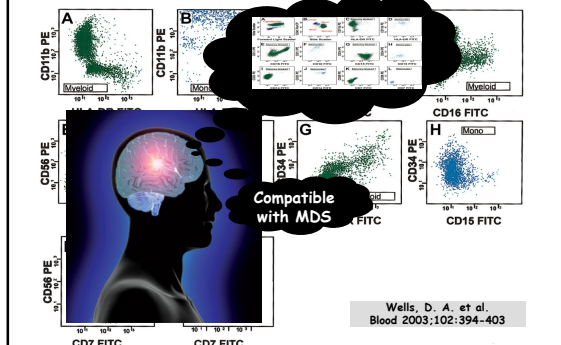


Immunophenotypic Myeloid Abnormalities in MDS



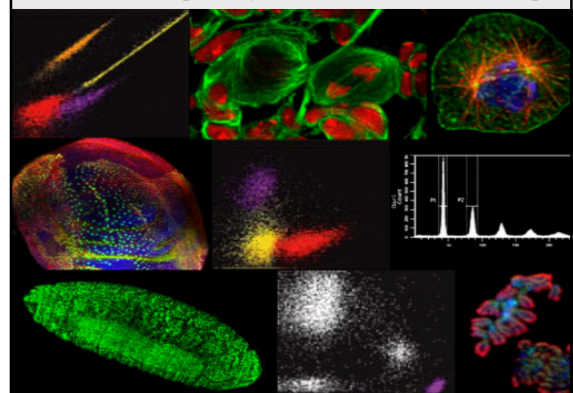
Stetler-Stevenson M et al, Blood 2001;98:979-987

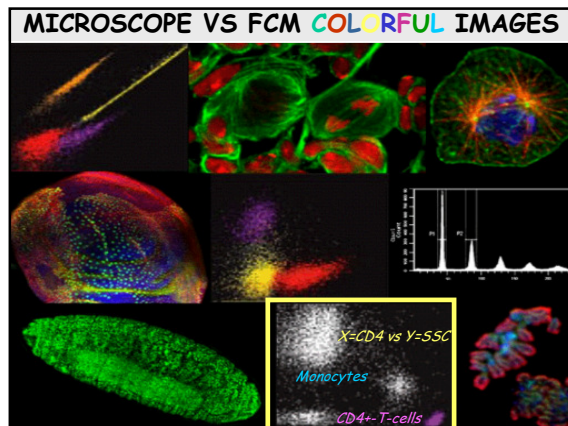
Examples of various myeloid and monocytic aberrant antigenic patterns in MDS



Wells, D. A. et al. Blood 2003;102:394-403

MICROSCOPE VS FCM COLORFUL IMAGES

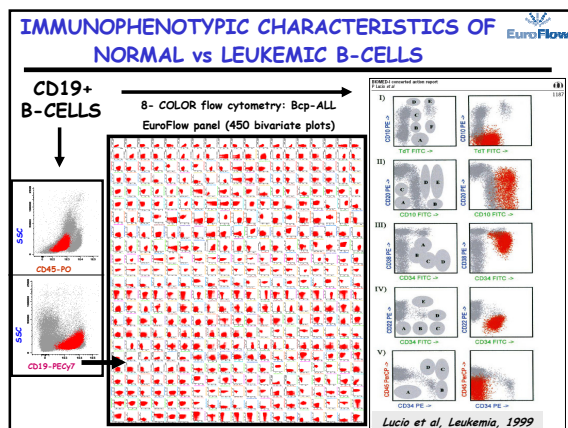




WHICH PROBLEMS ARE WE FACING ?

- Many reagents: costly and complex panels
- Need expertise in normal (reference) cell populations
- Time consuming
- Technical limitations
- Many (my) suboptimal strategies to reach a similar result
- Not standardized: reproducibly harmonized?
- Partial and more limited clinical utility than expected

EuroFlow



REQUIRED DEVELOPMENTS IN FLOW CYTOMETRY (status in 2005)

Immunobeads

- introduce combined cellular/immunobead assays
- special immunobead for leukemias

Novel antibodies

- test new (academic) antibodies for application in intracellular stainings
- development of new antibodies against oncoproteins and aberrant signalling pathways

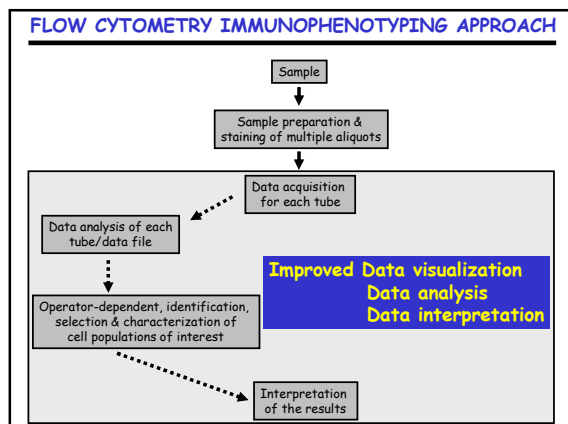
Multicolor flow cytometry: ≥8 color comprehensive panels

- inclusion of solid state violet laser
- selection of appropriate fluorochromes
- compare conjugated antibodies (multiple companies)

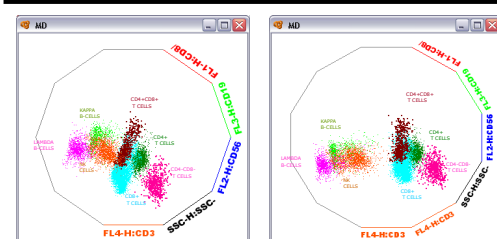
Development of novel software for complex pattern recognition

- combining multiple tubes: calculate data & multivariate analyses
- mapping of diagnosis and follow-up leukemia samples against templates of reference "normal/control" samples

EuroFlow



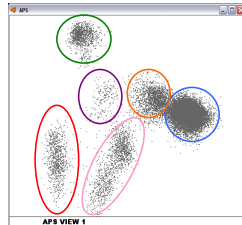
INCREASING DISCRIMINATION POWER N-multiplication of individual parameters



Duplication of CD3 allows a better discrimination between CD8+ T-cells and NK-cells

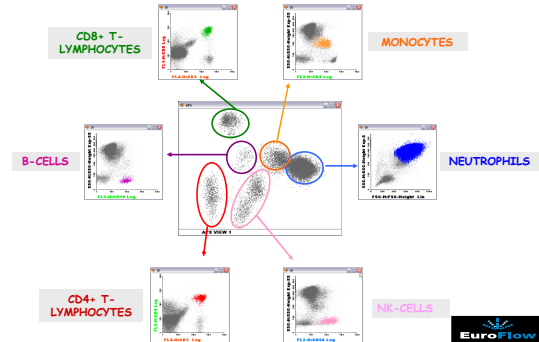
EuroFlow

AUTOMATED SEPARATION AMONG DIFFERENT CELL POPULATIONS (APS view)



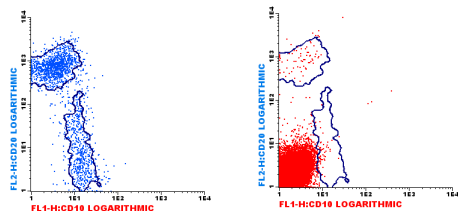
EuroFlow

AUTOMATED SEPARATION AMONG DIFFERENT CELL POPULATIONS (APS view)



EuroFlow

FCM DATA OVERLAYED ON REFERENCE IMAGES

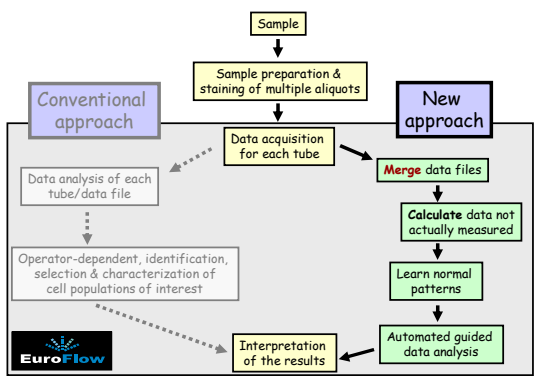


EuroFlow

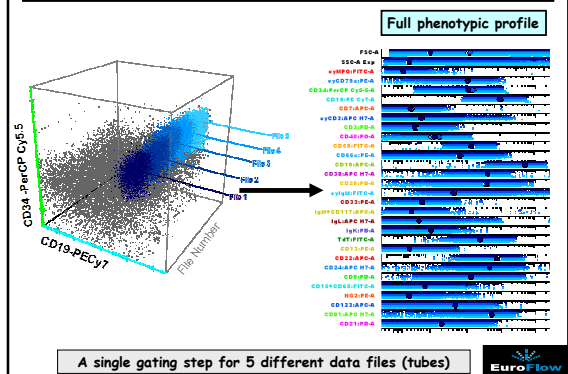
HOW TO SIMPLIFY DATA ANALYSIS

- Construct reference data files for normal and pathologic cell populations (e.g.: per disease category)
- Multi-n-dimensional comparison of normal vs pathologic cell populations (e.g.: at diagnosis and follow-up):
 - Automated PCA-guided approach for homogeneous cell populations (e.g. Blood lymphocyte subsets)
 - Maturation tools for heterogeneous cell populations (e.g. Maturing BM B-cell precursors)

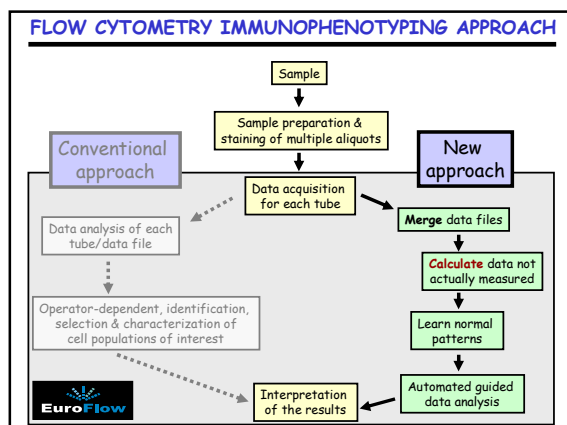
FLOW CYTOMETRY IMMUNOPHENOTYPING APPROACH



MERGED DATA FILES FOR SINGLE STEP GATING



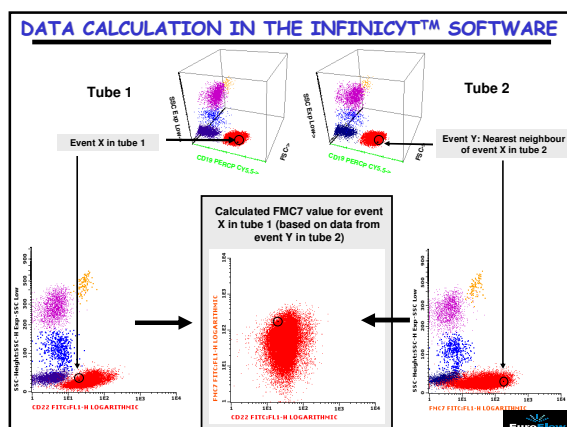
EuroFlow



DATA IN A MERGED DATA FILE

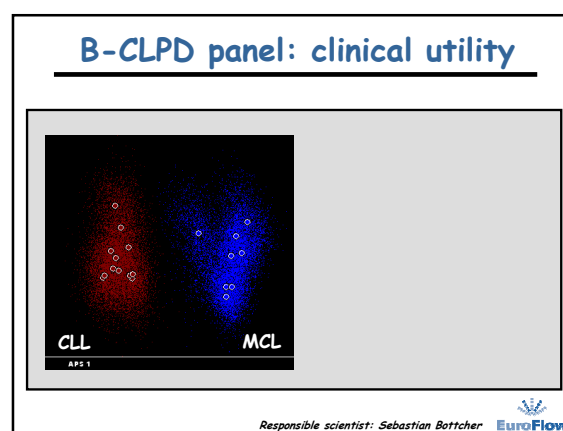
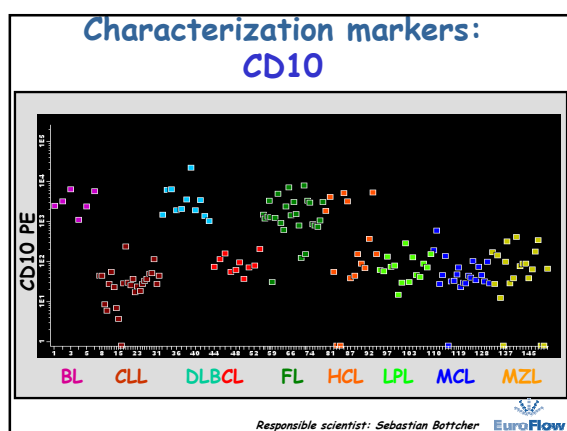
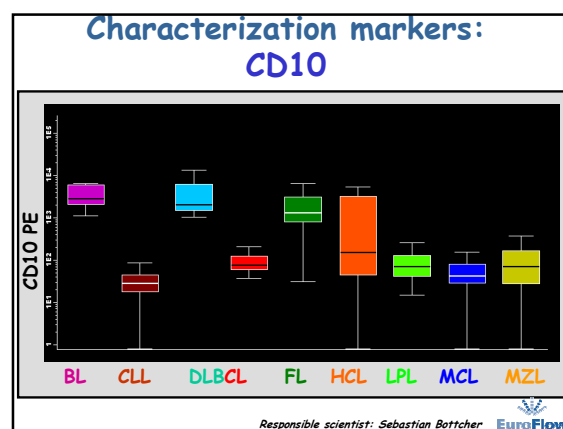
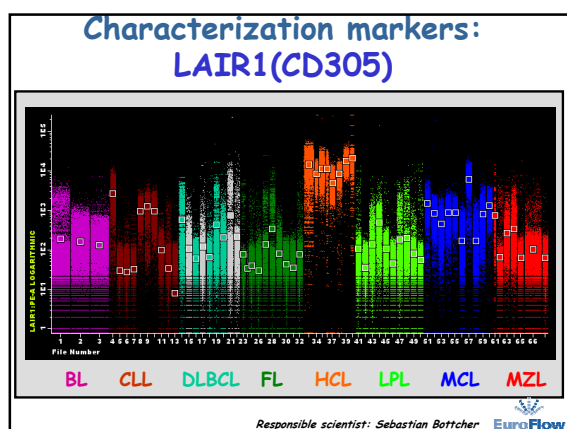
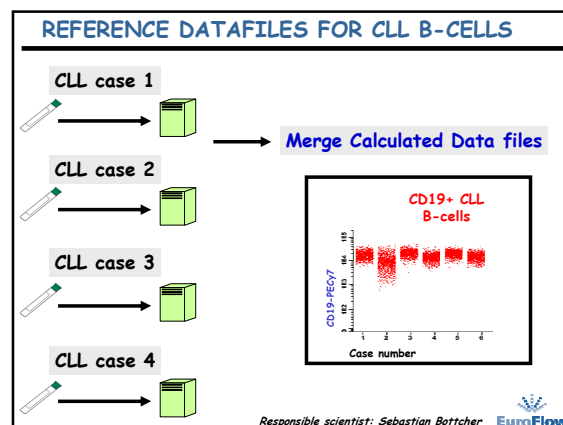
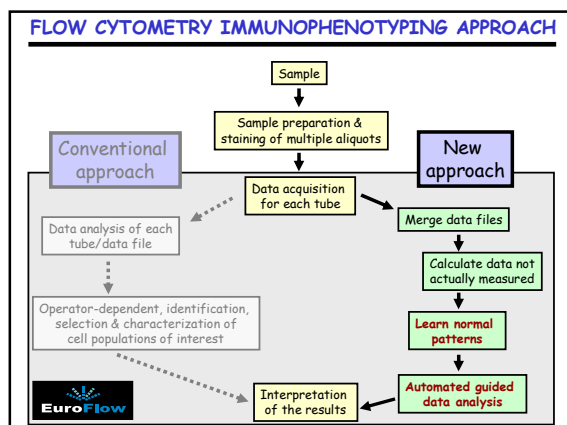
PARAMETER	TUBE No								
	1	2	3	4	5	6	7	8	9
FSC-H-HEIGHT									
SSC-H-HEIGHT									
CD11b-FITC									
CD13-PE									
CD45-PerCP									
CD34-APC									
CD2-FITC									
CD56-PE									
HLADR-FITC									
CD117-PE									
CD123-PE									
CD15-FITC									
CD16-PE									
CD22-FITC									
CD25-PE									
CD65-FITC									
7.1-PE									
CD61-FITC									
CD33-PE									
CD71-FITC									
GlypAin-PE									

The table shows a grid of parameters across 9 tubes. Parameters are color-coded: green for 'Common', red for 'Measured', and blue for 'Calculated'. The EuroFlow logo is at the bottom right.

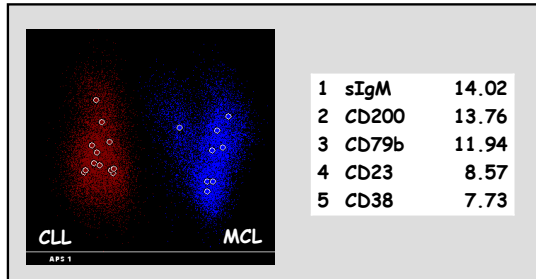


MERGED & CALCULATED LISTMODE DATA FILE

Parameters/File		Files					
		1	2	3	4	5	6
FSC-A		C	C	C	C	C	C
SSC-A		C	C	C	C	C	C
KAPPA-FITC-A		E	E	E	E	E	E
LA1		R	E	E	E	E	E
CD1	6 Files x	R	R	R	R	R	E
CD2		C	C	C	C	C	C
CD3	10 parameters x	R	E	E	E	E	E
CD4		C	C	C	C	C	C
CD5	100,000 events	C	C	C	C	C	C
CD6		C	C	C	C	C	C
CD7		C	C	C	C	C	C
CD8		C	C	C	C	C	C
CD9		C	C	C	C	C	C
CD10		C	C	C	C	C	C
CD11		E	E	E	E	E	E
CD12		E	R	E	E	E	E
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CD224		E	R	E	E	E	E
CD225		E	R	E	E	E	E
CD226		E	R	E	E	E	E
CD227		E	R	E	E	E	E
CD228		E	R	E	E	E	E
CD229		E	R	E	E	E	E
CD230		E	R	E	E	E	E
CD231		E	R	E	E	E	E
CD232		E	R	E	E	E	E
CD233		E	R	E	E	E	E
CD234		E	R	E	E	E	E
CD235		E	R	E	E	E	E
CD236		E	R	E	E	E	E
CD237		E	R	E	E	E	E
CD238		E	R	E	E	E	E
CD239		E	R	E	E	E	E
CD240		E	R	E	E	E	E
CD241		E	R	E	E	E	E
CD242		E	R	E	E	E	E
CD243		E	R	E	E	E	E
CD244		E	R	E	E	E	E
CD245		E	R	E	E	E	E
CD246		E	R	E	E	E	E
CD247		E	R	E	E	E	E
CD248		E	R	E	E	E	E
CD249		E	R	E	E	E	E
CD250		E	R	E	E	E	E
CD251		E	R	E	E	E	E
CD252		E					



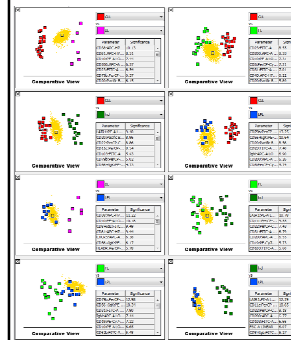
B-CLPD panel: clinical utility



Responsible scientist: Sebastian Bottcher



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



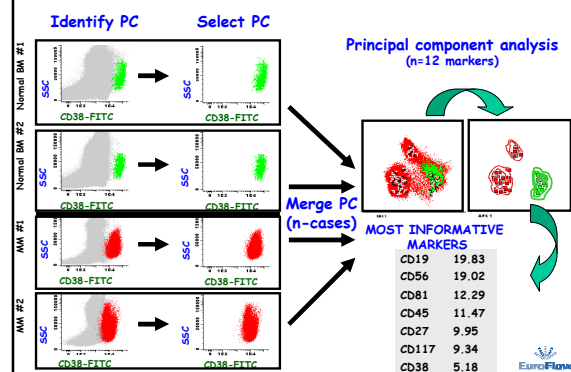
Responsible scientists: Sebastian Bottcher

Costa et al, Leukemia, 2010

HOW TO SIMPLIFY MRD STRATEGIES

- Improve the design of MRD panels for a greater efficiency and higher reproducibility.
- **Construct reference data files** for normal and neoplastic cells (e.g.: per disease category)
- Multi-n-dimensional comparison of normal vs neoplastic cell populations (e.g.: at diagnosis and follow-up):
 - Automated PCA-guided approach for homogeneous cell populations (e.g. lymphoid)
 - Maturation tools for heterogeneous cell populations (e.g. myeloid)

CONSTRUCTION OF EuroFlow MRD PANELS: MM



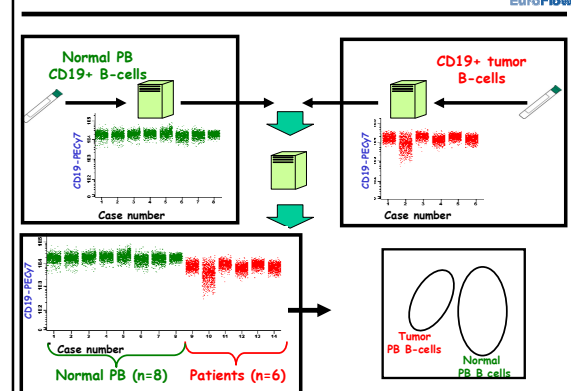
Aim of Euroflow MRD approach for B-CLPD

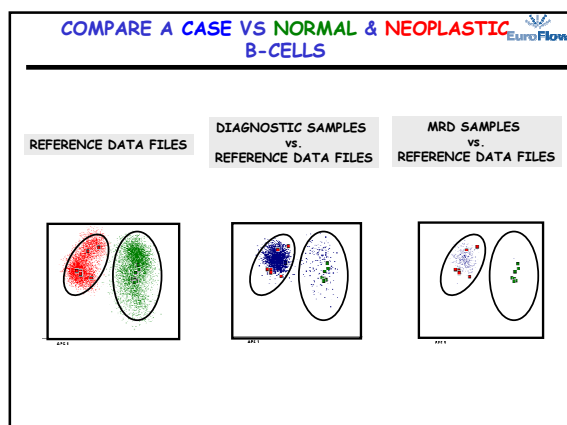
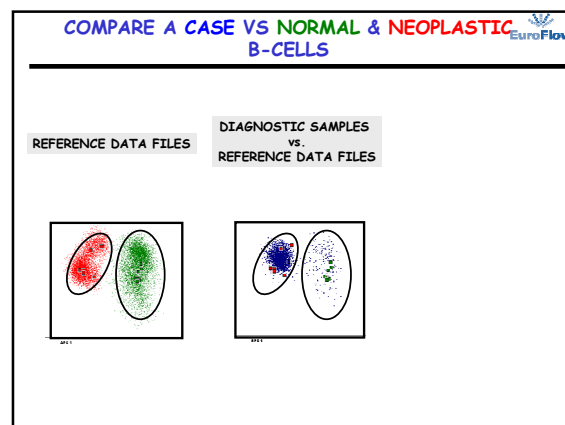
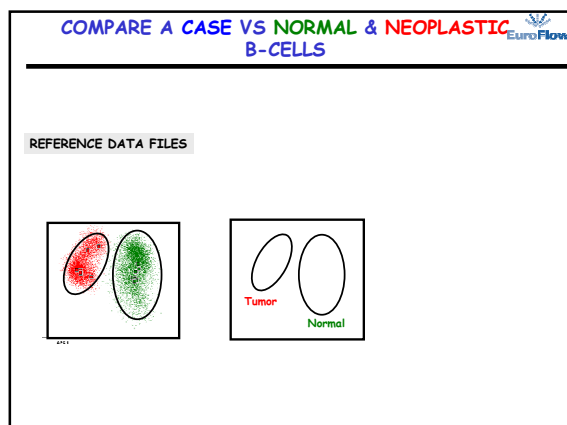
- To find a marker combination to **discriminate all neoplastic B-cells in every B-CLPD case** vs all normal/reactive PB and BM mature B-cells/hematogones

NOT an individualized approach **per patient**

the approach should **work without** knowledge of the exact **initial immunophenotype**

REFERENCE DATAFILES: NORMAL vs. TUMOR B-CELLS



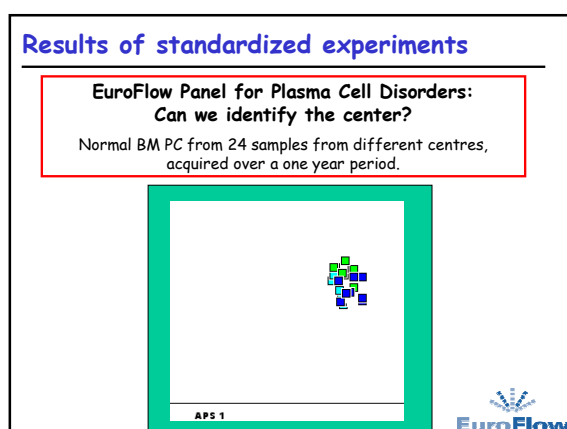


UTILITY OF THE NEW SOFTWARE TOOLS

Standardization of FCM immunophenotyping

- How to get optimal and comparable measurements?
- Which are the most appropriate fluorochromes?
- What is the optimal sample preparation protocol?

REPRODUCIBLE & OBJECTIVE RESULTS



www.euroflow.org EuroFlow participants

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**MUCHAS
GRACIAS**