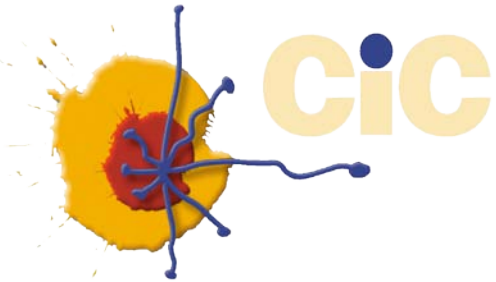
# Novel Strategies for Immunophenotypic Diagnosis and Classification in Acute Leukemias

VNiVERSiDAD D SALAMANCA

EuroFlow

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

# Diagnostics in 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 (ALL, AML, CML)

# IMMUNOPHENOTYPING OF ACUTE LEUKEMIAS: Technical information

## Leukemic vs normal cells:

- Identification
- Enumeration
- Characterization

# IMMUNOPHENOTYPIC CHARACTERIZATION OF LEUKEMIC CELLS

## HOW SIMILAR ARE LEUKEMIC CELLS TO NORMAL CELLS ?

- Reflect cell lineage and maturation stage.

## IN WHAT DO LEUKEMIC CELLS DIFFER FROM NORMAL CELLS ?

- Reflect derailment of protein expression (underlying genetic abnormalities?)

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- Reflect derailment of protein expression (underlying genetic abnormalities?)

# EGIL: DEFINITION OF BAL

| Score | B-Lineage | T-Lineage | Myeloid lineage |
|---|---|---|---|
| 2 | cCD79a<br>cIgM<br>cCD22 | c/mCD3<br>TCR | MPO<br>Lisozyme |
| 1 | CD19, CD10<br>CD20 | CD2, CD5<br>CD8, CD10 | CD13, CD33<br>CD117, CDw65 |
| 0.5 | Tdt, CD24 | Tdt, CD7<br>CD1a | CD14, CD15<br>CD64 |

**Criteria: > 2 points**

*Bene et al; Leukemia 1995; 9: 1783*

# Clasificación inmunológica de las neoplasias de precursores de linfocitos B

## CD19+ y cytCD79a+

| | | |
|---|---|---|
| BI | LLA-pro B | CD10- |
| BII | LLA-común | CD10+, Ig- (sup y cyt) |
| BIII | LLA-preB | Cyt Igμ+ |
| BIV | LLA-B | sIg+ |

Bene et al; Leukemia 1995; 9: 1783

# Clasificación inmunológica de las leucemias linfoblásticas agudas de línea T

## CD7+ y CD3+ en cit. y/o membrana

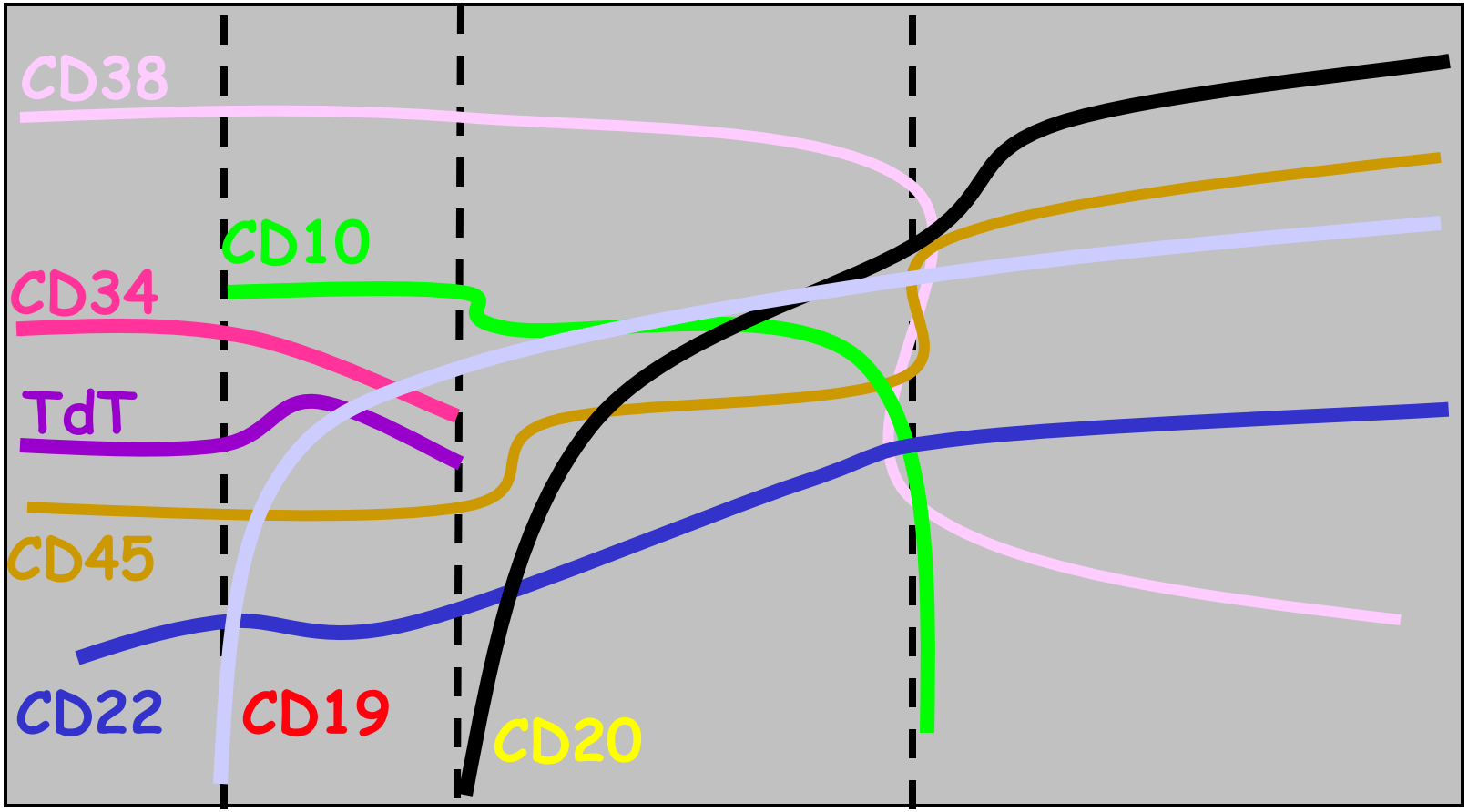
| | | | |
|---|---|---|---|
| *LLA pro- T* | TI | LLA-pro T | **No expresión de otros** marcadores asociados a línea T |
| *LLA tímicas* | TII | LLA-pre T | **CD2+ y/o CD5+ y/o CD8+** CD1a- y mCD3- |
| | TIII | LLA-T cortical | **CD1a+ y mCD3-/+** |
| | TIV | LLA-T madura<br>**LLA-Tαβ**<br>**LLA-Tγδ** | CD1a- y CD3+ de membrana<br>TCRαβ+<br>TCRγδ+ |

Suelen ser TdT+, HLA-DR-, CD34-, pero estos marcadores no se consideran para el dco o clasificación de la enfermedad

*Bene et al; Leukemia 1995; 9: 1783*

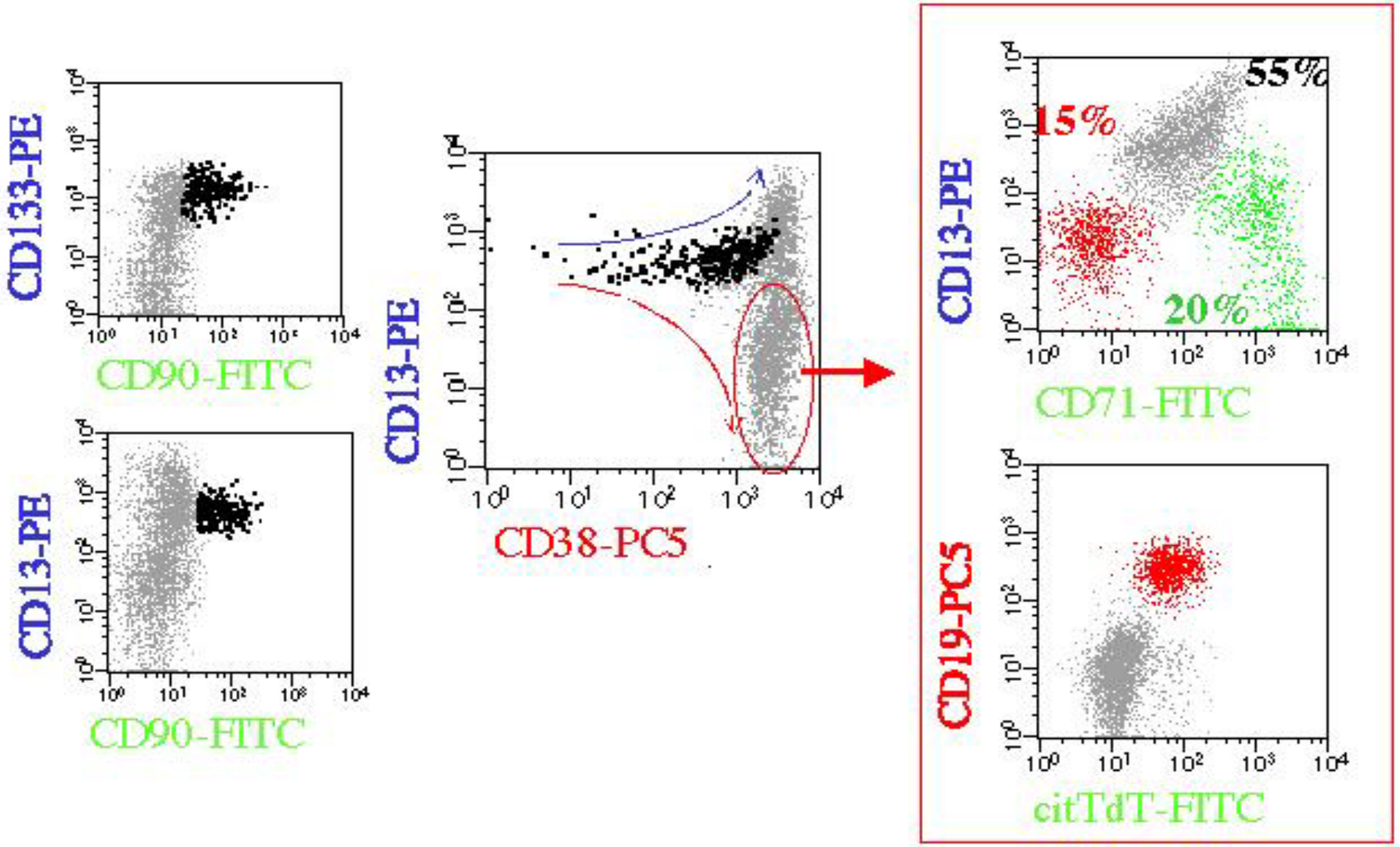
# IMMUNOPHENOTYPE OF NORMAL B-CELL PRECURSORS

## B-cell maturation in normal BM



# ERYTHROID & B-LYMPHOID DIFFERENTIATION IN BM

## GATE OF CD34$^{+}$ HPC



# IMMUNOPHENOTYPE OF CD34+ MYELOID-COMMITTED HPC

| CELL LINEAGE | SSC | IMMUNOPHENOTYPE |
|---|---|---|
| Erythroid | stable | CD36+, CD64-, CD45lo |
| Megakaryocytic | high | CD61+, CD45lo |
| Neutrophil | high | cMPO+, CD13hi |
| Eosinophil | high | cMPO-, CD15/65+, EPO+ |
| Basophil | low | CD123hi, HLADRlo, CD117lo CD45hi |
| Monocytic | stable | MPO-, HLADR+, CD117+/lo |
| Mast cell | low | CD117hi, HLADRlo, CD45hi |
| pDC | stable | CD123hi, HLADRhi, CD4+ |

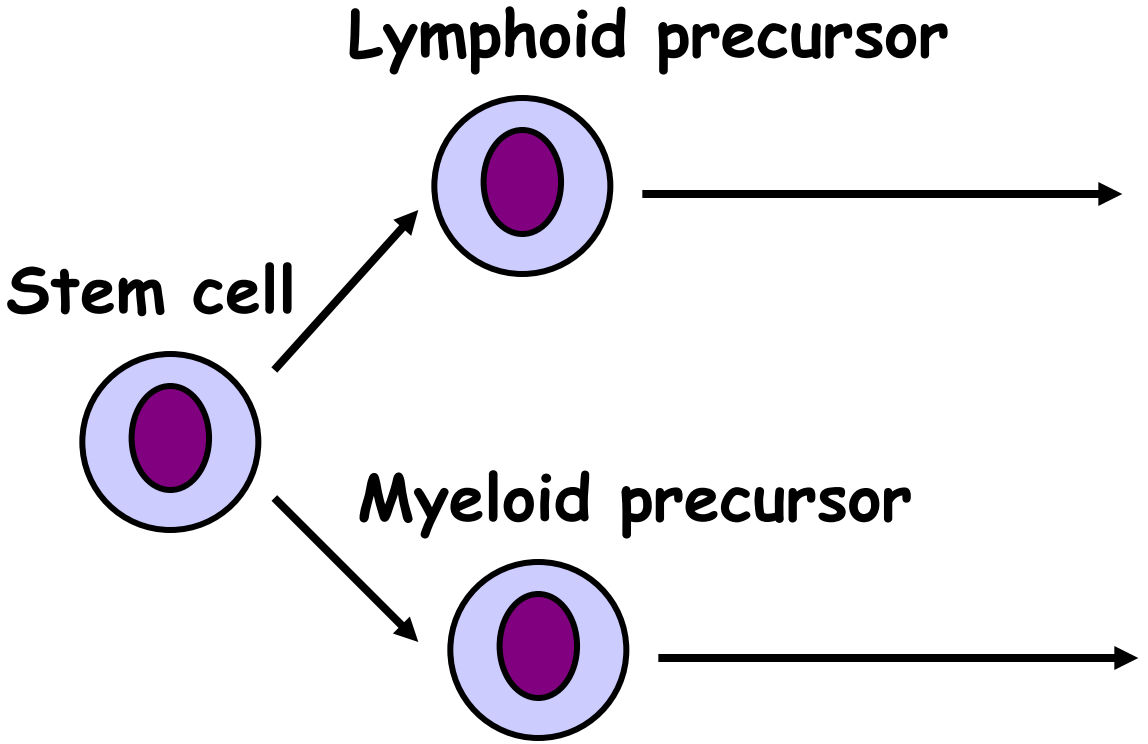
# AML: 4-COLOR PANEL

| - FITC | PE | PerCP/Cy5.5 | APC |
|---|---|---|---|
| - cCont. | cCont. | CD45 | CD34 |
| - nTdt | cMPO | CD45 | CD34 |
| - cCD3 | CD7 | CD45 | CD34 |
| - CD19 | cCD79a | CD45 | CD34 |
| - sCont | sCont | CD45 | CD34 |
| - HLADR | CD117 | CD45 | CD34 |
| - HLADR | CD123 | CD45 | CD34 |
| - CD11b | CD13 | CD45 | CD34 |
| - CD15 | CD16 | CD45 | CD34-CD10 |
| - CD36 | CD64 | CD45 | CD34-CD14 |
| - CD33 | CD61 | CD45 | CD34 |
| - CD71 | GphA | CD45 | CD34 |
| - CD65 | 7.1 | CD45 | CD34 |
| - CD2 | CD56 | CD45 | CD34 |

# UTILIDAD CLÍNICA DEL ANÁLISIS INMUNOFENOTÍPICO DE LAS HEMOPATÍAS MALIGNAS

- **Leucemias agudas:**
  - Diagnóstico de línea (mieloide vs linfoide B y T)
  - Diagnóstico de leucemias bifenotípicas/mixtas linfoide/mieloide
  - Clasificación fenotípica de LLA de precursores B
  - Clasificación fenotípica de LLA T
  - Subclasificación de línea de LMA (p.ej.: LMA megacariocítica)
- Síndromes linfoproliferativos crónicos:
  - Diagnóstico de clonalidad T y B
  - Clasificación fenotípica de SLPC-T/NK
  - Clasificación fenotípica de SLPC-B
  - Estratificación pronóstica de la LLC-B (e.g.: CD38 y zap70)
- Diagnóstico de otras hemopatías clonales:
  - Diagnóstico y clasificación de HPN
  - Diagnóstico y clasificación de mastocitosis

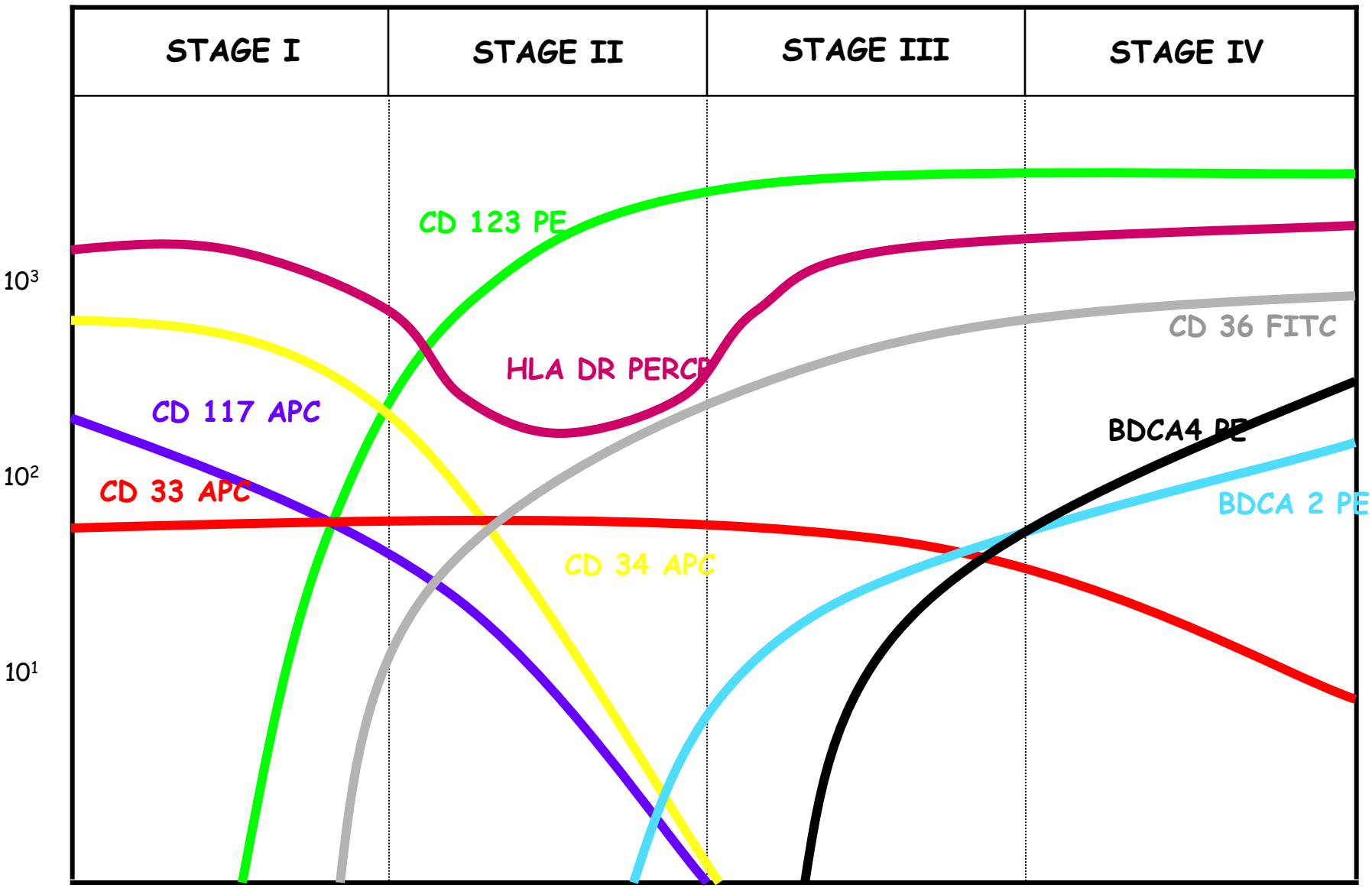
# HEMATOPOIESIS



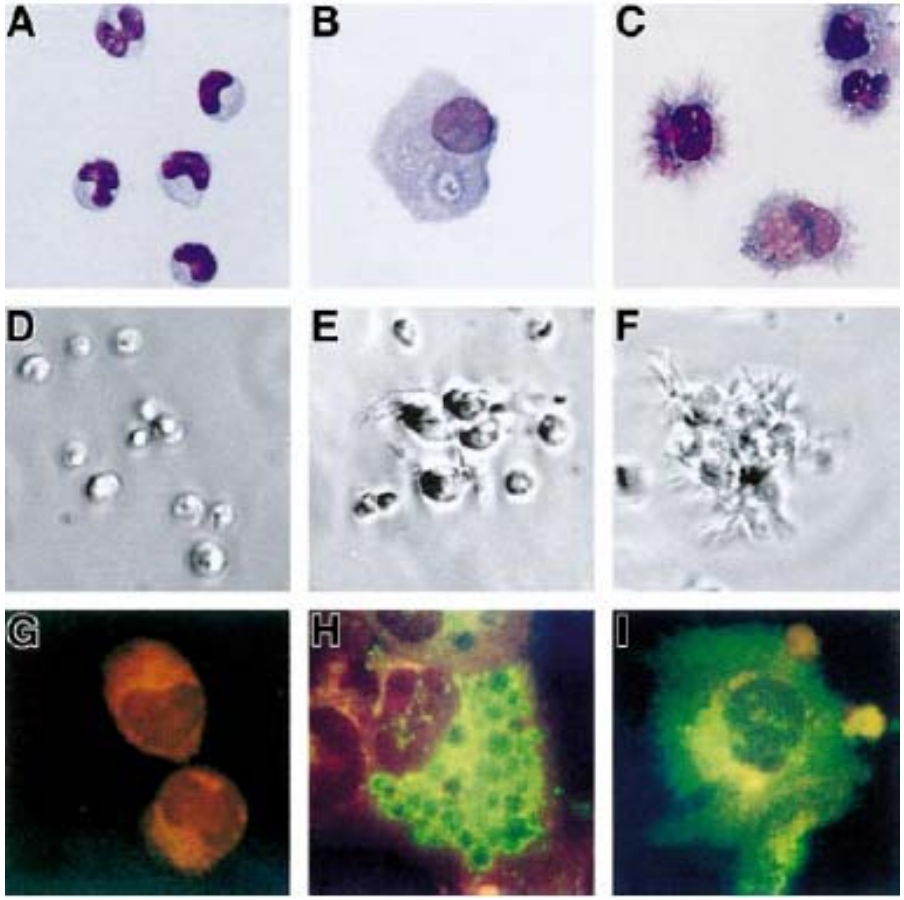

T-lymphocytes
B-lymphocytes
NK-cells
Plasmacytoid DC

Erythroid cells
Megakaryocytic cells
Neutrophils
Monocytes
Monocytic DC
Eosinophils
Basophils
Mast cells
Myeloid DC

# PHENOTYPIC CHANGES DURING NORMAL MATURATION OF PLASMACYTOID DENDRITIC CELLS IN BM

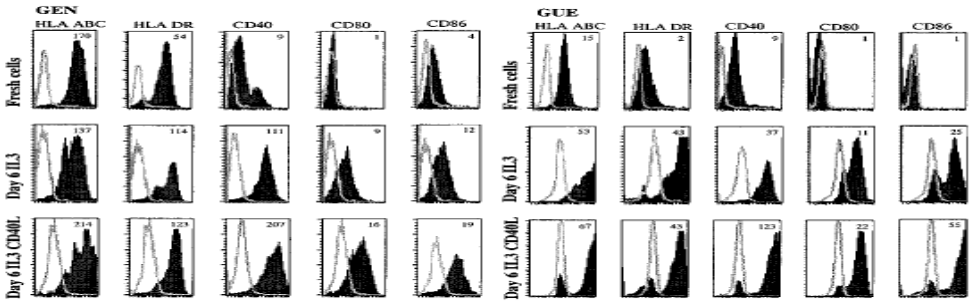


# The leukemic counterpart of the plasmacytoid dendritic cells

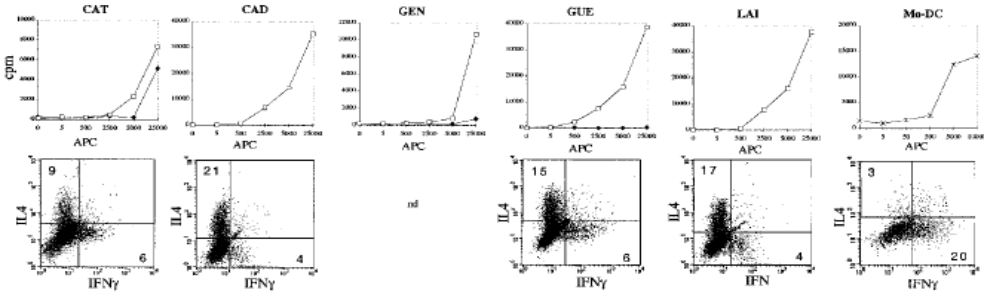



*IL3 induces differentiation of neoplastic cells*

*Leukemic cells up-regulate co-stimulatory molecules upon IL3 and CD40L stimulation*




*Leukemic cells stimulate T cells and induce Th2 polarization*




*Chaperot et al, Blood 2001*

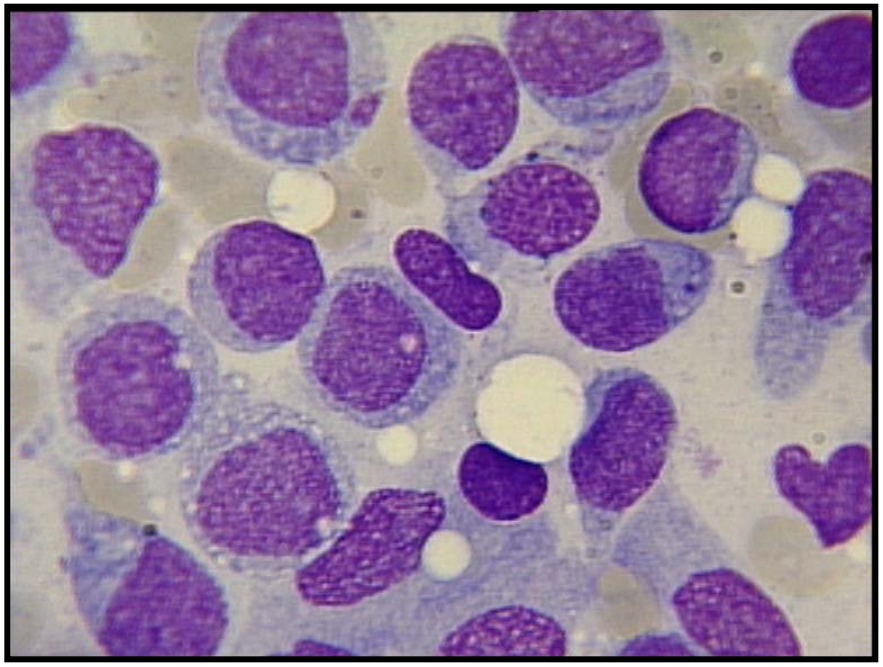
# CD123$^{hi}$ DC NEOPLASIAS:CLINICAL FEATURES

| Reason for consulting | |
|---|---|
| Fever | 12/57 (21%) |
| Constitutional syndrome | 5/57 (9%) |
| Anaemic syndrome | 27/57 (47%) |
| Organ infiltration | 34/57 (60%) |
| Severe renal failure | 1/57 (2%) |
| Bone pain | 5/57 (9%) |
| Hemorraghe | 7/57 (12%) |

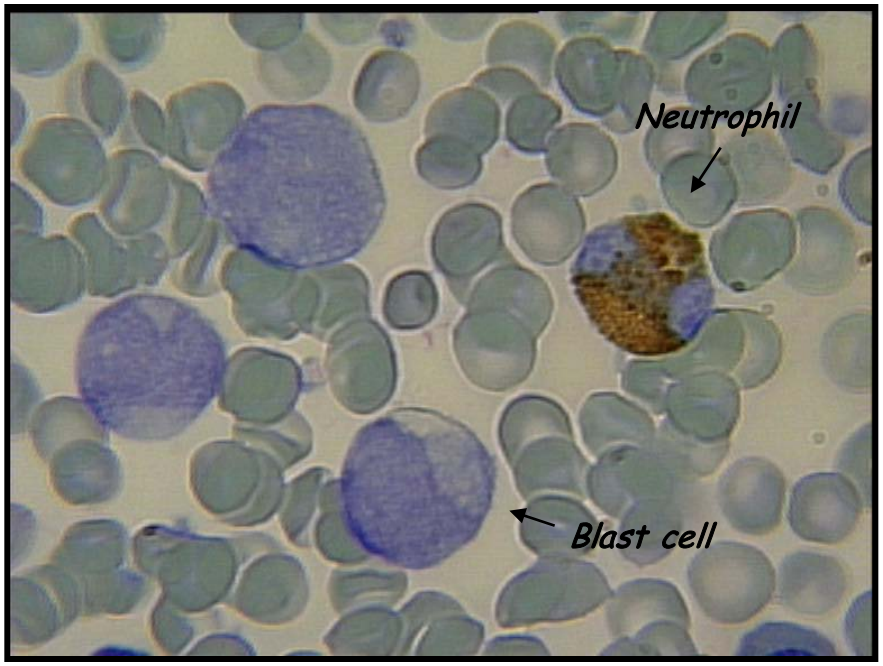
| Physical examination | |
|---|---|
| Adenopathies | 29/57 (50%) |
| Splenomegaly | 22/57 (39%) |
| Hepatomegaly | 15/57 (27%) |
| Skin lesions | 30/57 (53%) |
| CNS involvement | 4/57 (7%) |
| Testis involvement* | 2/44 (5%) |
| Other | 2/57 (4%) |

*Both cases were <11 years

# MORPHOLOGY OF NEOPLASTIC pDCs

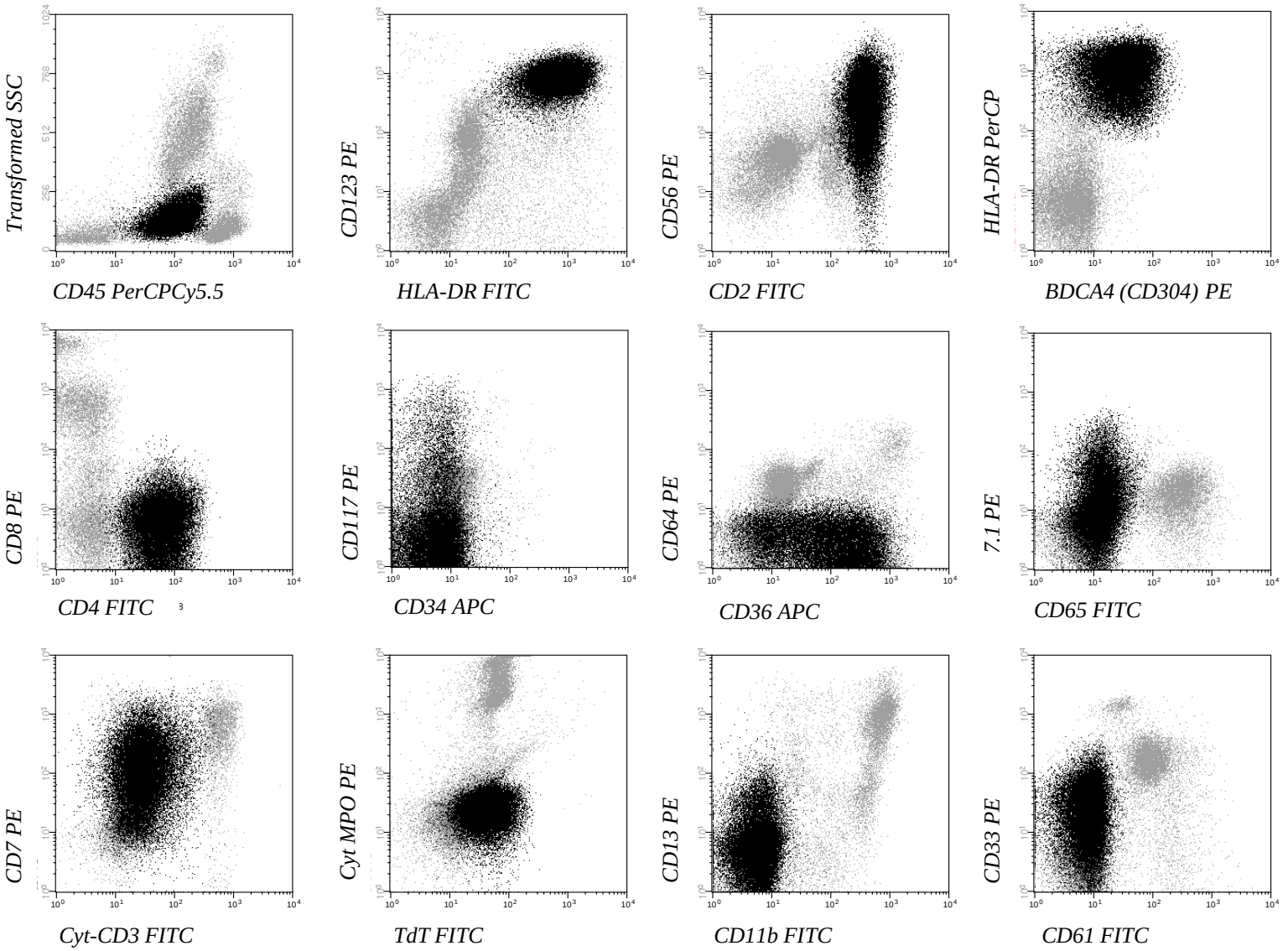


May-Grünwald Giemsa (x1000)

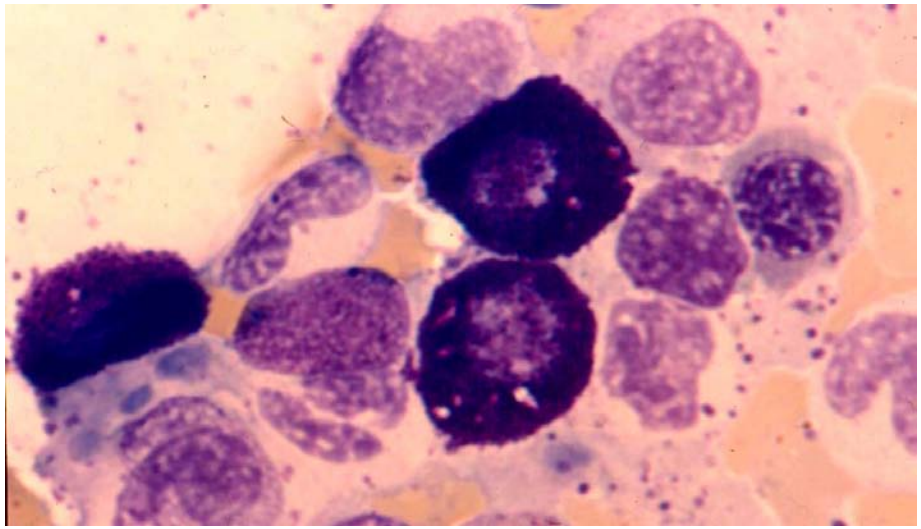


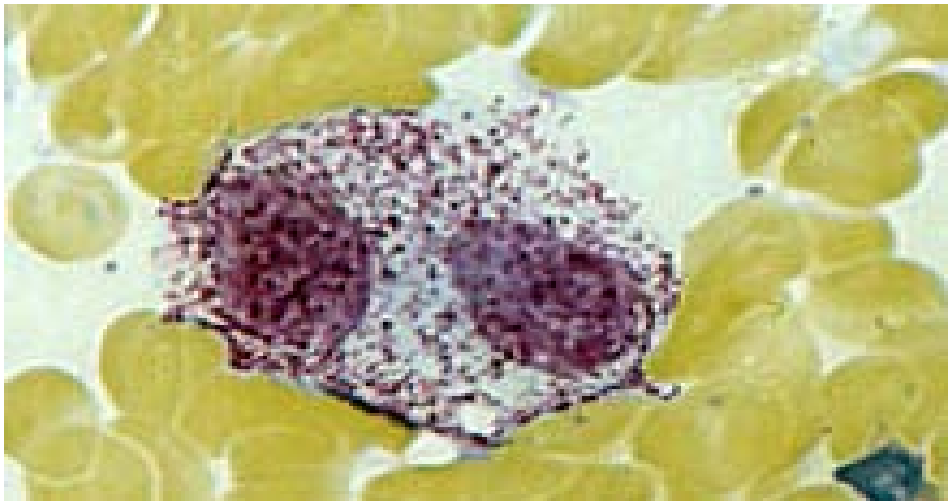

MPO immunocytochemical staining

# Blastic plasmacytoid dendritic cell neoplasm (WHO 2008): Immunophenotypic features

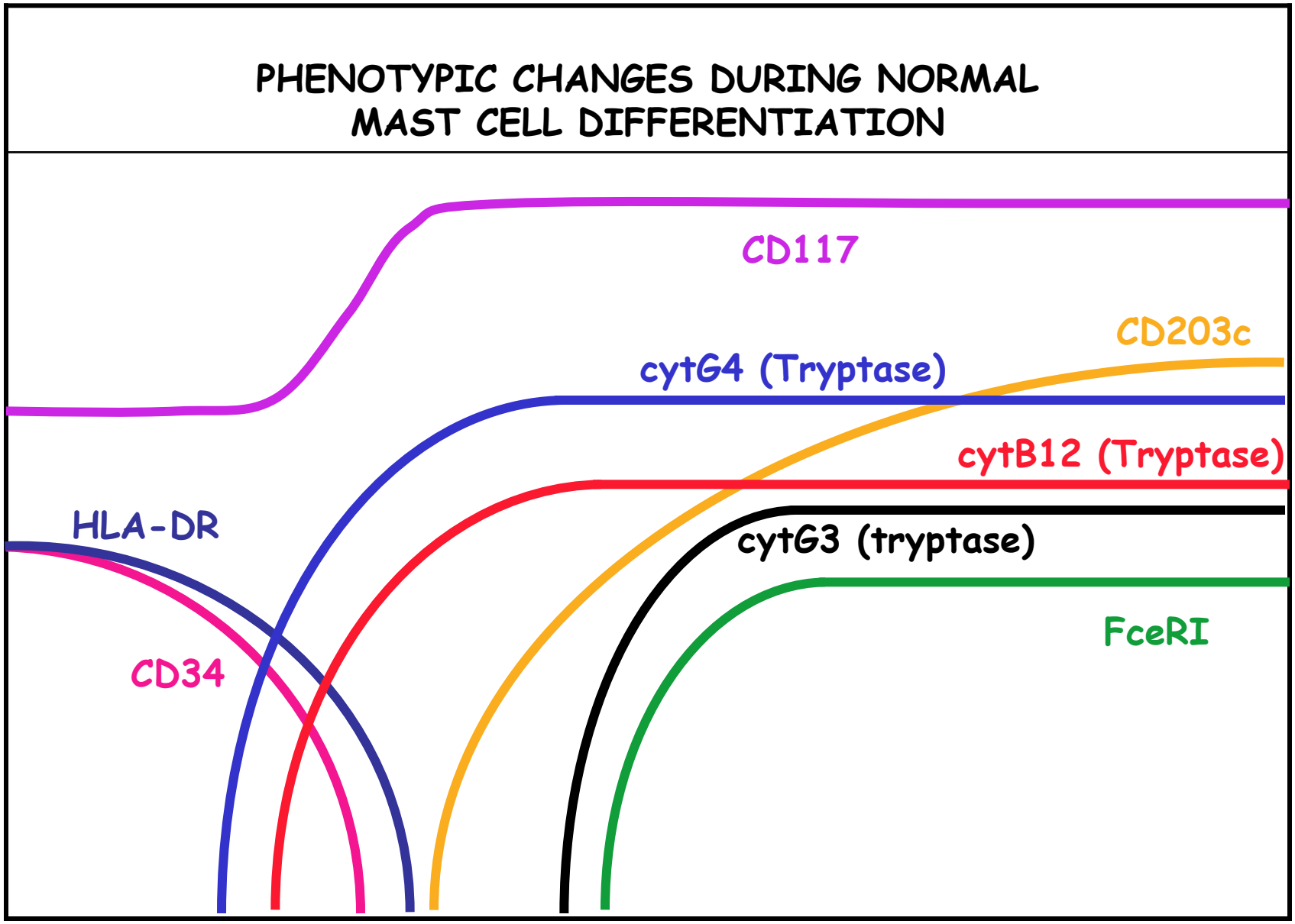


# MAST CELLS

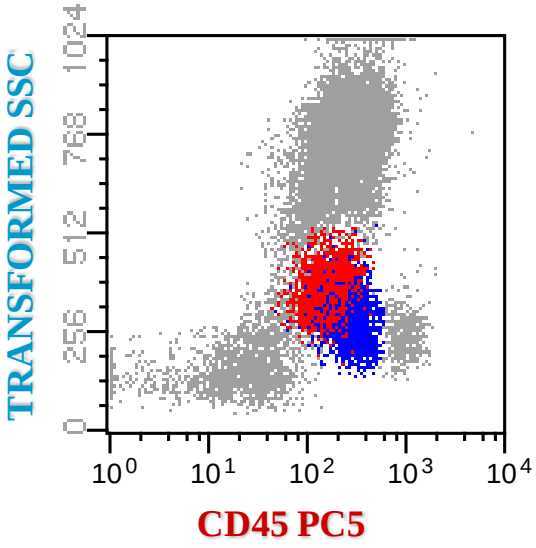


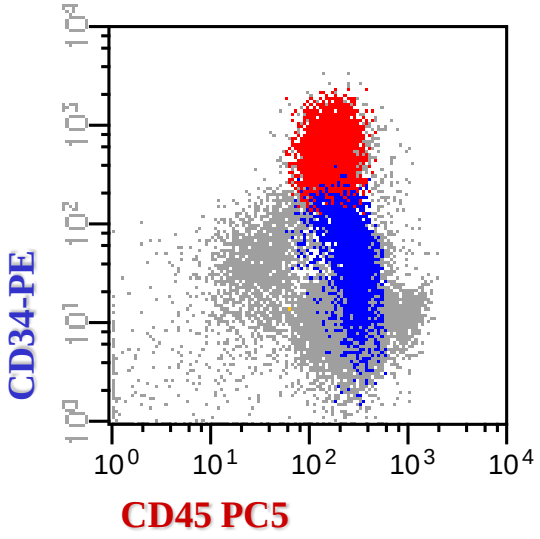


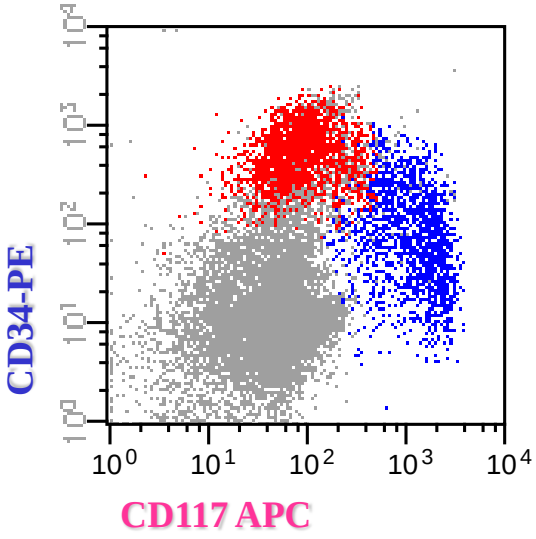
# PHENOTYPIC CHANGES DURING NORMAL MAST CELL DIFFERENTIATION



# ACUTE MAST CELL LEUKEMIA



# ACUTE LEUKEMIAS OF MAST CELLS

**Phenotypic characteristics:**

- CD33++, CD7-/+, CD34-/+, tryptase-/+, CD117+/+++, FCRεI-/+, HLADR-/+

**Associations:**

- Chronic myeloid leukaemia
- Systemic mastocytosis
- "De novo" AML: inv(16), AML1/ETO+

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- Reflect cell lineage and maturation stage.

IN WHAT DO LEUKEMIC CELLS DIFFER FROM NORMAL CELLS ?

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# Acute promyelocytic leukemia: AML with t(15;17)

# IMMUNOPHENOTYPE OF AML CASES WITH t(15;17)

| Phenotypic pattern | M3-PML/ RAR-alfa+ N=34 | Non-M3 PML/RARalfa- N=68 | Univariate analysis p-value | Multivariate analysis p-value |
|---|---|---|---|---|
| Typical CD34/CD15 | 100% | 21% | < 0.00001 | < 0.00001 |
| CD13+ heterogeneous | 100% | 34% | < 0.00001 | < 0.00001 |
| One blast cell population | 100% | 56% | < 0.00001 | < 0.002 |
| HLADR- | 91% | 24% | <0.00001 | < 0.03 |
| CD33+ homogeneous | 82% | 34% | <0.00001 | < 0.19 |

# IMMUNOPHENOTYPIC SCORE OF AML CASES WITH t(15;17)

| Phenotypic score | M3-PML/ RAR-alfa+ | Non-M3 PML/RARalfa+ | M3-PML/ RARalfa- | Non-M3/ PML/RARalfa- |
|---|---|---|---|---|
| 0 | - | - | 1 | 15 |
| 1 | - | 2 | 1 | 38 |
| 2 | - | 1 | 1 | 14 |
| 3 | 34 | 2 | 1 | 1 |
| Total cases | 34 | 5 | 4 | 68 |

*Orfao et al. Haematologica 1999; 84: 405*

# AML WITH t(15;17): DISCREPANT CASES

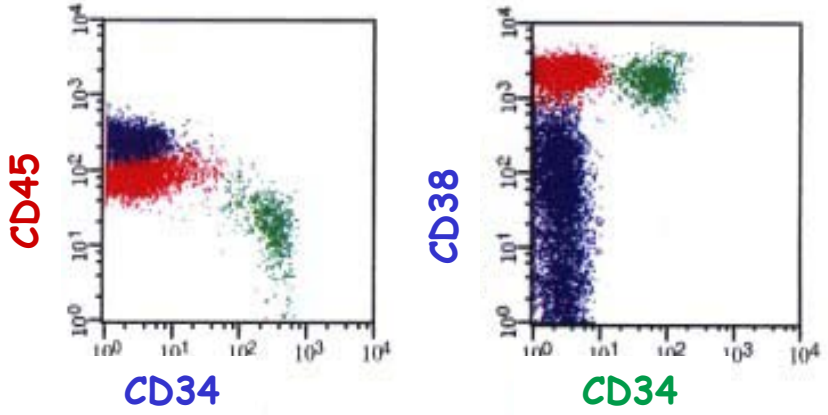
| Case | IF Score | FAB | FISH | Confirmatory PCR |
|---|---|---|---|---|
| M3/PML-RARalfa- | | | | |
| 23308 | 0 | M3c | - | - |
| 26810 | 1 | M3c | - | - |
| 20968 | 2 | M3c | - | - |
| 26584 | 3 | M3c | + | - * |
| Non-M3/PML-RARalfa+ | | | | |
| 18711 | 1 | M1 | - | - |
| 21339 | 1 | M0 | - | - |
| 21303 | 2 | M0 | - | + |
| 21365 | 3 | M2 | + | + |
| 28012 | 3 | M2 | + | + |

* Positive by Southern Blot hybridization

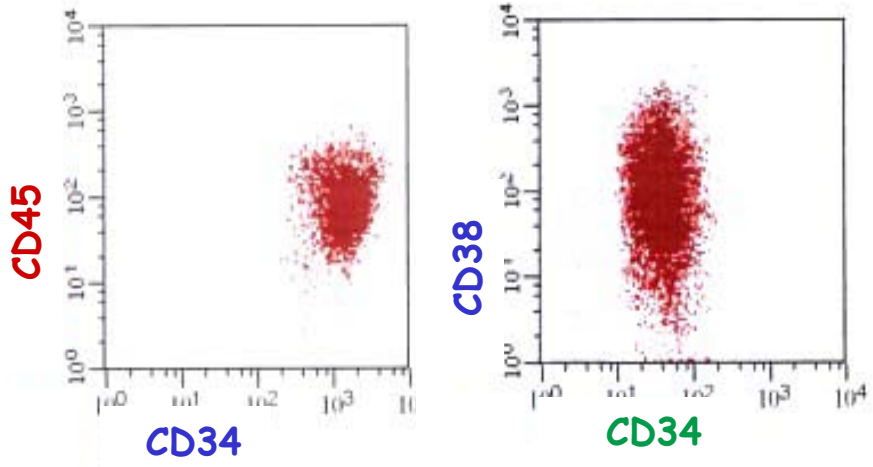
# PRECURSOR B-ALL: IMMUNOPHENOTYPE OF BCR/ABL+ CASES

# IMMUNOPHENOTYPIC PATTERNS OF NORMAL VS BCR/ABL+ PRECURSOR-B ALL BM B-CELLS

Normal BM




Precursor-B ALL

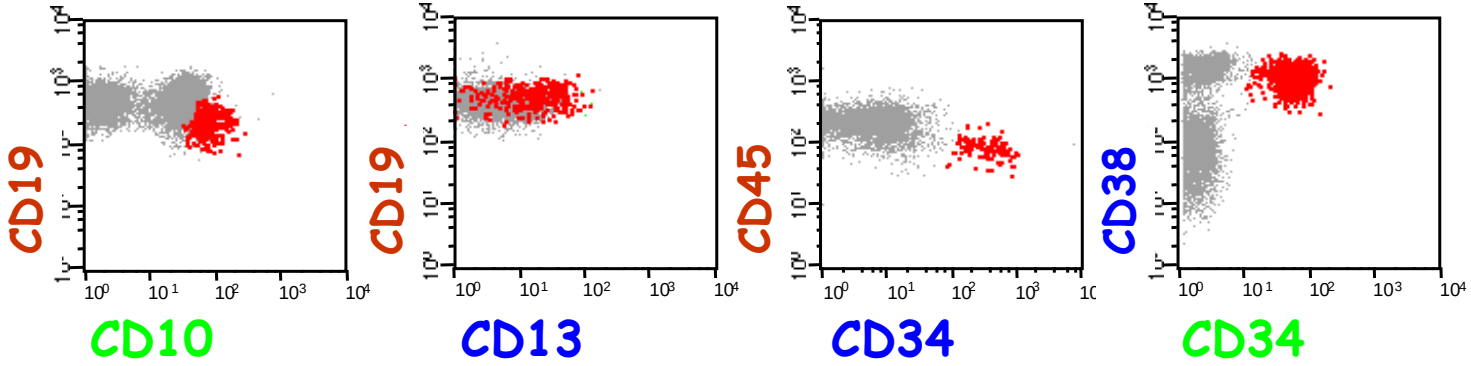


# PHENOTYPE OF B-ALL: Bcr/Abl+ vs Bcr/Abl- CASES

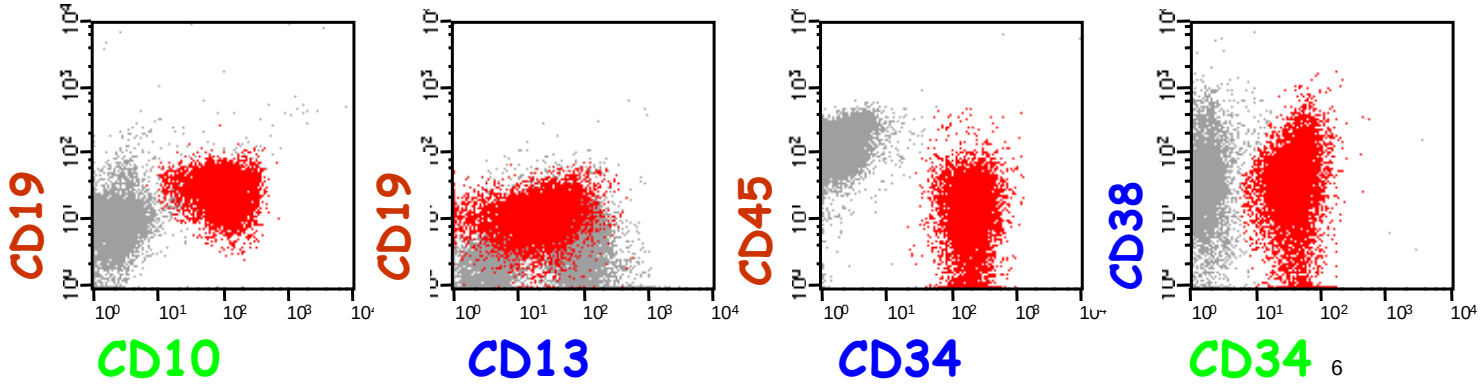
| Phenotypic marker | Bcr/Abl+ n=12 | Bcr/Abl- n=70 | p-value |
|---|---|---|---|
| CD34 PE ++hm (MFI > 100 & CV <85%) | 100% | 29% | < 0.0001 |
| CD38 PE -/lo int (MFI <450 & CV>60% & <170%) | 100% | 33% | < 0.0001 |
| CD10 FITC + hm (MFI > 11 % CV < 100%) | 100% | 56% | = 0.003 |
| CD13 PE lo int (MFI > 3 & CV > 50% & < 300%) | 100% | 52% | = 0.015 |

# ADULT PRECURSOR B-ALL: IMMUNOPHENOTYPE OF BCR/ABL+ CASES

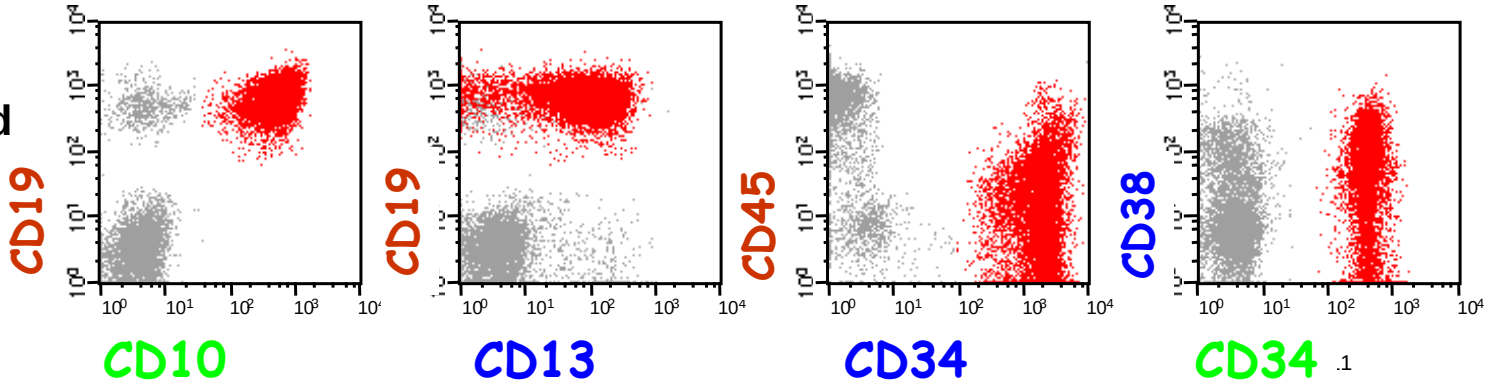
**Normal BM CD34+ B-cells**




**DNA diploid Bcr/abl+ ALL**




**DNA aneuploid Bcr/abl+ ALL**



# PHENOTYPIC SCORE OF B-ALL: Bcr/Abl+ vs Bcr/Abl- CASES

| Phenotypic score | Bcr/Abl+ n=12 | Bcr/Abl- n=70 |
|---|---|---|
| 0 | 0% | 8% |
| 1 | 0% | 33% |
| 2 | 0% | 33% |
| 3 | 0% | 20% |
| 4 | 100% | 6% |

# B-ALL: UTILITY OF PHENOTYPE TO SCREEN FOR Bcr/Abl+ CASES

**$CD34^{++hm}$ / $CD38^{-/lo\ int}$ / $CD10^{+hm}$ / $CD13^{lo\ int}$**

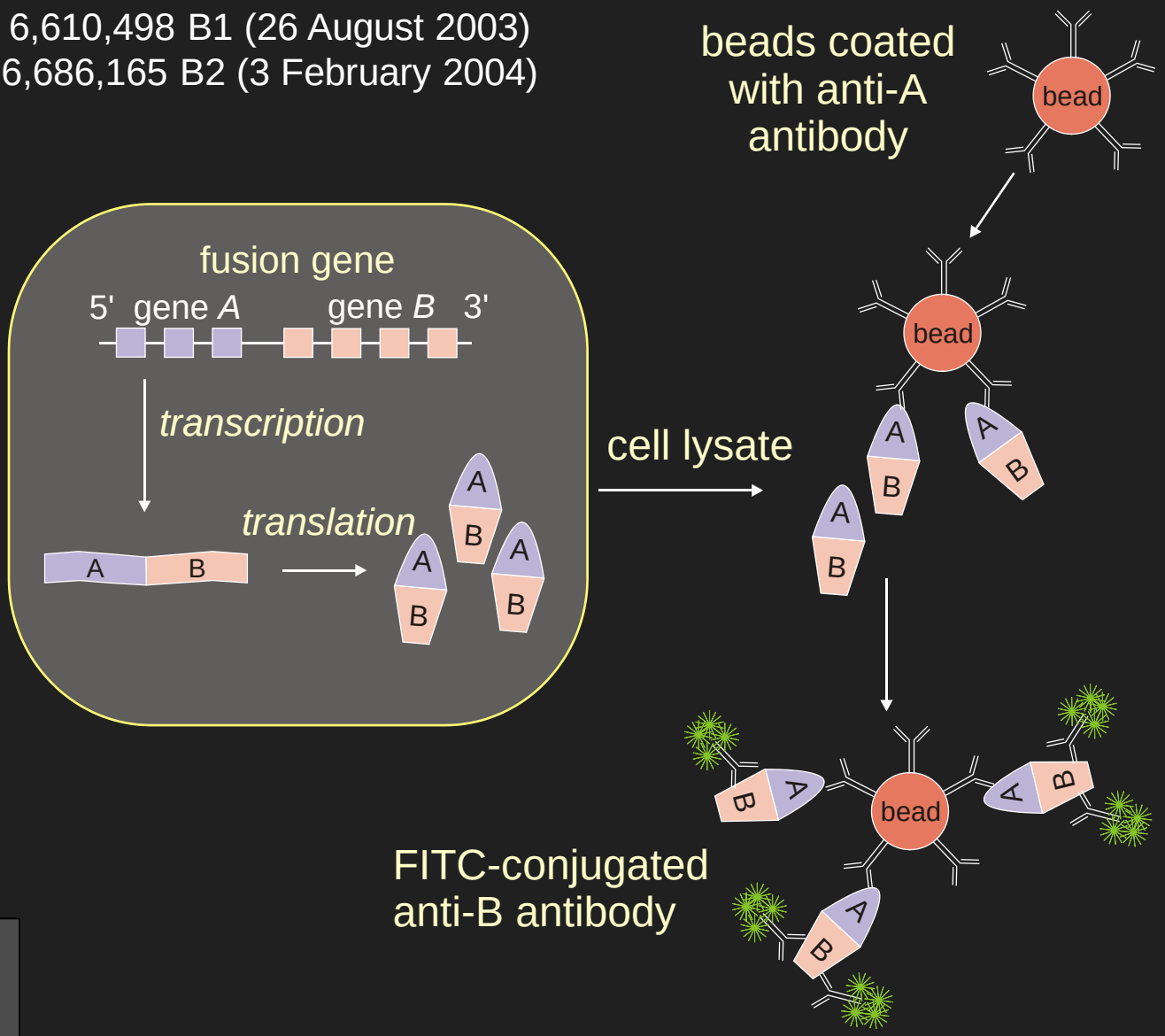
| | | |
|---|---|---|
| False + | 3/82 | (4%) |
| False - | 0/82 | (0%) |
| Sensitivity | 100% | |
| Specificity | 94% | |
| Efficacy | 95% | |

# AL: GENOTYPIC-PHENOTYPIC ASSOCIATIONS

| Diagnosis | Genetic lesion | Aberrant immunophenotype |
|---|---|---|
| BCP-ALL | t(9;22)* | $CD34^{hi}$, CD10+, $CD38^{lo}$, $CD13^{lo}$ |
| | t(12;21) | $CD34^{het}$, CD10+, $CD20^{-}$, $CD13^{lo}$ |
| | 11q23 | $CD34^{+}$, $CD10^{-}$, $7.1^{+}$, $CD15^{+}$ |
| AML | t(15;17) | $CD34^{-/+}$, $CD15^{-/lo}$, $CD2^{-/lo}$, $CD13^{het}$ |
| | Inv(16) | $MPO^{hi}$, $CD2^{-/lo}$ |
| | t(8;21) | $CD19^{+}$, $CD56^{+}$ |
| | 11q23 | $CD56^{+}$, $7.1^{-/+}$, $CD19^{-/lo}$, $CD2^{-/+}$ |

# Bead-based flowcytometric assay for detection of fusion proteins

*Patents:* US 6,610,498 B1 (26 August 2003)
US 6,686,165 B2 (3 February 2004)

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# BCR-ABL RUO KIT: CONCLUSIONS *(Leukemia, 2009)*

.- High sensitivity & specificity:

- Absolute concordance with PCR resuls:
  12/41 BCP-ALL (all adults)
  12/12 CML
  0/29 AML & T-ALL

.- Amount of bcr/abl protein:

- Heterogeneous pattern with two groups of positive cases:
  - High expression: IMF ≥ 2.000
  - Lower expression: IMF ≥ 500 y < 2.000
- MFI of negative samples: < 150

.- Different pattern of expression in BCP-ALL vs CML:

BCP-ALL: 83% (10/12) displayed high expression
CML: 75% (9/12) showed dim/intermediate expression*

# PANEL DE MICROSPHERE TUBES FOR THE CLASSIFICATION OF ACUTE LEUKEMIAS

| Precursor-B-ALL | AML | 'MLL' | T-ALL |
|---|---|---|---|
| BCR-ABL | PML-RARA | MLL-AF4 | CALM-AF10 |
| TEL-AML1 | AML1-ETO | MLL-AF9 | LMO2 |
| E2A-PBX1 | CBFB-MYH11 | MLL-AF10 | HOX11L2 |
| ( MLL-AF4) | | MLL-ENL | TAL1 |
| | | MLL-AF6 | |

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# CLINICAL APPLICATIONS OF FLOW CYTOMETRY

**Microscopy** — **Flow cytometry**

*70s-90s*

Hybridoma technology
Monoclonal antibodies
Fluorochrome-conjugates

From research laboratories to clinical diagnostics

*XXI century*

Digital instruments
>4 color flow cytometers
Higher analytical speed

Exponentially growing amount of complex information/data

# 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

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# Identification of leukemia-associated immunophenotypes

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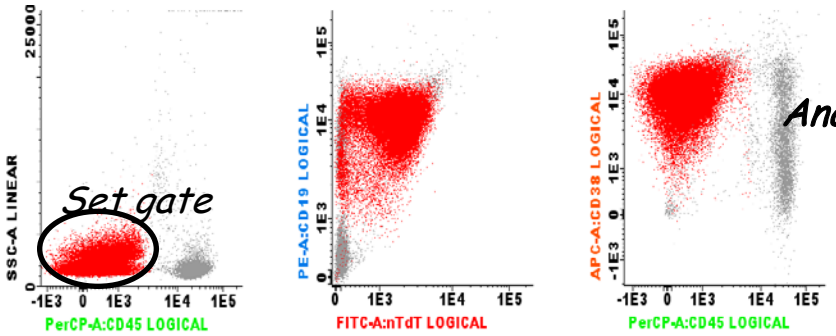
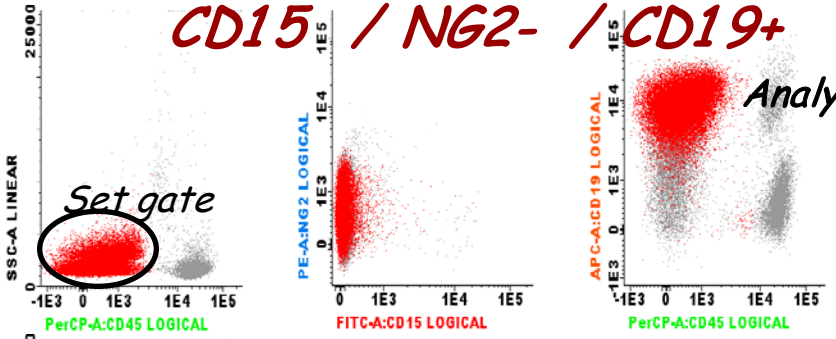
## TdT+ / CD19+ / CD38+

Tube



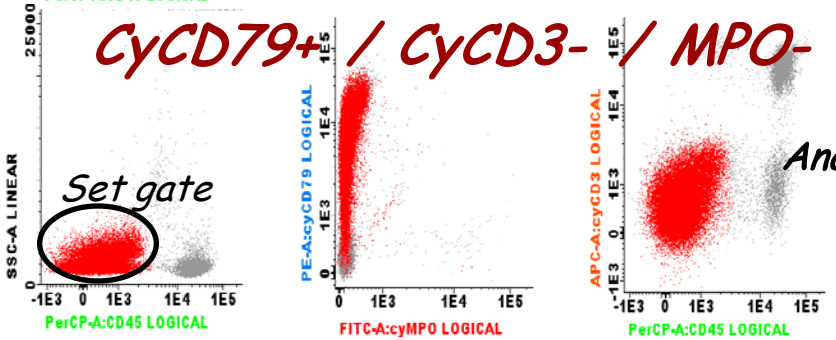

## CD15 / NG2- / CD19+

Tube

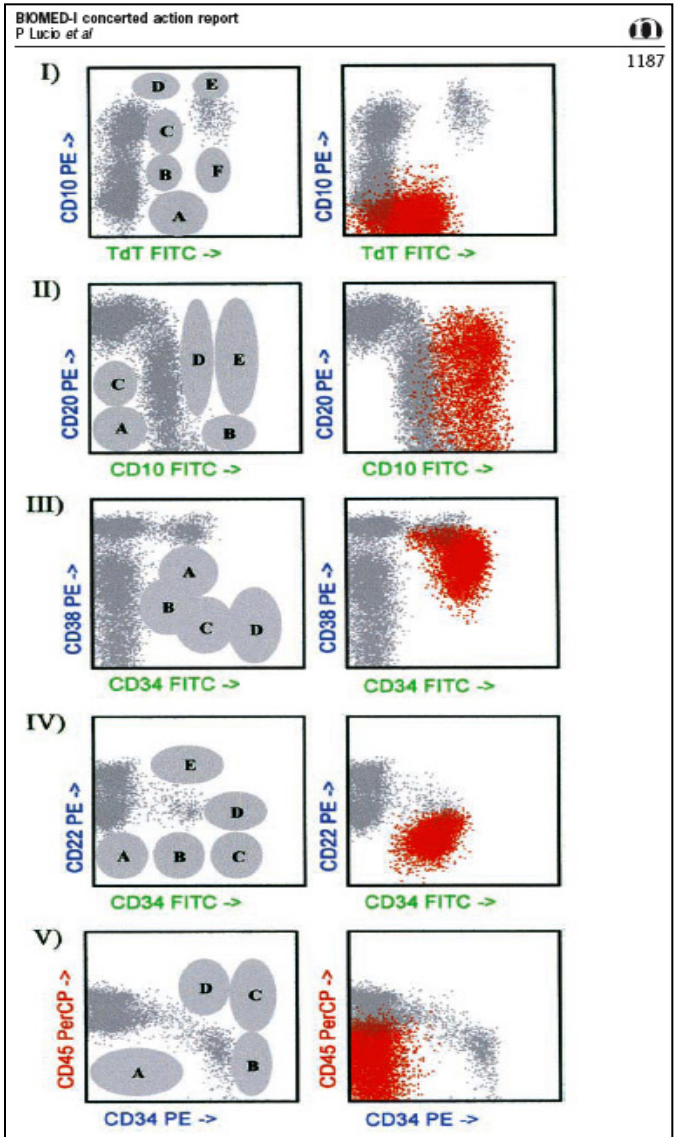



## CyCD79+ / CyCD3- / MPO-

Tube




JJM van Dongen Department of Immunology, Erasmus MC




Lucio et al, Leukemia, 1999

# Identification of leukemia-associated immunophenotypes

EuroFlow

TdT+ / CD19+ / CD38+

Precursor - B- ALL

LAIP: CD45- Tdt^lo

Tube

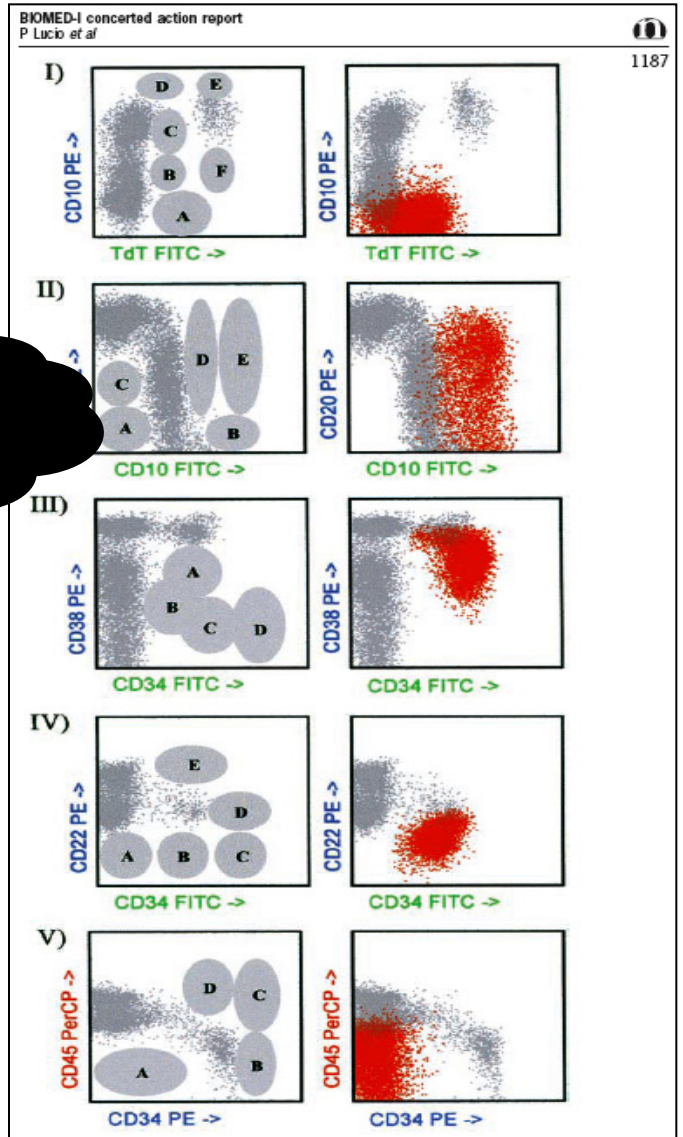
Tube

Tube

Analyse (2D)

Analyse (2D)


JJM van Dongen Department of Immunology, Erasmus MC




Lucio et al, Leukemia, 1999

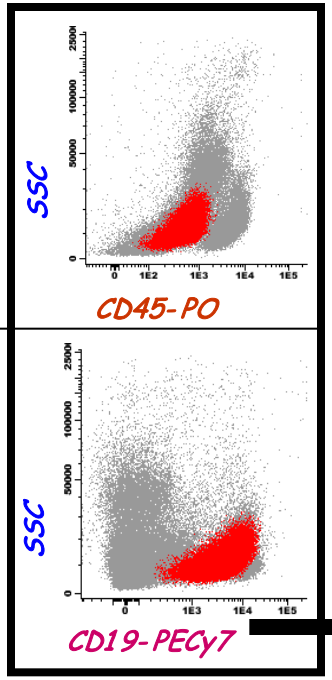
# Identification of leukemia-associated immunophenotypes

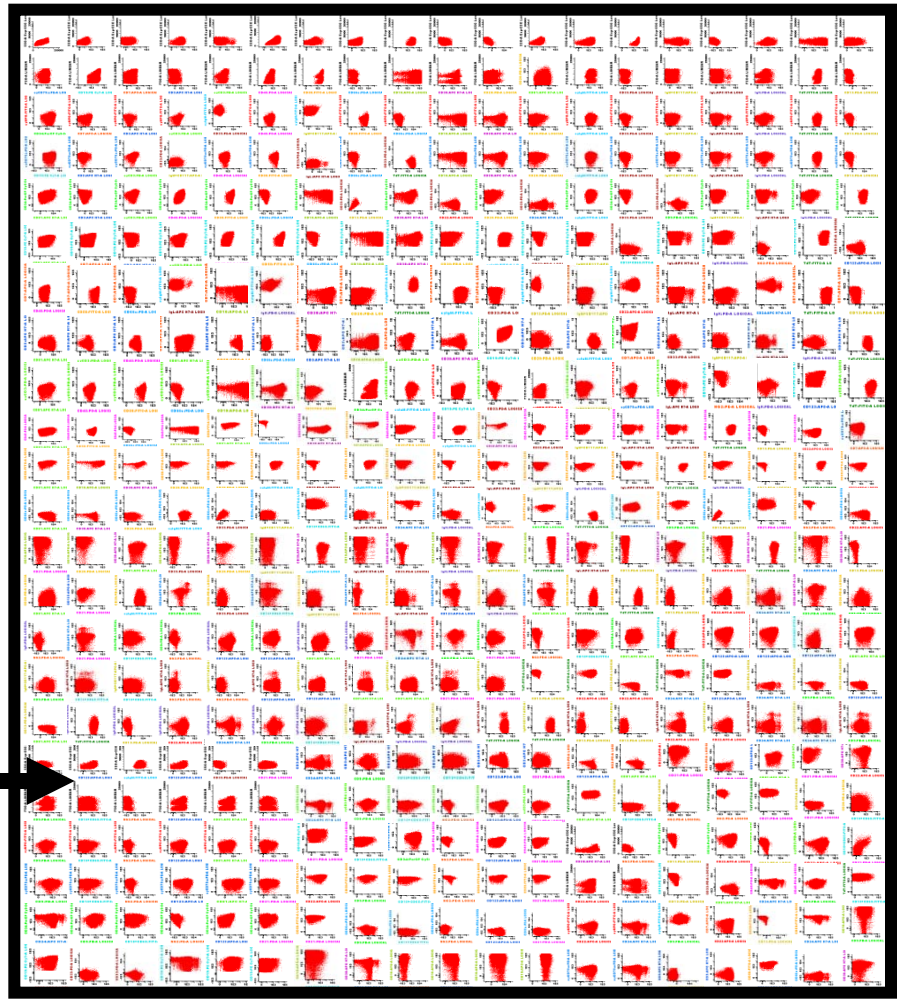
EuroFlow

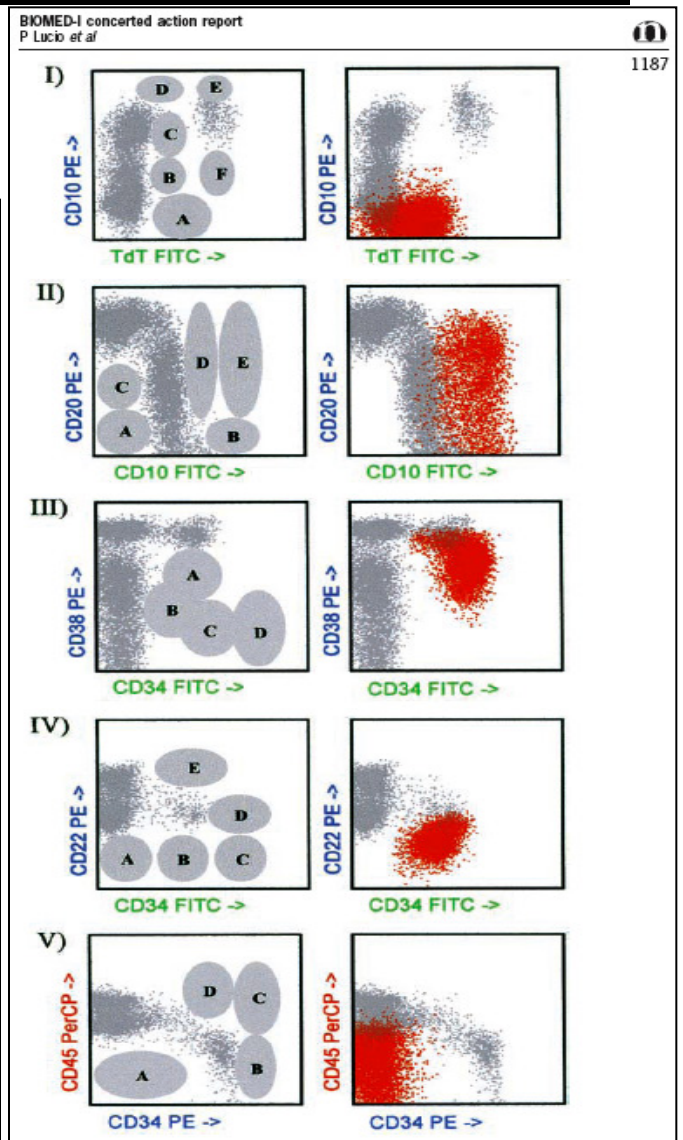
CD19+ B-CELLS

8- COLOR flow cytometry: Bcp-ALL

EuroFlow panel (450 bivariate plots)





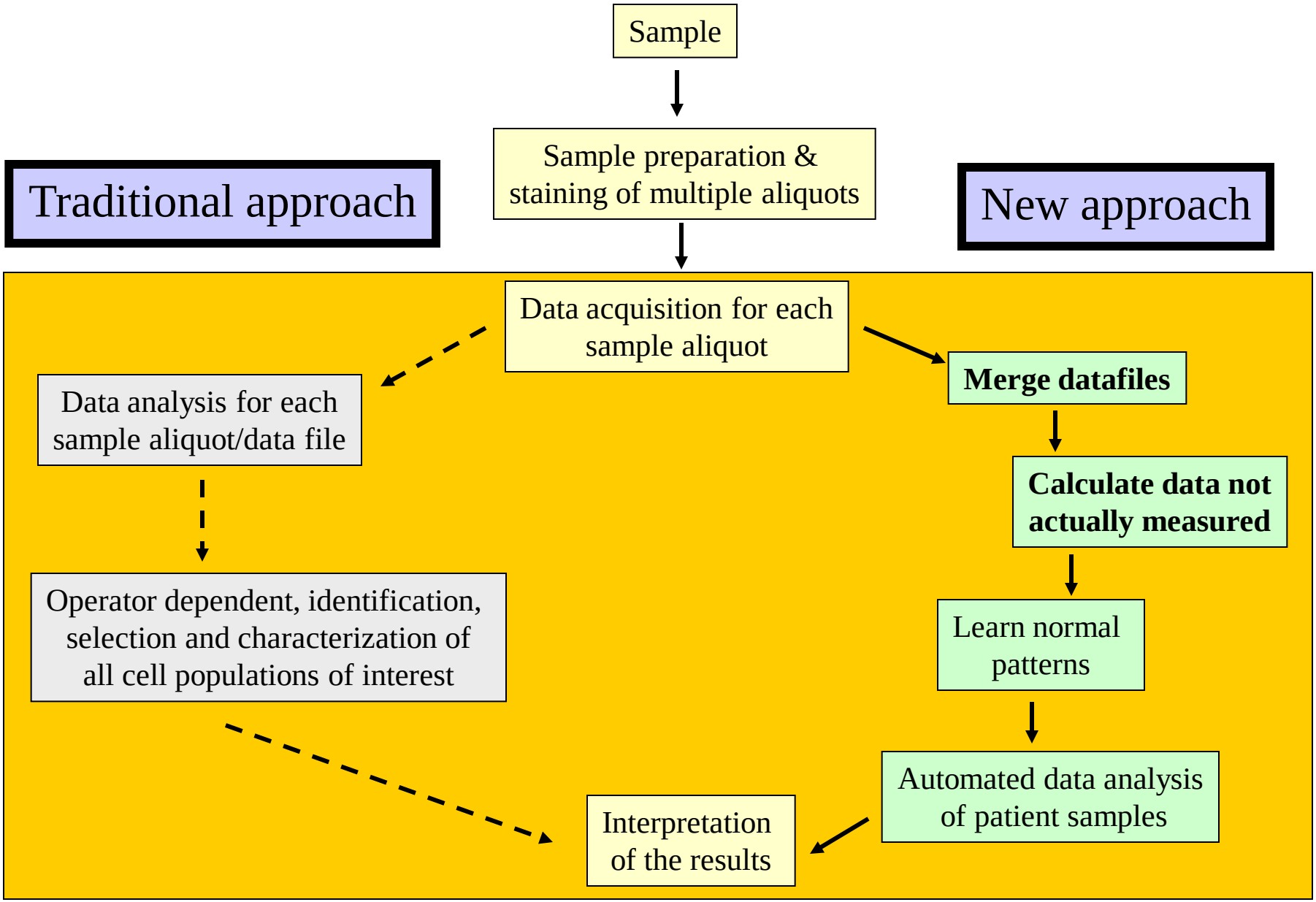
# EuroFlow


## EuroFlow consortium aims at innovation in flow cytometry

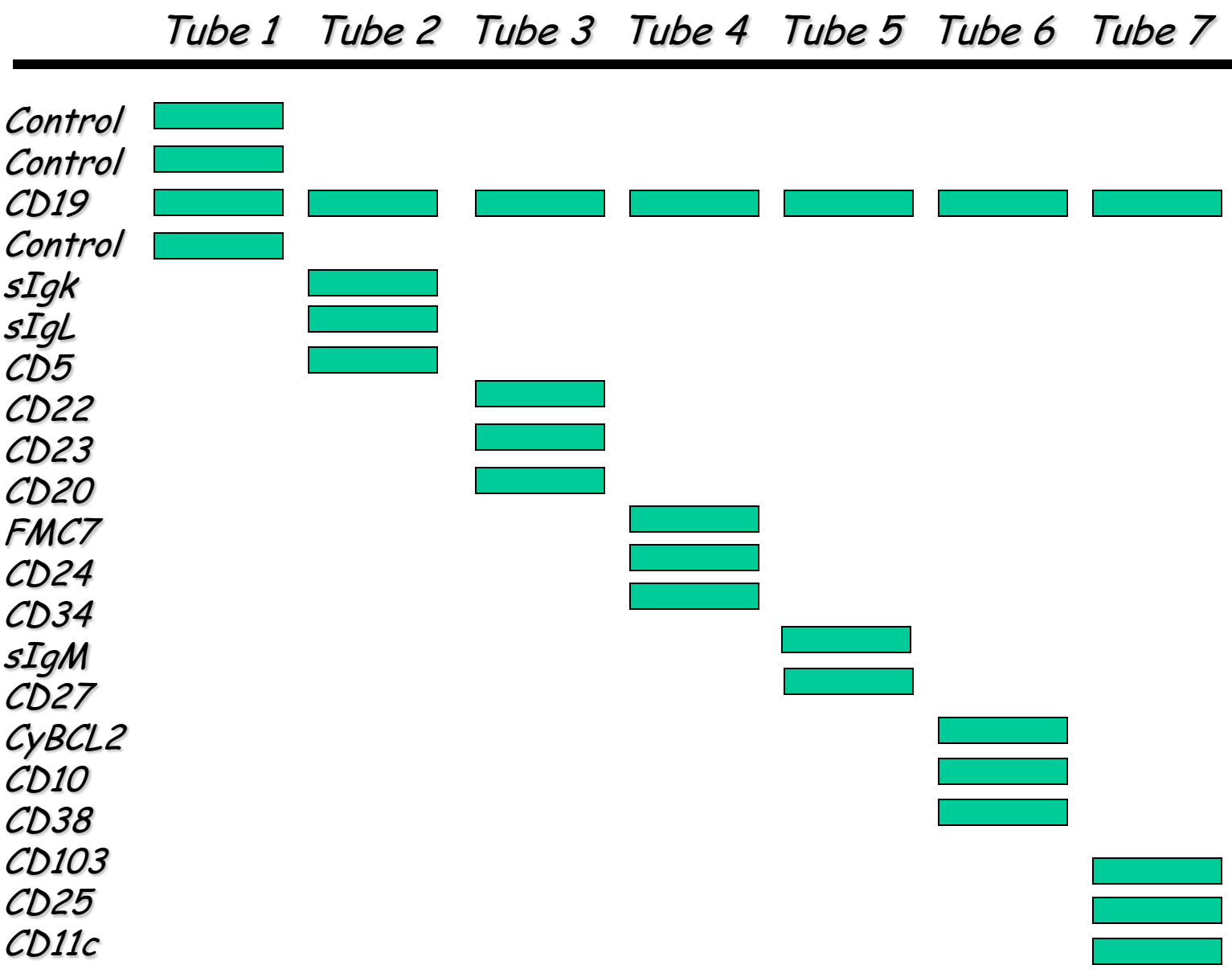
www.euroflow.org

*1st EuroFlow meeting, Salamanca (Spain), April 2006*

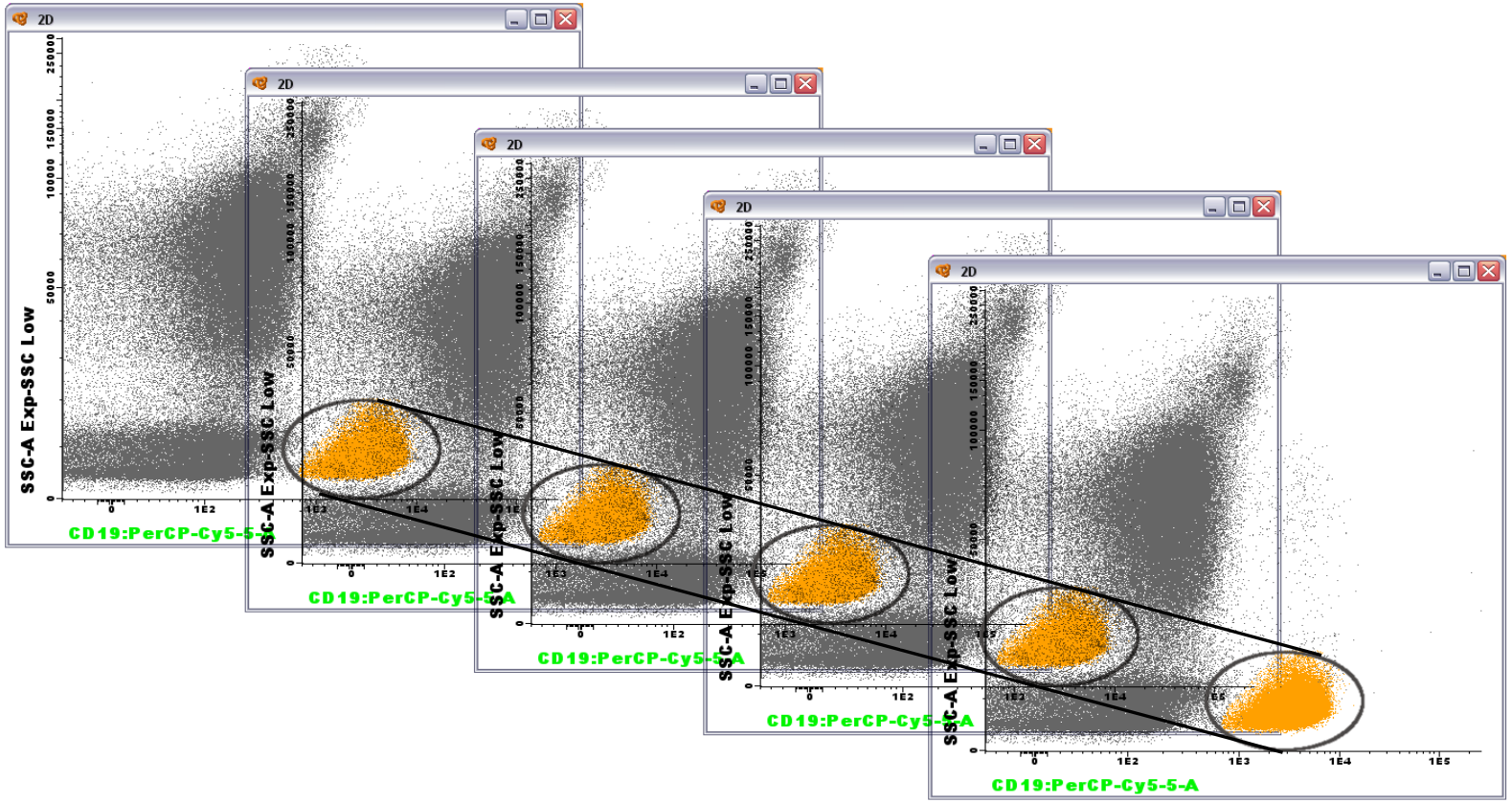
# FLOW CYTOMETRY IMMUNOPHENOTYPING



# B-CLPD: FCM PHENOTYPIC ARRAY



# MERGE OF DIFFERENT DATA FILES: Single gating step

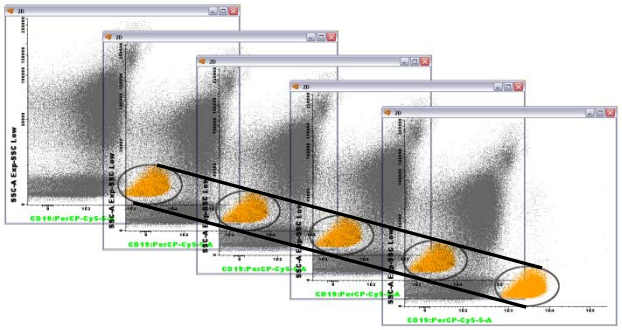


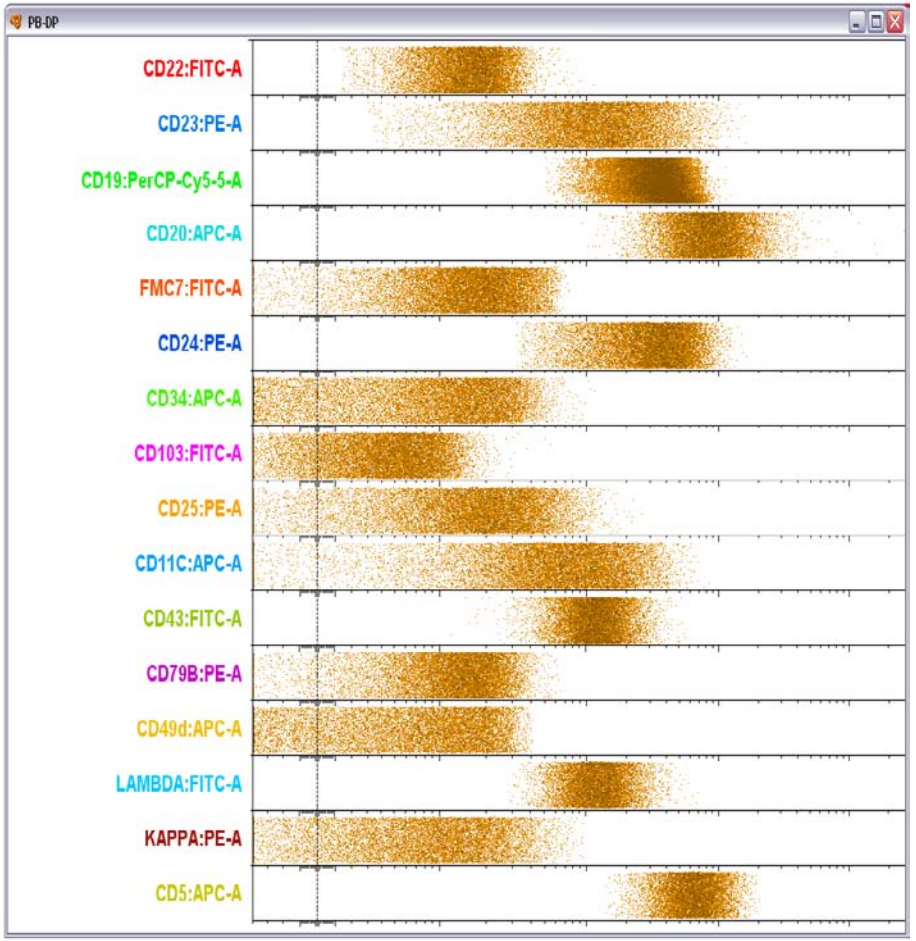

A single gating step for 5 different data files

(sample aliquots)

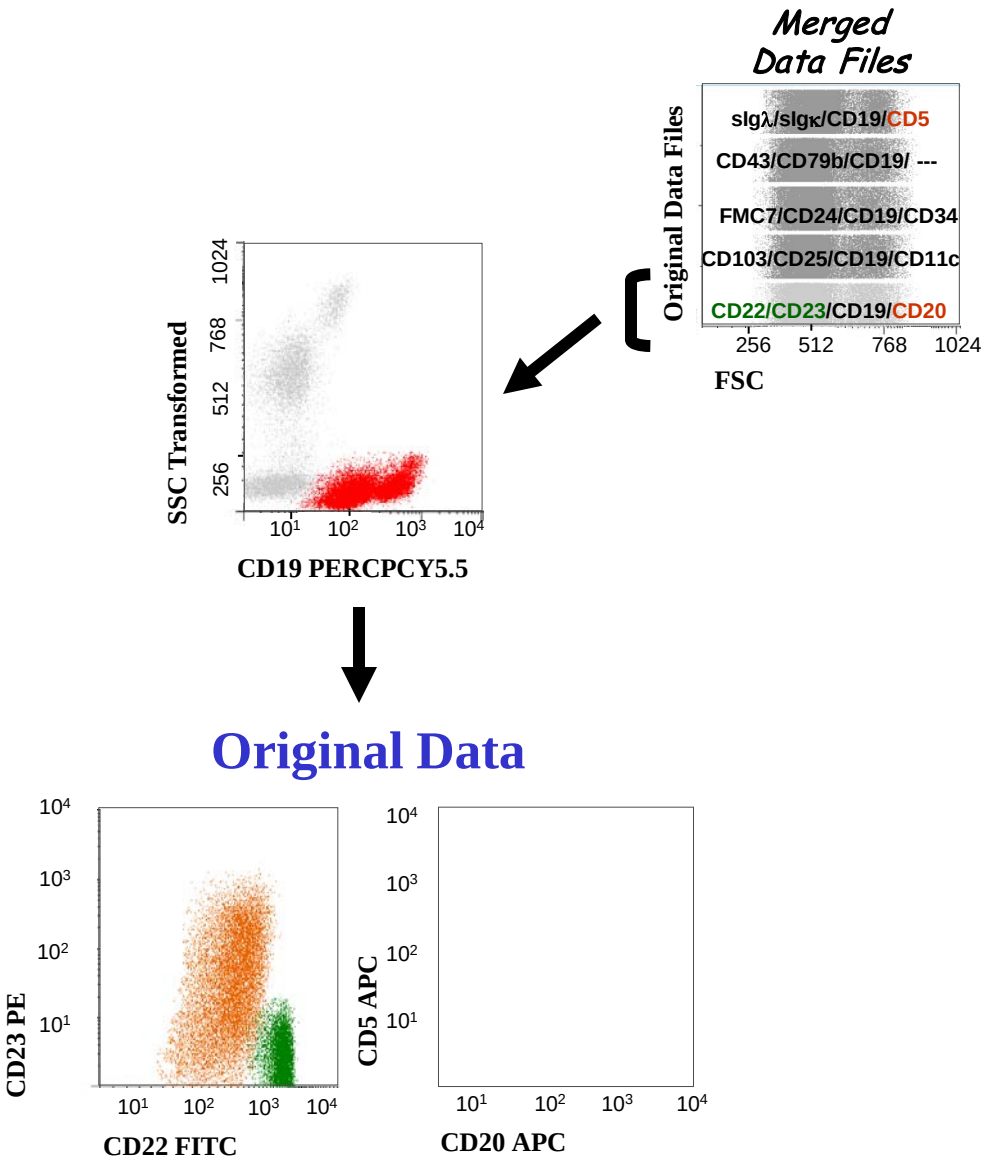
# MERGED DATA FILES:

## Simultaneous display of the immunophenotype of the gated cell population





# MULTIPARAMETER DISPLAY OF MERGED DATA FILES:

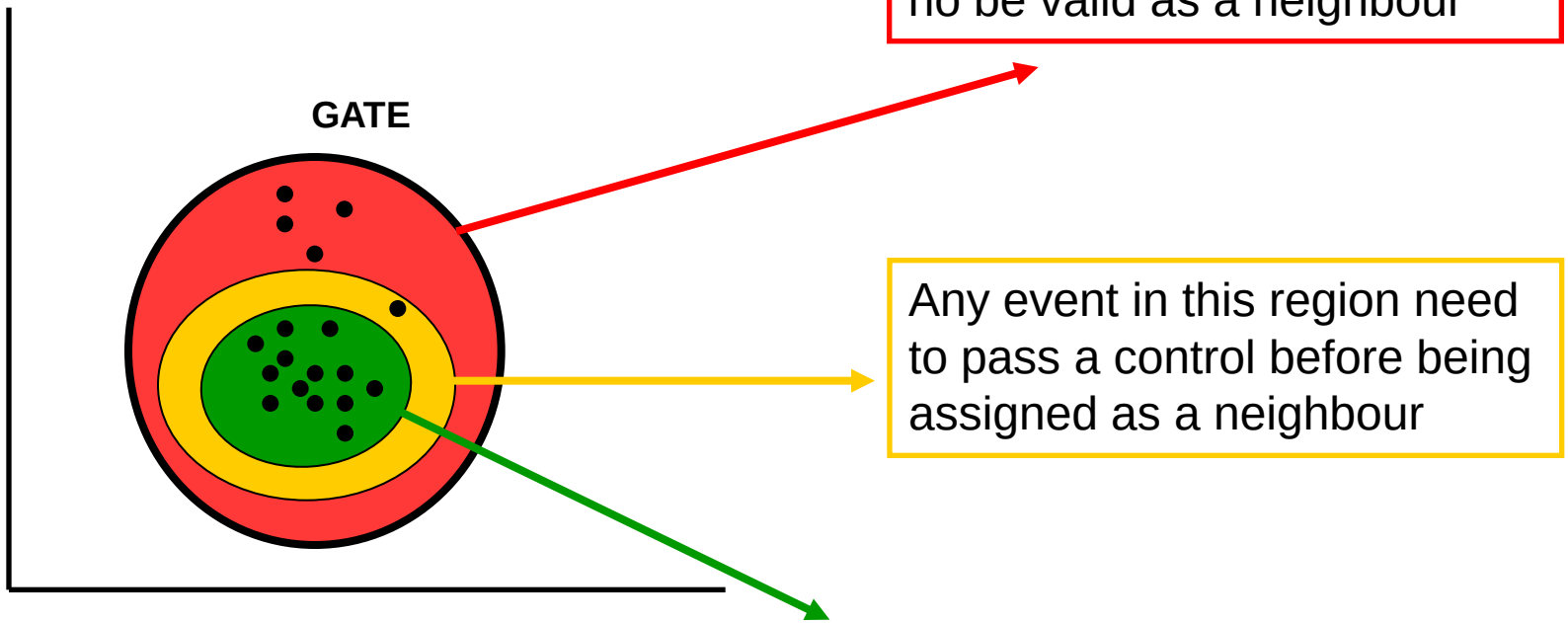


# CALCULATE DATA

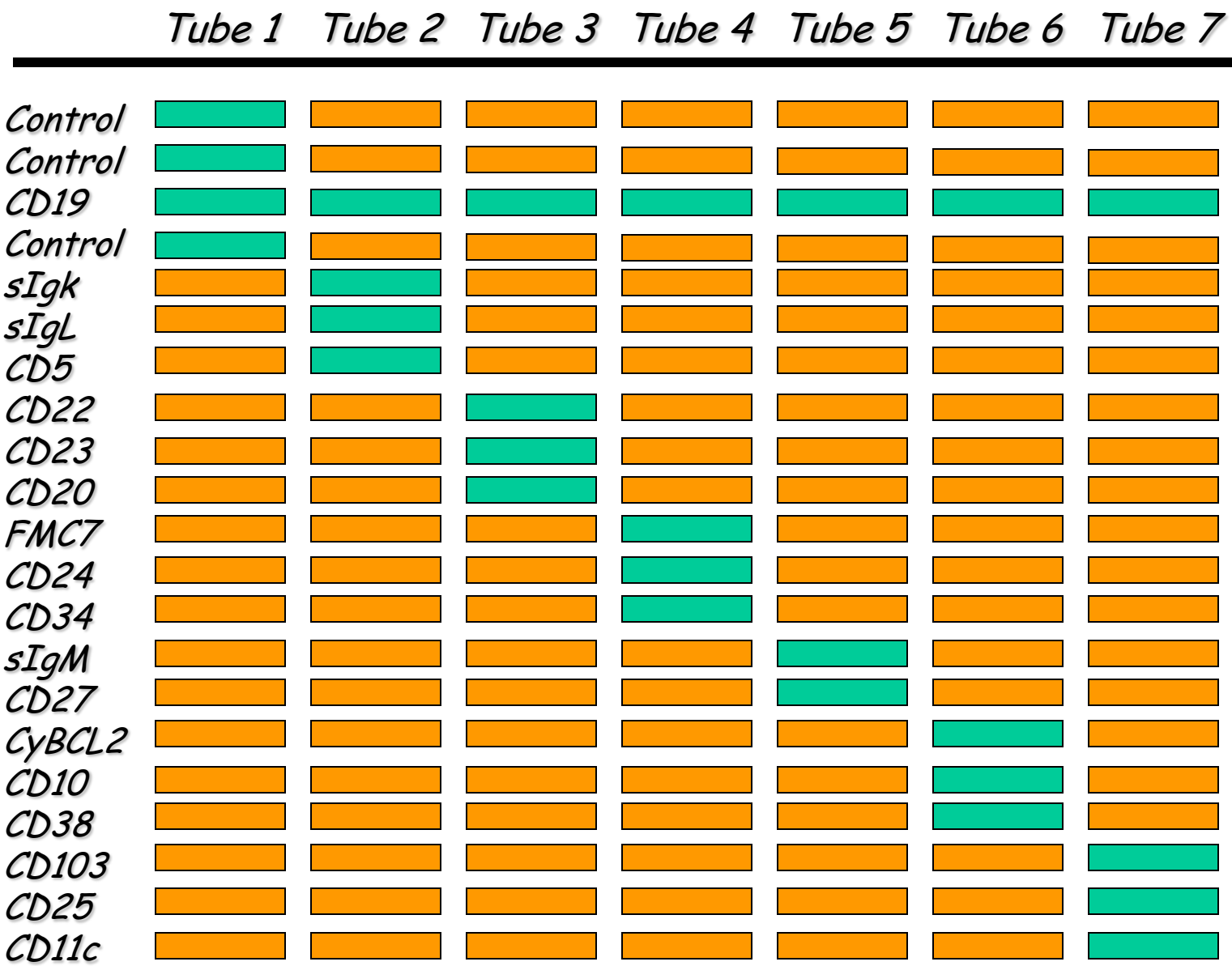
**For each event measured:** Calculation of the information about **each individual parameter** not actually measured in an individual event for the overall panel of markers analyzed

Based on nearest-neighbour statistical tools

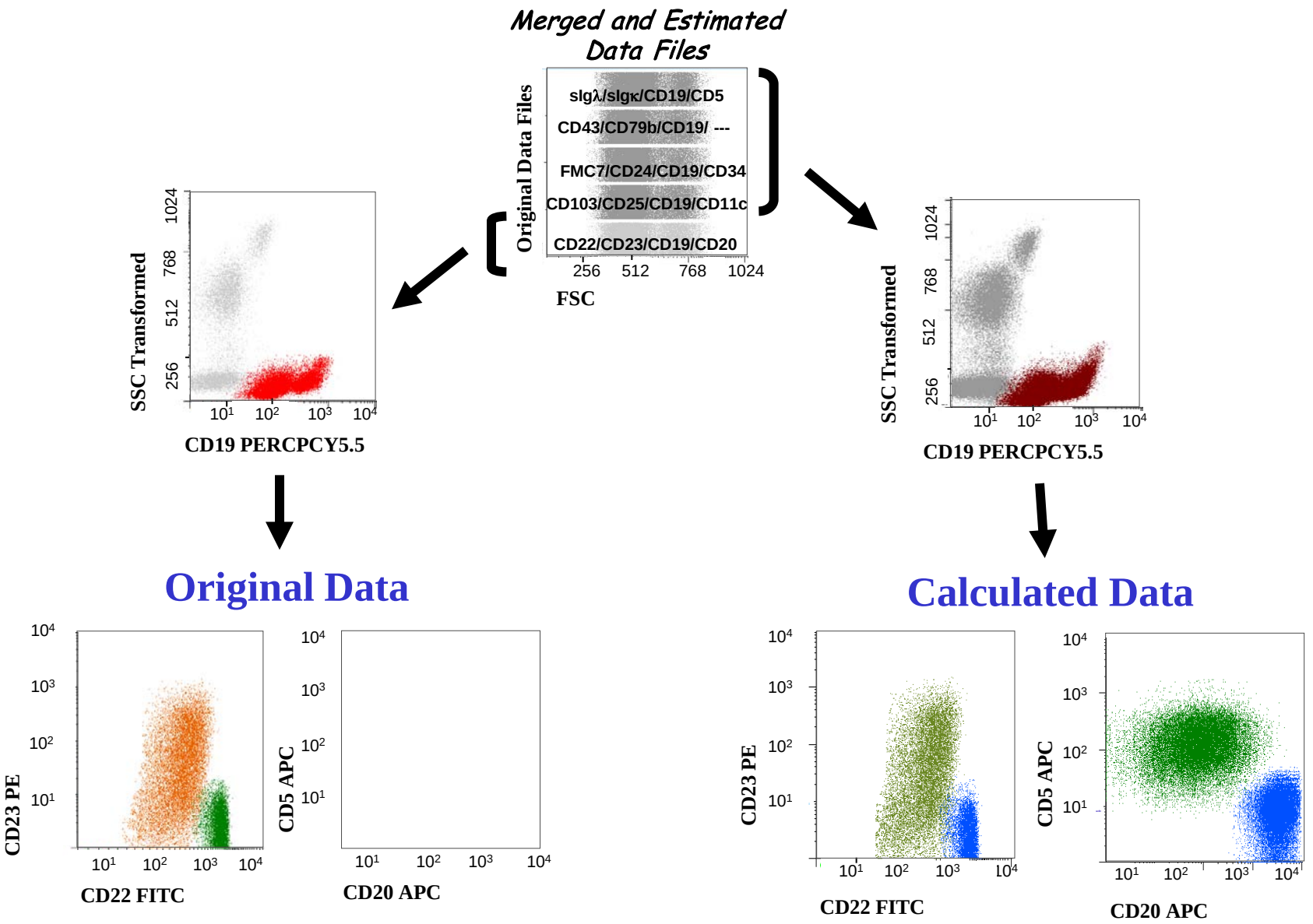
**NEIGHBOUR TUBE**



# B-CLPD: FCM PHENOTYPIC ARRAY



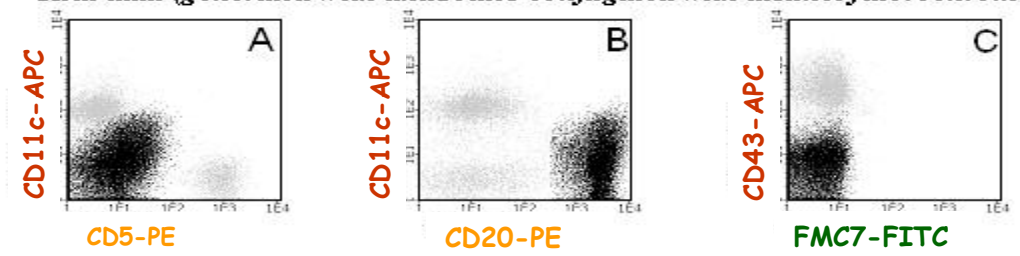
# INFINICYT: Calculation of merged data



# CALCULATED VS REAL DATA

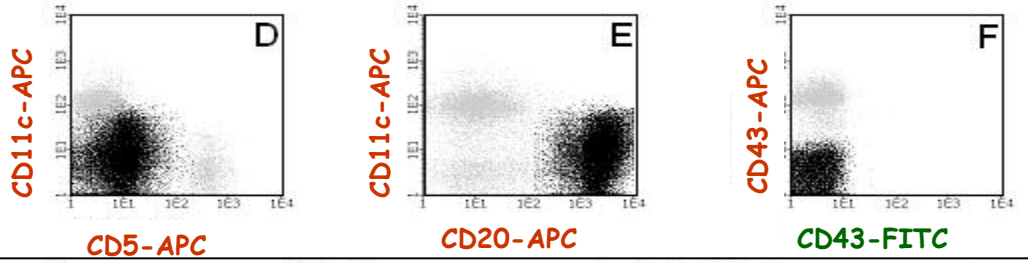
**Real**

*Real data (generated with antibodies conjugated with distinct fluorochrome)*



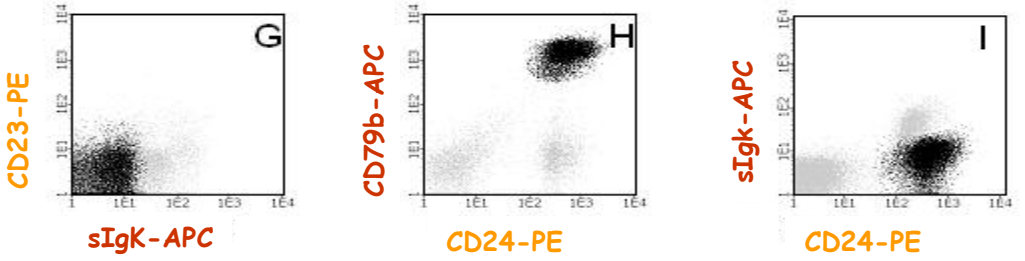

**Calculated**

*Calculated data (Originally impossible 2-dimensional dot-plot representations)*



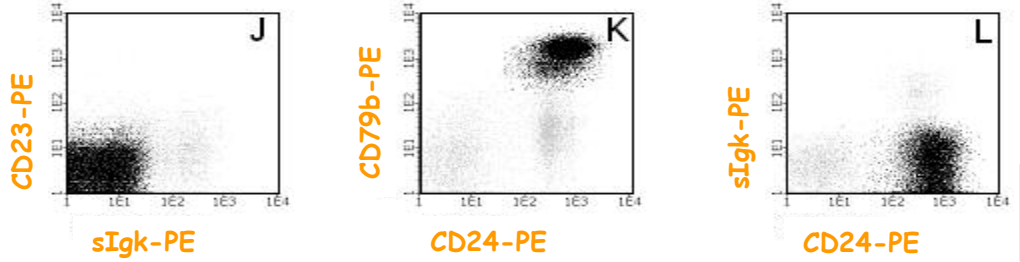

**Real**

*Real data (generated with antibodies conjugated with distinct fluorochrome)*




**Calculated**

*Calculated data (Originally impossible 2-dimensional dot-plot representations)*




Pedreira et al, Cytometry 2008

# MERGE AND CALCULATION OF LISTMODE DATA FROM DIFFERENT SAMPLE ALIQUOTS

Files

Parameters

| Parameters\File | 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 | R | E | E | E | E | E |
| LAM | R | E | E | E | E | E |
| CD5 | R | R | R | R | R | E |
| CD | C | C | C | C | C | C |
| IgM | R | E | E | E | E | E |
| CD | C | C | C | C | C | C |
| CD | C | C | C | C | C | C |
| CD45-PC7:AmCyan-H | C | C | C | C | C | C |
| CD103:FITC-A | E | R | E | E | E | E |
| CD10:PE-A | E | R | E | E | E | E |
| CD43:APC-A | E | R | E | E | E | E |
| CD81:FITC-A | E | E | R | E | E | E |
| CD79b:PE-A | E | E | R | E | E | E |
| CD2 | E | E | R | E | E | E |
| CD3 | E | E | E | R | E | E |
| CD6 | E | E | E | R | E | E |
| CXC | E | E | E | R | E | E |
| CD2 | E | E | E | E | R | E |
| LAIP | E | E | E | E | R | E |
| CD11a:APC-A | E | E | E | E | R | E |
| CD38:FITC-A | E | E | E | E | E | R |
| CD25:PE-A | E | E | E | E | E | R |
| CD138:PerCP-Cy5-5-A | E | E | E | E | E | R |

6 Files x

10 parameters x

100.000 events

1 File x

25 parameters x

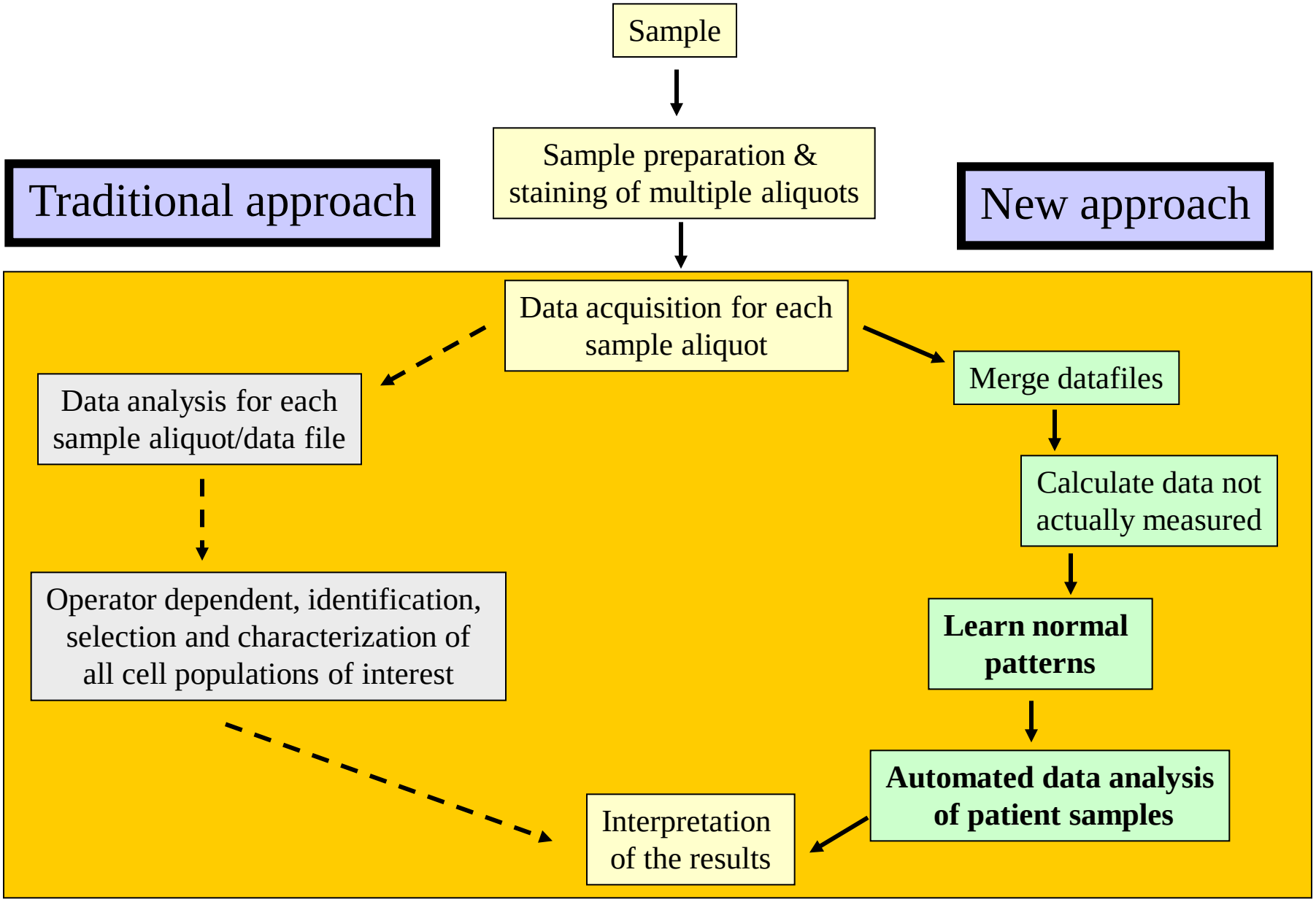
600.000 events

C = Common

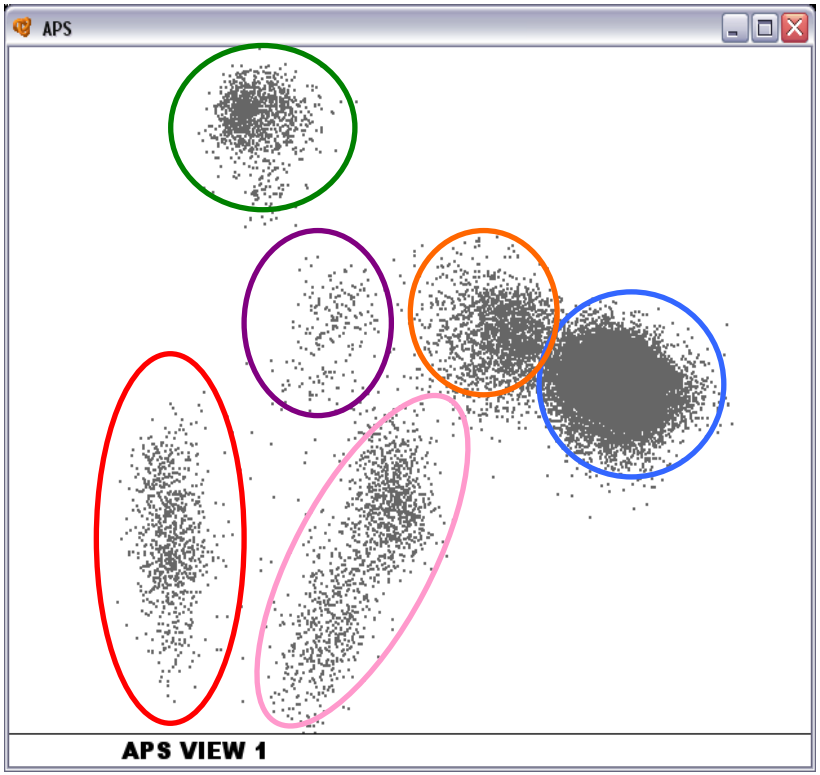
R = Real

E =Calculated

# FLOW CYTOMETRY IMMUNOPHENOTYPING

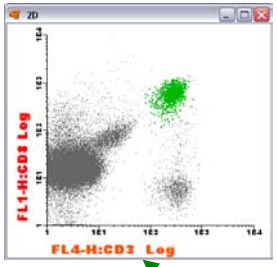


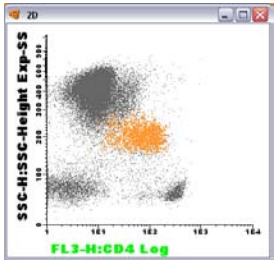
# AUTOMATED SEPARATION AMONG DIFFERENT CELL POPULATIONS (APS view)



# AUTOMATED SEPARATION AMONG DIFFERENT CELL POPULATIONS (APS view)

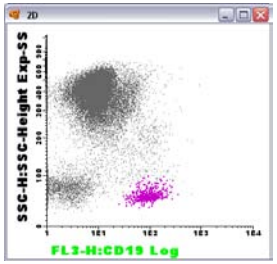
CD8+ T-LYMPHOCYTES

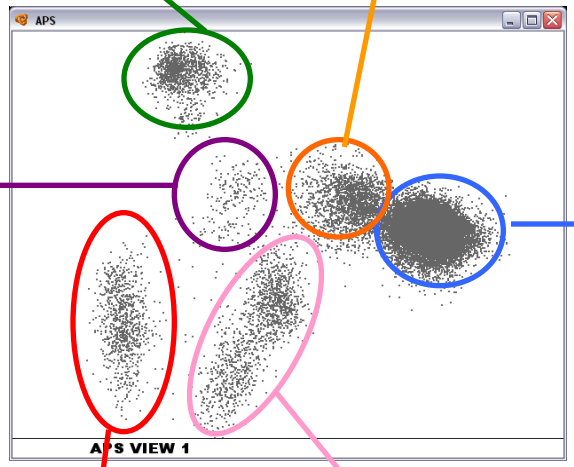


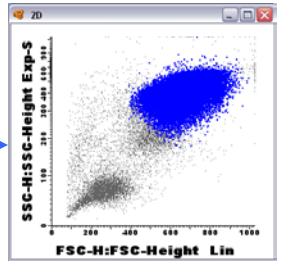




MONOCYTES

B-CELLS

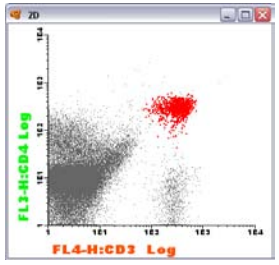


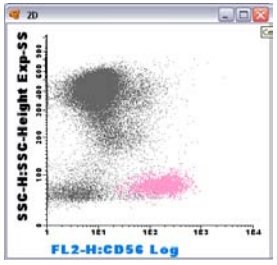




NEUTROPHILS

CD4+ T-LYMPHOCYTES

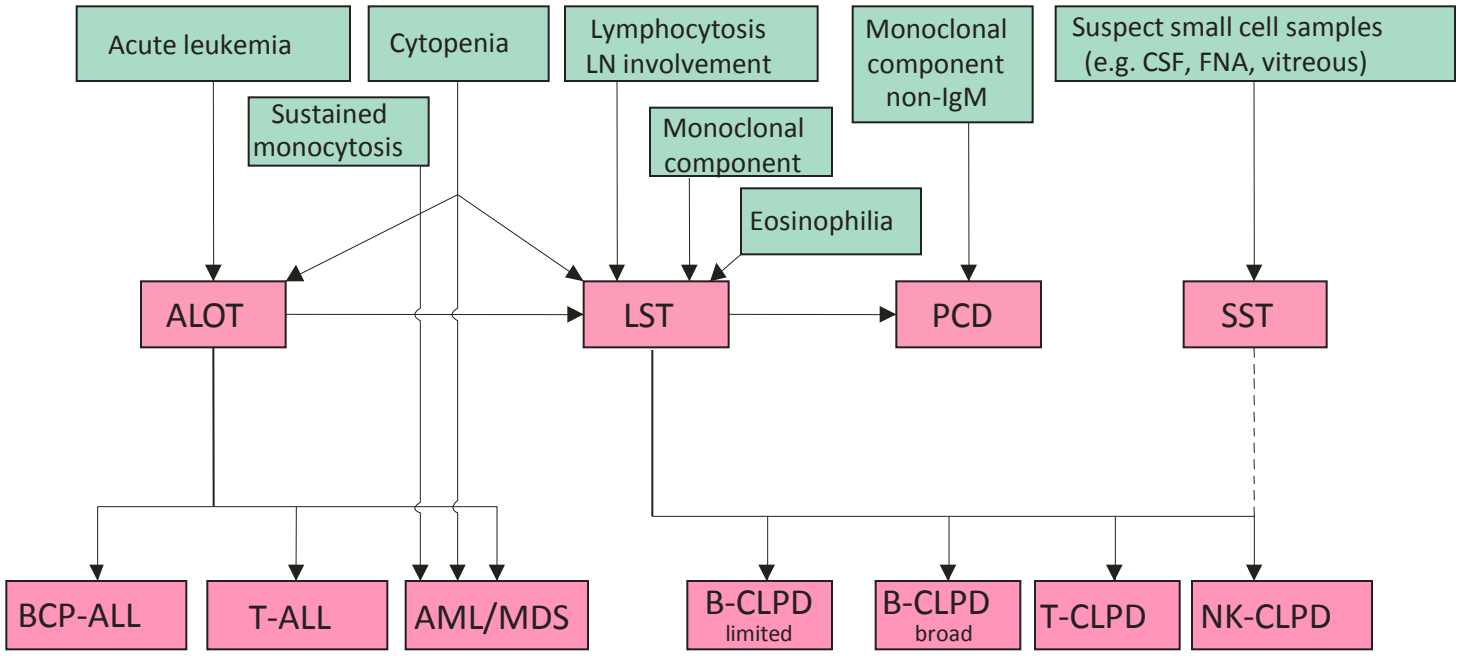



NK-CELLS

EuroFlow

# CONSTRUCTION OF EUROFLOW PANELS: MEDICAL INDICATION ENTRIES AND ORIENTATION/SCREENING PANELS

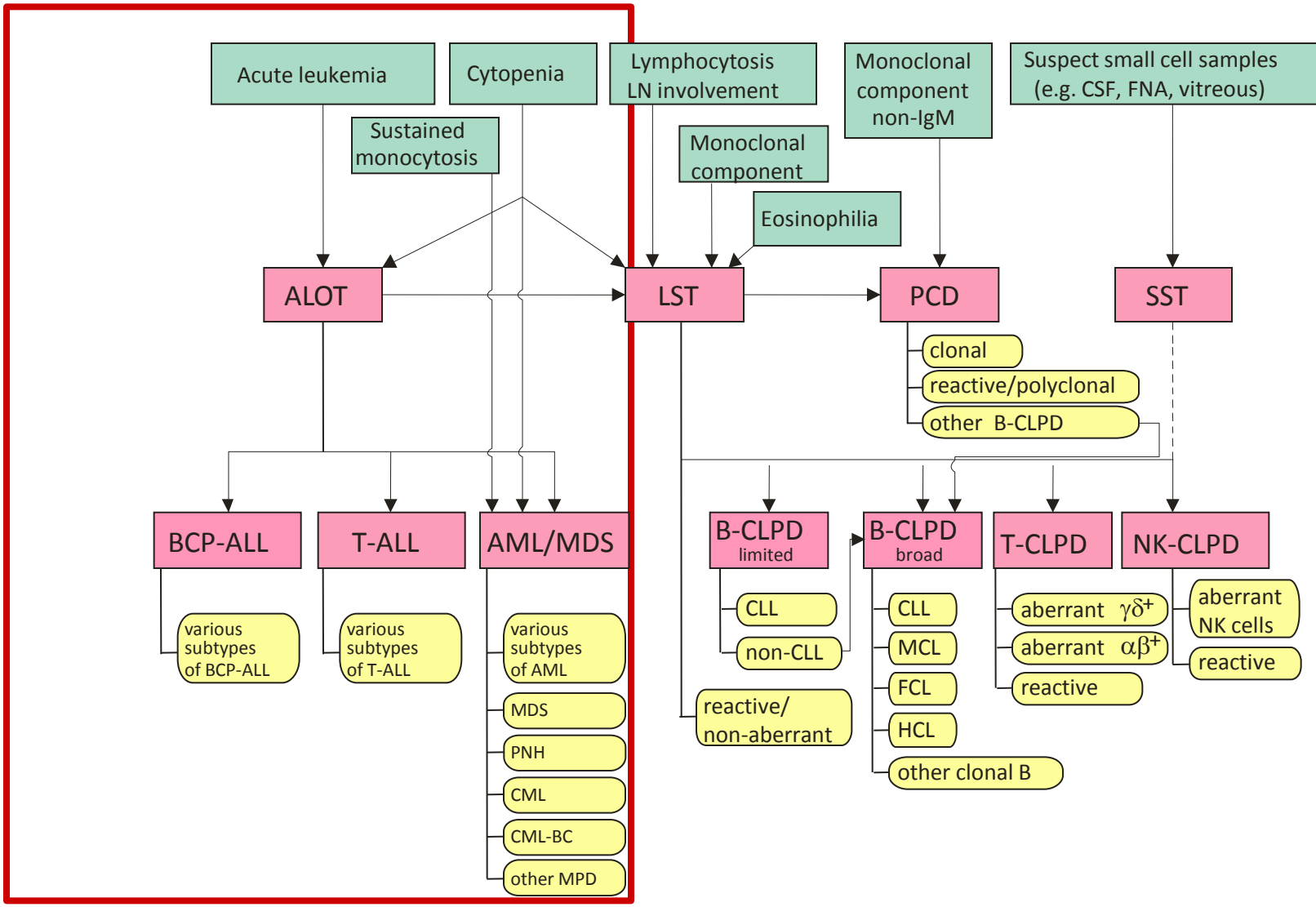



**Comprehensive network of panels aimed at the phenotypic diagnosis and characterization of the major WHO entities**

Van Dongen et al: EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. To be published in: Leukemia 2010;

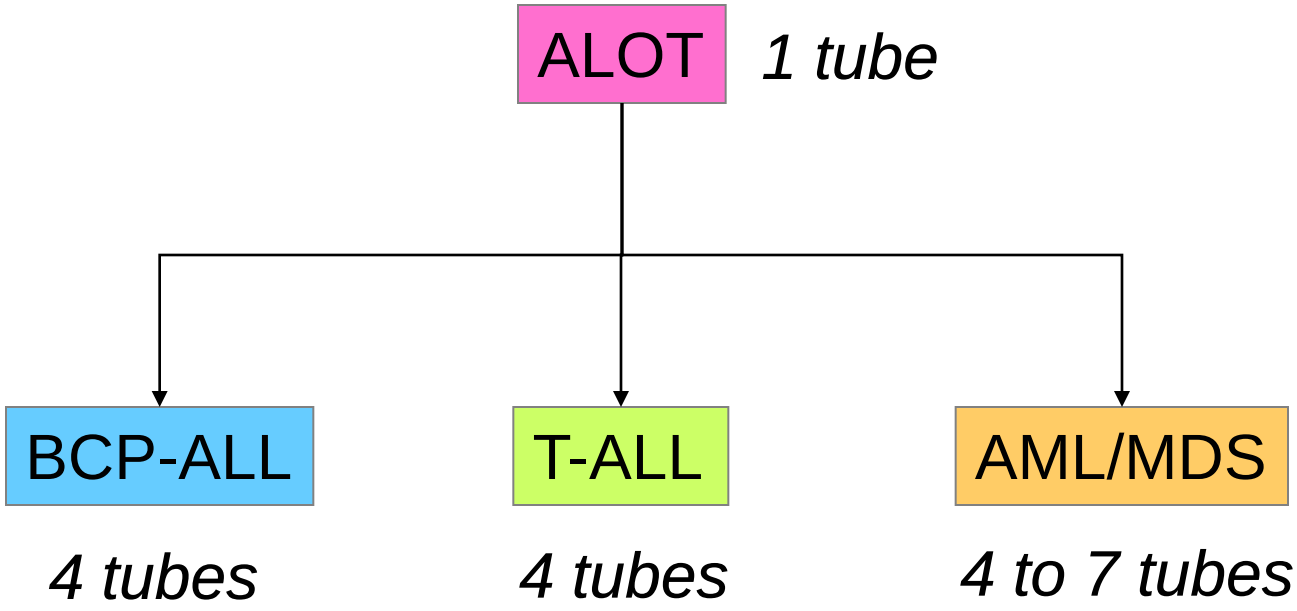
EuroFlow

# CONSTRUCTION OF EUROFLOW PANELS: MEDICAL INDICATION ORIENTATION/SCREENING & CLASSIFICATION PANELS




*Van Dongen et al: EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. To be published in: Leukemia 2010;*

EuroFlow




EuroFlow

# Single tube EuroFlow screening tube for acute leukemias

## Acute Leukemia Orientation Tube (ALOT)*

Responsible scientist: L Lhermitte

| Pacific Blue | Pacific Orange | FITC | PE | PerCP-Cy5.5 | PE-Cy7 | APC | APC-H7 |
|---|---|---|---|---|---|---|---|
| **cyCD3** | **CD45** | cyMPO | cyCD79a | **CD34** | **CD19** | CD7 | **smCD3** |

* Backbone markers are indicated in bold; cy= cytoplasmic; sm= surface membrane.

EuroFlow

# ALOT *(Acute Leukemia Orientation Tube)*

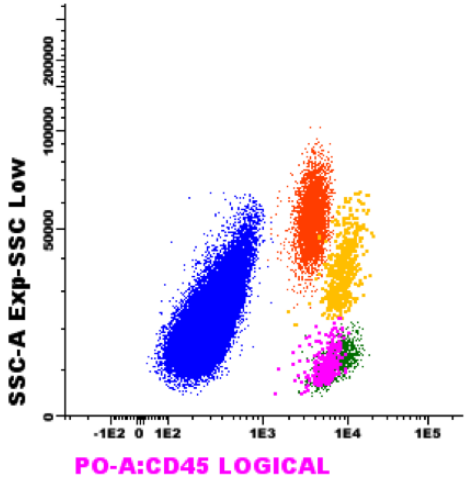
| Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|
| cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |

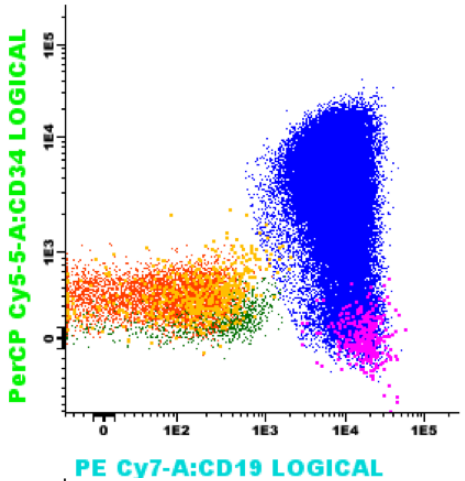
- **Designed for assessment of the nature of immature blast cell populations in acute leukemia samples**

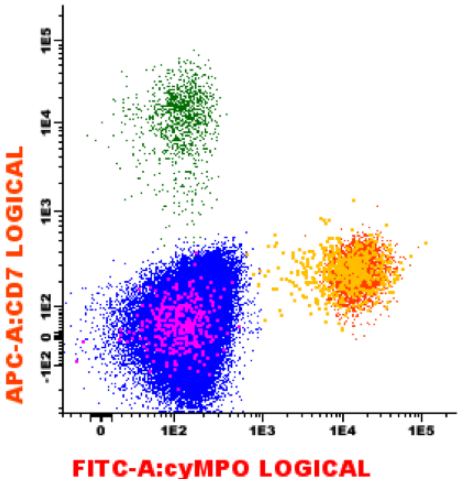
- **Designed to choose appropriate immunophenotypic panel(s)**

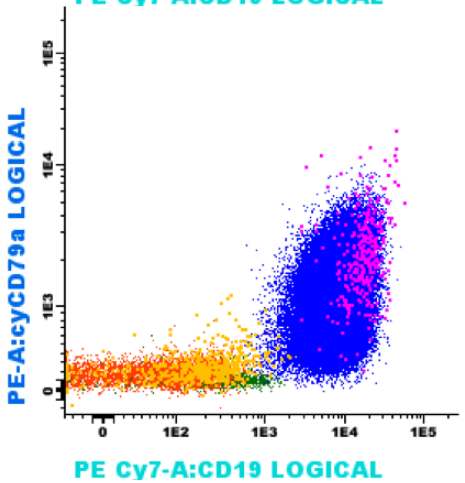
| *Acute Leukemia Orientation Tube (AL0T)* | | | | | |
|---|---|---|---|---|---|
| Target Antigen | Fluorochrome conjugate | Gating markers (first level) | Gating Markers (second level) | Immaturity markers | Lineage markers |
| cyMPO | FITC | | X | | My |
| cyCD79a | PE | | X | | B, T |
| CD34 | PerCP Cy5.5 | X | | X | - |
| CD19 | PE CY7 | | X | | B, My |
| CD7 | APC | | X | X | T, My |
| smCD3 | APC H7 | | X | | T |
| cyCD3 | Pacific Blue | | X | | T |
| CD45 | PO | X | | X | - |

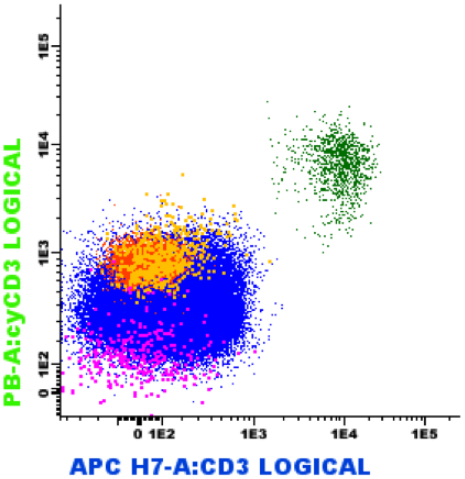
EuroFlow

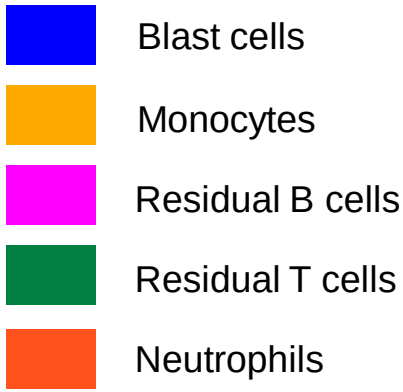

SSC-A Exp-SSC Low
PO-A:CD45 LOGICAL


PerCP Cy5-5-A:CD34 LOGICAL
PE Cy7-A:CD19 LOGICAL

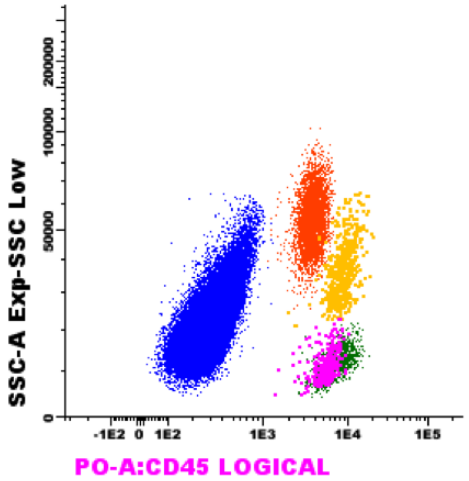

APC-A:CD7 LOGICAL
FITC-A:cyMPO LOGICAL

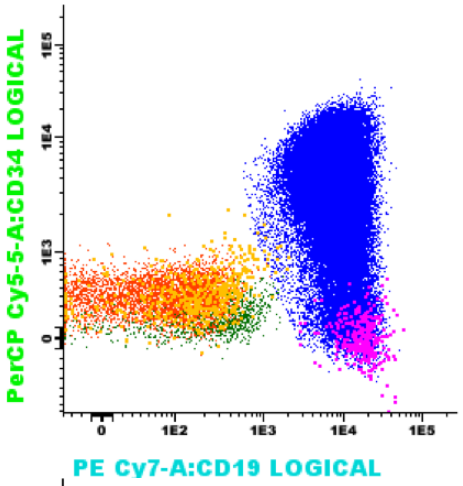

PE-A:cyCD79a LOGICAL
PE Cy7-A:CD19 LOGICAL

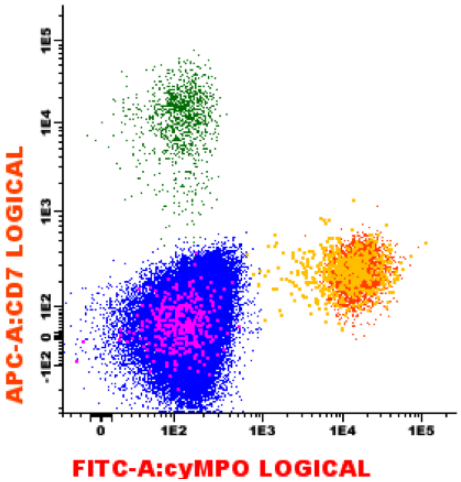

PB-A:cyCD3 LOGICAL
APC H7-A:CD3 LOGICAL

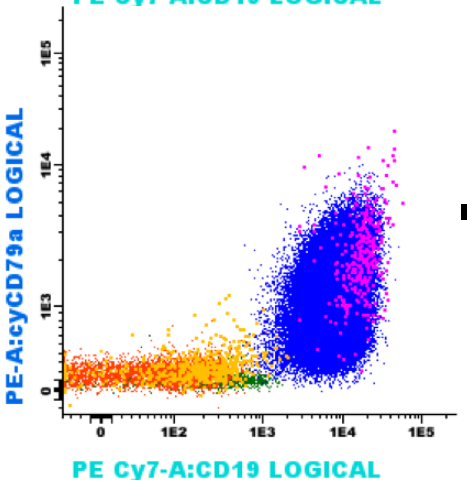

Blast cells
Monocytes
Residual B cells
Residual T cells
Neutrophils

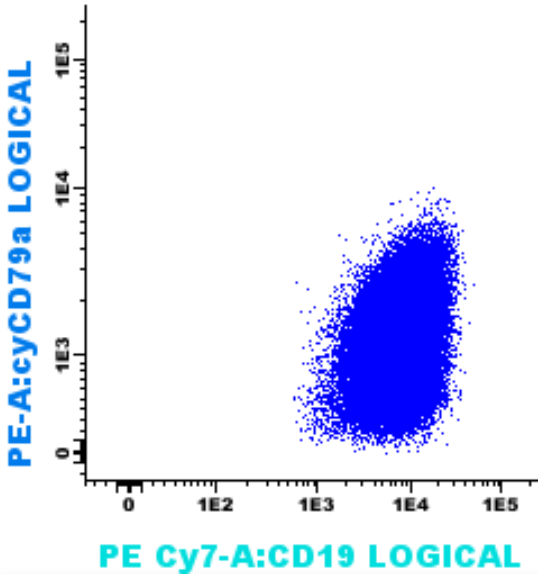
EuroFlow

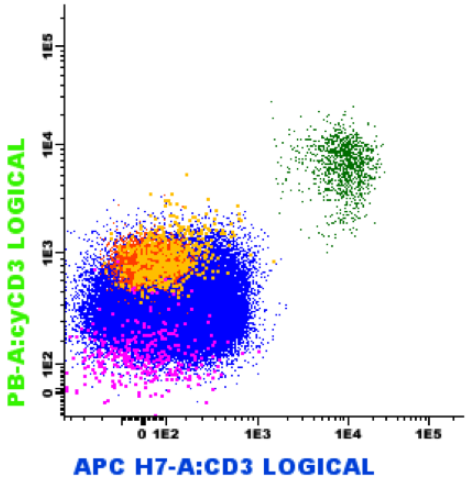



Blast cells

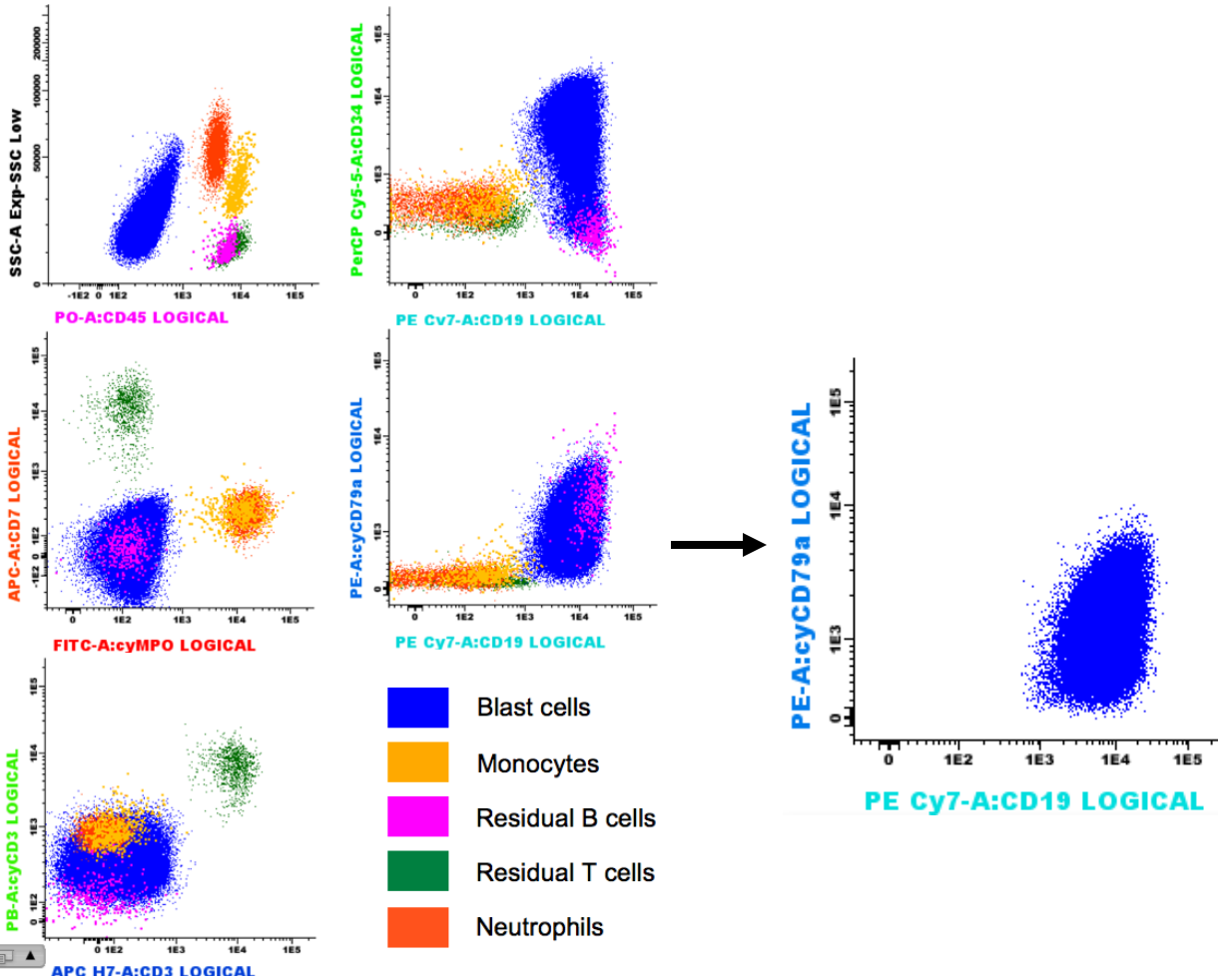
Monocytes

Residual B cells

Residual T cells

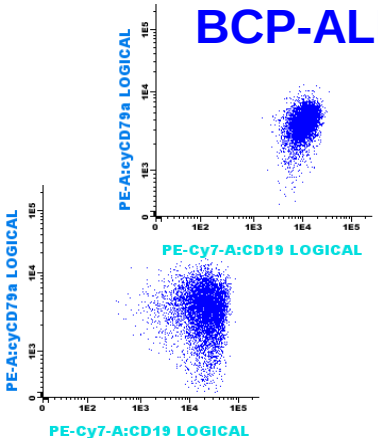
Neutrophils

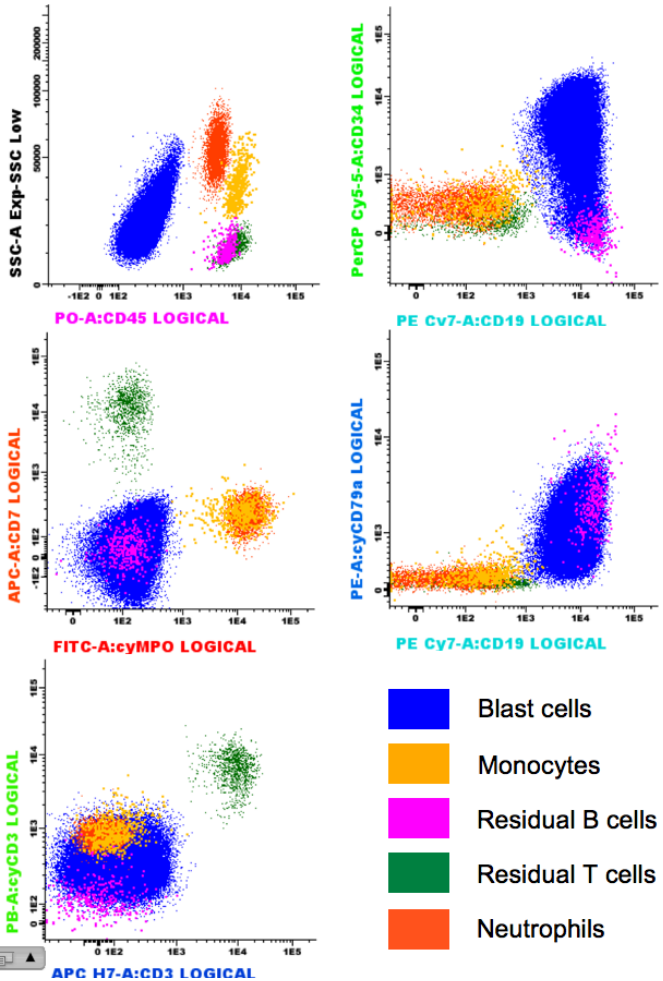
EuroFlow

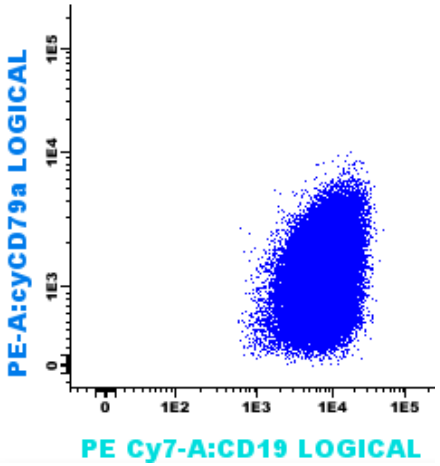

SSC-A Exp-SSC Low
PO-A:CD45 LOGICAL
PerCP Cy5-5-A:CD34 LOGICAL
PE Cy7-A:CD19 LOGICAL
APC-A:CD7 LOGICAL
FITC-A:cyMPO LOGICAL
PE-A:cyCD79a LOGICAL
PE Cy7-A:CD19 LOGICAL
PB-A:cyCD3 LOGICAL
APC H7-A:CD3 LOGICAL
Blast cells
Monocytes
Residual B cells
Residual T cells
Neutrophils
PE-A:cyCD79a LOGICAL
PE Cy7-A:CD19 LOGICAL

EuroFlow

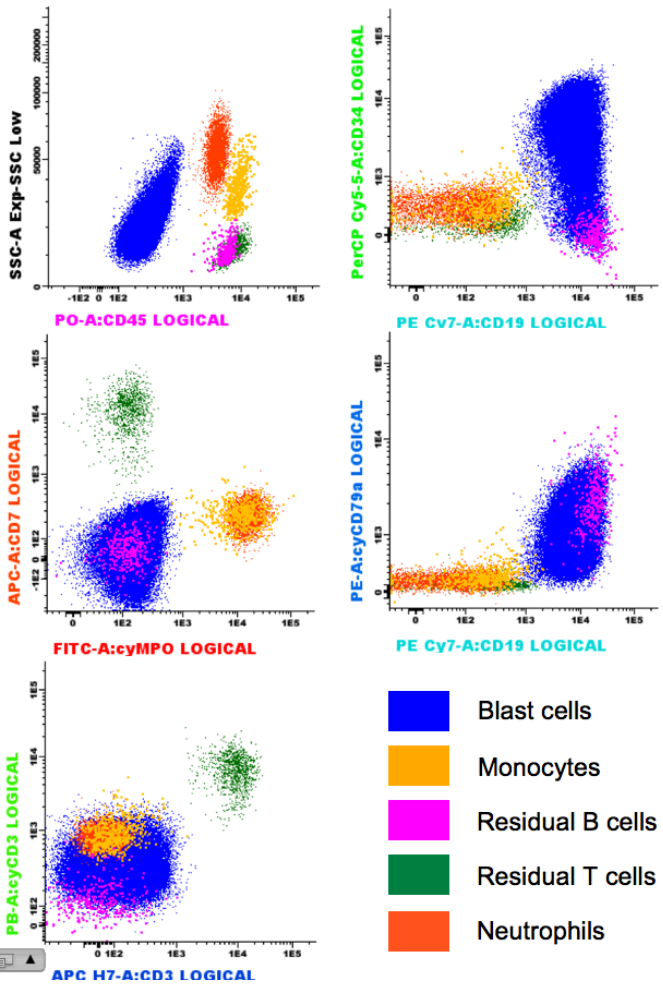
# BCP-ALL

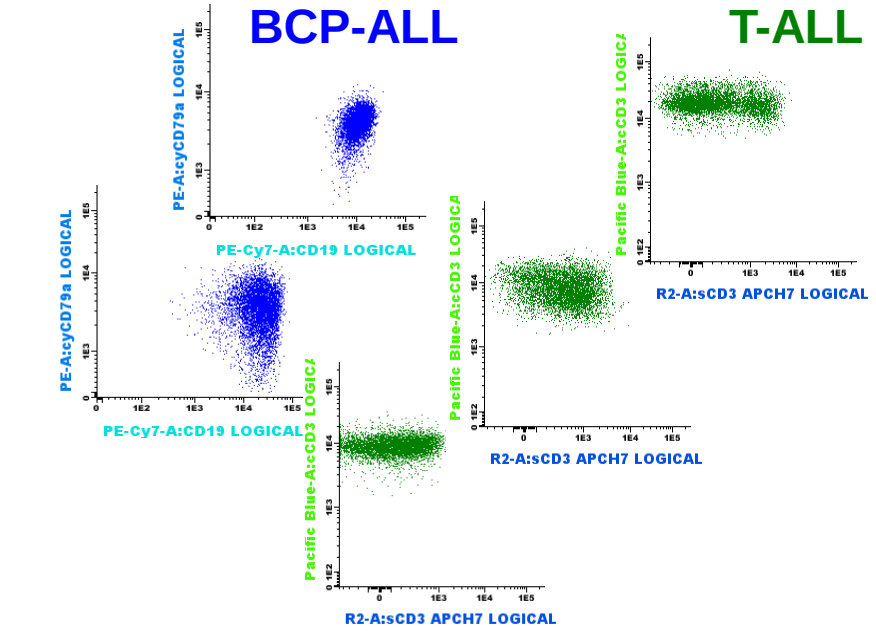


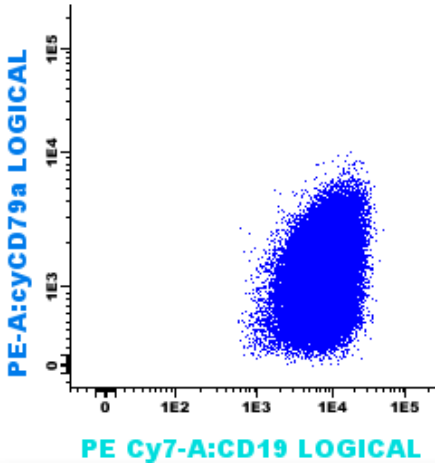



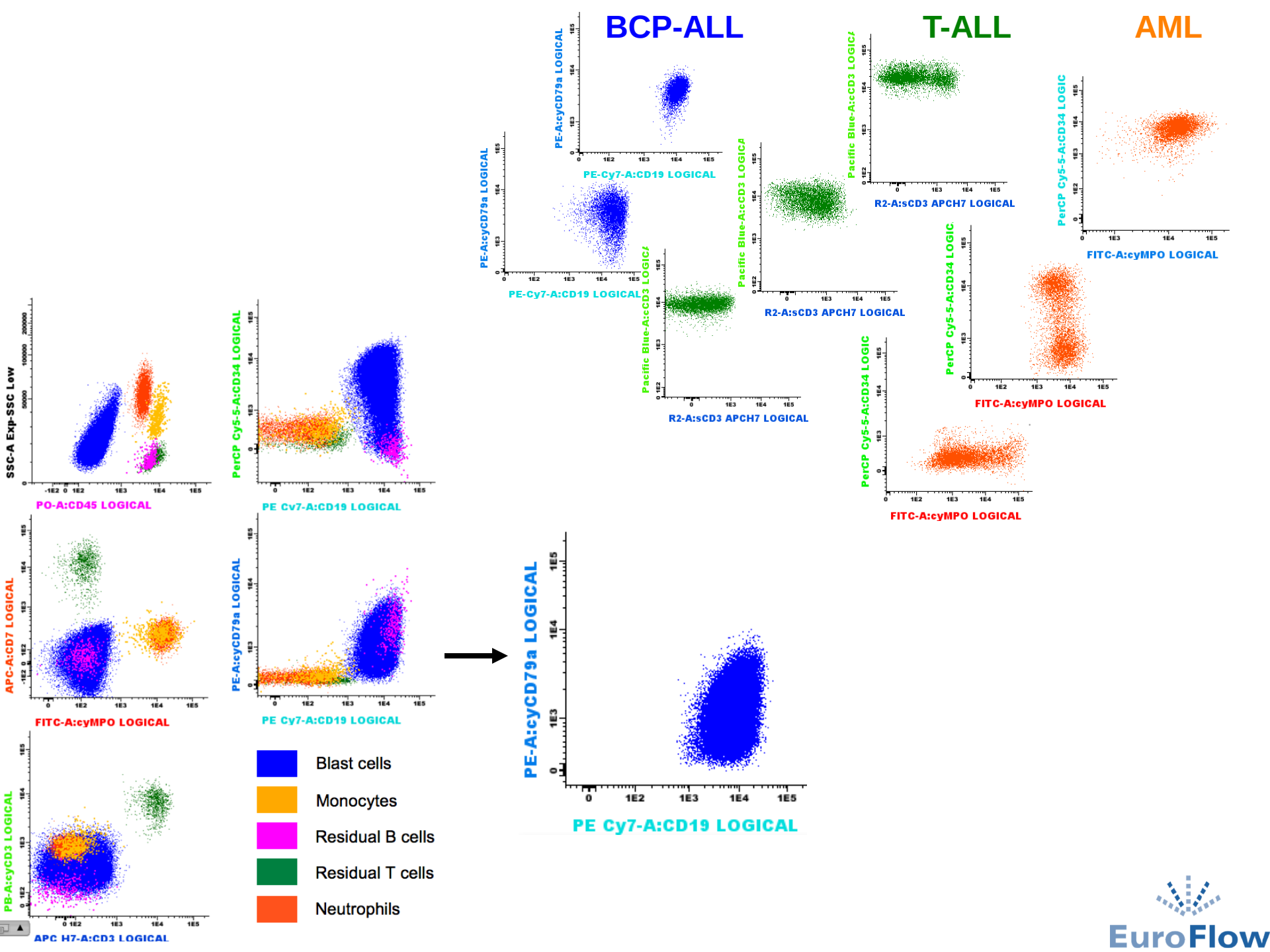

EuroFlow


SSC-A Exp-SSC Low
PO-A:CD45 LOGICAL
PerCP Cy5-5-A:CD34 LOGICAL
PE Cy7-A:CD19 LOGICAL
APC-A:CD7 LOGICAL
FITC-A:cyMPO LOGICAL
PE-A:cyCD79a LOGICAL
PE Cy7-A:CD19 LOGICAL
PB-A:cyCD3 LOGICAL
APC H7-A:CD3 LOGICAL
Blast cells
Monocytes
Residual B cells
Residual T cells
Neutrophils

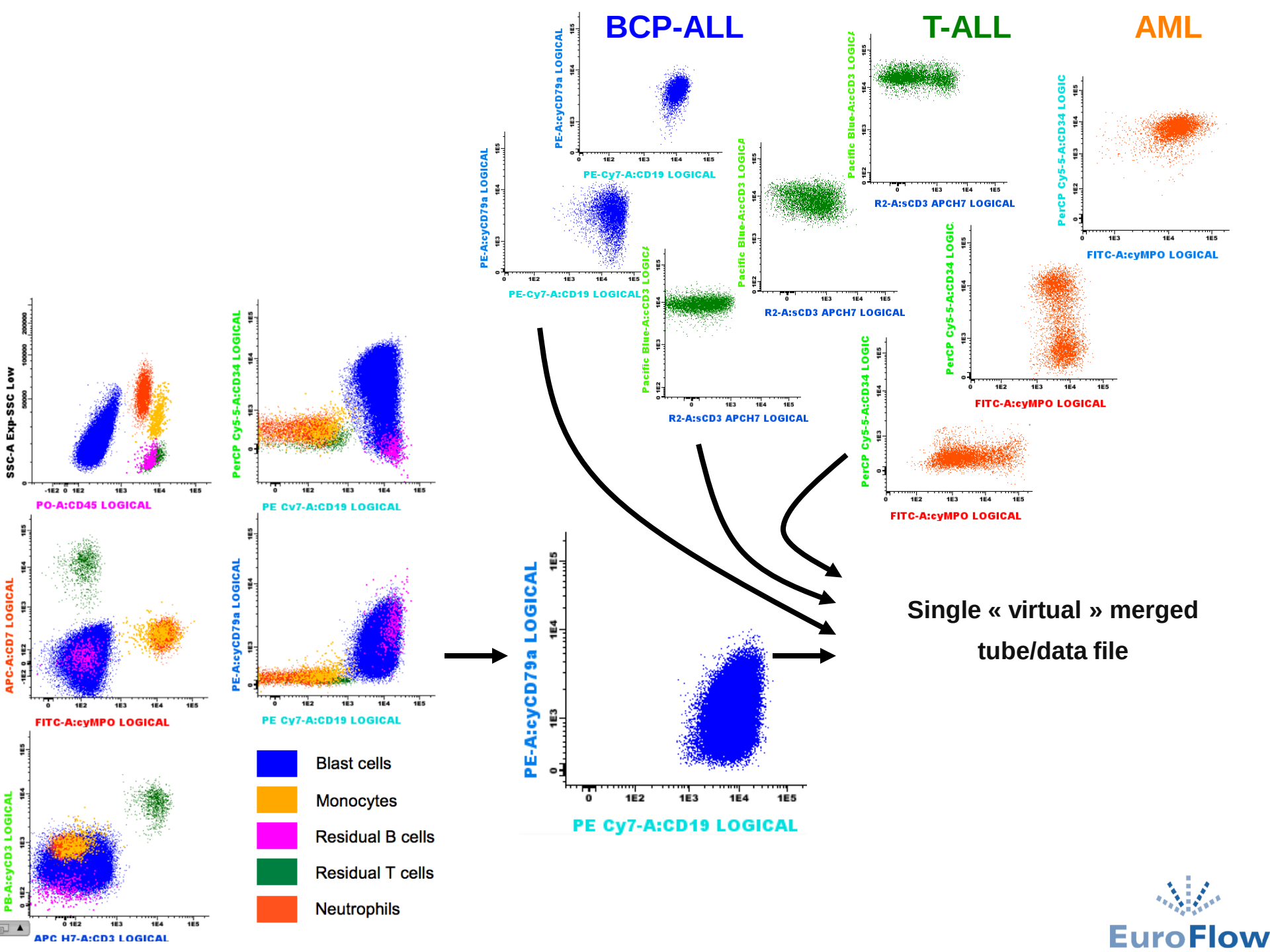

BCP-ALL
T-ALL
PE-A:cyCD79a LOGICAL
PE-Cy7-A:CD19 LOGICAL
Pacific Blue-A:cCD3 LOGICA
R2-A:sCD3 APCH7 LOGICAL


PE-A:cyCD79a LOGICAL
PE Cy7-A:CD19 LOGICAL

EuroFlow

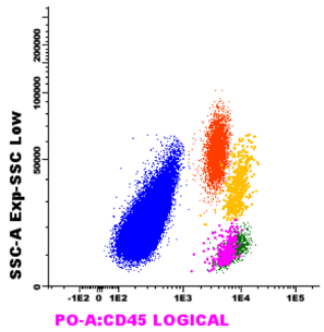

BCP-ALL
T-ALL
AML
SSC-A Exp-SSC Low
PO-A:CD45 LOGICAL
PerCP Cy5-5-A:CD34 LOGICAL
PE Cy7-A:CD19 LOGICAL
APC-A:CD7 LOGICAL
FITC-A:cyMPO LOGICAL
PE-A:cyCD79a LOGICAL
PE Cy7-A:CD19 LOGICAL
PB-A:cyCD3 LOGICAL
APC H7-A:CD3 LOGICAL
Blast cells
Monocytes
Residual B cells
Residual T cells
Neutrophils
PE-A:cyCD79a LOGICAL
PE Cy7-A:CD19 LOGICAL
PE-A:cyCD79a LOGICAL
PE-Cy7-A:CD19 LOGICAL
Pacific Blue-A:cCD3 LOGICAL
R2-A:sCD3 APCH7 LOGICAL
PerCP Cy5-5-A:CD34 LOGIC
FITC-A:cyMPO LOGICAL

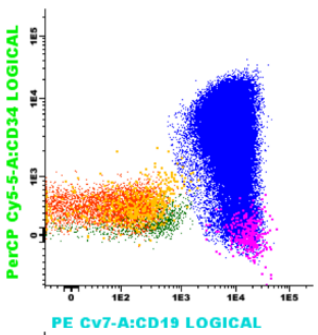
EuroFlow

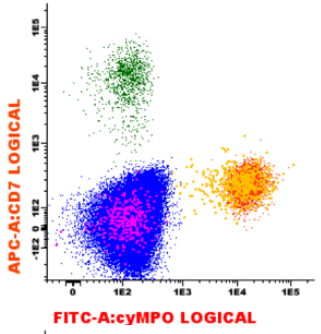

BCP-ALL
T-ALL
AML
SSC-A Exp-SSC Low
PO-A:CD45 LOGICAL
PerCP Cy5-5-A:CD34 LOGICAL
PE Cv7-A:CD19 LOGICAL
APC-A:CD7 LOGICAL
FITC-A:cyMPO LOGICAL
PE-A:cyCD79a LOGICAL
PE Cy7-A:CD19 LOGICAL
PB-A:cyCD3 LOGICAL
APC H7-A:CD3 LOGICAL
Blast cells
Monocytes
Residual B cells
Residual T cells
Neutrophils
PE-Cy7-A:CD19 LOGICAL
Pacific Blue-A:cCD3 LOGIC
R2-A:sCD3 APCH7 LOGICAL
Single « virtual » merged tube/data file

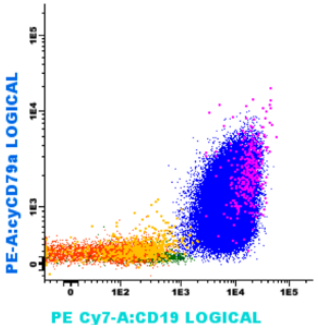
EuroFlow

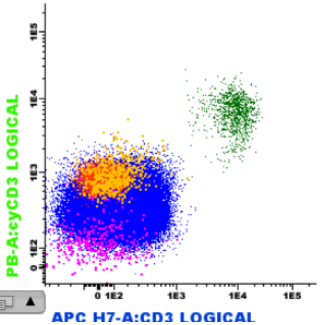
EuroFlow



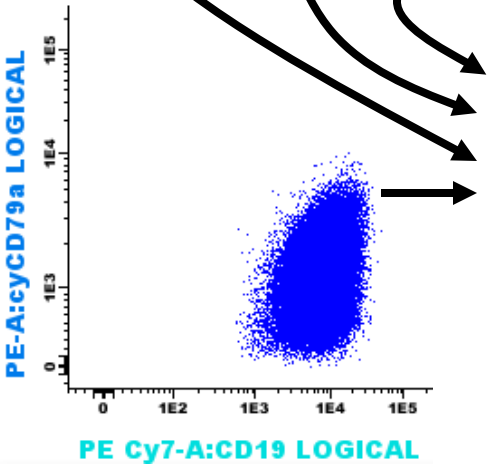



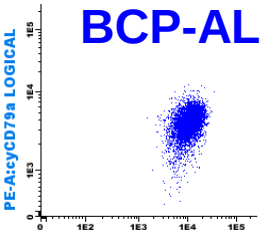



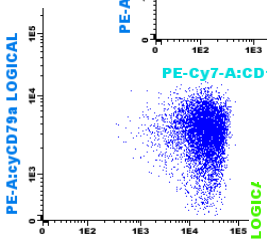




Blast cells
Monocytes
Residual B cells
Residual T cells
Neutrophils

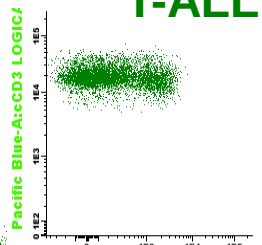


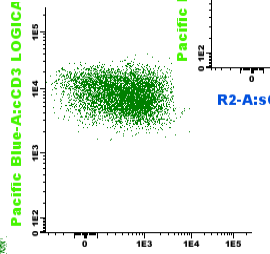

BCP-ALL

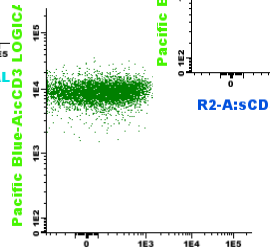



T-ALL

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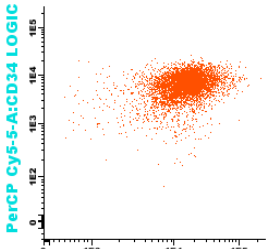
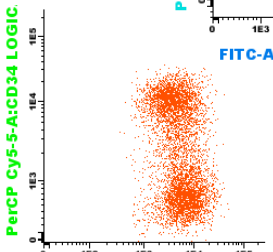
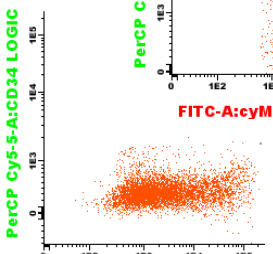
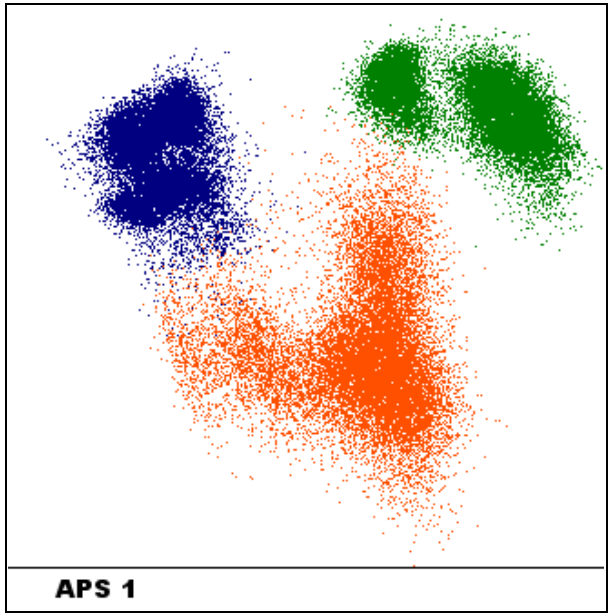
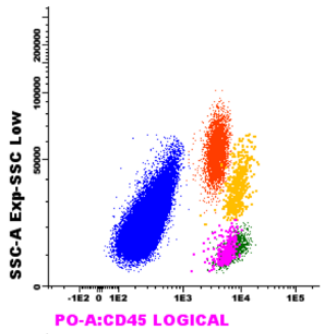


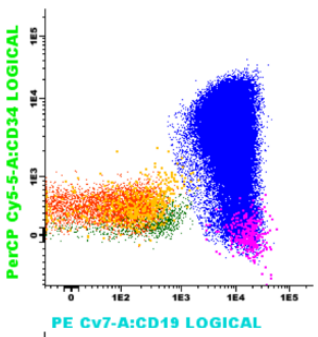



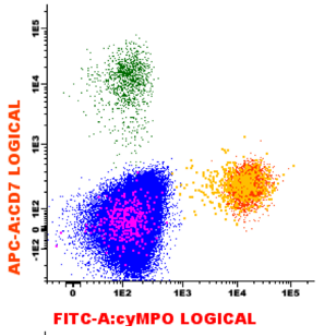



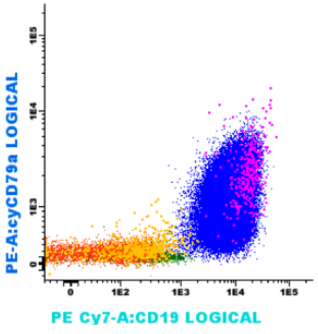



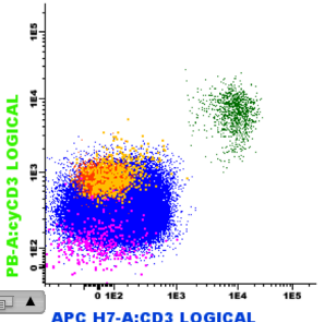
EuroFlow



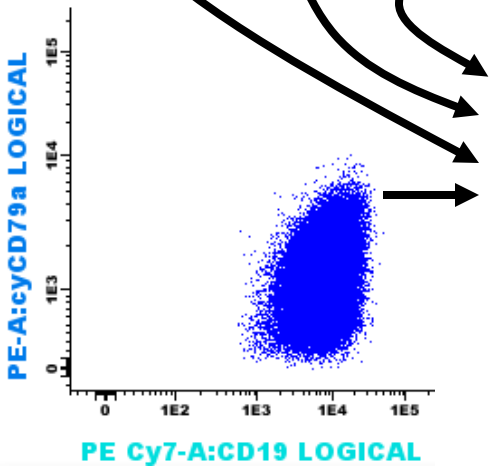



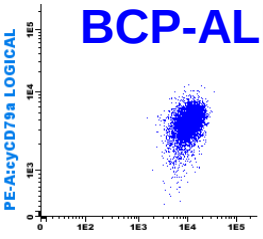



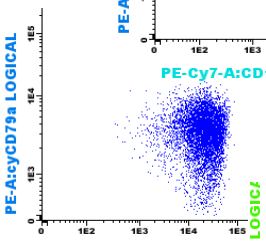




Blast cells
Monocytes
Residual B cells
Residual T cells
Neutrophils

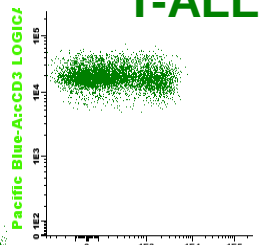


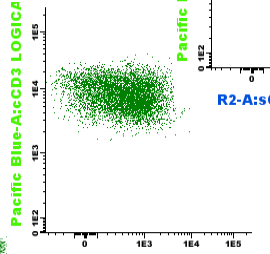

BCP-ALL

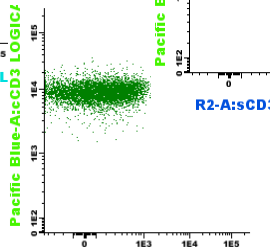



T-ALL

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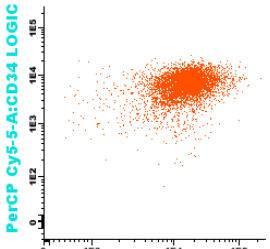
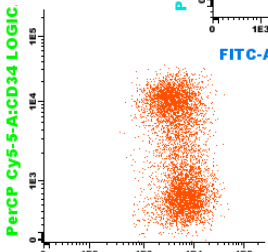
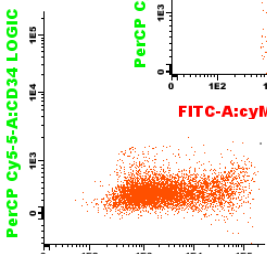
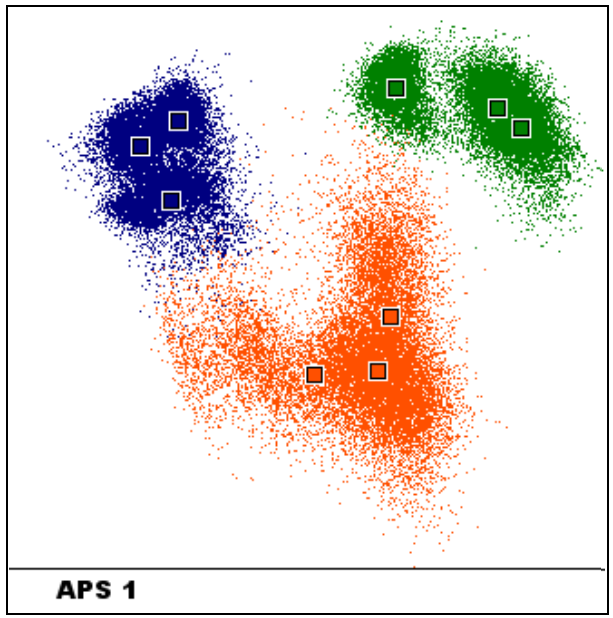
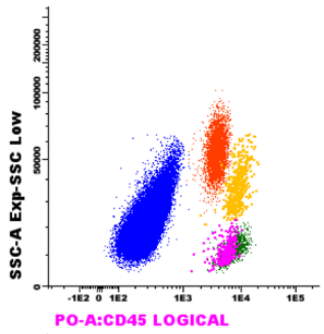


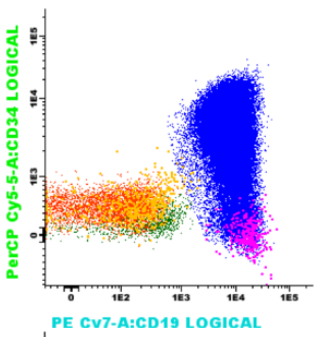



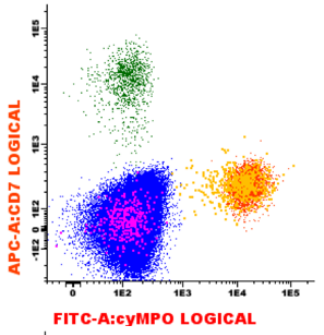



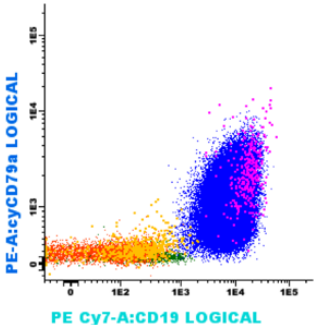



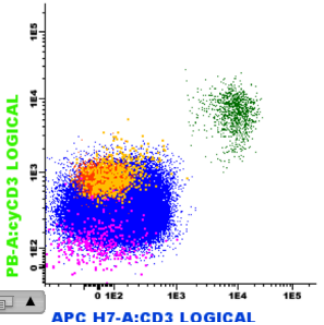
EuroFlow



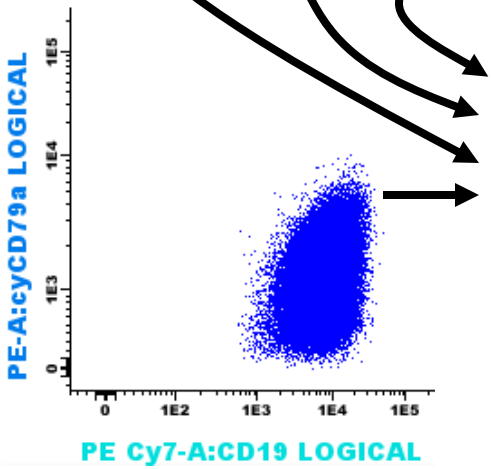



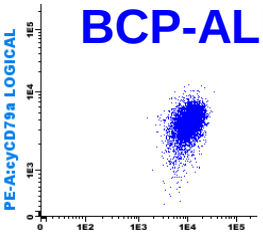



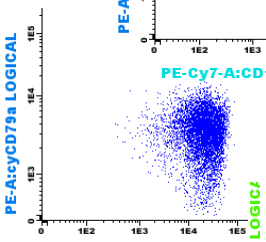




Blast cells
Monocytes
Residual B cells
Residual T cells
Neutrophils

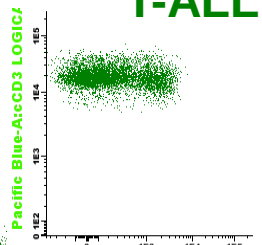


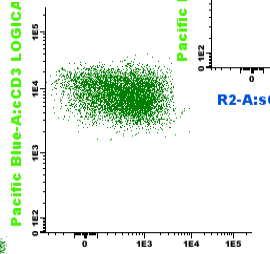

BCP-ALL

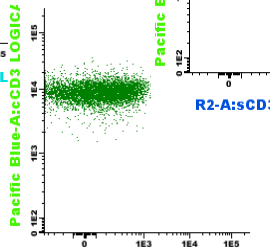



T-ALL

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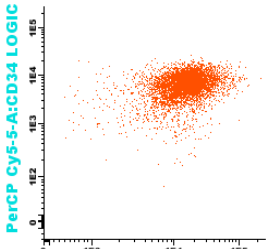
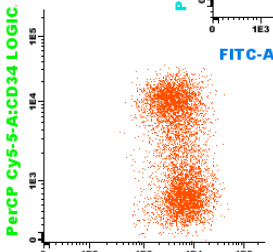
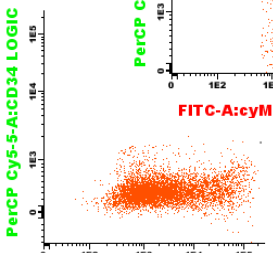
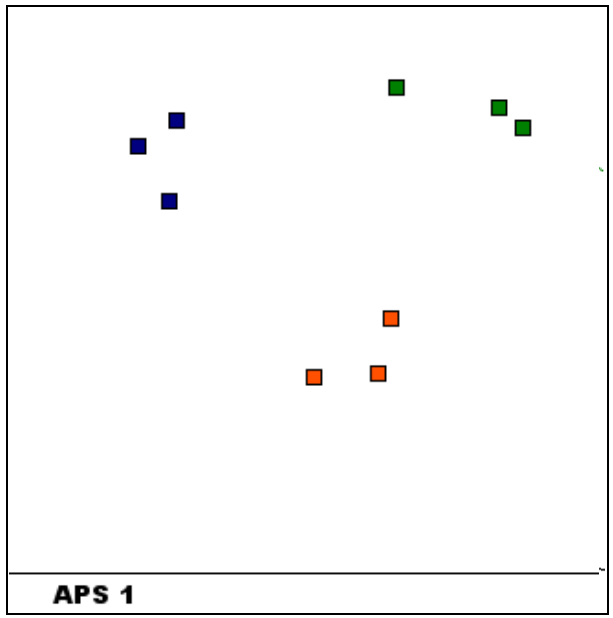
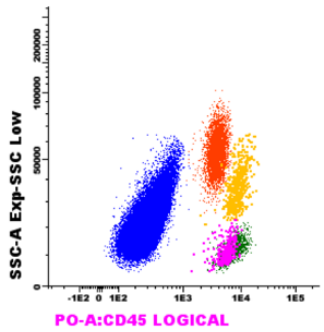


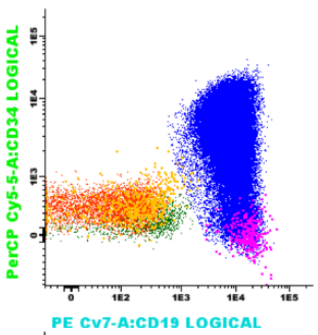



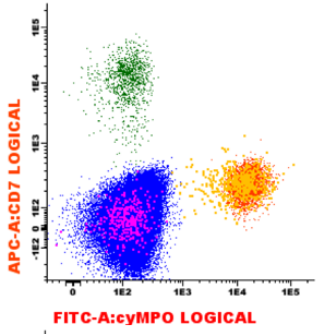



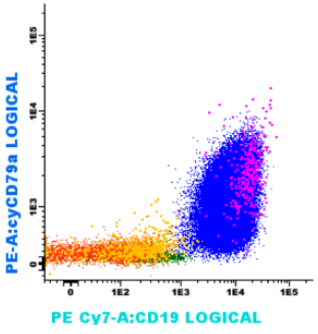



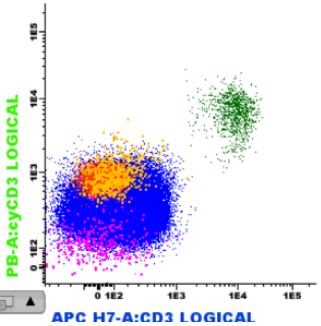
EuroFlow



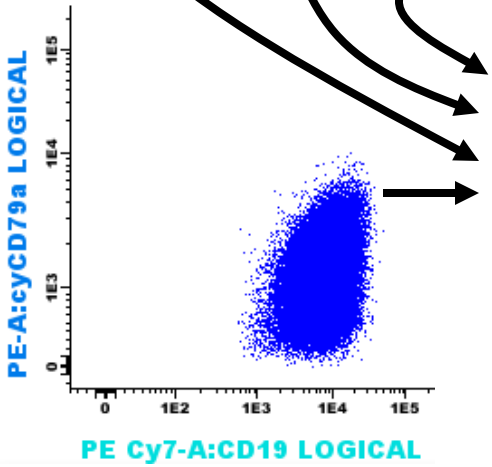



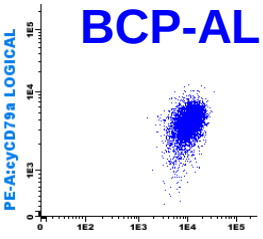



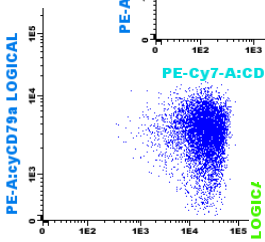



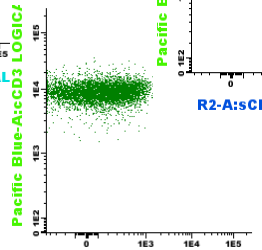

Blast cells
Monocytes
Residual B cells
Residual T cells
Neutrophils



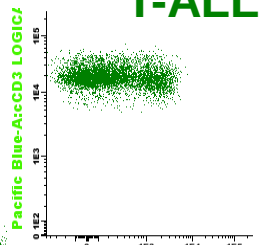

BCP-ALL

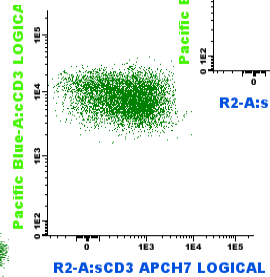


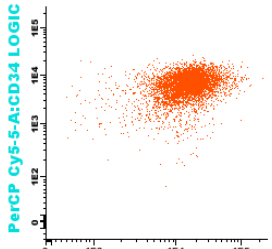



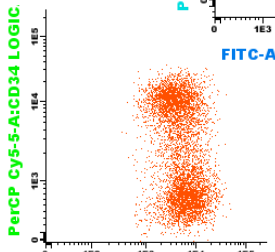

T-ALL

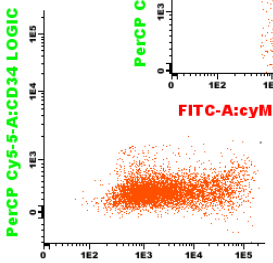


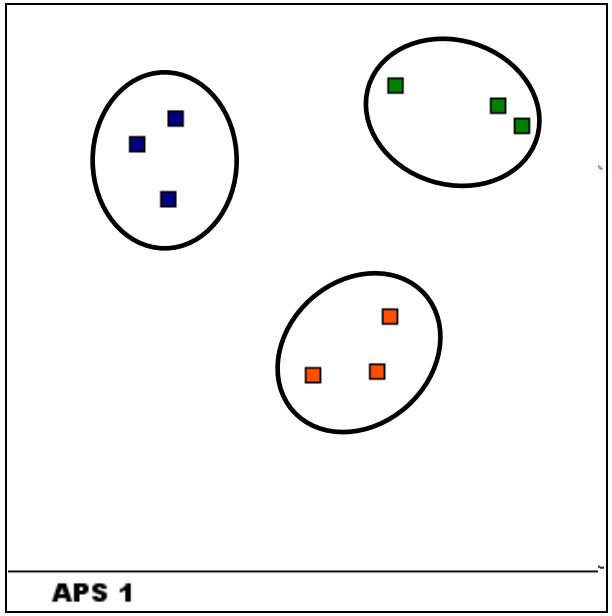




AML

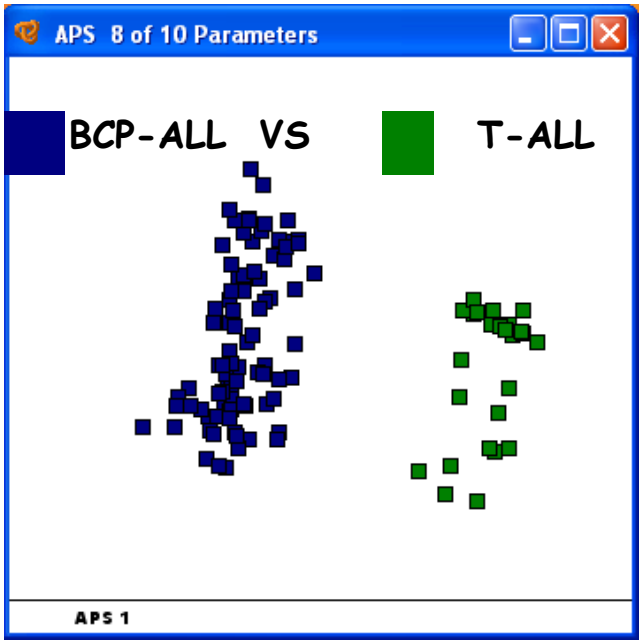


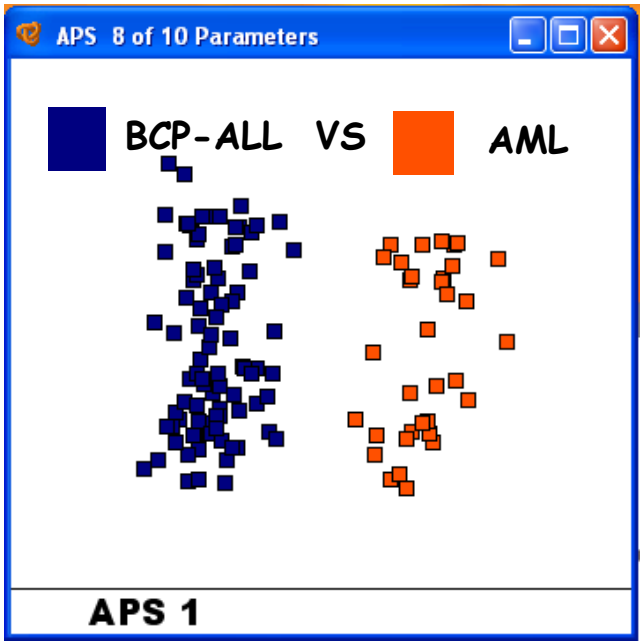



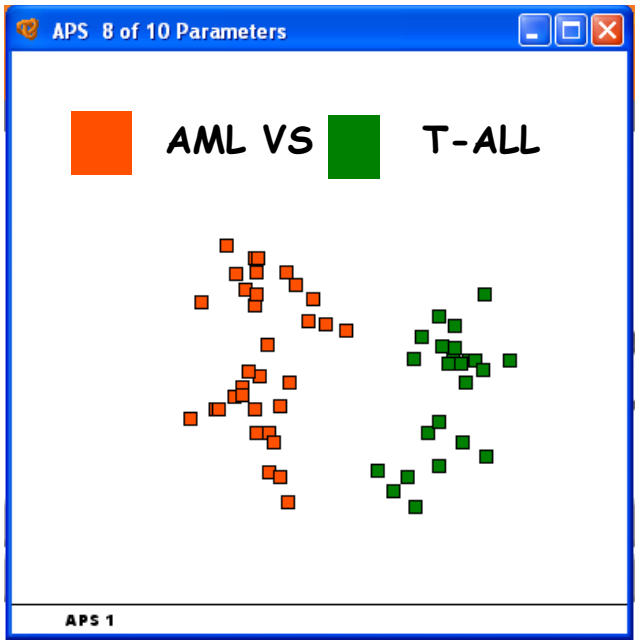



# LINEAGE ASSESSMENT IN ACUTE LEUKAEMIAS (N=158)

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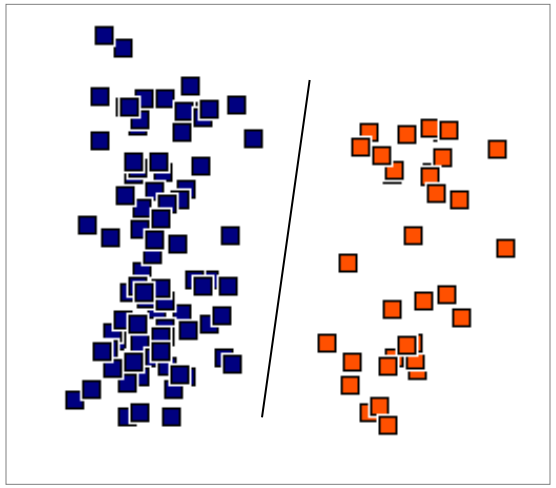
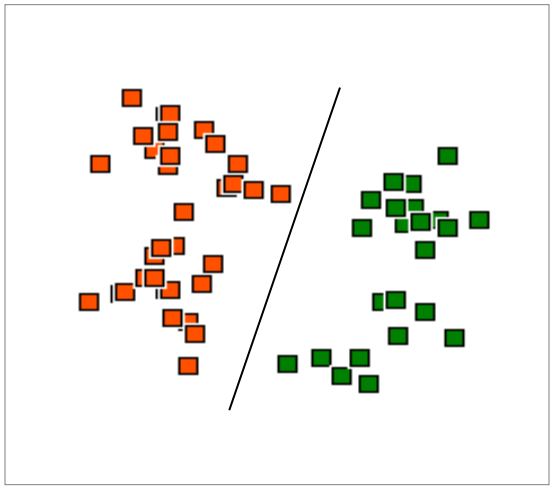
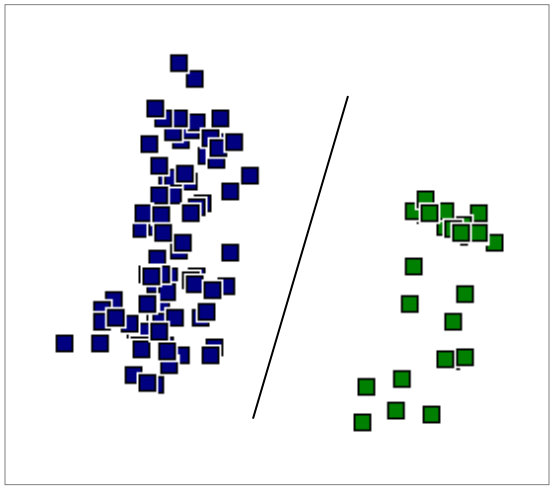
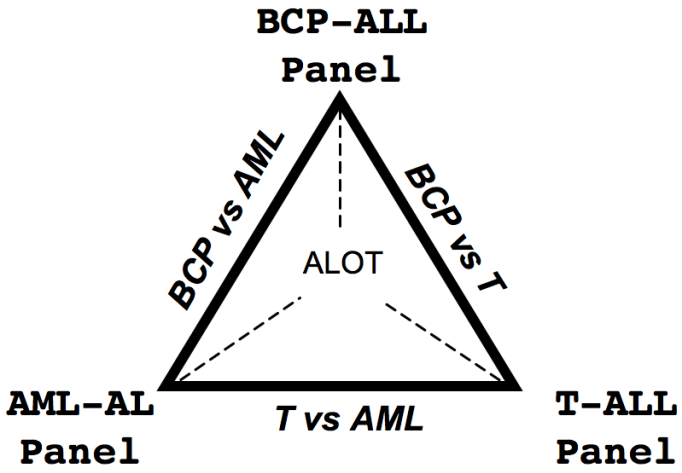
# ALOT *(Acute Leukemia Orientation Tube)*










BCP-ALL cases (n=89)

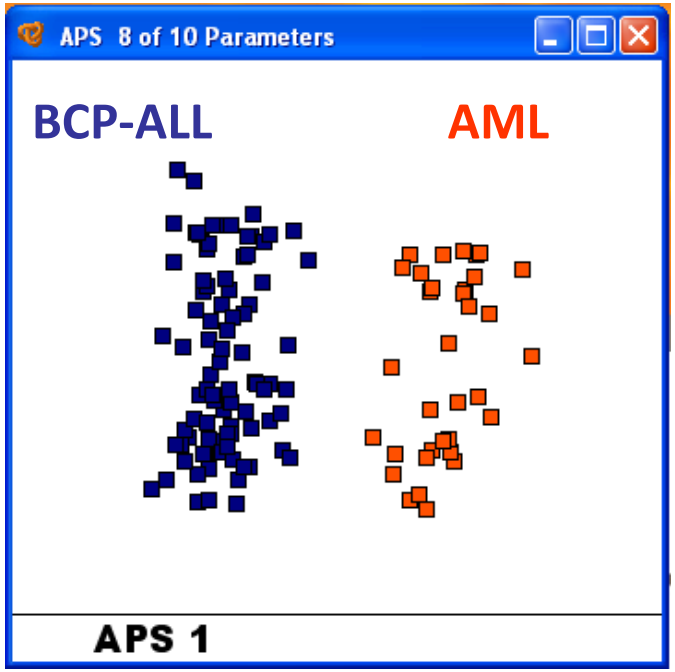
T-ALL cases (n=27)

AML cases (n=36)

EuroFlow

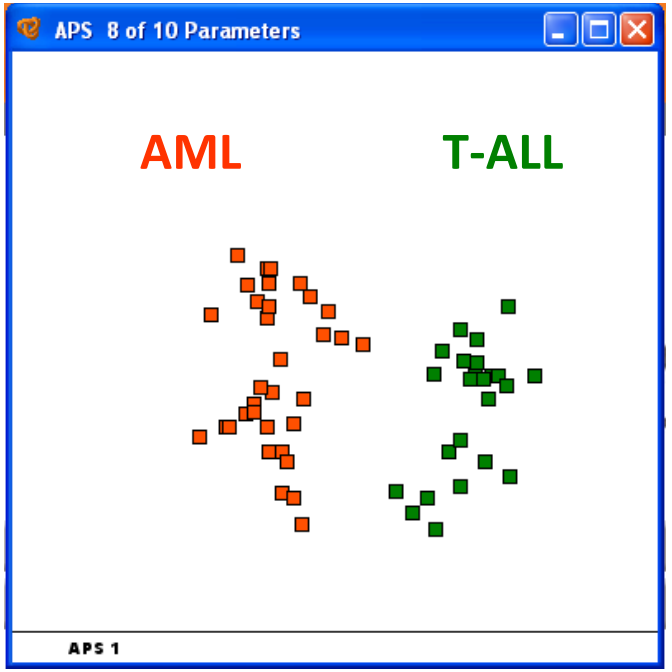
# ALOT – Acute Leukemia Orientation Tube

## BCP vs AML




Principal Component 1
(1 31.967915) : CD19:PE-Cy7-A LOGICAL
(2 25.360966) : cMPO:FITC-A LOGICAL
(3 21.53262) : CD79a:PE-A LOGICAL
(4 8.655562) : CD45 PO LOGICAL
(5 5.808244) : cCD3:Pacific Blue-A LOGICAL
(6 4.827642) : CD7:APC-A LOGICAL
(7 1.3671329) : CD34:PerCP-Cy5-5-A LOGICAL
(8 0.47992325) : sCD3:APC-H7-A LOGICAL

## AML vs T-ALL




Principal Component 1
(1 24.395567) : cMPO:FITC-A LOGICAL
(2 22.343613) : cCD3:Pacific Blue-A LOGICAL
(3 21.838257) : CD7:APC-A LOGICAL
(4 14.833124) : CD34:PerCP-Cy5-5-A LOGICAL
(5 12.695069) : sCD3:APC-H7-A LOGICAL
(6 1.4593897) : CD45 PO LOGICAL
(7 1.3203148) : CD79a:PE-A LOGICAL
(8 1.1146643) : CD19:PE-Cy7-A LOGICAL

Ludovic Lhermitte : EuroFlow York Meeting Presentation 2009 (non-published data)

# BCP-ALL panel

BCP-ALL

| Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|
| CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

EuroFlow

# BCP-ALL panel

BCP-ALL

| Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|
| CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

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# BCP-ALL panel

ALOT

| Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|
| cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |

BCP-ALL

| Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|
| CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

EuroFlow

# BCP-ALL panel

| | Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|---|
| ALOT | cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |
| BCP-ALL | CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| | smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| | CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| | CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

EuroFlow

# BCP-ALL panel

| | Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|---|
| ALOT | cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |
| BCP-ALL | CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| | smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| | CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| | CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

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# BCP-ALL panel

| | Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|---|
| ALOT | cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |
| BCP-ALL | CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| | smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| | CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| | CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

## Positive Diagnosis

EuroFlow

# BCP-ALL panel

| | Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|---|
| ALOT | cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |
| BCP-ALL | CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| | smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| | CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| | CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

*Differential Diagnosis*

*&*

*Ambiguous lineage acute leukemia*

EuroFlow

# BCP-ALL panel

| | Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|---|
| ALOT | cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |
| BCP-ALL | CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| | smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| | CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| | CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

## Maturation stage (EGIL)

EuroFlow

# BCP-ALL panel

| | Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|---|
| ALOT | cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |
| BCP-ALL | CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| | smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| | CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| | CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

*Alternative classification*

*Immunophenotypic features associated with well-defined molecular aberrations*

EuroFlow

# BCP-ALL panel

| | Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|---|
| ALOT | cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |
| BCP-ALL | CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| | smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| | CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| | CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

## *Prognosis markers*

EuroFlow

# BCP-ALL panel

| | Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|---|
| ALOT | cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |
| BCP-ALL | CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| | smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| | CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| | CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

## LAP markers

EuroFlow

# BCP-ALL panel

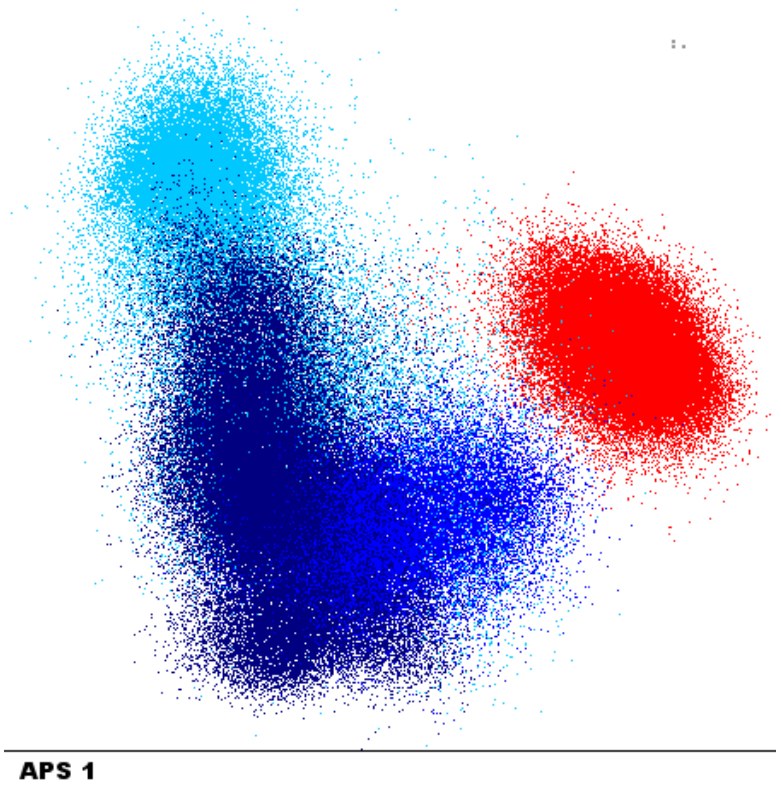
| | Pac Blue | Pac Orange | FITC | PE | PerCP Cy5.5 | PE Cy7 | APC | APC H7 |
|---|---|---|---|---|---|---|---|---|
| ALOT | cyCD3 | CD45 | cyMPO | cyCD79a | CD34 | CD19 | CD7 | smCD3 |
| BCP-ALL | CD20 | CD45 | CD58 | CD66c | CD34 | CD19 | CD10 | CD38 |
| | smIgK | CD45 | cyIgM | CD33 | CD34 | CD19 | smIgM +CD117 | smIgL |
| | CD9 | CD45 | TdT | CD13 | CD34 | CD19 | CD22 | CD24 |
| | CD21 | CD45 | CD15 +CDw65 | NG2 | CD34 | CD19 | CD123 | CD81 |

*LS CD45 CD19 CD34 cyCD3 cyMPO cyCD79a CD7 smCD3 CD20 CD58 CD66C CD10 CD38 smIgK cyIgM CD33 smIgM+CD117 smIgL CD9 TdT CD13 CD22 CD24 CD21 CD15+65 NG2 CD123 CD81*

*31 parameters*

EuroFlow

# BCP-ALL panel



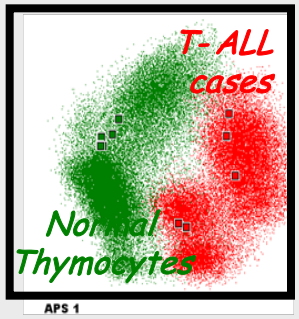
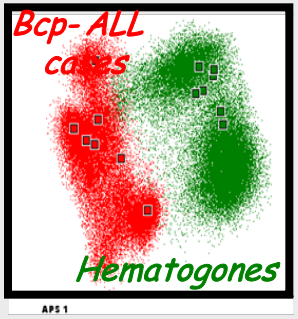
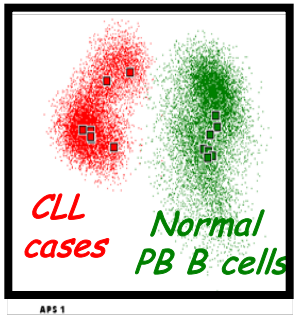
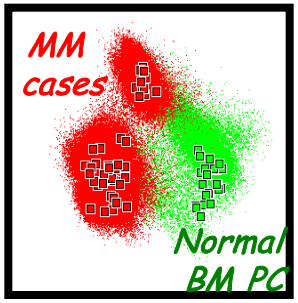
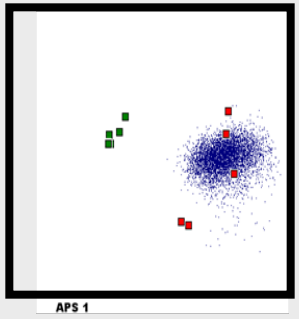
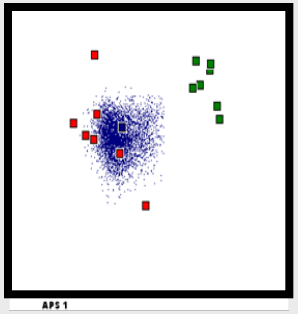
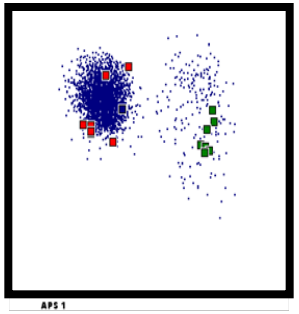
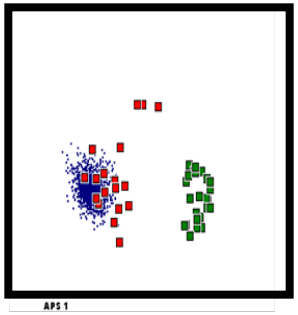
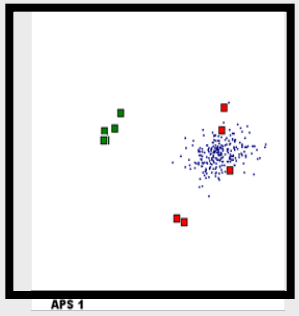
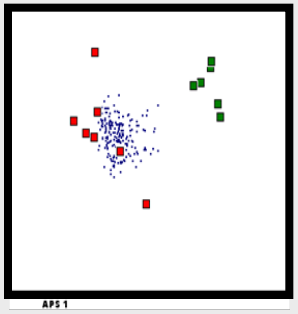
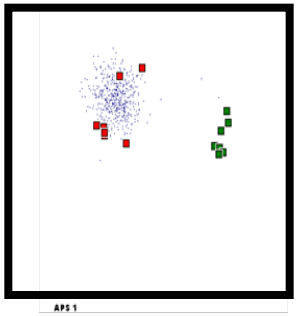
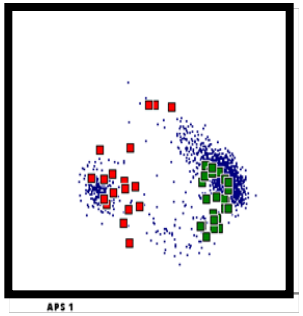

*Mix of 3 different regenerating B cell populations (Haematogones)*

*BCP-ALL blast cells*

EuroFlow

# COMPARE A CASE VS NORMAL & NEOPLASTIC 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 |   |   |   |   |

# Multi-tube EuroFlow classification panel for AML/MDS

Responsible scientist: VHJ van der Velden

| Tube | Pacific Blue | Pacific Orange | FITC | PE | PerCP-Cy5.5 | PE-Cy7 | APC | APC-H7 | Aim** |
|---|---|---|---|---|---|---|---|---|---|
| **AML/ MDS** | | | | | | | | | |
| 1 | **HLADR** | **CD45** | CD16 | CD13 | **CD34** | **CD117** | CD11b | CD10 | Diagnosis and subclassification of AML and PNH especially focused on neutrophilic lineage |
| 2 | **HLADR** | **CD45** | CD35 | CD64 | **CD34** | **CD117** | IREM2 | CD14 | Diagnosis and subclassification of AML and PNH especially focussed on monocytic lineage |
| 3 | **HLADR** | **CD45** | CD36 | CD105 | **CD34** | **CD117** | CD33 | CD71 | Diagnosis and subclassification of AML especially focused on erythroid lineage |
| 4 | **HLADR** | **CD45** | nuTdT | CD56 | **CD34** | **CD117** | CD7 | CD19 | Aberrant expression of lymphoid-associated markers and abnormal lymphoid maturation |

* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; nu= nuclear.
** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

# Multi-tube EuroFlow classification panel for AML/MDS *(Part 2)*

Responsible scientist: VHJ van der Velden

| Tube | Pacific Blue | Pacific Orange | FITC | PE | PerCP-Cy5.5 | PE-Cy7 | APC | APC-H7 | Aim** |
|---|---|---|---|---|---|---|---|---|---|
| **AML** | | | | | | | | | |
| 5 | **HLADR** | **CD45** | CD15 | NG2 | **CD34** | **CD117** | CD22 | CD38 | Aberrant expression of markers; detection of stem cells |
| 6 | **HLADR** | **CD45** | CD42a and CD61 | CD203c | **CD34** | **CD117** | CD123 | CD4 | Diagnosis and subclassification of AML especially focused on megakaryocytic, basophilic, and plasmacytoid dendritic lineages |
| **AML-M7** | | | | | | | | | |
| 7 | **HLADR** | **CD45** | CD41 | CD25 | **CD34** | **CD117** | CD42b | CD9 | Characterization of AML-M7, mastocytosis |

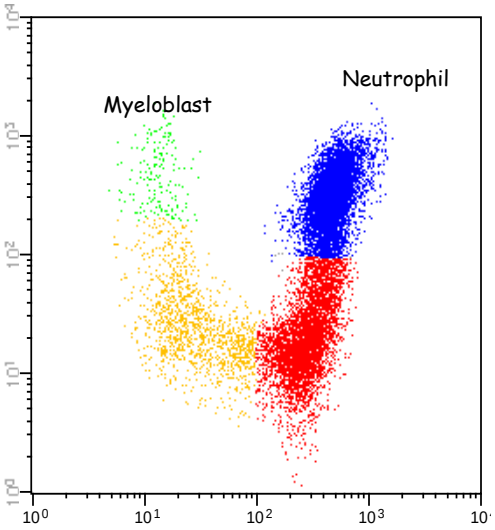
* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; nu= nuclear.

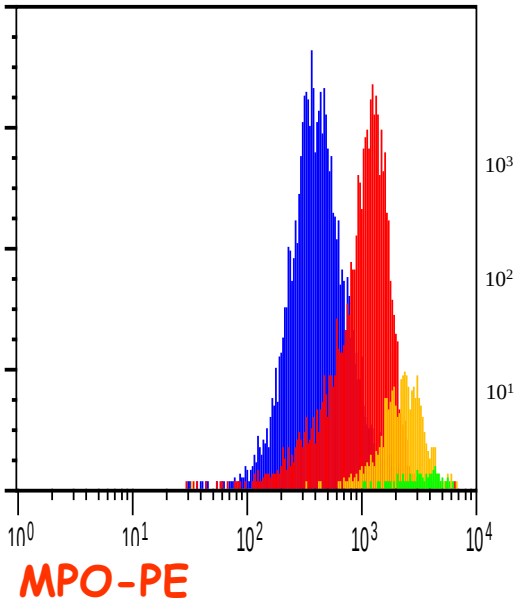
****** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

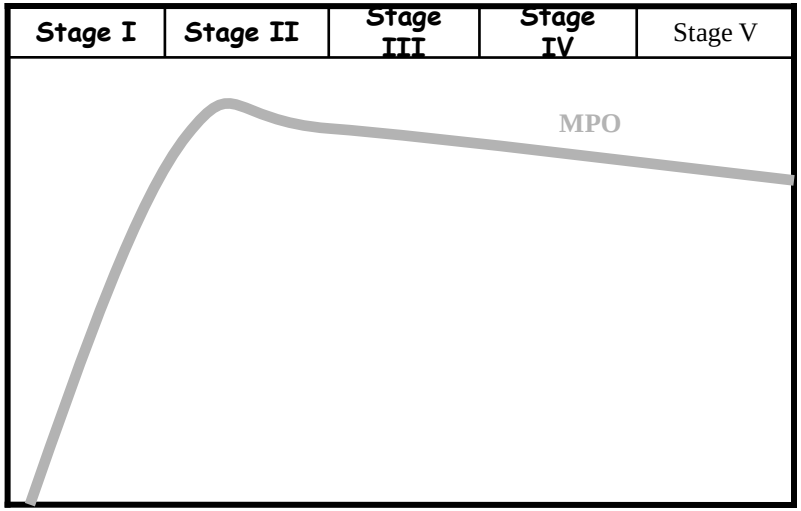
EuroFlow

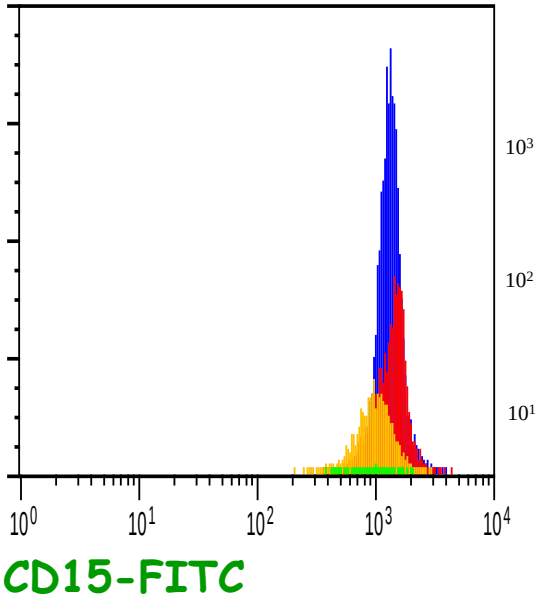
# New analytical tools for FCM immunophenotyping of myeloid neoplasias

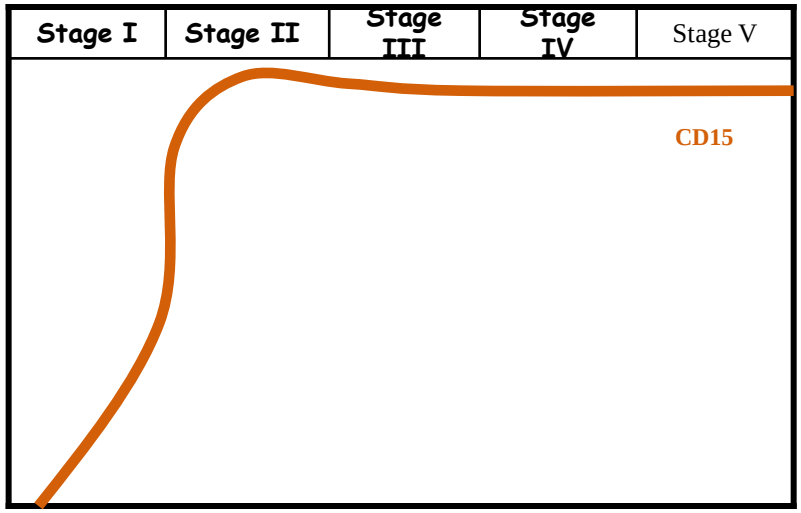
# NEUTROPHIL MATURATION



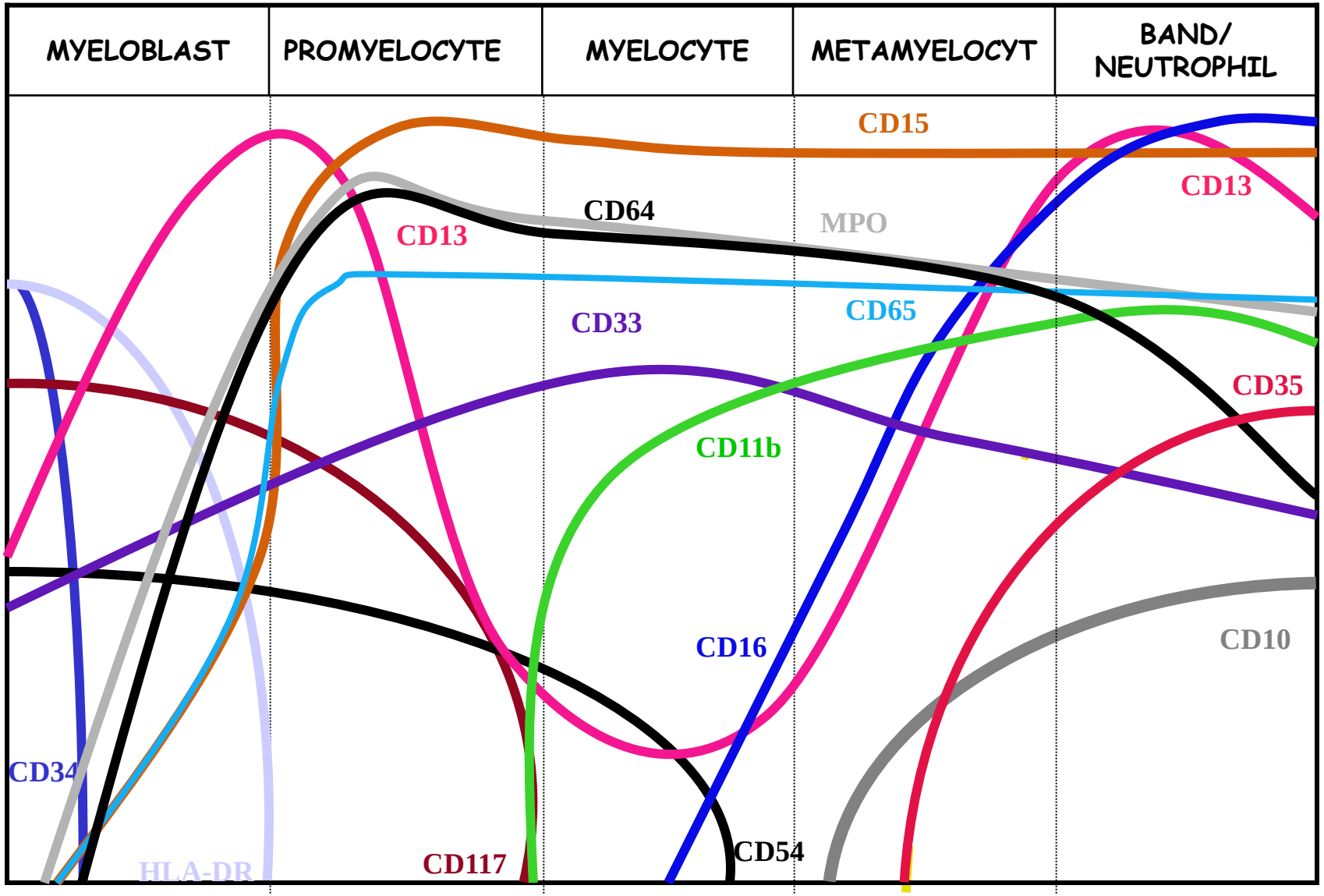



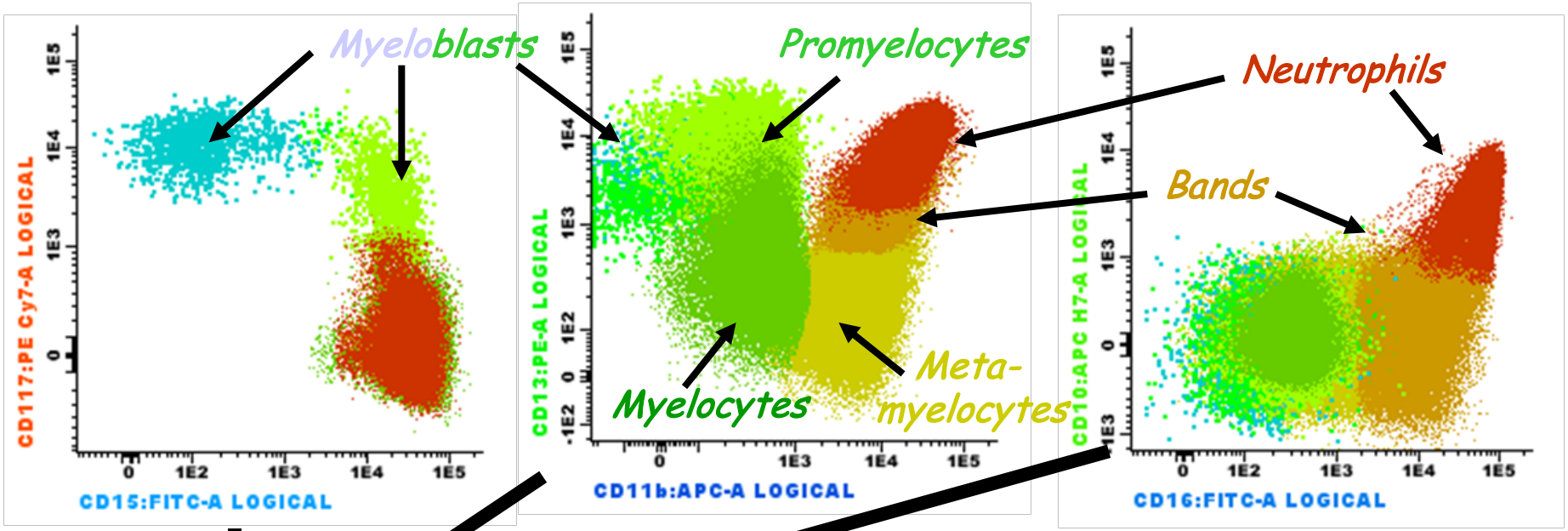



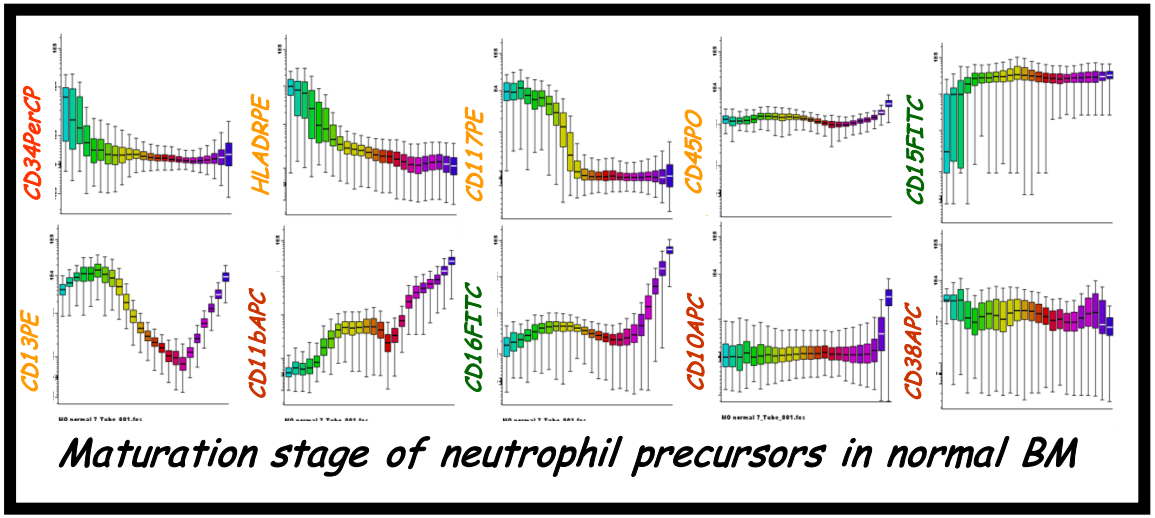
# PHENOTYPIC CHANGES DURING NORMAL NEUTROPHIL DIFFERENTIATION



# N-DIMENSIONAL NEUTROPHIL MATURATION IN NORMAL BM

EuroFlow

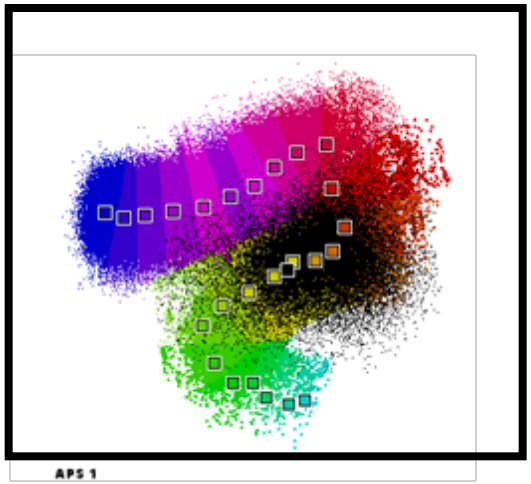


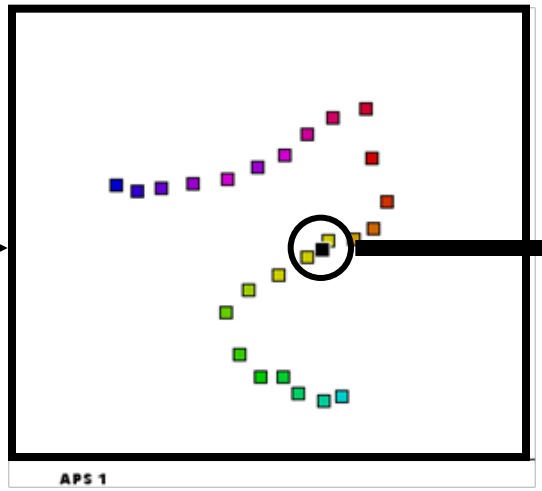



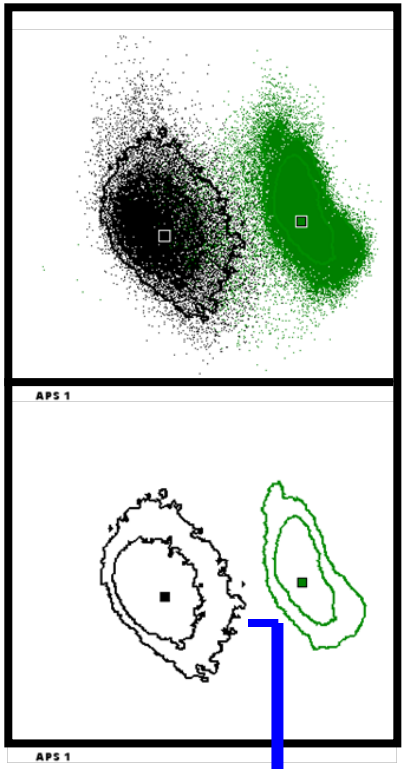
# N-DIMENSIONAL NEUTROPHIL MATURATION IN NORMAL BM VS AML

EuroFlow

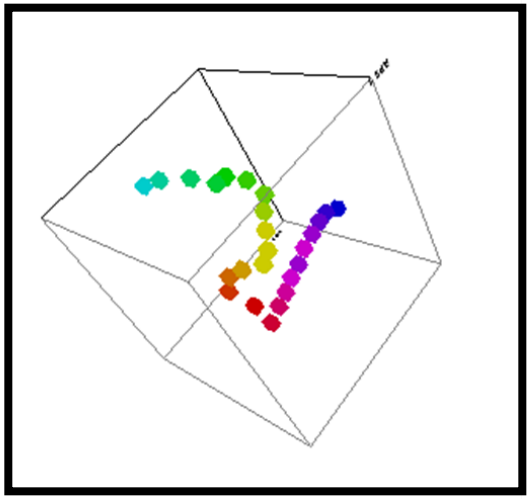
## Maturation stage of AML blasts

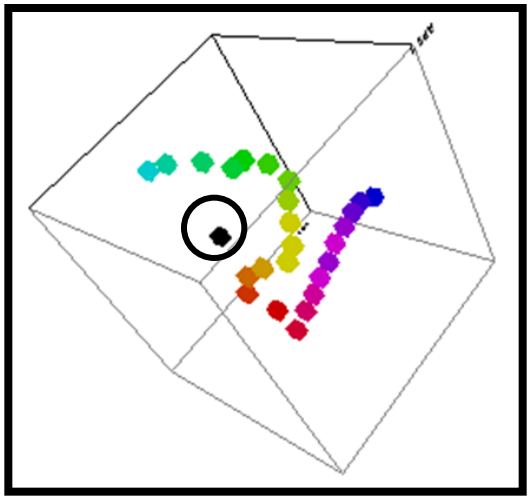



## Aberrant phenotype of AML blasts



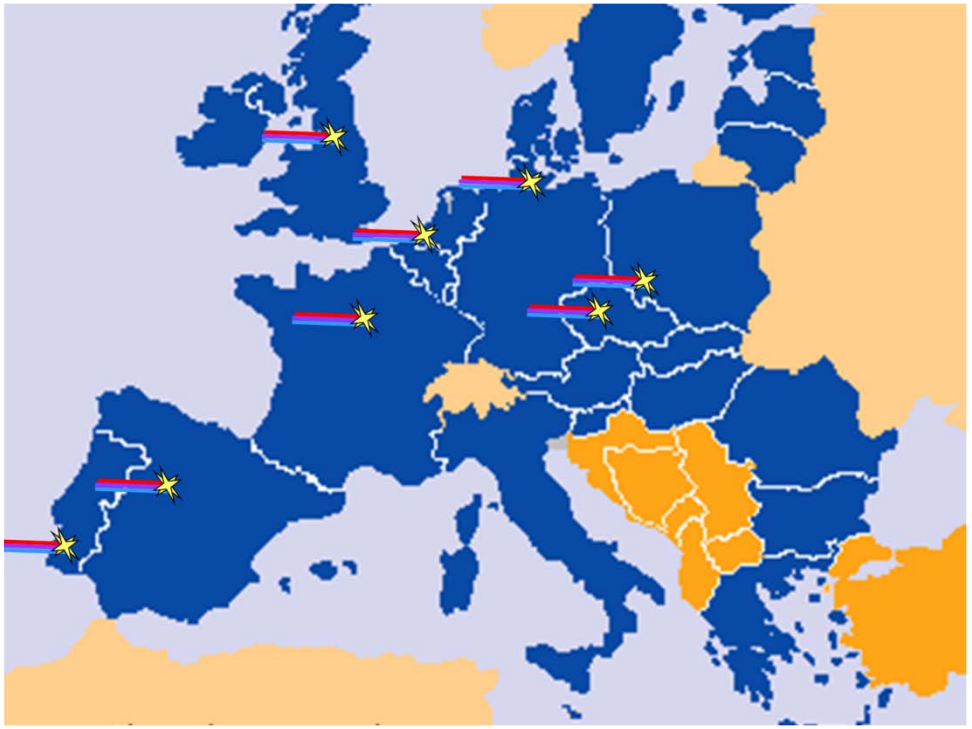


### Aberrant markers

| | |
|---|---|
| CD15 | 26.6 |
| CD117 | 19.4 |
| SSC | 19.2 |
| FSC | 19.0 |
| CD11b | 6.0 |

# 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?

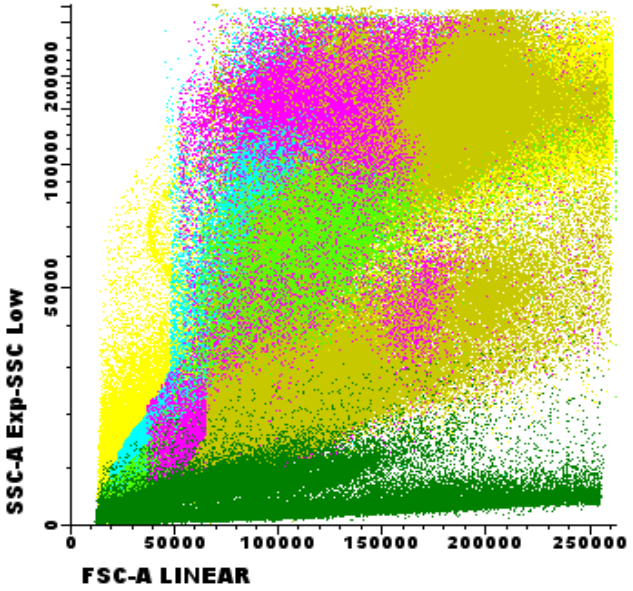


REPRODUCIBLE & OBJECTIVE RESULTS

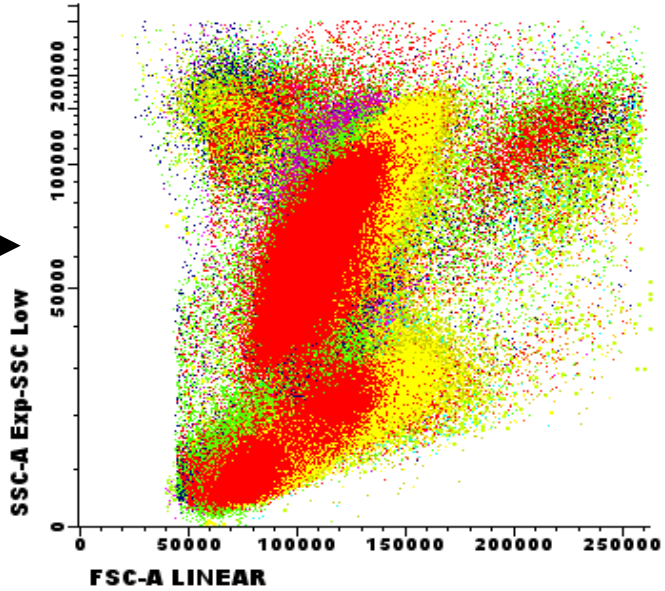
EuroFlow

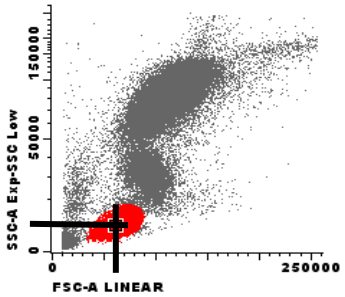
# Synchronized light scatter experiments

## "Local" settings




## EuroFlow settings



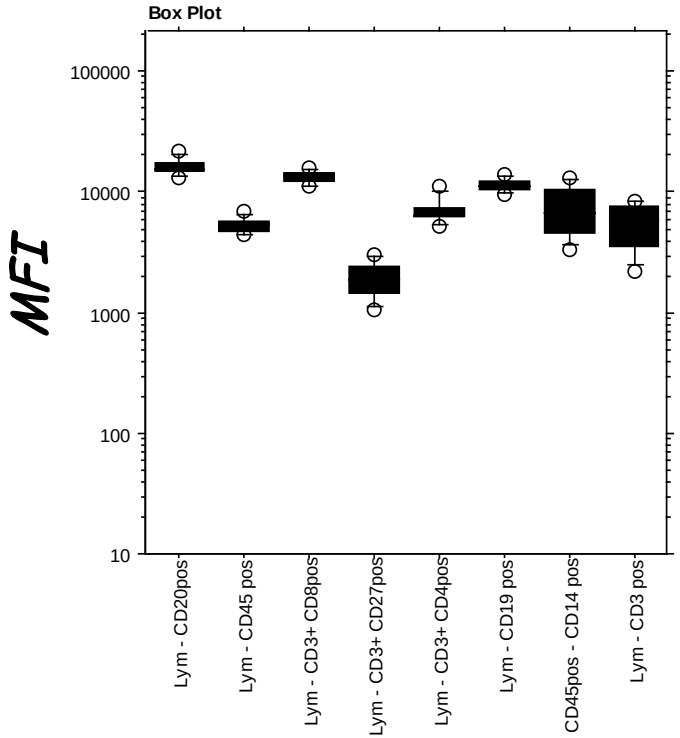

*7 different normal PB samples acquired in 7 different centers*

*Normal PB samples processed according to EuroFlow sample preparation protocol*

EuroFlow

# Results of synchronized experiments

Paciifc Blue
Pacific Orange
FITC
PE
PerCP-Cy5.5
PC7
APC
APC-H7




Stabilized donor

## Interlaboratory MFI CV of gated cell subsets

| Cell subset | CV |
|---|---|
| Lym - CD20pos | 15,25% |
| Lym - CD45 pos | 13,90% |
| Lym - CD19 pos | 11,13% |
| Lym - CD3 pos | 38,67% |
| CD45pos - CD14 pos | 43,77% |
| Lym - CD3+ CD4pos | 24,68% |
| Lym - CD3+ CD8pos | 11,35% |
| Lym - CD3+ CD27pos | 32,09% |

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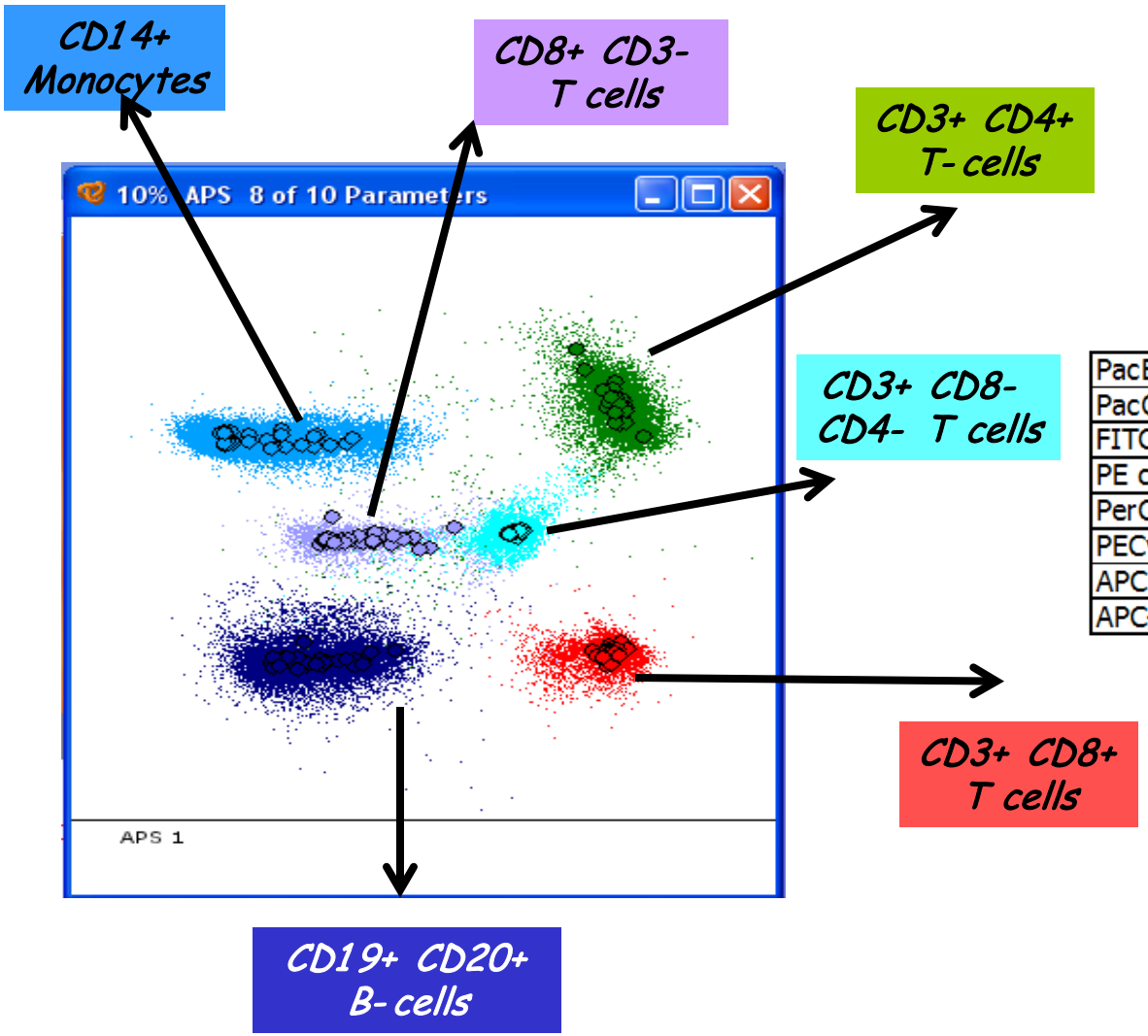
# Results of synchronized experiments:

## APS view of 30 merged data files from different centers




Local normal donors

CV MFI subset

| | | |
|---|---|---|
| PacBlue channel | Lym - CD20pos | 16,90% |
| PacOr channel | Lym - CD45 pos | 15,52% |
| FITC channel | Lym - CD3+ CD8pos | 16,93% |
| PE channel | Lym - CD3+ CD27pos | 27,95% |
| PerCPCy55 chann | Lym - CD3+ CD4pos | 28,39% |
| PECy7 channel | Lym - CD19 pos | 15,40% |
| APC channel | CD45pos - CD14 pos | 22,68% |
| APC-H7 channel | Lym - CD3 pos | **48,35%** |

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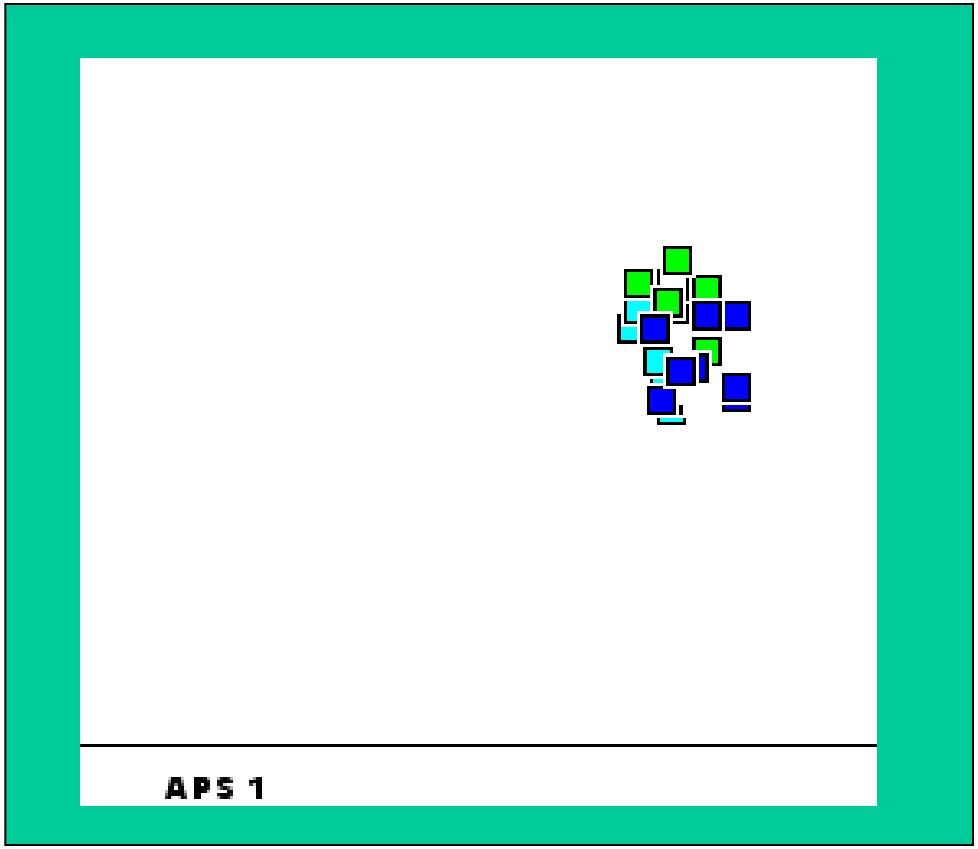
# Results of standardized experiments

**EuroFlow Panel for Plasma Cell Disorders:**
**Can we identify the center?**

Normal BM PC from 24 samples from different centres, acquired over a one year period.




EuroFlow

# EuroFlow


## EuroFlow consortium aims at innovation in flow cytometry

www.euroflow.org