Curso Avanzado de Actualización en Onco-hematología por Citometría de flujo,
Buenos Aires, 30 de mayo de 2011

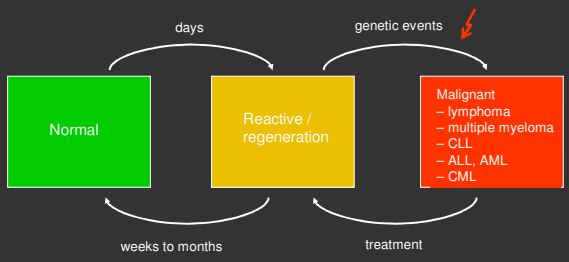
Proyecto EuroFlow: objetivos, resultados y perspectivas futuras

Un nuevo concepto en diagnóstico or citometria de flujo

Jacques J.M. van Dongen & Alberto Orfao
por el grupo

EuroFlow

Discrimination between normal and malignant immune cells in blood, bone marrow and lymphoid tissues



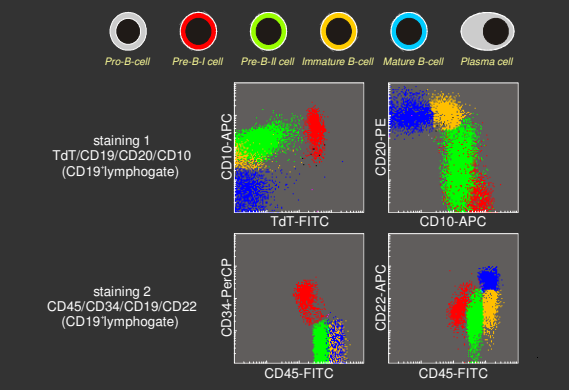
Laboratory methods:
- Cytomorphology and immunophenotyping
- Molecular diagnostics, e.g. PCR-based clonality diagnostics and molecular classification via detection of oncogenetic defects

Diagnostics for 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)

Identification of different B-cell subpopulations in childhood BM



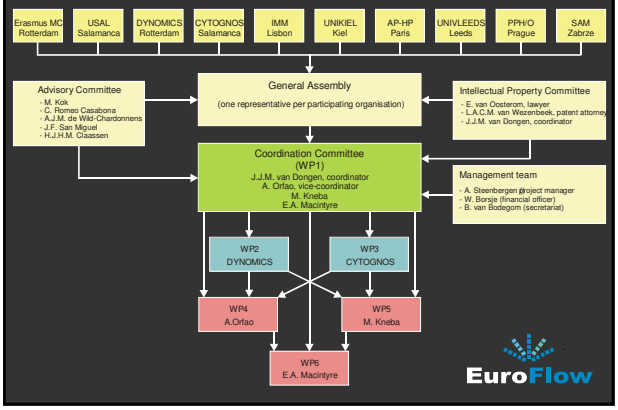
E.G. van Lochem et al., Cytometry Part B 2004; 60B: 1-13.

Flow cytometric immunophenotyping of normal and malignant leukocytes
Gaps and areas for improvement (Status in 2005)

- no technical standardization in flow cytometry
- no guidelines for selection of the appropriate antibody clones
- virtually no new markers introduced over a decade (stand-still in development)
- many oncoproteins (including fusion proteins) not yet included in immunostaining protocols;
- 3- and 4-color flow cytometry has many limitations: limited sensitivity and limited specificity
- management of large data files from multiple samples is complex and time-consuming; new software needed for:
  - fast and easy analysis of data;
  - automated patient reports;
  - introduction of flow data into electronic hospital systems.

→ International collaboration between academia and industry

Management structure of EuroFlow Consortium



5th EuroFlow Educational Workshop, Paris, FR, 9 March 2011

Achievements of the EuroFlow Consortium

Multicolor flow cytometry (≥8 colors) with full technical standardization
- inclusion of violet laser and selection of appropriate fluorochromes
- standardization of instrument settings and laboratory protocols
- detailed testing and comparison of antibody clones and conjugated antibodies (multiple companies)

Implementation and development of novel software
- fast and easy handling of large data files (including automated pattern recognition)
- combining multiple tubes: calculation and APS view
- mapping of diagnosis and follow-up leukemia samples against templates of "normal/control" samples

Development of 8-color antibody protocols for diagnosis, classification and monitoring of hematological malignancies
- screening tubes (include recognition of normal leukocyte subsets)
- multi-tube panels for diagnosis and classification per disease category
- special tubes for MRD monitoring per disease category

Standardization in diagnostic flow cytometry

Standardization according to literature generally refers to:
- lists of CD codes and markers per disease category
- rarely a specific antibody is recommended and (almost) never a fluorochrome is proposed

HOWEVER: Standardization according to GLP guidelines demands for much higher levels of standardization

EuroFlow standardization aims at:
- usage of comparable flow cytometers (3 lasers and ≥ 8 colors)
- full standardization of instrument settings (e.g. based on standard beads)
- standardized laboratory protocols and immunostaining procedures (SOP's)
- careful selection of optimal antibody clones per marker/CD code
- selection of optimal 8-color antibody combinations and fluorochromes
- design of combinations of multiple 8-color tubes: estimation and APS view
- new software for fast and easy data analysis with automated pattern recognition
- recognition of normal and abnormal leukocyte subsets (complete differentiation pathways) with the same immunostaining protocols
- mapping of new patient samples against large data base of earlier collected patient samples, analyzed with the same immunostaining protocol

Fluorochromes for 8-color flow cytometric immunophenotyping

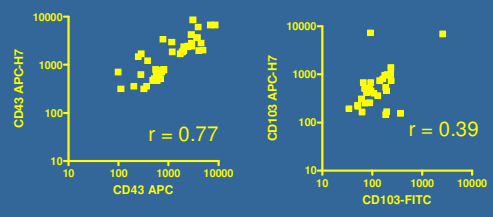
Fluorochrome	Excitation Peak (nm)	Emission Peak (nm)	Lasers: Violet	Lasers: Argon	Lasers: Helium-Neon
Pacific Blue	405	455	+		
AmCyan	405	490	+		
Pacific Orange	405	550	+		
Marina Blue	365	460		+	
FITC	495	520		+	
Phycocrythrin (PE)	565	575		+	
PE Texas Red	565	615		+	
PerCP	488	678		+	
PerCP-Cy5.5	488	695		+	
PE-Cy7	565	770		+	
Allophycocyanin (APC)	650	660			+
Alexa 700	635	720			+
APC-H7	650	770			+

Fluorochromes for 8-color flow cytometric immunophenotyping

Fluorochrome	Excitation Peak (nm)	Emission Peak (nm)	Lasers: Violet	Lasers: Argon	Lasers: Helium-Neon
Pacific Blue/Horizon	405	455	+		
AmCyan	405	490	+		
Pacific Orange/Horizon	405	550	+		
Marina Blue	365	460		+	
FITC	495	520		+	
Phycoerythrin (PE)	565	575		+	
PE Texas Red	565	615		+	
PerCP	488	678		+	
PerCP-Cy5.5	488	695		+	
PE-Cy7	565	770		+	
Allophycocyanin (APC)	650	660			+
Alexa 700	635	720			+
APC-H7	650	770			+

Construction of EuroFlow panels
Fluorochrome conjugates, antibody panels, and antibody combinations

Fluorochrome's choice

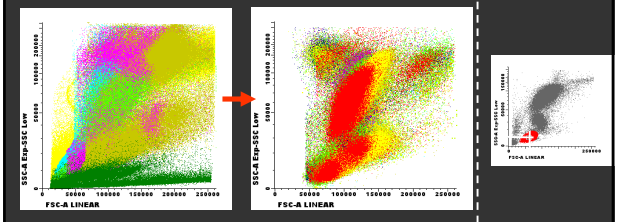


Responsible scientist: Sebastian Bottcher

Synchronized light scatter experiments

"Local" settings

EuroFlow settings



7 different normal PB samples acquired in 7 different centers

Normal PB samples processed according to the standardized EuroFlow sample preparation protocol

5th EuroFlow Educational Workshop,
Paris, FR, 9 March 2011

EuroFlow

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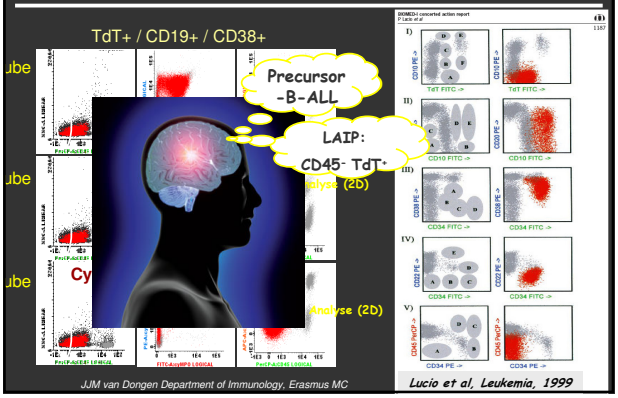
Immunophenotypic classification & identification of LAIP

EuroFlow



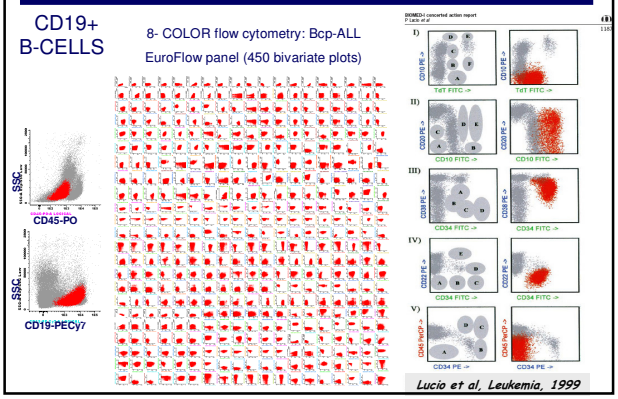
IMMUNOPHENOTYPIC CHARACTERISTICS OF NORMAL vs LEUKEMIC B-CELLS

EuroFlow

CD19+ B-CELLS

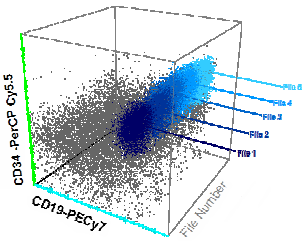
8- COLOR flow cytometry: Bcp-ALL

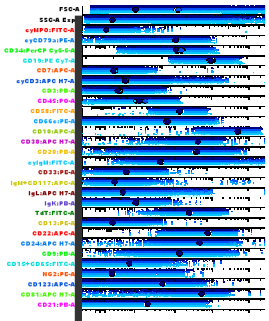
EuroFlow panel (450 bivariate plots)



MERGED DATA FILES FOR SINGLE STEP GATING

Full phenotypic profile





A single gating step for 5 different data files (tubes)

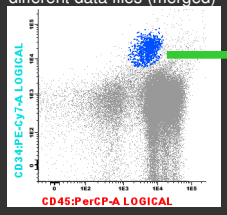
EuroFlow

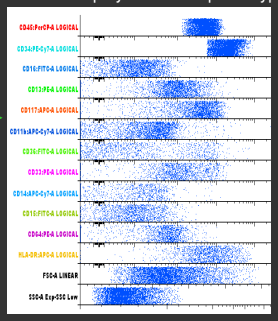
Merging of data files

EuroFlow

Simultaneous display of immunophenotype

A single gating step for five different data files (merged)

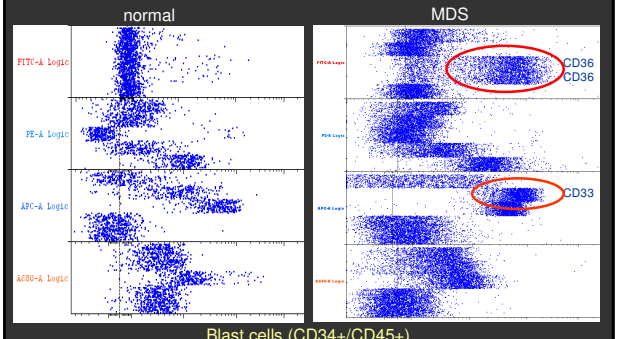




Pedreira et al, Cytometry 2008;73:834

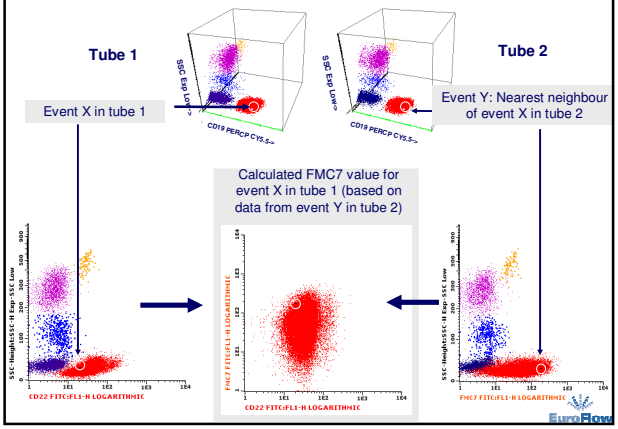
Multicolor analysis: "bar code"

EuroFlow

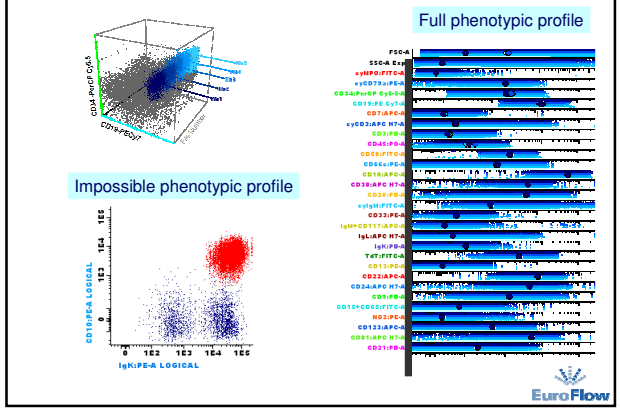


Blast cells (CD34+/CD45+)

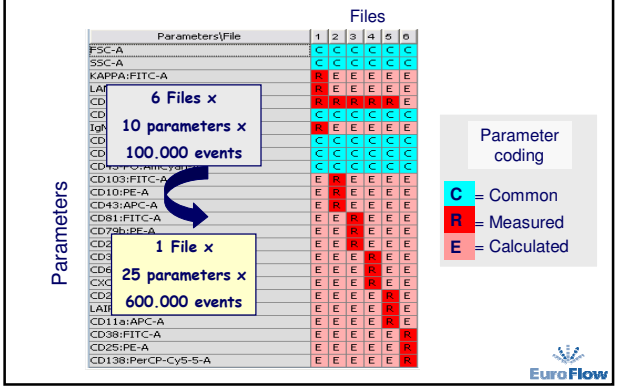
DATA CALCULATION IN THE INFINICYT™ SOFTWARE



MERGED AND CALCULATED DATA FILE



MERGED & CALCULATED LISTMODE DATA FILE



" Merging" by Infinicyt™ program

Integration of results from multiple tubes based on 4 to 6 common parameters per tube

Colors	No. of tubes	Total no. of antibodies	No. of common parameters: antibodies	No. of common parameters: Scatter	End result no. of parameters
4-colors	8	32	2	2	20 (18+2)
6-colors	6	36	3	2	23 (21+2)
8-colors	4	32	4	2	22 (20+2)
8-colors	4	32	3	2	25 (23+2)

→ Development of 8-color multi-tube antibody protocols (3 or 4 antibodies in common per tube in each protocol)

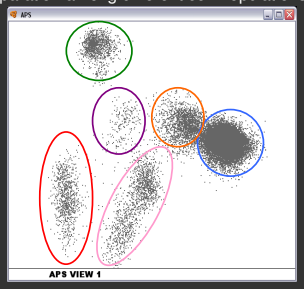
EuroFlow

Automatic identification of populations

EuroFlow

Multidimensional analysis:

Automated Separation among different cell Populations (APS view)



www.infinicyt.com

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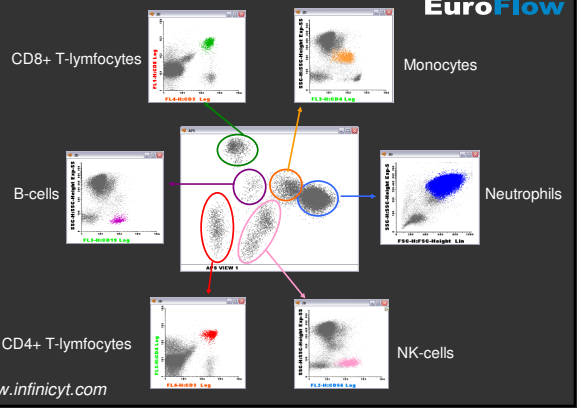
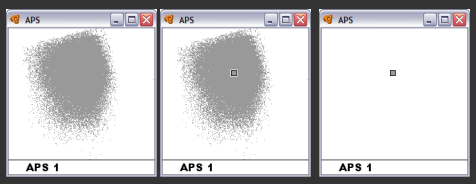


www.infinicyt.com

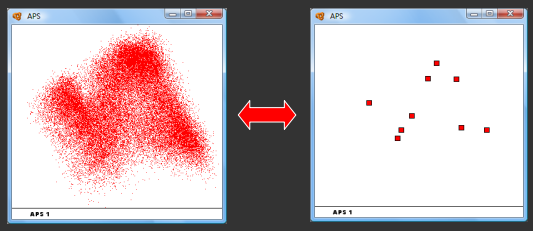
APS Procedure for AUTOMATIC ANALYSIS

VISUALIZATION OPTIONS



Dots | Dots / Mean | Mean

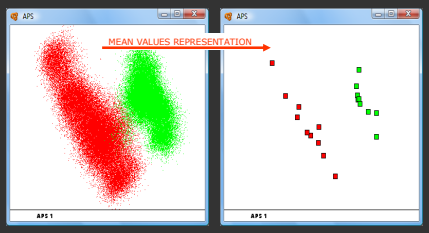
APS Procedure for groups of patients



APS Dots View | APS Means View

Group of patients with the same panel/protocol applied and same disease category

APS Procedure for groups of patients

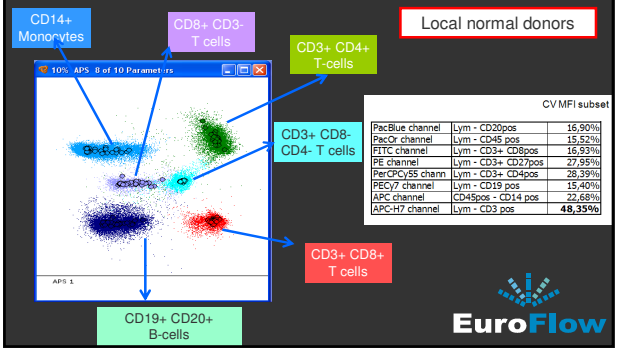


Each dot = patient/sample

Group of patients with same panel/protocol applied and 2 different disease categories

Results of synchronized experiments

APS view of 30 merged data files from different centers



EuroFlow

5th EuroFlow Educational Workshop, Paris, FR, 9 March 2011

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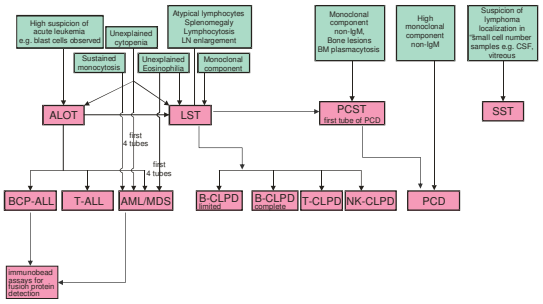
EuroFlow antibody protocols

EuroFlow

Development of 8-color multi-tube antibody protocols
(3 or 4 antibodies in common per tube in each protocol)

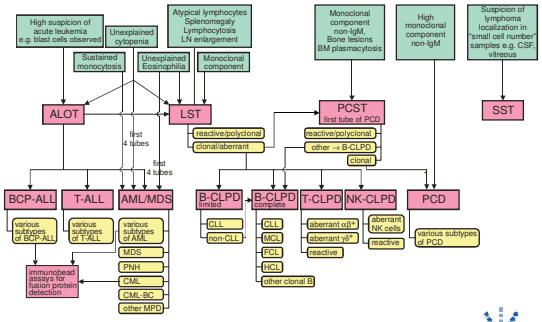
1. Screening tubes (include recognition of normal leukocyte subsets)
   - Acute leukemia orientation tube (ALOT): 1 tube (L Lhermitte)
   - Lymphoid screening tube (LST): 1 tube (J Flores Montero)
   - Small sample screening tube (SST): 1 tube (AW Langerak)
   - Plasma cell dyscrasia tubes (PCD): 2 tubes (J Flores Montero)
2. Multi-tube panels for characterization per disease category
   - B-cell precursor ALL (BCP-ALL) protocol: 4 tubes (L Lhermitte)
   - T-cell ALL (T-ALL) protocol: 4 tubes (V Asnafi)
   - AML/MDS protocol: 7 tubes (VHJ van der Velden)
   - B chronic lymphoproliferative diseases (B-CLPD): 5 tubes (S Böttcher)
   - T chronic lymphoproliferative diseases (T-CLPD): 6 tubes (J Almeida)
   - NK chronic lymphoproliferative diseases (NK-CLPD): 3 tubes (J Almeida)

Algorithm for EuroFlow antibody panels in hemato-oncology



EuroFlow

Algorithm for EuroFlow antibody panels in hemato-oncology



EuroFlow

Acute leukemia orientation tube (ALOT)

ALOT *1 tube*

BCP-ALL *4 tubes* | T-ALL *4 tubes* | AML/MDS *4 to 7 tubes*

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

EuroFlow

Responsible scientist: L Lhermitte

Single tube EuroFlow screening tube for acute leukemias

Acute Leukemia Orientation Tube (ALOT)*

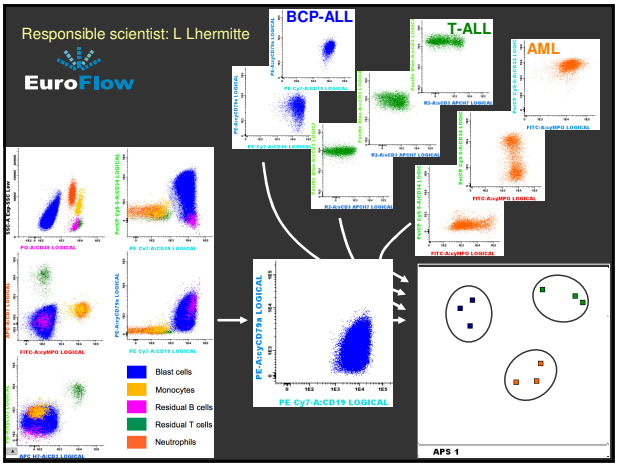
Responsible scientist: L Lhermitte

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

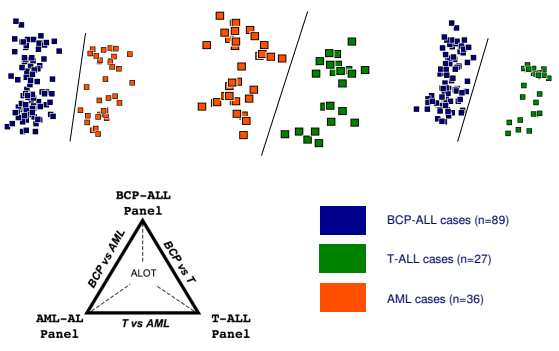
* Backbone markers are indicated in bold; cy= cytoplasmic; sm= surface membrane.

EuroFlow

Responsible scientist: L Lhermitte



ALOT *(Acute Leukemia Orientation Tube)*



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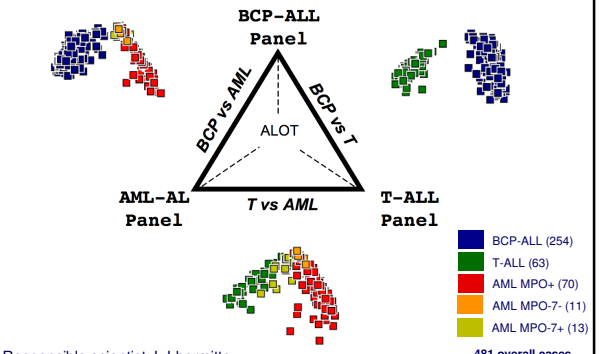
ALOT *(Acute Leukemia Orientation Tube)*



Responsible scientist: L Lhermitte

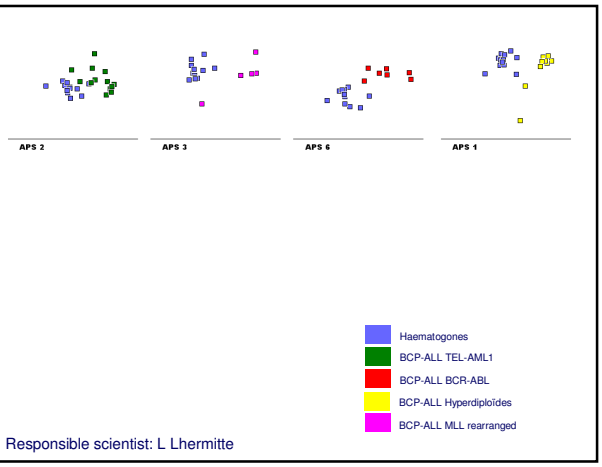
Multi-tube EuroFlow classification panel for B-cell precursor ALL (BCP-ALL)*

Responsible scientist: L Lhermitte

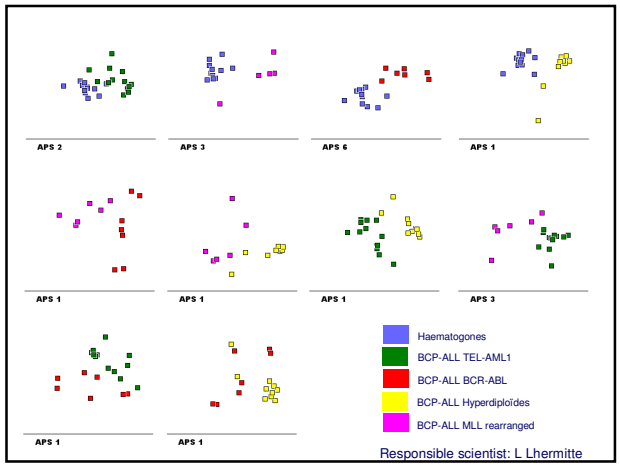
Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
1	CD20	**CD45**	CD58	CD66c	**CD34**	**CD19**	CD10	CD38	Diagnosis and classification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations
2	smIgκ	**CD45**	cyIgμ	CD33	**CD34**	**CD19**	smIgμ and CD117	smIgλ	Diagnosis and classification of BCP-ALL;
3	CD9	**CD45**	nuTdT	CD13	**CD34**	**CD19**	CD22	CD24	Diagnosis and classification of BCP-ALL; Detection of phenotypes associated with molecular aberrations; Detection of LAP markers
4	CD21	**CD45**	CD15 and CDw65	NG2	**CD34**	**CD19**	CD123	CD81	Subclassification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations

* Backbone markers are indicated in bold; cy= cytoplasmic.
** The described marker combinations can also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics).

EuroFlow



Responsible scientist: L Lhermitte



Responsible scientist: L Lhermitte

Multi-tube EuroFlow classification panel for T-ALL.*

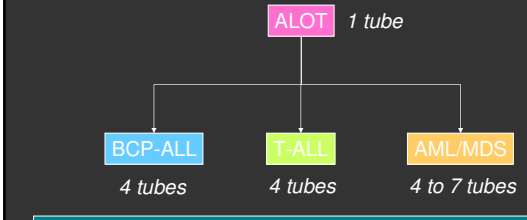
Responsible scientist: V Asnafi

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
1	**cyCD3**	**CD45**	nuTdT	CD99	CD5	CD10	CD1a	**smCD3**	Diagnosis and classification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations
2	**cyCD3**	**CD45**	CD2	CD117	CD4	CD8	CD7	**smCD3**	Diagnosis and classification of BCP-ALL;
3	**cyCD3**	**CD45**	TCRγδ	TCRαβ	CD33	CD56	cyTCRβ	**smCD3**	Diagnosis and classification of BCP-ALL; Detection of phenotypes associated with molecular aberrations; Detection of LAP markers
4	**cyCD3**	**CD45**	CD45	CD13	HLADR	CD45RA	CD123	**smCD3**	Subclassification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations

* Backbone markers are indicated in bold; cy= cytoplasmic; sm= surface membrane; nu= nuclear.
** The described marker combinations can also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics).

EuroFlow

Acute leukemia orientation tube (ALOT)

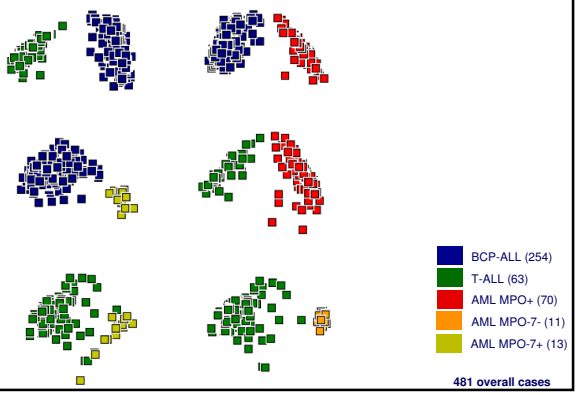


Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
cyCD3	**CD45**	cyMPO	cyCD79a	**CD34**	**CD19**	CD7	**smCD3**

EuroFlow

Responsible scientist: L Lhermitte

ALOT *(Acute Leukemia Orientation Tube)*



Multi-tube EuroFlow classification panel for B-cell precursor ALL (BCP-ALL)*

Responsible scientist: L Lhermitte

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
1	CD20	**CD45**	CD58	CD66c	**CD34**	**CD19**	CD10	CD38	Diagnosis and classification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations
2	smIgκ	**CD45**	cyIgμ	CD33	**CD34**	**CD19**	smIgμ and CD117	smIgλ	Diagnosis and classification of BCP-ALL;
3	CD9	**CD45**	nuTdT	CD13	**CD34**	**CD19**	CD22	CD24	Diagnosis and classification of BCP-ALL; Detection of phenotypes associated with molecular aberrations; Detection of LAP markers
4	CD21	**CD45**	CD15 and CDw65	NG2	**CD34**	**CD19**	CD123	CD81	Subclassification of BCP-ALL; Detection of LAP markers; Detection of phenotypes associated with molecular aberrations

* Backbone markers are indicated in bold; cy= cytoplasmic.
** The described marker combinations can also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

EuroFlow

Acute leukemia orientation tube (ALOT)

ALOT *1 tube*

BCP-ALL *4 tubes* | T-ALL *4 tubes* | AML/MDS *4 to 7 tubes*

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

EuroFlow

Responsible scientist: L Lhermitte

5th EuroFlow Educational Workshop, Paris, FR, 9 March 2011

EuroFlow

Achievements of the EuroFlow Consortium

Multicolor flow cytometry (≥8 colors) with full technical standardization
- inclusion of violet laser and selection of appropriate fluorochromes
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Implementation and development of novel software
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Development of 8-color antibody protocols for diagnosis, classification and monitoring of hematological malignancies
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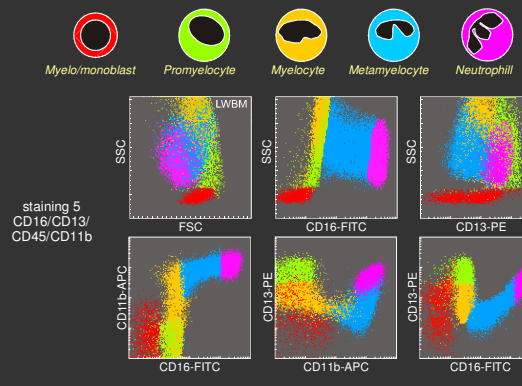
- special tubes for MRD monitoring per disease category

Identification of different granulocytic subpopulations in childhood BM



E.G. van Lochem et al., Cytometry Part B 2004; 60B: 1-13.

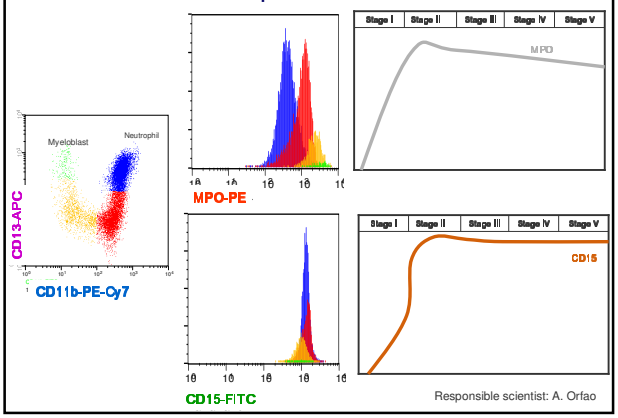
Multi-tube EuroFlow classification panel for AML/MDS

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 focused 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

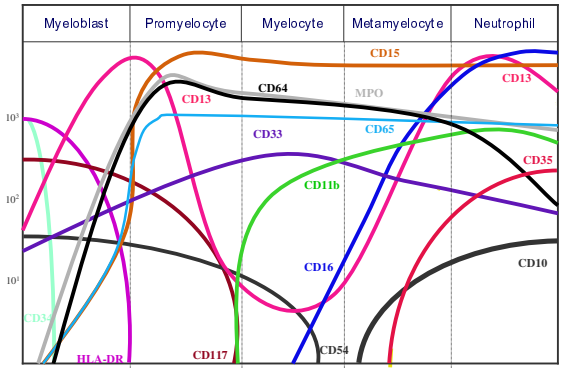
* Further information about the markers and the availability of hybridoma clones is summarized in Appendix A. Backbone markers are indicated in bold; nu= nuclear.
** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

Neutrophil maturation



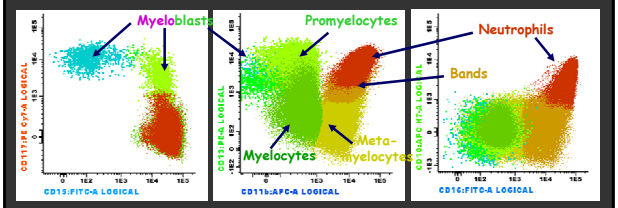
Responsible scientist: A. Orfao

Phenotypic changes during normal neutrophil differentiation

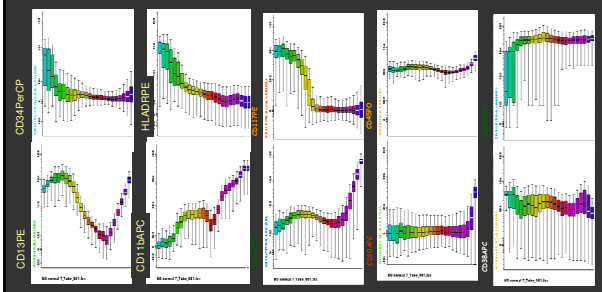


N-DIMENSIONAL NEUTROPHIL MATURATION IN NORMAL BM

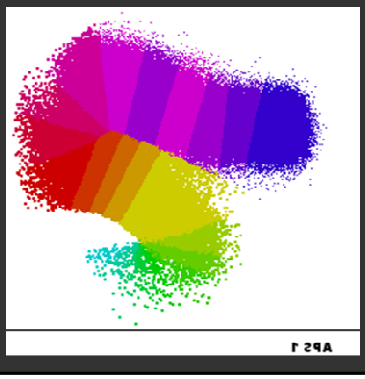
EuroFlow



Maturation stage of neutrophil precursors in normal BM



Maturation stage of neutrophil precursors in normal BM



5th EuroFlow Educational Workshop, Paris, FR, 9 March 2011

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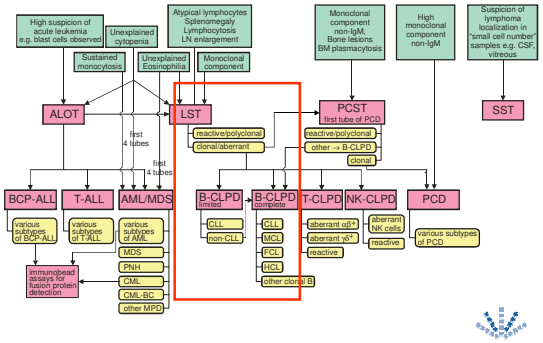
- special tubes for MRD monitoring per disease category

Algorithm for EuroFlow antibody panels in hemato-oncology



EuroFlow

Multi-tube EuroFlow classification combinations for B-cell chronic lymphoproliferative diseases (B-CLPD) *

Responsible scientist: S Böttcher supported by A Rawstron and J Flores Montero

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
1	**CD20** and CD4	**CD45**	CD8 and smIgλ	CD56 and smIgκ	CD5	**CD19** and TCRγδ	smCD3 8	CD38	LST tube; detection of B-CLPD
2	**CD20**	**CD45**	CD23	CD10	CD79b	**CD19**	CD200	CD43	Identification of CLL vs other B-CLPD cases, when combined with LST
3	**CD20**	**CD45**	CD31	LAIR1	CD11c	**CD19**	smIgμ	CD81	Further subclassification of non-CLL B-CLPD, e.g. HCL, MCL, FL, MZL, LPL, DLBCL and other B-CLPD
4	**CD20**	**CD45**	CD103	CD95	CD22	**CD19**	CXCR5	CD49d	
5	**CD20**	**CD45**	CD62L	CD39	HLADR	**CD19**	CD27		

* Backbone markers are indicated in bold; sm= surface membrane.
** The described marker combinations might also be applied for disease staging and monitoring of treatment effectiveness (MRD diagnostics)

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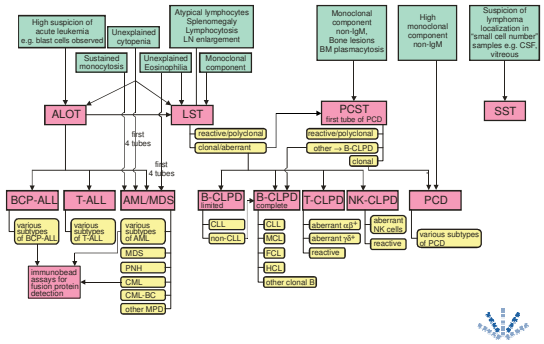

EuroFlow

Technical aspects of EuroFlow protocols: instrument settings, fluorochrome choice, standardization

T. Kalina[1], J. Flores-Montero[2], Q. Lecrevisse[2], M. Cullen[3],L. Lhermitte[4], L. Sedek[5], A. Mendonca[6], S. Bötcher[7], J. te Marvelde[8], Mejstříková, O. Hrušák[1], J.J.M. van Dongen[8], and A. Orfao[2]

On behalf of the EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708)

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3, St. James University Hospital, Leeds, UK;
4, Department of Hematology, Hôpital Necker, Paris, FR
5, Department of Pediatric Hematology and Oncology, Medical University of Silesia, Zabrze, PL;
6, Department of Hematology, Instituto Portugues de Oncologia , Lisbon, PT;
7, 2nd Department of Medicine, University Klinik Schleswig-Holstein, Kiel, DE;
8, Department of Immunology, Erasmus MC, Rotterdam, NL;

To be published in: Leukemia 2011; 25: xxxx-xxxx

EuroFlow

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

J.J.M. van Dongen, L. Lhermitte, S. Böttcher, J. Almeida, V.H.J. van der Velden, J. Flores-Montero, A. Rawstron, V.Asnafi, Q. Lécrevisse, P. Lucio, E. Mejstrikova, T. Szczepański, T. Kalina, R. de Tute, M. Brüggemann, L. Sedek, M. Cullen, A.W. Langerak, A. Mendonça, E. Macintyre, M. Martin-Ayuso,O. Hrusak, M.B. Vidriales, and A. Orfao

On behalf of the EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708)

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5, St. James University Hospital, Leeds, UK;
6, Department of Hematology, Instituto Portugues de Oncologia , Lisbon, PT;
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To be published in: Leukemia 2011; 25: xxxx-xxxx

EuroFlow

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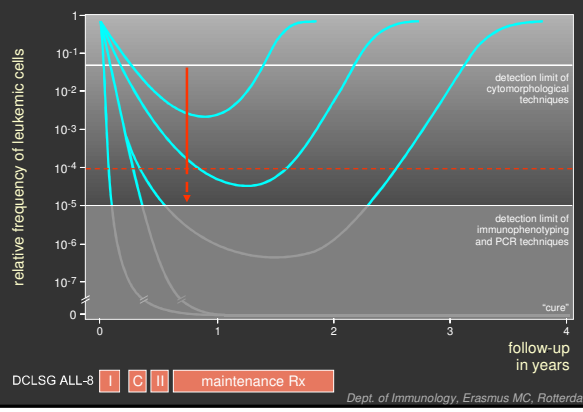
Detection of minimal residual disease (MRD)



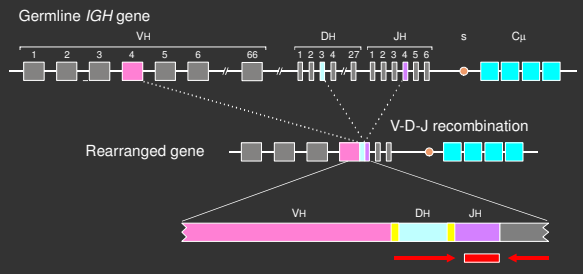
DCLSG ALL-8 | I | C | II | maintenance Rx

Dept. of Immunology, Erasmus MC, Rotterdam

Detection of minimal residual disease in ALL

Technique	Applicability	Detection limit	Remark
Flow cytometry (4 colors)	BCP-ALL: 85% T-ALL: 90%	(10^{-3}-) 10^{-4}	Fast, but variable sensitivity because of similarities between normal (regenerating) cells and malignant cells
PCR of Ig/TCR genes	BCP-ALL: 95% T-ALL: 95%	10^{-4}-10^{-5}	Time consuming and relatively expensive (junctional region sequencing), but applicable in ≥ 95% of lymphoid malignancies
PCR of fusion transcripts	BCP-ALL: 40% T-ALL: 25%	10^{-4}-10^{-6}	Limited applicability in ALL, but potentially useful in specific subgroups, e.g. BCR-ABL cases in specific protocols

PCR analysis of Ig/TCR genes



Dept. of Immunology, Erasmus MC, Rotterdam

EuroMRD: European Study Group on MRD detection (ESG-MRD-ALL)

EuroMRD

AIMS of EuroMRD (focus on PCR ananlysis of Ig/TCR genes):

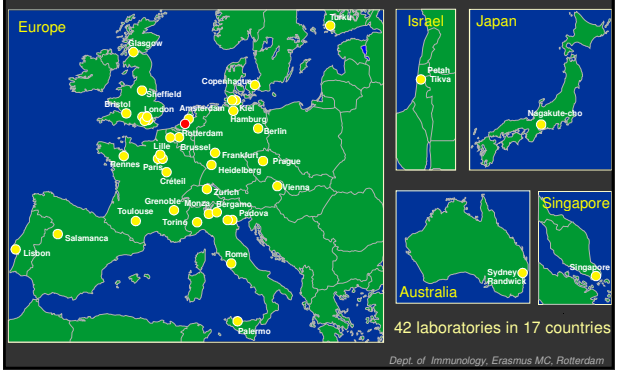
1. Quality Control Program: 2 times per year: - February / March
   - August / September
2. Educational meetings including evaluation of quality control rounds:
   2 times per year: - May / June
   - October / November
3. Standardization of MRD techniques
   - standardization techniques within each treatment protocol
   - guidelines for interpretation of RQ-PCR results
4. Collaborative development and clinical evaluation of new MRD strategies and new MRD techniques

→ Basis for accreditation of MRD diagnostics

EuroMRD

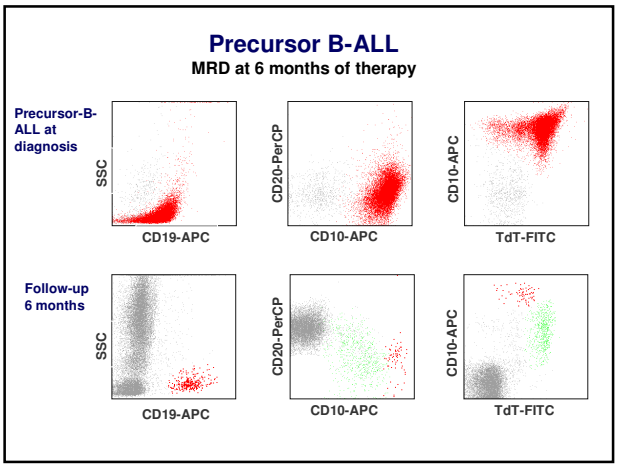
European Study Group on MRD detection

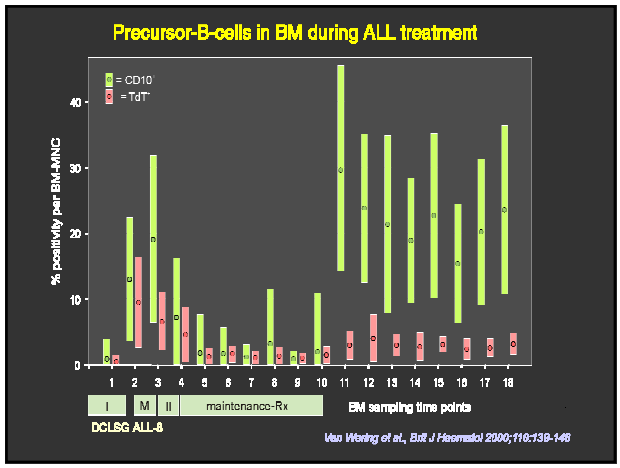
Chairman: J.J.M. van Dongen

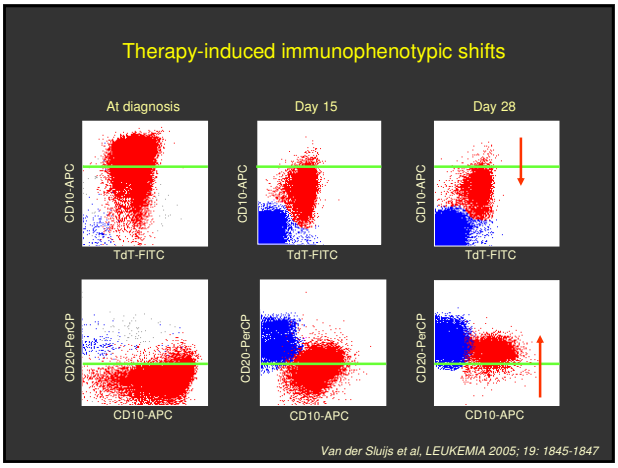


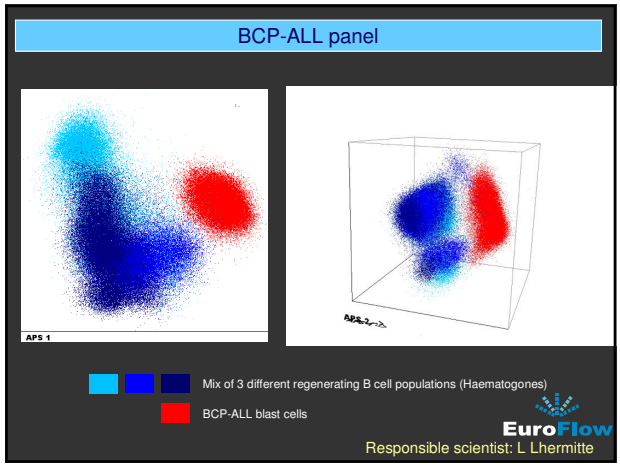
42 laboratories in 17 countries

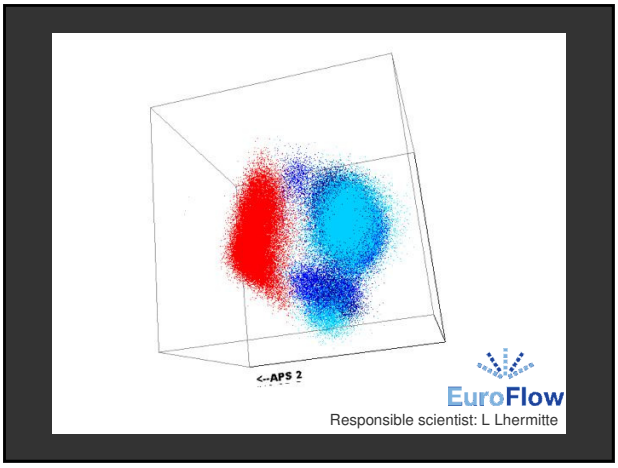
Dept. of Immunology, Erasmus MC, Rotterdam











Development of 8-color MRD panels

EuroFlow

Single-tube antibody EuroFlow MRD protocols under evaluation

1. Acute leukemias (includes recognition of normal precursors)
   - Acute myeloid leukemia panel (AML-MRD): 1 tube per pathway (A. Orfao)
   - B-cell precursor (BCP-ALL-MRD): 1 tube (V. van der Velden, E. Mejstrikova)
   - T-cell ALL (T-ALL-MRD): 1 tube (V.Asnafi)

2. Chronic lymphoproliferative disorders (includes recognition of normal cells)
   - Chronic lymphocytic leukemia (CLL-MRD): 1 tube (A. Langerak)
   - Hairy cell leukemia (HCL-MRD): 1 tube (E. MacIntyre)
   - Mantle cell lymphoma (MCL-MRD): 1 tube (S. Böttcher)
   - Follicular lymphoma (FL-MRD): 1 tube (S. Böttcher)
   - Marginal zone lymphoma (MZL-MRD): 1 tube (P.Lucio)
   - Lymphoplasmacytic lymphoma (LPL-MRD): 1 tube (P. Lucio)
   - Diffuse large B-cell lymphoma (DLBCL-MRD): 1 tube (P. Lucio)
   - Burkitt lymphoma (BL): 1 tube (L.Lhermitte)
   - T-chronic lymphoproliferative diseases (T-CLPD-MRD): 1 tube (J. Almeida)
   - Multiple myeloma (MM): 1 tube (J. Flores)

Achievement of the EuroFlow Consortium: New concept in diagnostic flow cytometry

1. Full technical standardization of multicolor flow cytometry (≥8 colors)
   - standardization of instrument settings and laboratory protocols
   - selection of fluorochromes and selection of antibody clones per marker
   - EuroFlow protocols work on all tested ≥8 colors flow cytometers:
     - DAKO Cyan, LSR-II, FACS Canto-II;
     - "late arrivals" (Navios and Gallios) still to be tested (new Workpackage)

2. Implementation and further development of novel software: Infinicyt
   - fast and easy data handling with automated pattern recognition
   - combining multiple tubes: calculation and APS (principle component analysis
   - mapping of diagnosis and follow-up leukemia samples against templates of "normal/control" samples

3. Development of 8-color antibody protocols for diagnosis, classification and monitoring of hematological malignancies
   - 8-color panels are based on recognition of normal cells & differentiation pathways
   - diagnosis and classification tubes are ready; MRD tubes in development
   - flexibility within panels: deletion and inclusion of markers and tubes is possible

4. Large EuroFlow data base linked to Infinicyt software

EuroFlow participants

www.euroflow.org

University Institutes / Medical Schools

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CYTOGNOS, Salamanca, ES	M. Martin, J. Bensadon, J. Hernandez, M. Muñoz a.o.

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Paulo Lucio
Andreia Mendonça
Evan Jensen
Tomek Szczepanski
Lukasz Sedek
Ondrej Hrusak
Tomas Kalina
Ester Mejstrikova
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Vincent van der Velden
Jeroen Te Marvelde
Alita van der Sluijs
Juan Flores-Montero
Julia Almeida
Quentin Lecrevisse

MUCHAS GRACIAS