20th International Leukemia Workshop
Mannheim, DE, 1-3 July 2011

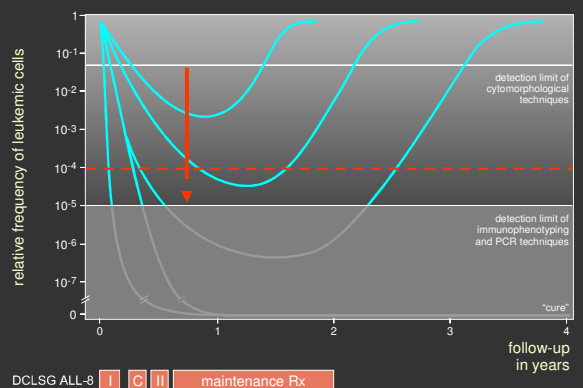
MRD detection in acute leukemia

Novel concepts and developments for flow cytometric MRD detection

Jacques J.M. van Dongen
on behalf of

EuroMRD & EuroFlow

Detection of minimal residual disease (MRD) in ALL

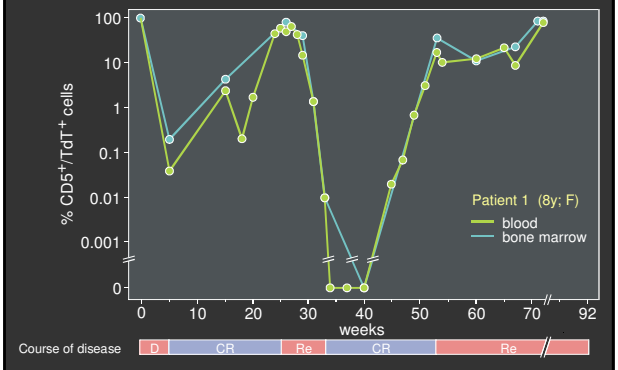


DCLSG ALL-8 | I | C | II | maintenance Rx

Dept. of Immunology, Erasmus MC, Rotterdam

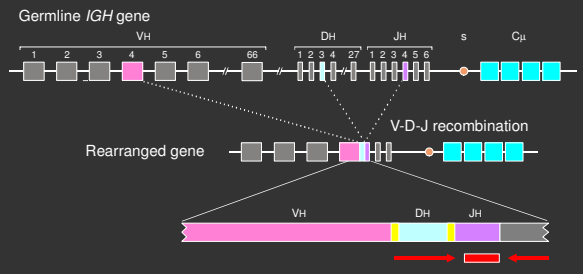
Monitoring of a T-ALL patient

1986



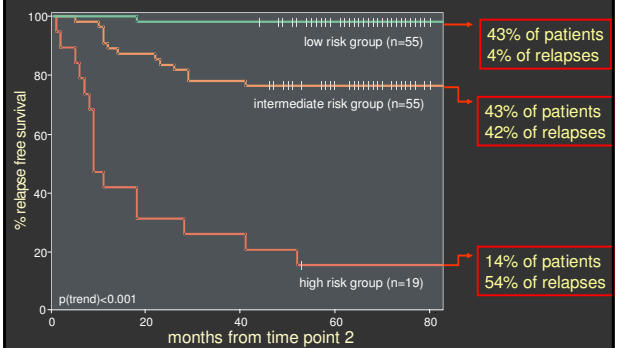
Dept. of Immunology, Erasmus MC, Rotterdam

PCR analysis of Ig/TCR genes



Dept. of Immunology, Erasmus MC, Rotterdam

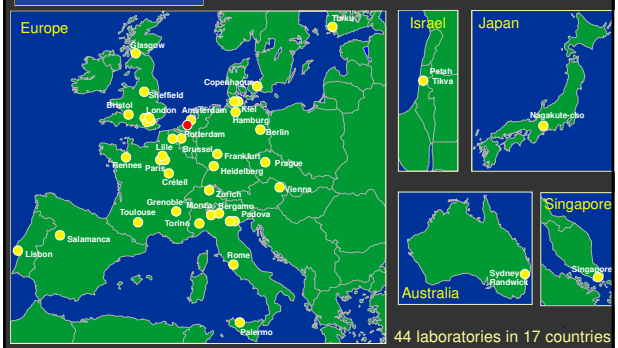
Relapse free survival in I-BFM-SG study according to the combined MRD information at time points 1 and 2 (n=129)



I-BFM-SG Report, J.J.M. van Dongen et al, Lancet 1998;352:1731-1738

EuroMRD

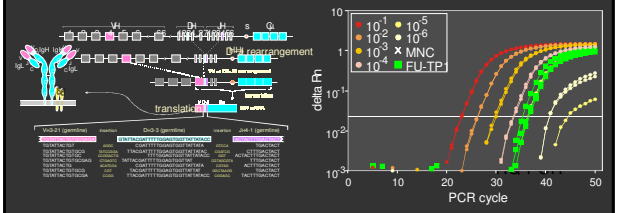
European Study Group on MRD detection
Chairman: J.J.M. van Dongen



44 laboratories in 17 countries

Supported by Leukaemia & Lymphoma Research, LeukemiaNet, and EuroClonality

Current MRD technique in lymphoid malignancies



Disadvantages of Ig/TCR-based MRD-PCR techniques:

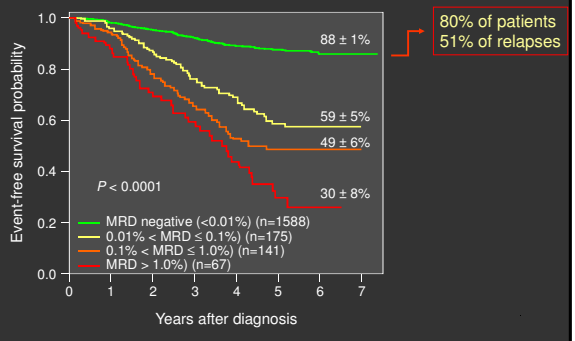
- labor intensive (junctional regions per patient);
- require specialized laboratories;
- time consuming (target identification: 4 to 6 weeks)

→ Faster technique needed: 8-color flow cytometry ?

Comparison between molecular techniques and flow cytometry in hematological malignancies

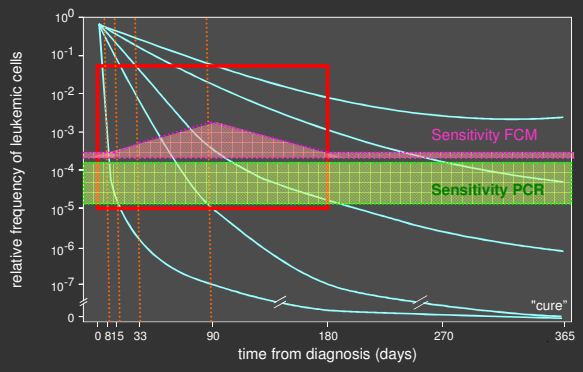
	Molecular techniques	Flow cytometry
Speed	2-3 days (up to weeks)	fast: 1-2 hours !!
Target	DNA or RNA (RNA is an instable target)	protein/cells ("end-product")
Applicability	depends on disease (chromosome aberrations)	broad
Multiplexing	technically demanding	relatively easy (even 25 to 100 tests per tube)
Accuracy	semi-quantitative	quantitative
Focus	all cells in sample (or: prior purification)	any subpopulation
Facilities	special laboratories needed (pre-PCR lab, PCR lab, etc)	only standard lab needed (+ flow cytometer)

EFS of MRD-based risk groups (FCM at day 29) in COG protocol



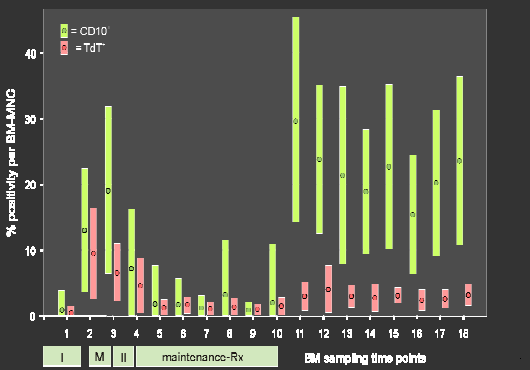
Borowitz et al., Blood 2008; 111: 5477-5485.

MRD window, time points, MRD techniques and QR & sensitivity



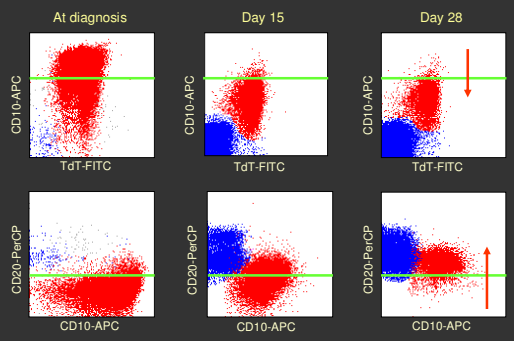
Dept. of Immunology, Erasmus MC, Rotterdam

Precursor-B-cells in BM during ALL treatment



Van Wering et al., Brit J Haematol 2000;110:139-148

Therapy-induced immunophenotypic shifts



Van der Sluijs et al, LEUKEMIA 2005; 19: 1845-1847

20th International Leukemia Workshop, Mannheim, DE, 1-3 July 2011

Standardization in diagnostic flow cytom

EuroFlow

Standardization according to literature gener
- lists of CD codes and markers per di
- rarely a specific antibody is recom lever a fluorochrome is proposed

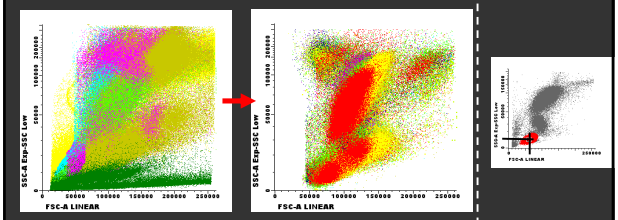
HOWEVER: Standardizat uidelines demands for much higher le

EuroFlow st
- usag ytometers (3 lasers and ≥ 8 colors)
- f strument settings (e.g. based on standard beads)
- sta tory protocols and immunostaining procedures (SO
- carefu tion 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

Full standardization and novel software (fast and easy)

Standardization is essential, e.g. Synchronized light scatter experiments

"Local" settings | EuroFlow settings



7 different normal PB samples acquired in 7 different centers

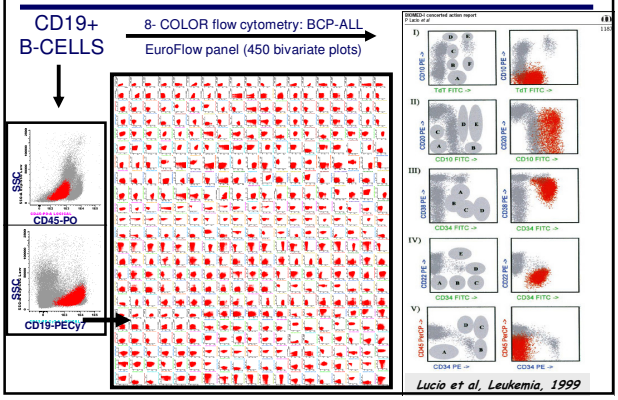
Normal PB samples processed according to the standardized EuroFlow sample preparation protocol

EuroFlow

Flow cytometric immunphenotyping of nomal and leukemic precursor-B-cells

EuroFlow

CD19+ B-CELLS → 8- COLOR flow cytometry: BCP-ALL → EuroFlow panel (450 bivariate plots)



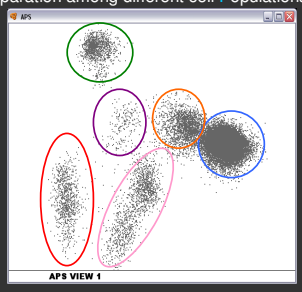
Lucio et al, Leukemia, 1999

Automatic identification of populations

EuroFlow

Multidimensional analysis:

Automated Separation among different cell Populations (APS view)



www.infinicyt.com

Automatic identification of populations

EuroFlow

CD8+ T-lymfocytes

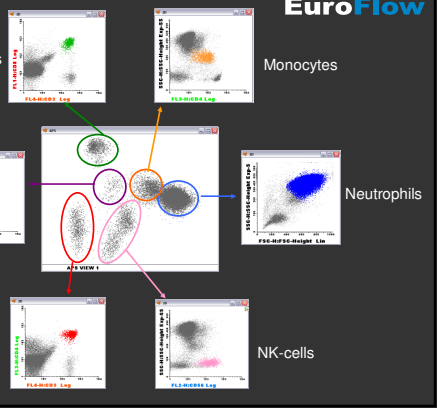
Monocytes

B-cells

Neutrophils

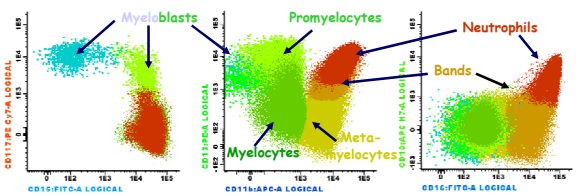
CD4+ T-lymfocytes

NK-cells



www.infinicyt.com

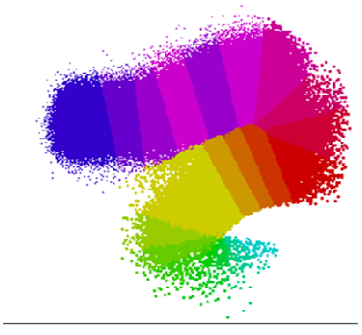
Maturation of neutrophil precursors in normal BM



Responsible scientist: V.H.J. van der Velden

EuroFlow

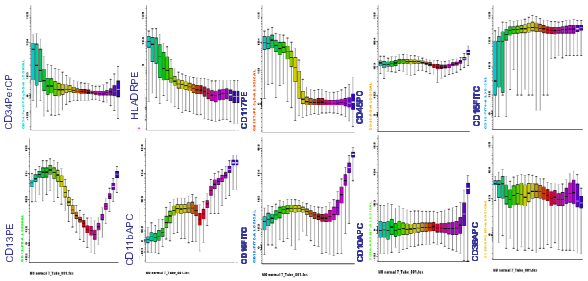
Maturation stage of neutrophil precursors in normal BM



Responsible scientist: A. Orfao

EuroFlow

Maturation stage of neutrophil precursors in normal BM

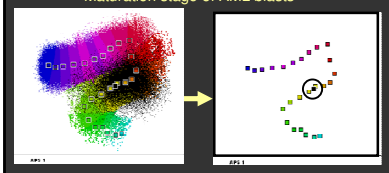


Responsible scientist: A. Orfao

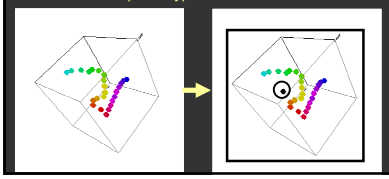
EuroFlow

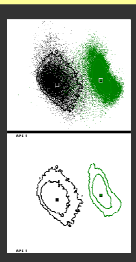
N-dimensional neutrophil maturation in normal BM versus AML

Maturation stage of AML blasts



Aberrant phenotype of AML blasts



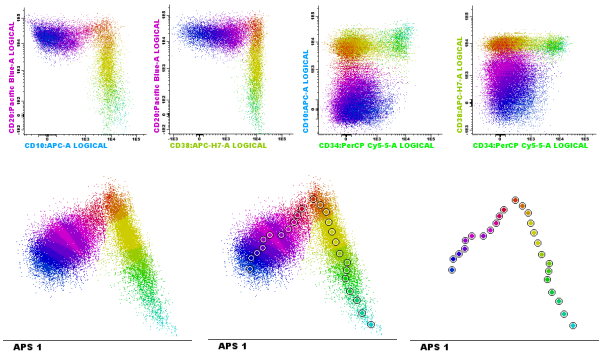


Aberrant markers

CD15	26.6
CD117	19.4
SSC	19.2
FSC	19.0
CD11b	6.0

Dissection of normal precursor-B-cell differentiation EuroFlow immunostaining 1

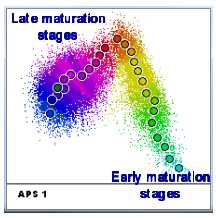
EuroFlow



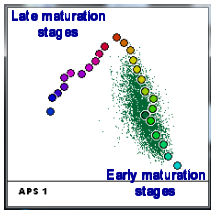
Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

Precursor B-cell differentiation in normal vs regenerating bone marrow in EuroFlow immunostaining 1

Normal BM



Normal vs Regenerating BM



CD19 Gated B-cells (excluding PC)

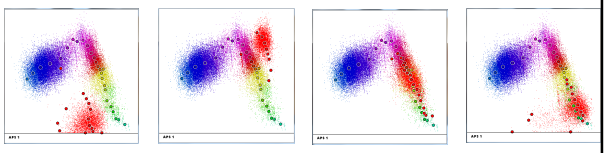
Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

EuroFlow

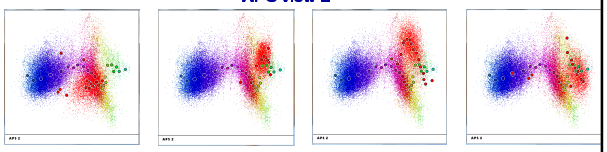
Four BCP-ALL cases vs normal precursor B-cells in EuroFlow immunostaining 1

EuroFlow

APS view 1



APS view 2

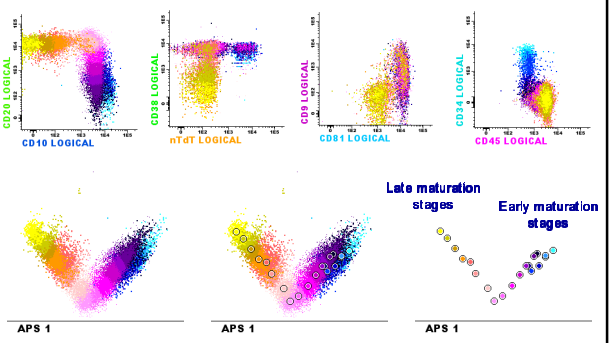


Case 1 **Case 2** **Case 3** **Case 4**

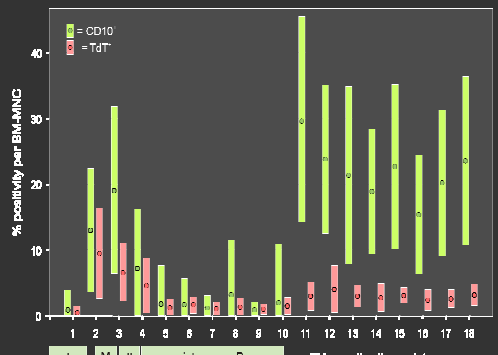
Responsible scientists: V.H.J. van der Velden and E. Mejstrikova

Designed by: A. Orfao & Q Lecrevisse

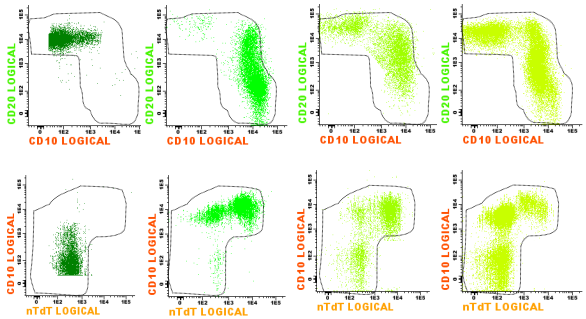
Dissection of normal precursor-B-cell differentiation
EuroFlow immunostaining 2



Precursor-B-cells in BM during ALL treatment

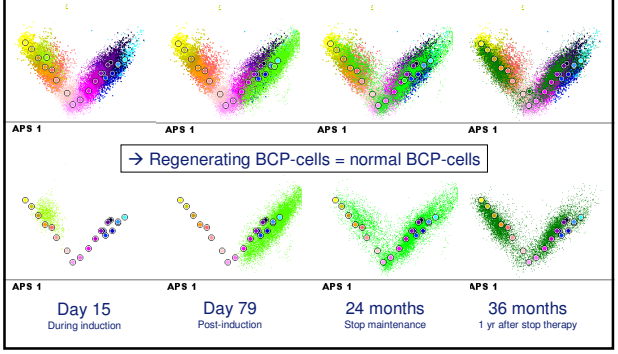


B-cell regeneration: time-point dependent



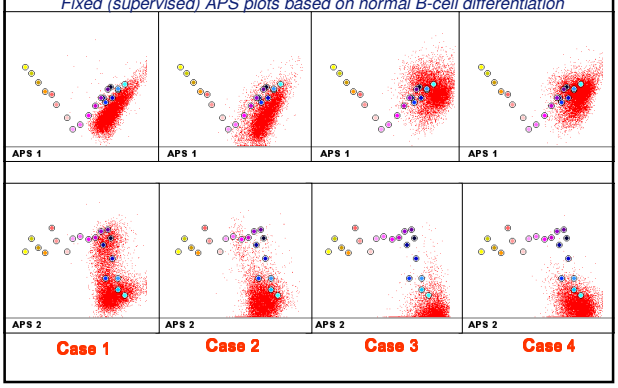
Regenerating B-cells: identical to normal B-cells
(in EuroFlow immunostaining 2)

Fixed (supervised) APS plots based on normal B-cell differentiation



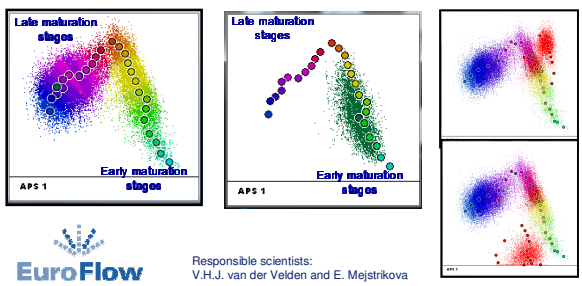
BCP-ALL versus normal B-cell differentiation
(in EuroFlow immunostaining 2)

Fixed (supervised) APS plots based on normal B-cell differentiation



New concept for flow cytometric MRD detection

Discrimination between normal-regenerating precursor-B-cells and aberrant ALL blast cells via flow cytometric PCA (automated population separation)



Responsible scientists:
V.H.J. van der Velden and E. Mejstrikova

20th International Leukemia Workshop, Mannheim, DE, 1-3 July 2011

Conclusions

1. PCR-based MRD diagnostics (IG/TCR genes or fusion genes) is currently the gold standard in most European ALL protocols
2. Differences in MRD value between ALL protocols are mainly caused by application of different non-standardized MRD techniques, which also differ in sensitivity (e.g. current flow cytometry does not reach ≤ 10^{-3}).
3. Novel concepts in ≥8-color flow cytometry can potentially replace PCR-based MRD diagnostics, based on discrimination of normal-regenerating precursor cells from aberrant blasts cells via PCA (see APS tool developments by EuroFlow)
4. Full standardization, regular Quality Control, and guidelines for data interpretation and data reporting are essential for international comparability of MRD results (within and between treatment protocols).

Collaborative international networks are essential for innovation

EuroFlow

EuroFlow is an independent scientific consortium, which aims at innovation in flow cytometry for improvement of diagnostic patient care

Chairmen:
J.J.M. van Dongen & A. Orfao

20 laboratories in 14 countries

www.euroflow.org