Flow Cytometry Workshop

19-21 SEPTEMBER 2018

ASTANA, KAZAKHSTAN

Advancements of the I-BFM FLOW network: Innovative solutions for diagnosis & MRD assessment in acute leukemias: B-ALL and others

Michael N. Dworzak

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Vienna, Austria, EU

IFCC International Federation of Clinical Chemistry and Laboratory Medicine

BECKMAN COULTER Life Sciences

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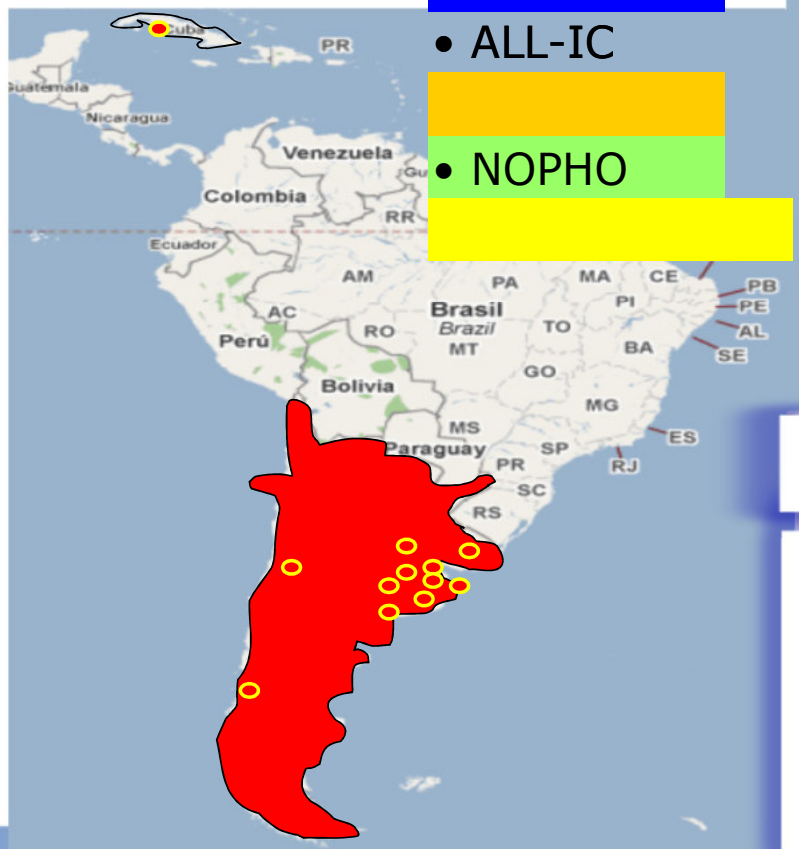
Conflict of interest disclosure:

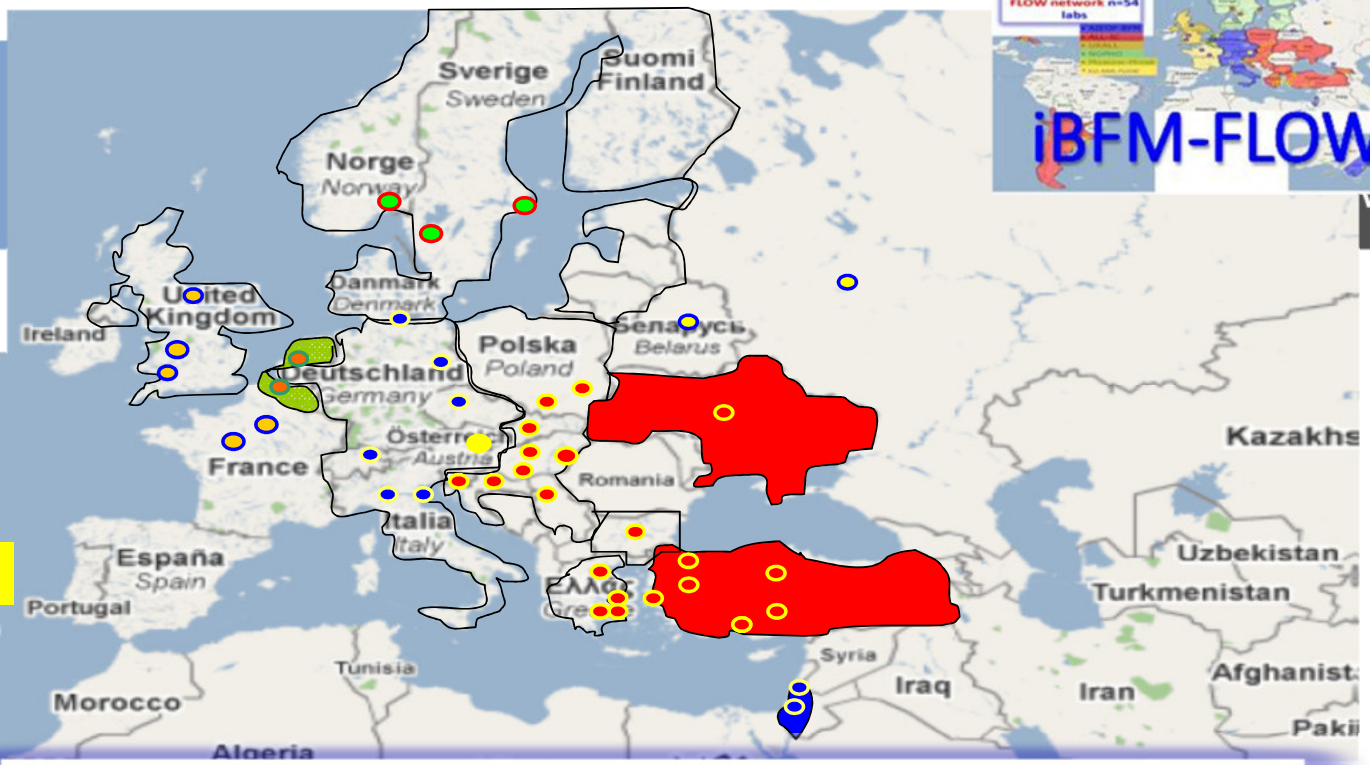
Non-binding research support from:

1. Beckman-Coulter
2. Becton Dickinson
3. Exbio

iBFM
FLOW network
n=56 labs

- ALL-IC
- NOPHO

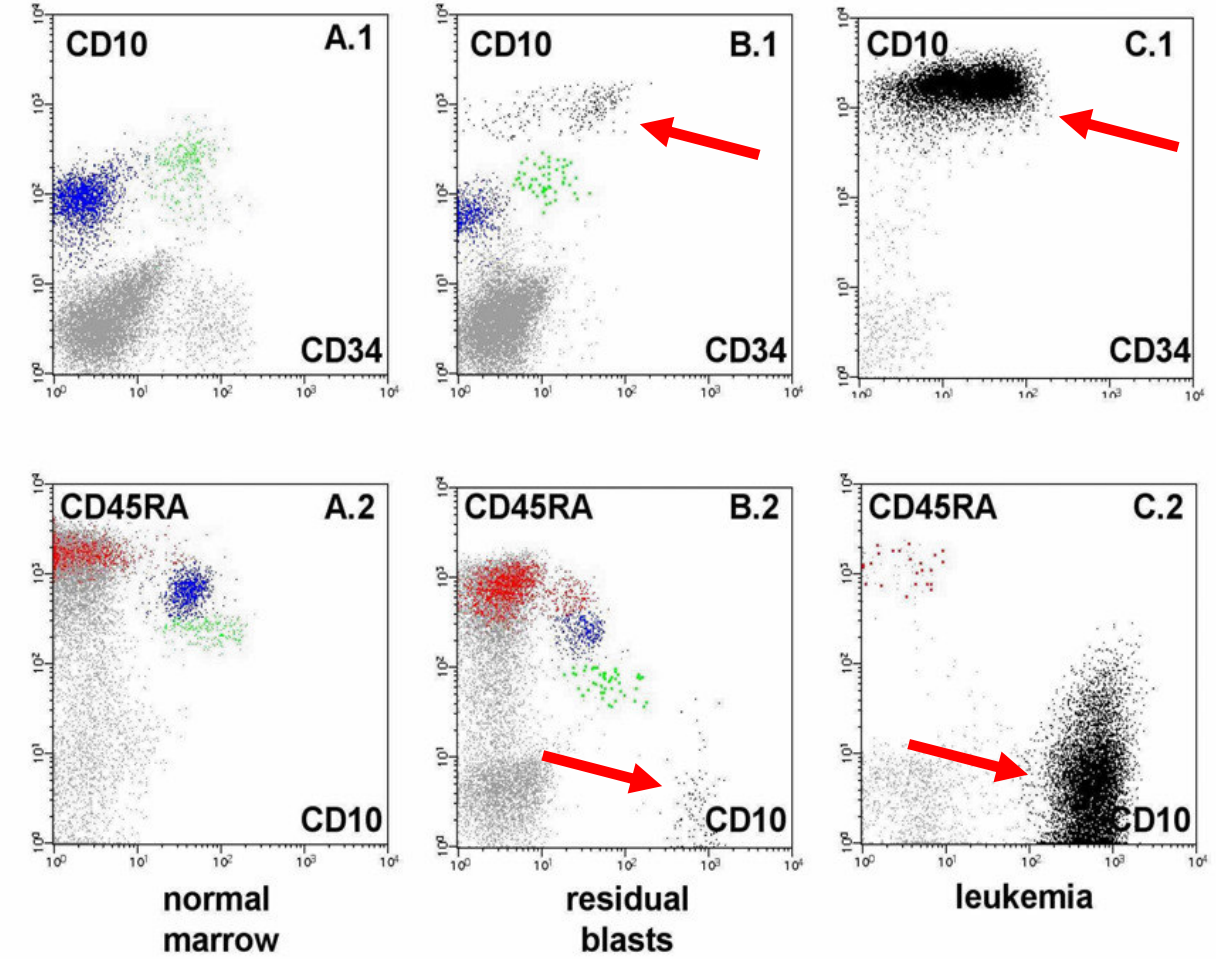




Coordination: Michael N. Dworzak

- assure quality
- make results comparable
- foster collaborative research

FLOW-MRD in pB-ALL

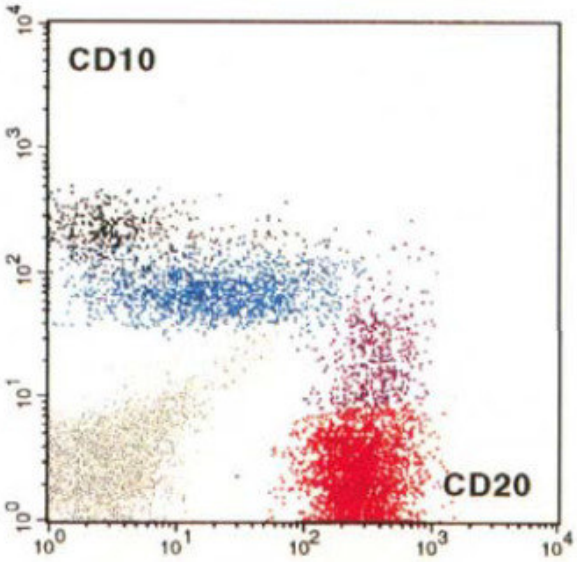


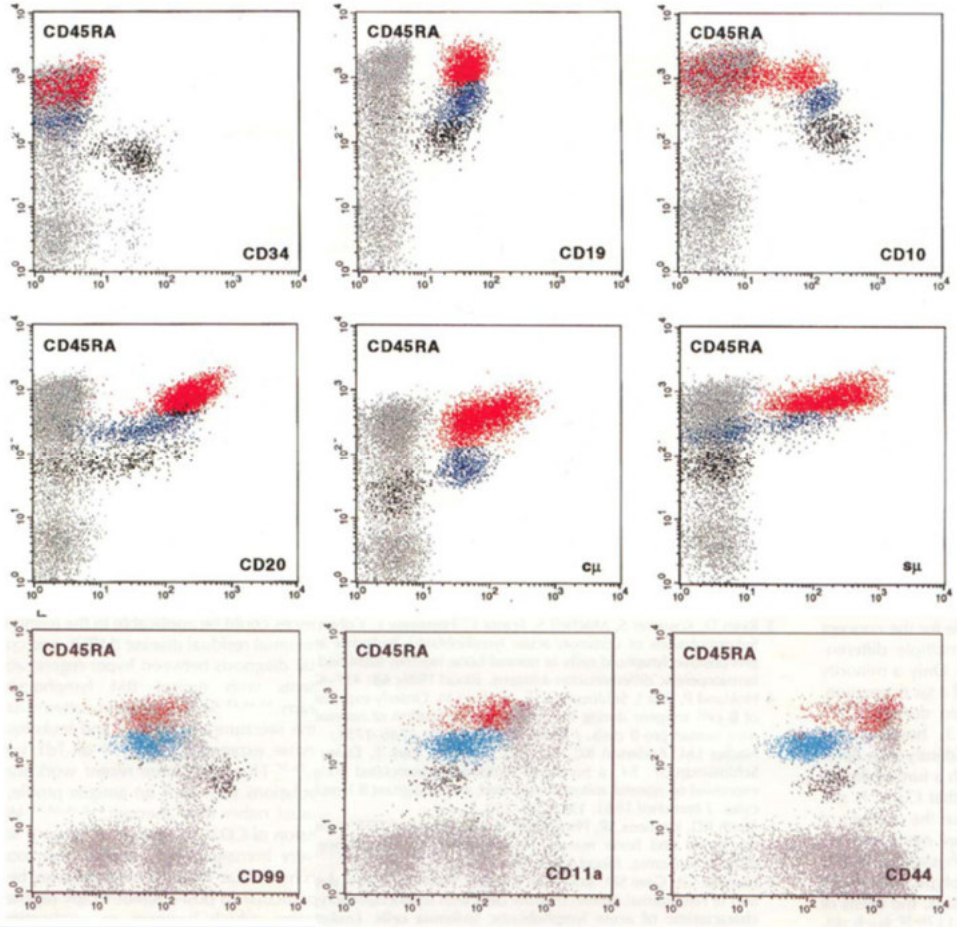
Leukemia (1997) 11, 1266–1273

Multiparameter phenotype mapping of normal and post-chemotherapy B lymphopoiesis in pediatric bone marrow

MN Dworzak, G Fritsch, C Fleischer, D Printz, G Fröschl, P Buchinger, G Mann and H Gadner

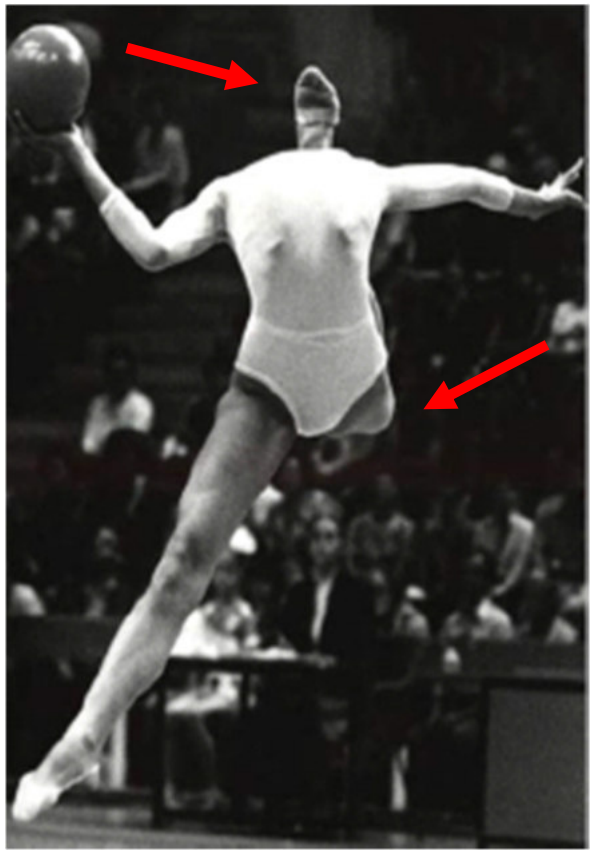
Children's Cancer Research Institute, St Anna Kinderspital, Kinderspitalgasse 6, A-1090 Vienna, Austria





FLOW-MRD in pB-ALL

FLOW-MRD in pB-ALL

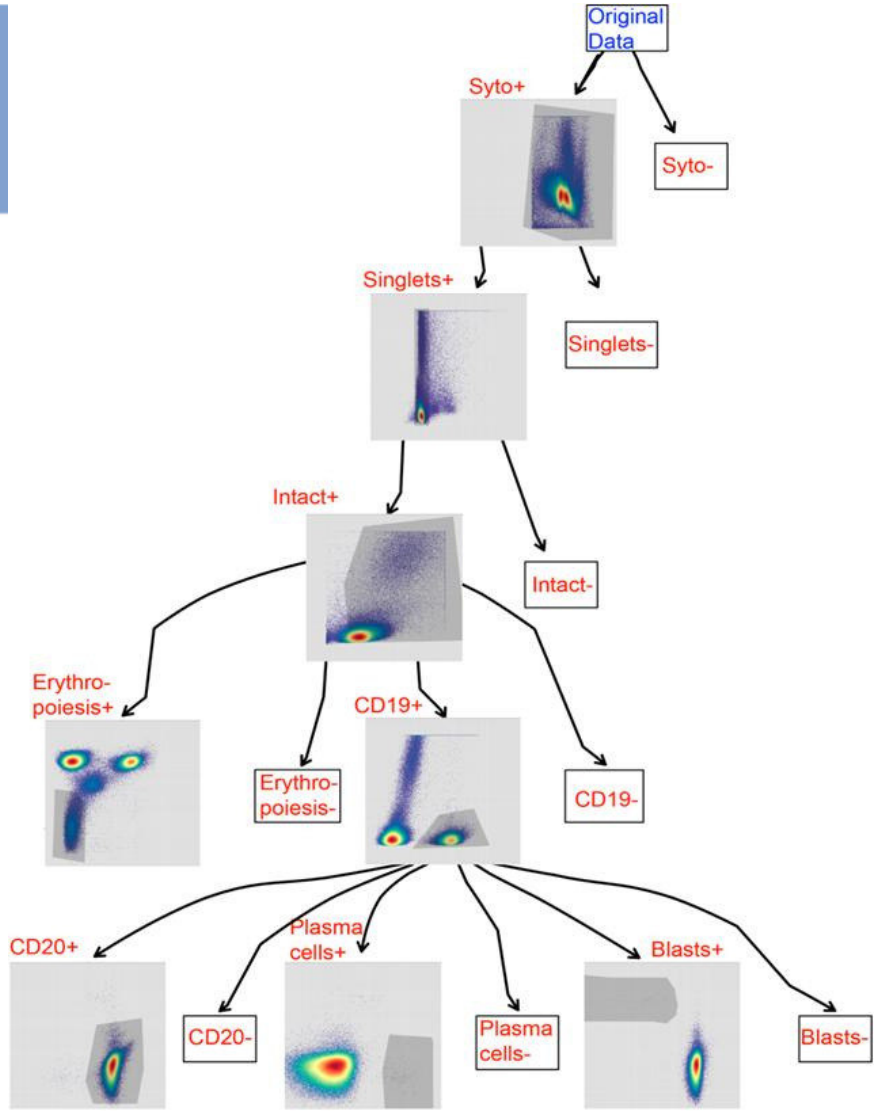


Watch out for weird phenotypes

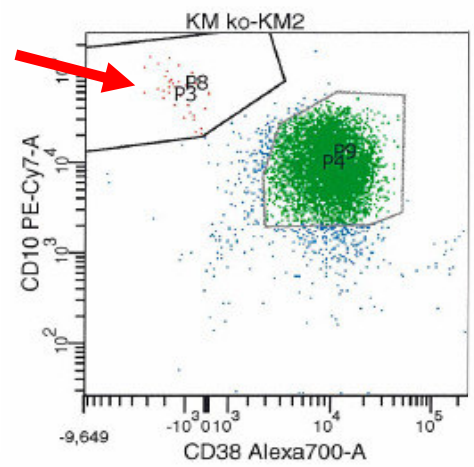
- LAIPs

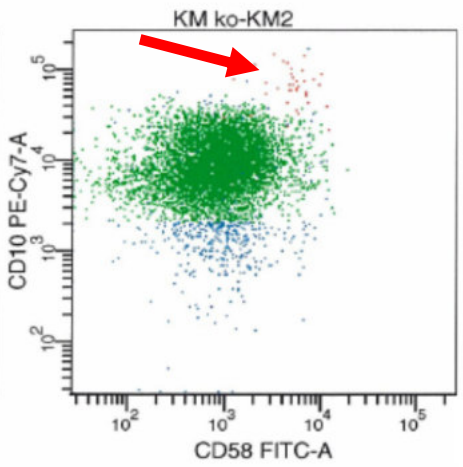
FLOW-MRD

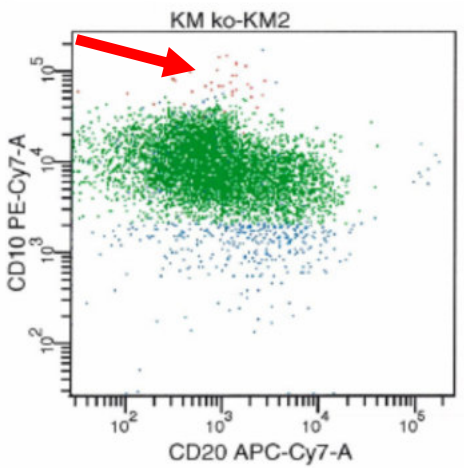
- Operator-based iterative process
- Visual inspection of a multitude of bi-dimensional plots

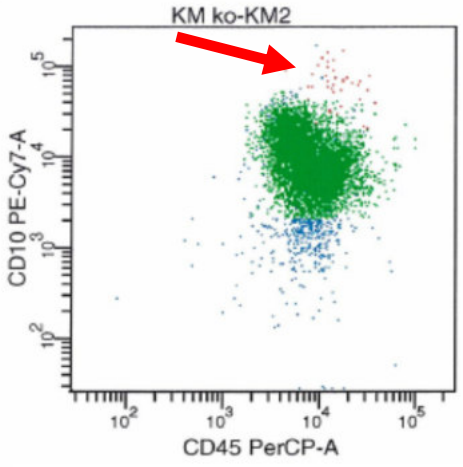


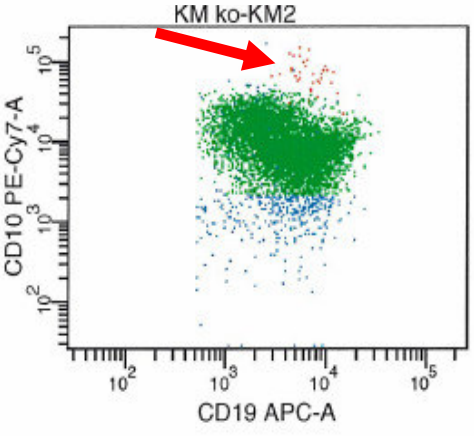
FLOW-MRD









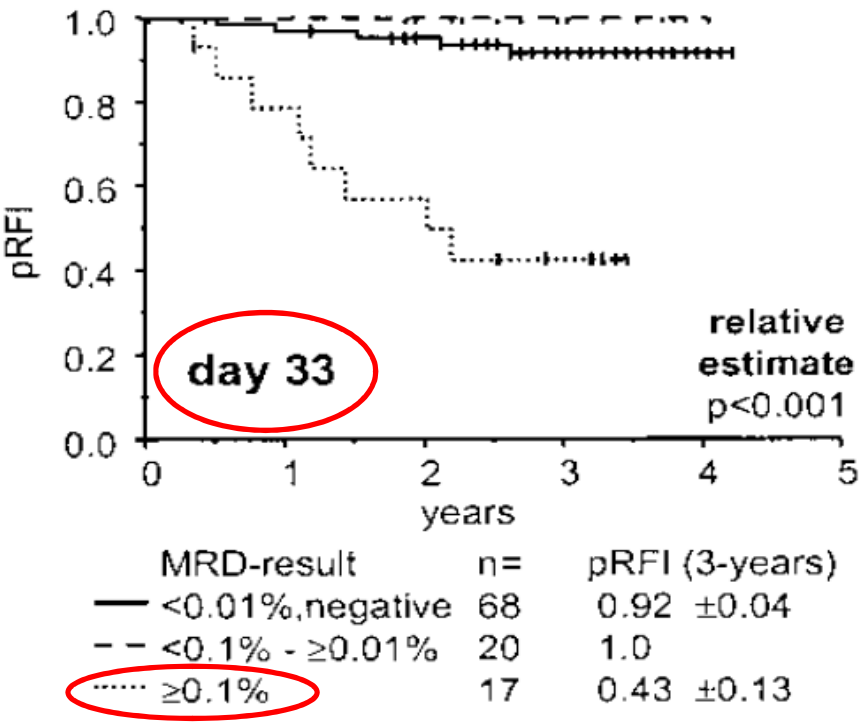


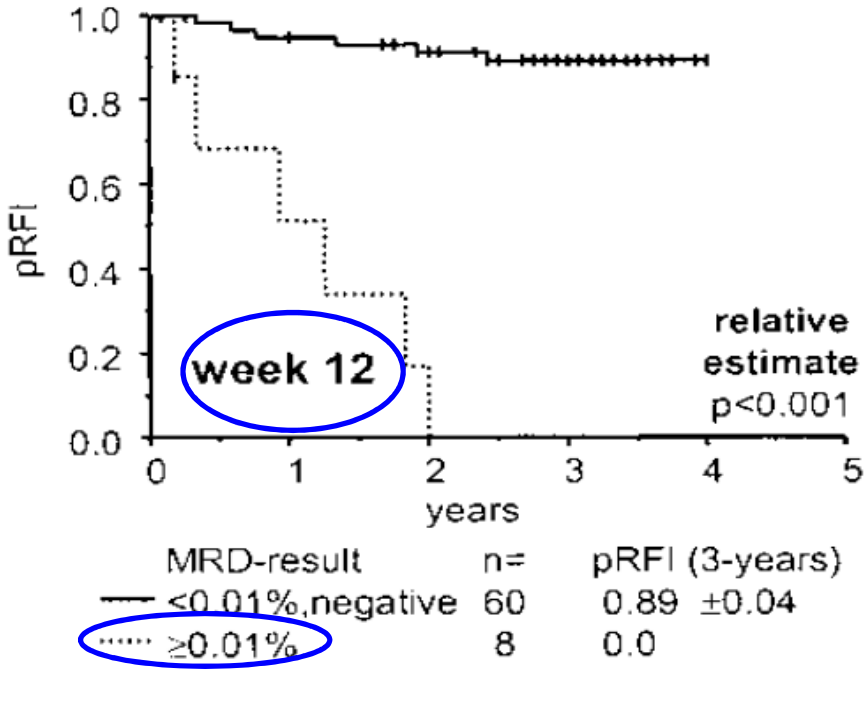
Population	#Events	%Parent	%Grand Par...
All Events	1,000,000	####	####
P1	999,995	99.9995	####
P2	7,954	0.7954	0.7954
P3	38	0.4777	0.0038
P4	7,408	93.1355	0.7408

CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS (Blood. 2002;99:1952-1958)

Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia ALL-BFM 95

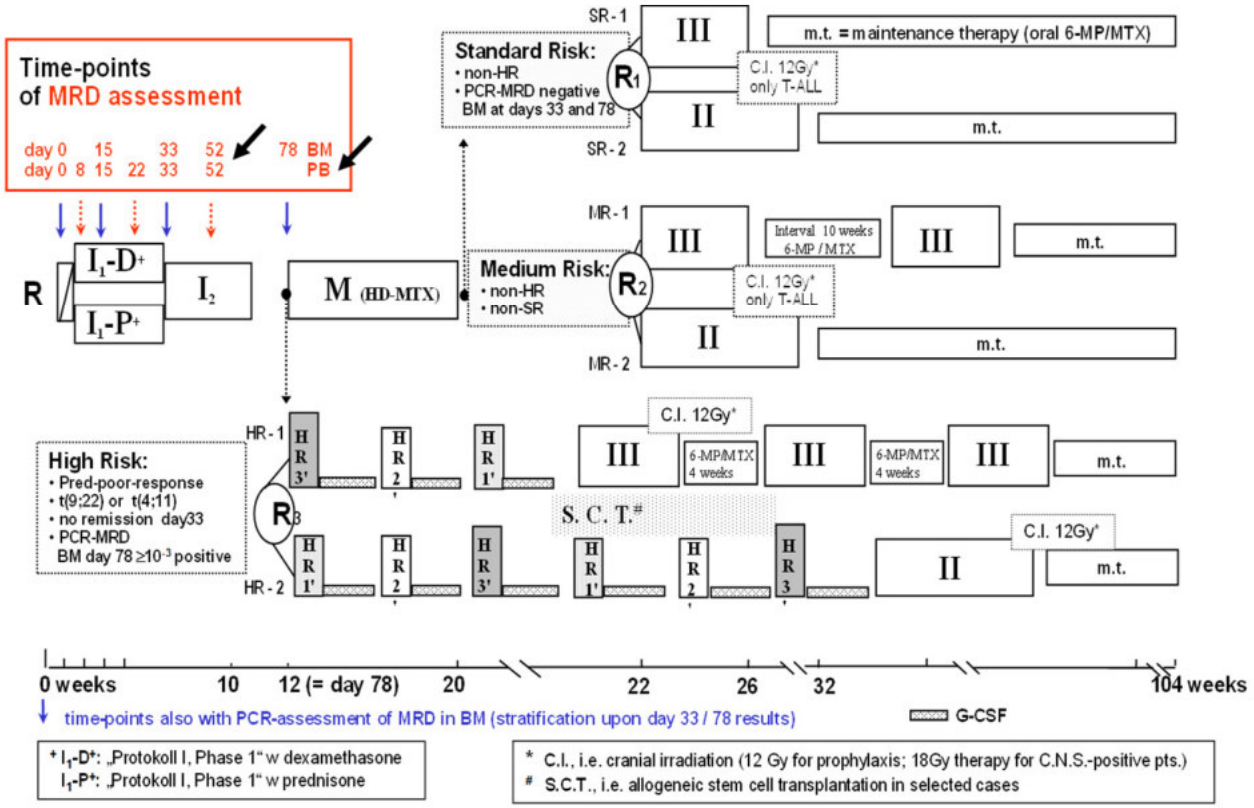
Michael N. Dworzak, Gertraud Fröschl, Dieter Printz, Georg Mann, Ulrike Pötschger, Nora Mühlegger, Gerhard Fritsch, and Helmut Gadner, for the Austrian Berlin-Frankfurt-Münster Study Group

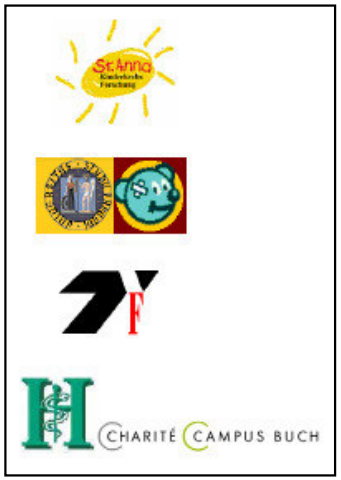


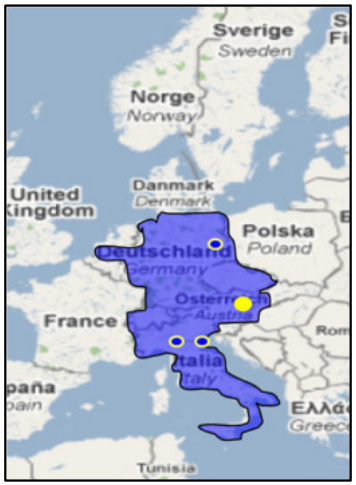


FLOW-MRD in trial AIEOP-BFM ALL 2000

Trial AIEOP-BFM ALL 2000





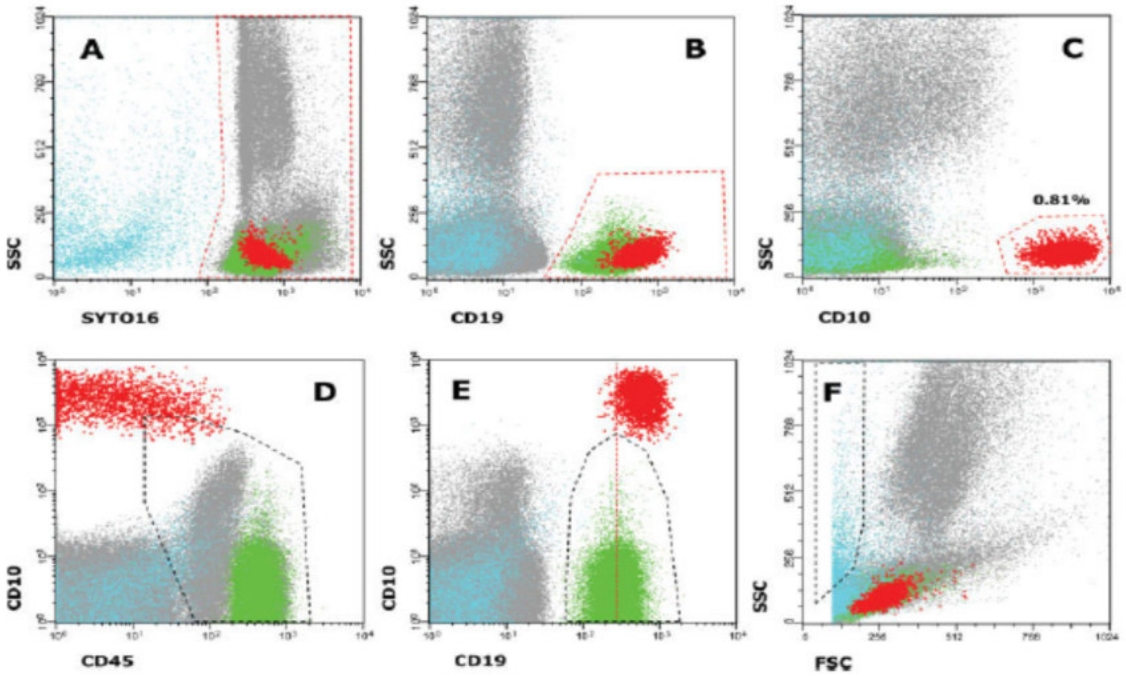


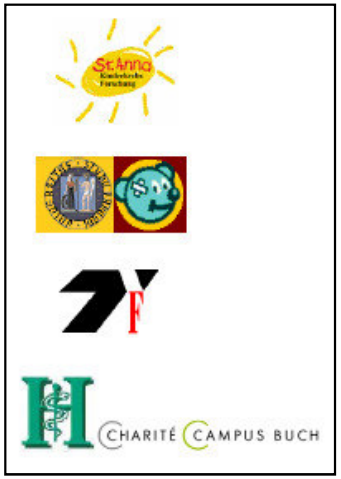
Cytometry Part B (Clinical Cytometry) 74B:331–340 (2008)

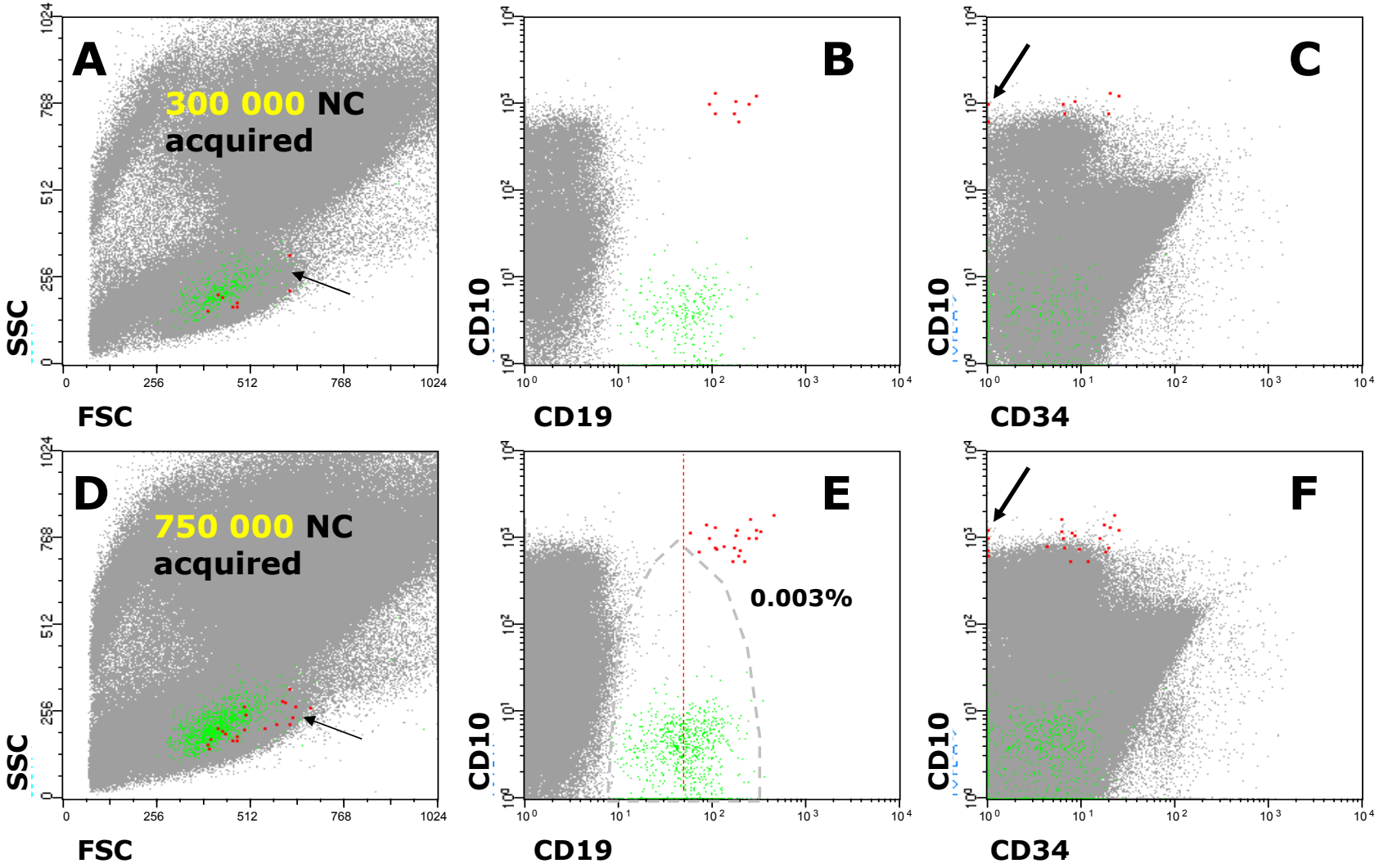
Original Articles

Standardization of Flow Cytometric Minimal Residual Disease Evaluation in Acute Lymphoblastic Leukemia: Multicentric Assessment Is Feasible

Michael Norbert Dworzak,[1*] Giuseppe Gaipa,[2] Richard Ratei,[3] Marinella Veltroni,[4,5] Angela Schumich,[1] Oscar Maglia,[2] Leonid Karawajew,[3] Allessandra Benetello,[4] Ulrike Pötschger,[1] Zvenyslava Husak,[1] Helmut Gadner,[1] Andrea Biondi,[2] Wolf-Dieter Ludwig,[3] and Giuseppe Basso[4]







- Cluster gating
- Positive:
  ≥ 10 events
- Quantifiable:
  ≥ 30 events

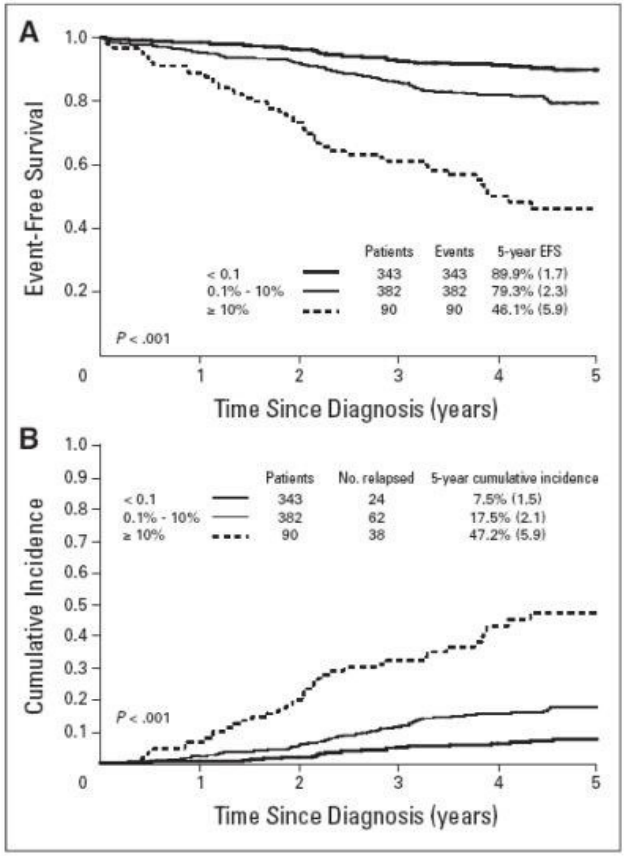
Cytometry Part B (Clinical Cytometry) 74B:331–340 (2008)

homogeneous appearance as a cluster: not with all markers!

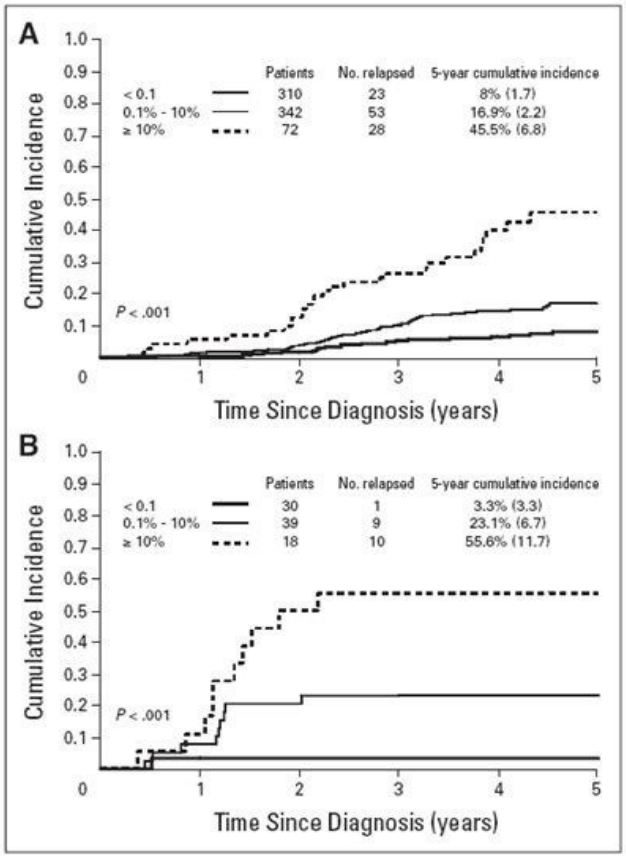
Risk of Relapse of Childhood Acute Lymphoblastic Leukemia Is Predicted By Flow Cytometric Measurement of Residual Disease on Day 15 Bone Marrow

Giuseppe Basso, Marinella Veltroni, Maria Grazia Valsecchi, Michael N. Dworzak, Richard Ratei, Daniela Silvestri, Alessandra Benetello, Barbara Buldini, Oscar Maglia, Giuseppe Masera, Valentino Conter, Maurizio Arico, Andrea Biondi, and Giuseppe Gaipa

total

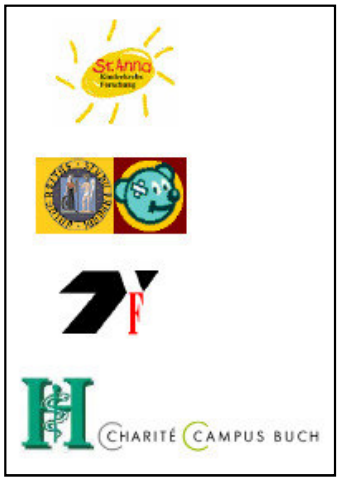


BCP



total

T



AIEOP-BFM ALL 2000 trial data

FLOW-MRD in pB-ALL

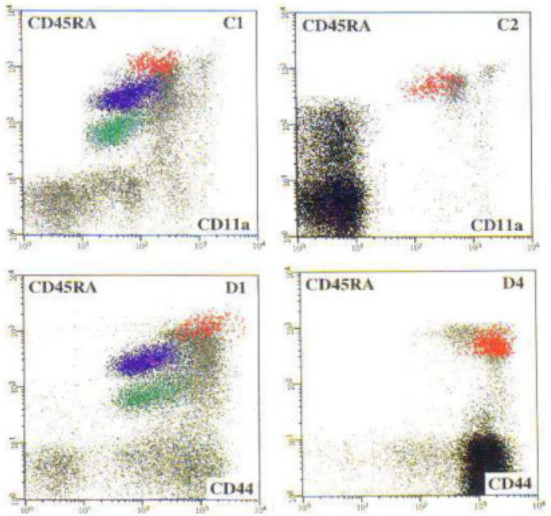
Antigen panel

Experimental Hematology 26:305–313 (1998)

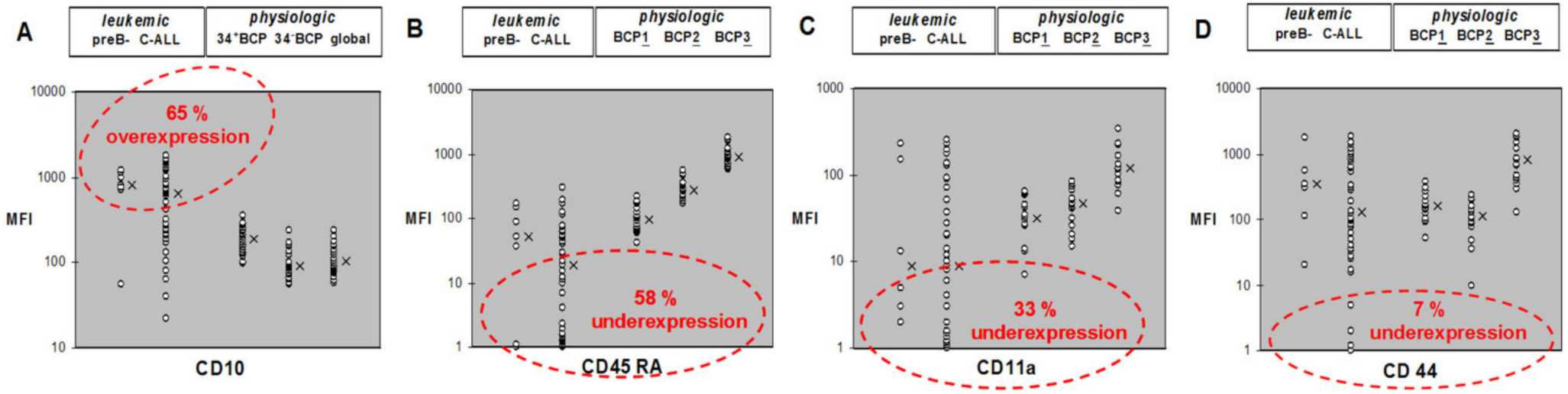
Comparative phenotype mapping of normal vs. malignant pediatric B-lymphopoiesis unveils leukemia-associated aberrations

Michael N. Dworzak, Gerhard Fritsch, Christine Fleischer, Dieter Printz, Gertraud Fröschl, Petra Buchinger, Georg Mann, Helmut Gadner

Children's Cancer Research Institute, St. Anna Kinderspital, Vienna, Austria



LAIPs in ALL: **generic** aberrations



New markers for MRD – are they necessary?

Aberrant Underexpression of CD81 in Precursor B-Cell Acute Lymphoblastic Leukemia

Utility in Detection of Minimal Residual Disease by Flow Cytometry

Tariq Muzzafar, MBBS,[1] L. Jeffrey Medeiros, MD,[1] Sa A. Wang, MD,[1] Archana Brahmandam, MS,[1] Deborah A. Thomas, MD,[2] and Jeffrey L. Jorgensen, MD, PhD[1]

Brief Communication

Cytometry Part B (Clinical Cytometry) 76B:150–158 (2009)

Overexpression of CD49f in Precursor B-cell Acute Lymphoblastic Leukemia: Potential Usefulness in Minimal Residual Disease Detection

Joseph A. DiGiuseppe,[1,2]* Sheila G. Fuller,[1] and Michael J. Borowitz[1]

Overexpression of CD123 correlates with the hyperdiploid genotype in acute lymphoblastic leukemia

Miroslav Djokic,[1*] Elisabet Björklund,[1] Elisabeth Blennow,[2] Joanna Mazur,[3] Stefan Söderhäll,[4] and Anna Porwit[1]

Leukemia (1999) 13, 558–567

http://www.stockton-press.co.uk/leu

A limited antibody panel can distinguish B-precursor acute lymphoblastic leukemia from normal B precursors with four color flow cytometry: implications for residual disease detection

EG Weir, K Cowan, P LeBeau and MJ Borowitz

CD9

CD304 Is Preferentially Expressed on a Subset of B-Lineage Acute Lymphoblastic Leukemia and Represents a Novel Marker for Minimal Residual Disease Detection by Flow Cytometry

Françoise Solly,[1,2] Fanny Angelot,[1,3] Richard Garand,[4] Christophe Ferrand,[1,3] Estelle Seillès,[1,3] Françoise Schillinger,[1] Agnès Decobecq,[1] Maryse Billot,[1] Fabrice Larosa,[5] Emmanuel Plouvier,[6] Eric Deconinck,[3,5] Faezeh Legrand,[5] Philippe Saas,[1,3] Pierre-Simon Rohrlich,[3,6] Francine Garnache-Ottou[1,3]*

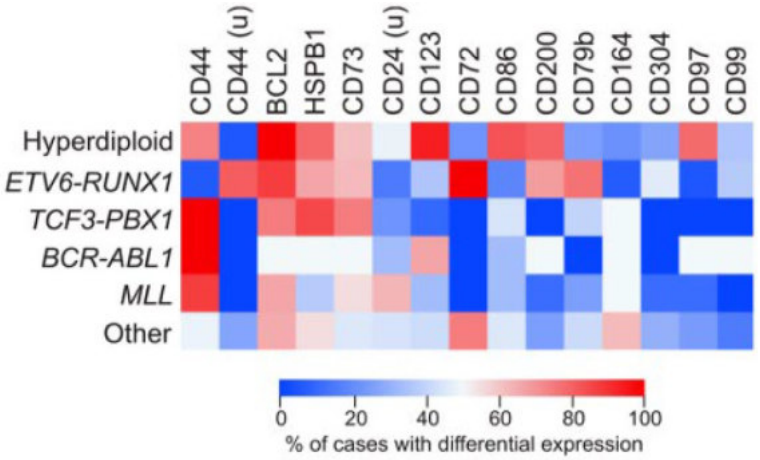
New markers for MRD – are they necessary?

blood

Prepublished online April 12, 2011;
doi:10.1182/blood-2010-12-324004

New markers for minimal residual disease detection in acute lymphoblastic leukemia

Elaine Coustan-Smith, Guangchun Song, Christopher Clark, Laura Key, Peixin Liu, Mohammad Mehrpooya, Patricia Stow, Xiaoping Su, Sheila Shurtleff, Ching-Hon Pui, James R. Downing and Dario Campana



Of the 30 markers, 22 (CD44, BCL2, HSPB1, CD73, CD24, CD123, CD72, CD86, CD200, CD79b, CD164, CD304, CD97, CD102, CD99, CD300a, CD130, PBX1, CTNNA1, ITGB7, CD69, CD49f) were differentially expressed in up to 81.4% of ALL cases; expression of some markers was associated with the presence of genetic abnormalities.

Minimal residual disease analysis by eight-color flow cytometry in relapsed childhood acute lymphoblastic leukemia

Leonid Karawajew,[1] Michael Dworzak,[2] Richard Ratei,[3] Peter Rhein,[1] Giuseppe Gaipa,[4] Barbara Buldini,[5] Giuseppe Basso,[5] Ondrej Hrusak,[6] Wolf-Dieter Ludwig,[3] Günter Henze,[1] Karl Seeger,[1] Arend von Stackelberg,[1] Ester Mejstrikova,[6] and Cornelia Eckert[1]

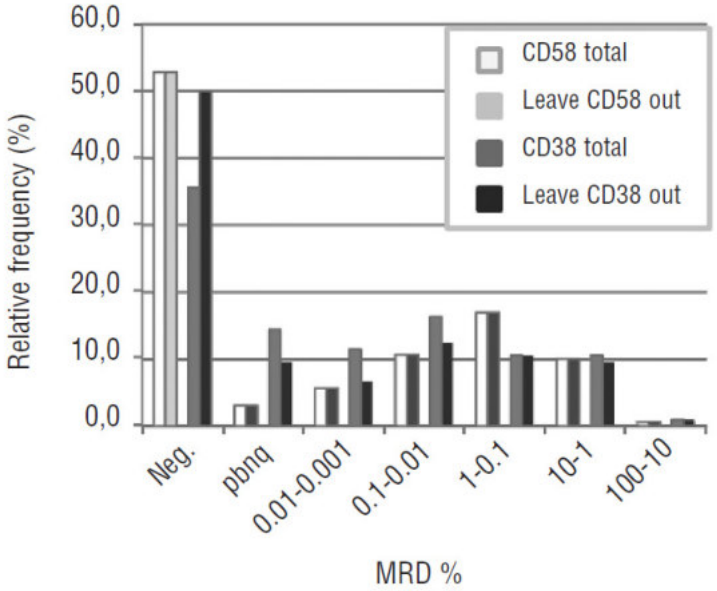
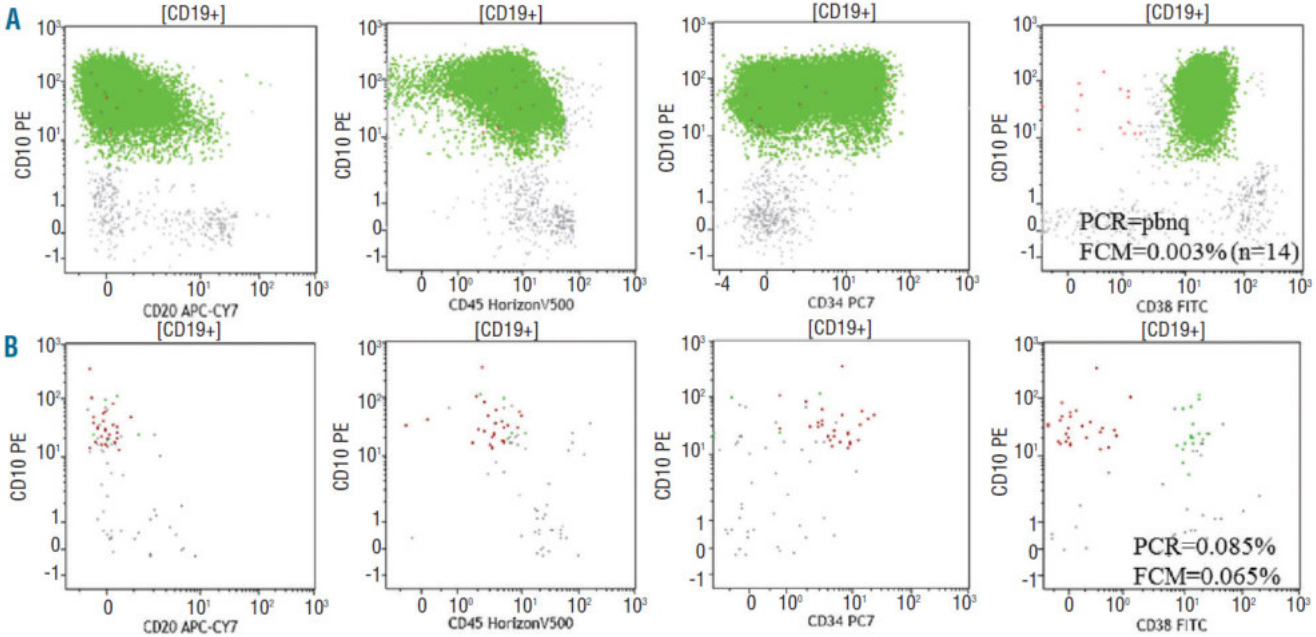


Figure 3. Histograms displaying the distribution of different MRD levels within testing series. The height of the bar (y-axis) corresponds to the relative frequency of the samples falling within the indicated MRD interval (x-axis). The series using experimental CD58-tubes comprised 159 samples, the series using the CD38-tube comprised 104 samples.



iBFM B-ALL-MRD 7-10-color panel

CD19 – CD10 – CD20 – CD34 – CD38 – CD45 – CD58
SYTO (41)

Additional markers (2 per tube) according to LAIP:
CD123 – CD371 – CD11a – CD22 – CD81 – CD99

DuraClone Reagents

DURACLONE

- **DuraClone** is BEC's proprietary line of **dry reagent cocktails** which can be used on several cytometry platforms.
- **Shelf-stable (at least 1 year) at room temperature**, don't require cold chain.
- These are unitized, ready-to-use, affordable and accurate.
- Simplified work flow, minimum hands-on-time and robust results

BECKMAN COULTER

DuraClone RE ALB
Tube, 25 Tests, RUO

REF C00163 – 25 tests

IFU- C00163-1.0

BECKMAN COULTER

	Specifications of Constituent 1	Specifications of Constituent 2	Specifications of Constituent 3	Specifications of Constituent 4	Specifications of Constituent 5	Specifications of Constituent 6	Specifications of Constituent 7
Specificity	CD58	CD34	CD10	CD19	CD38	CD20	CD45
Clone	AICD58	581	ALB1	J3-119	LS198-4-3	B9.E9 (HRC20)	J.33
Immunogen	PHA blasts	Human CD34+ cells	Human Leukemia cells	SKLY 18 lymphoma cells	Human T cell line HUT 78	DAUDI cell line (Human B Lymphoblastoid)	Laz 221 cell line
Isotype	IgG2a	IgG1	IgG1	IgG1	IgG1	IgG2a	IgG1
Species	Mouse						
Source	Ascites fluid or supernatant of in vitro cultured hybridoma cells.						
Purification	Affinity chromatography						
Fluorochrome	Fluorescein isothiocyanate (FITC)	ECD	R Phycoerythrin-Cyanine 5.5 (PC5.5)	R Phycoerythrin-Cyanine 7 (PC7)	Allophycocyanin Alexa Fluor 700 (APC-A700)	Allophycocyanin Alexa Fluor 750 (APC-A750)	Krome Orange (KrO)
Excitation	488 nm	488 nm	488 nm	488 nm	633 nm	633/638 nm	405 nm
Emission	525 nm	613 nm	692 nm	770 nm	720 nm	775 nm	528 nm

PE-drop-in

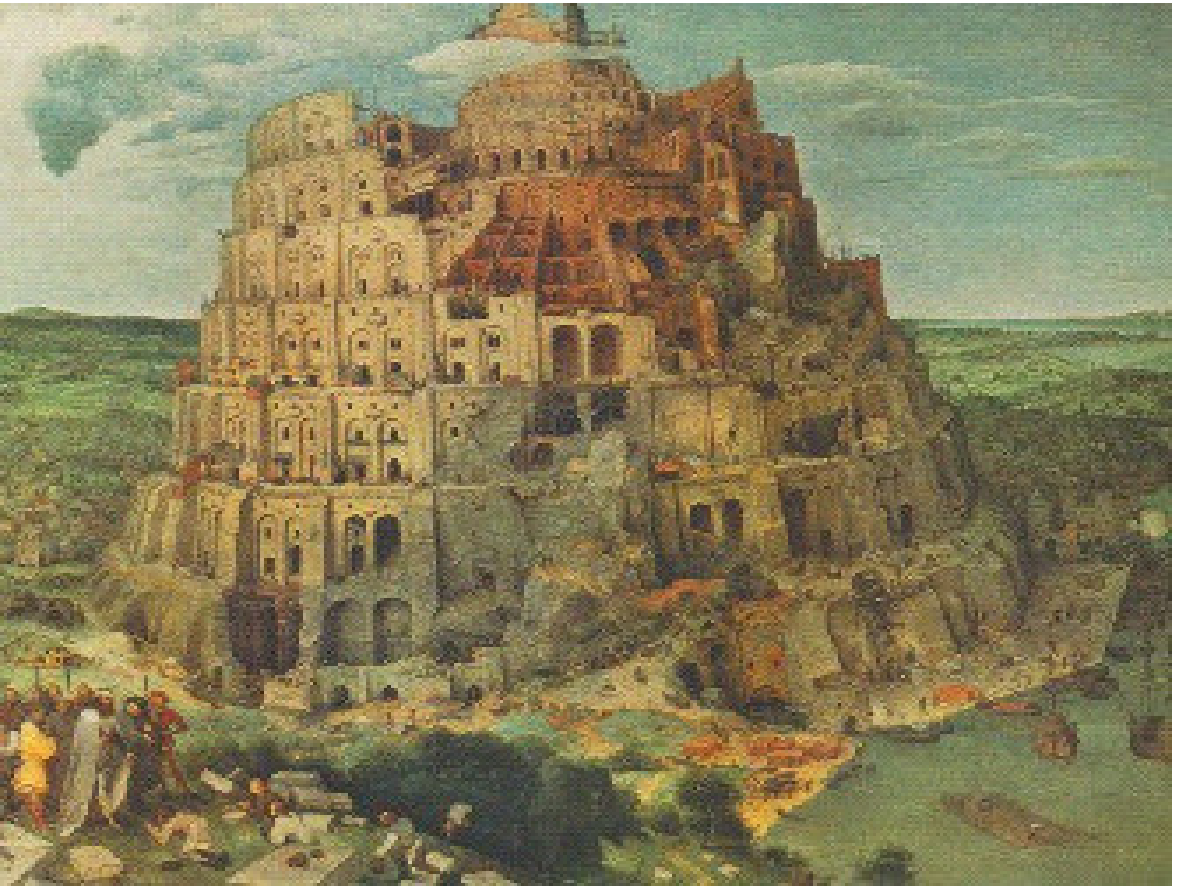
CD11a
CD123
CD371 (CLL1)

APC-drop-in

CD22
CD81
CD99

SYTO41

Steps of harmonization



- Procedure harmonization - SOP
- Discussion, meetings, education
- Cytometer performance monitoring
- Sample quality monitoring
- External QC-QA program

Standard Operating Procedure

≥6-color FLOW-MRD detection in ALL

AIEOP – BFM-A/G/S – CPH – ISPHO - NSW

version "International" 1.5 of November 17, 2010

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ISPHO, Petach-Tikva, ISRAEL

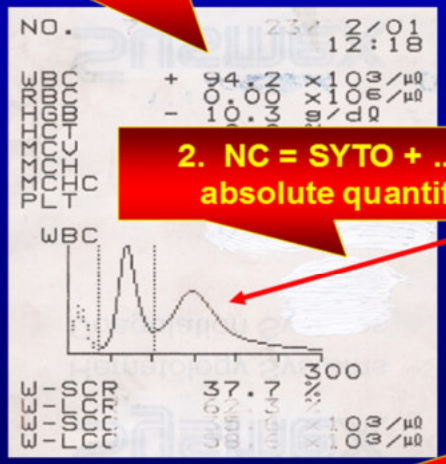
Dr. Mary Sartor (PI)
NSW, Sydney, AUSTRALIA

CONTENTS

Note: **Deliverables in BLUE**

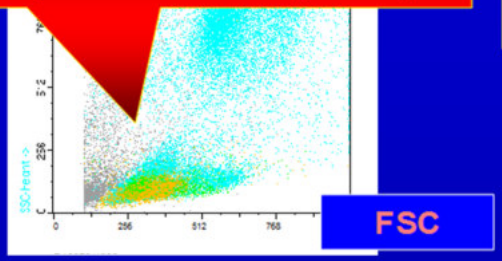
FCM-tools for correct quantification of MRD

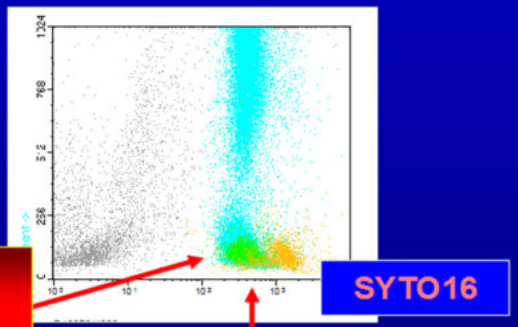
1. hematologic cell count (NC) of BM or PB

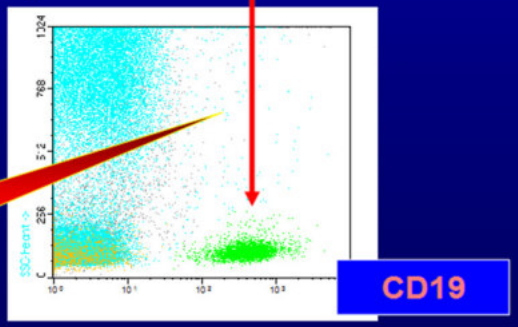


2. NC = SYTO + ... use for absolute quantification

3. NC among events... separation not always easy

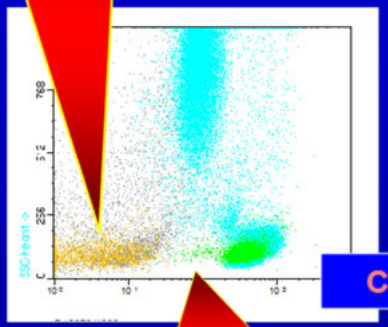




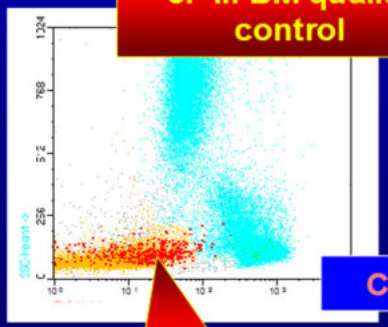


4. ...calculate blast-% via CD19+ among SYTO16 +

CD45-negative SYTO16 + (NC) = erythroid precursors



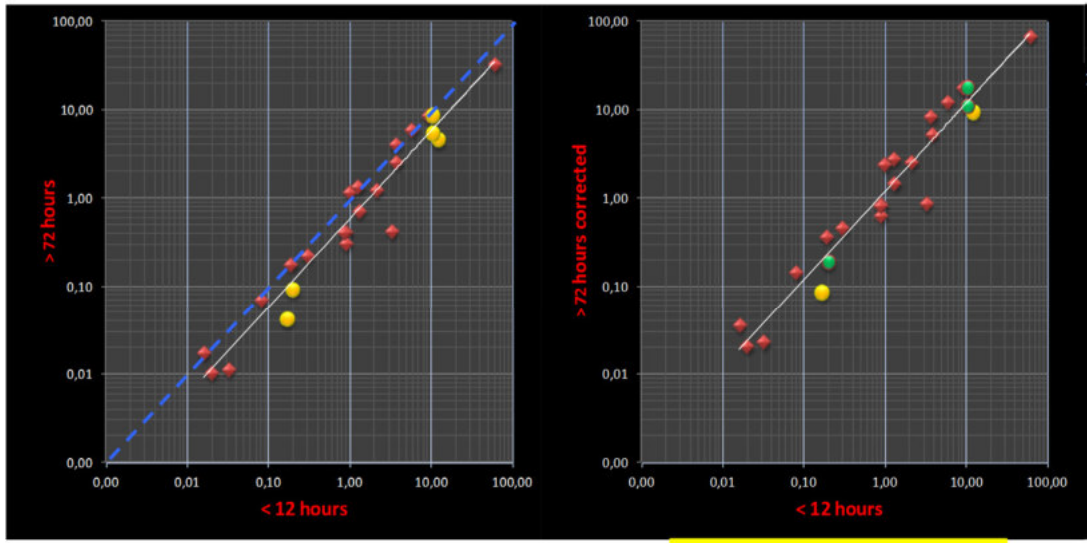
5. ... BM quality control



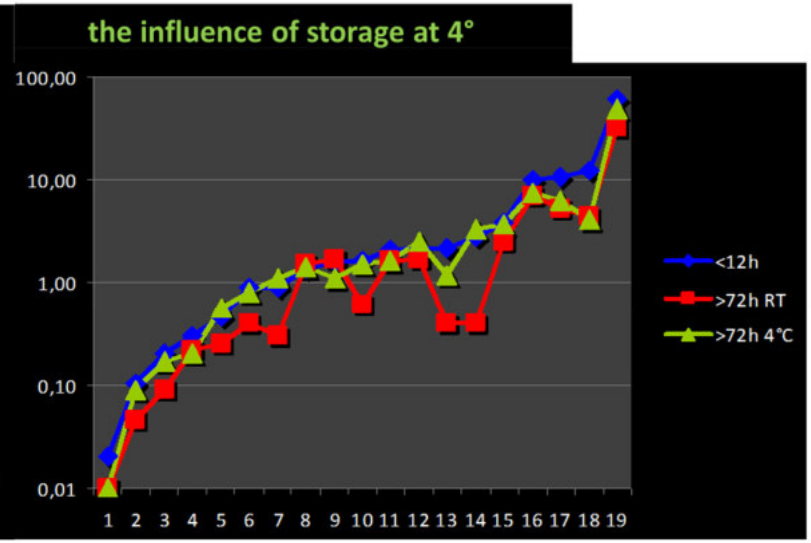
...or leukemic blasts

Time delay may hamper correct MRD-quantification

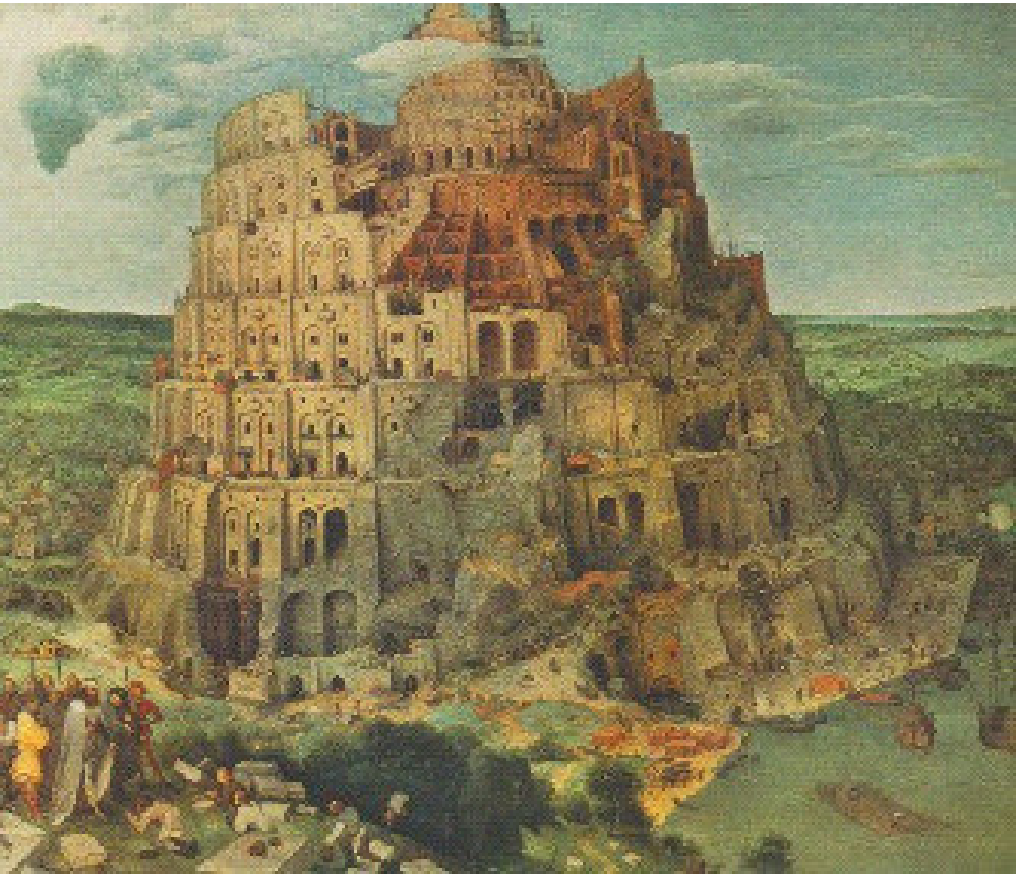
experiments comparing time from sampling to processing:
< 12 hours vs. > 72 hours



with correction factor 2.0
(mean ratio 12h/72h)



External QC & QA in the iBFM-FLOW-nw



- Educational rounds (wet-lab, plot review)
- Twinning – Maturation program
- UK Neqas trials (spiked sample send-arounds)
- LMD-file send-arounds
- Independent data survey

- Pre-acquisition standardization (cocktail tubes)
- Operator-independent analysis

EU Research Projects

AUTOFLOW

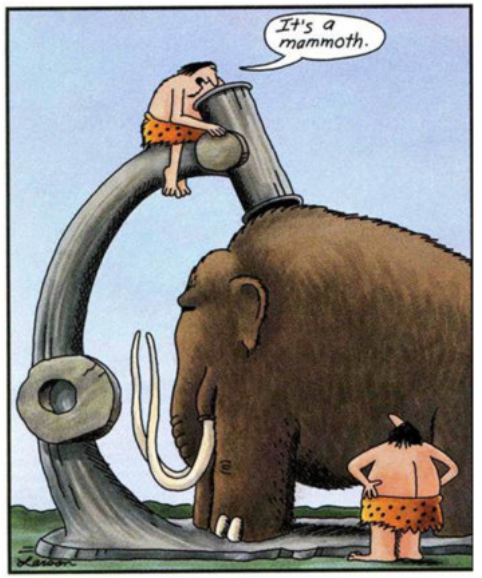
Automation of Flow Cytometric Analysis for Quality-Assured Follow-up Assessment to Guide Curative Therapy for Acute Lymphoblastic Leukaemia in Children

From 2014-02-01 to 2018-01-31 | AUTOFLOW website

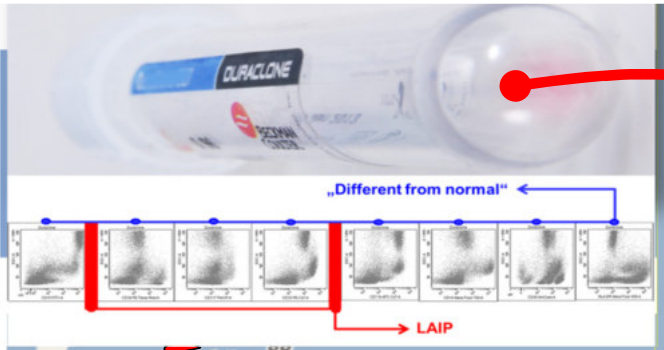
Innovative software-based pattern recognition for automated MRD-assessment

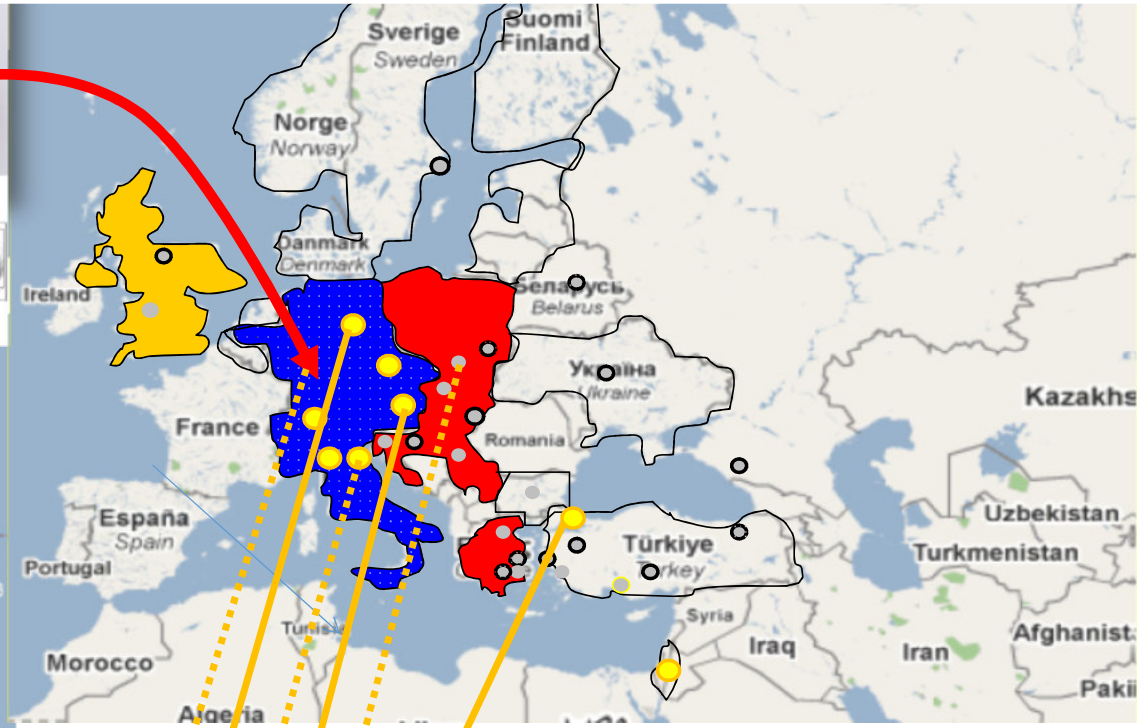
Objectives at a glance

- Reduce subjectivity caused by manual operator gating; increase result comparabiliy and reproducibility through automation and standardization
- Develop a software-based tool for operator assistance in daily practice (automated FLOW-data analysis supporting system) >> „TWINNING automate“ on WWW

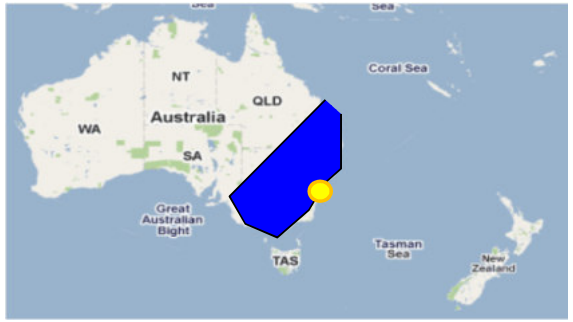


Early microscope





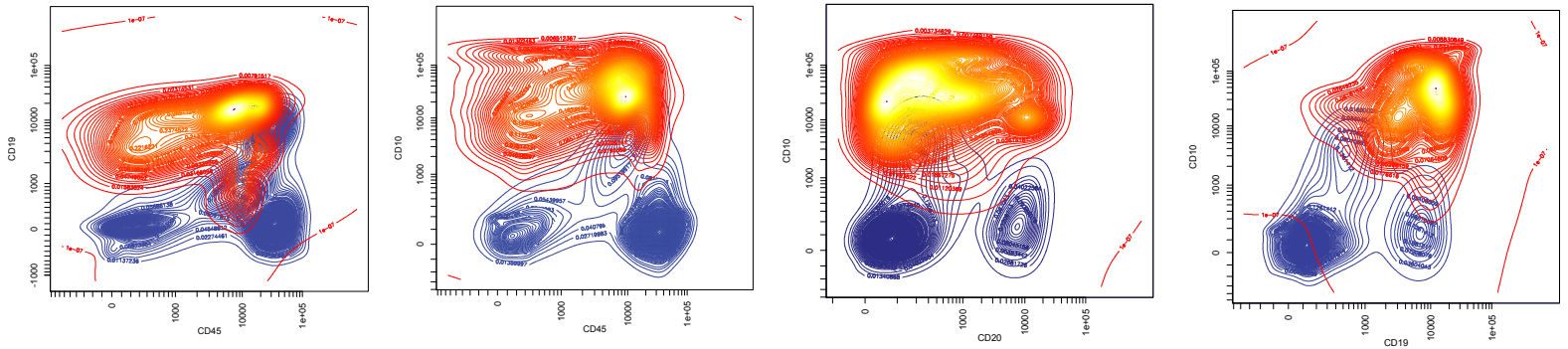
MRD-data cloud



The CLOUD concept – what cloud?

Cumulative clouds of experted annotated samples
to hold against new and unknown samples

- clouds of normal background cells >> „different-from-normal“-concept
- clouds of leukemic cells >> leukemia-associated phenotypes (LAIPs)



iBFM/pan-EU pedAML-MRD 10-color panel

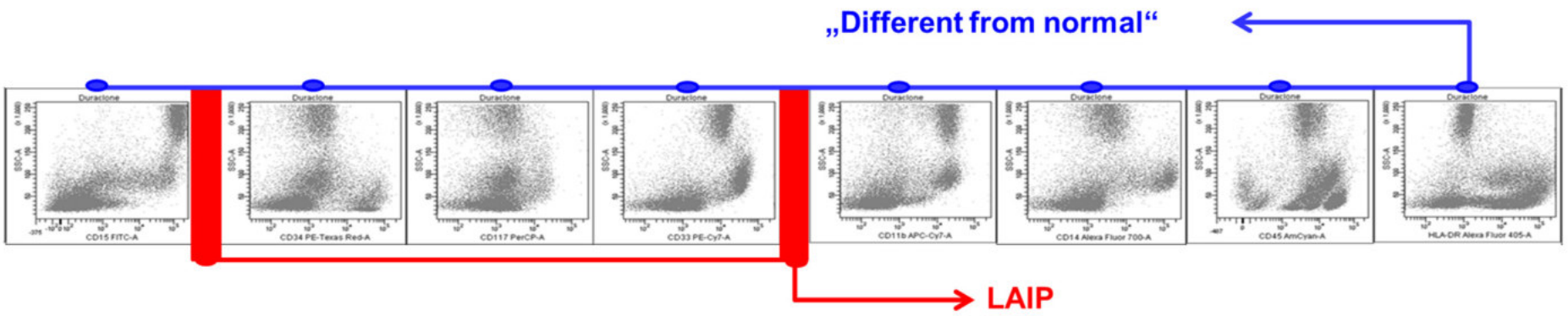
Per patient >> 2 MRD-tubes: LAIP & CFU (=stem cell tube) plus 1 SYTO-tube (gross population overview tube)

Dura-clone	FITC	PE	ECD	PC5.5	PE-Cy7
LAIP	CD15	CD-PE	CD34	CD117	CD33
CFU	CD38	CD-PE	CD34	CD117	CD33

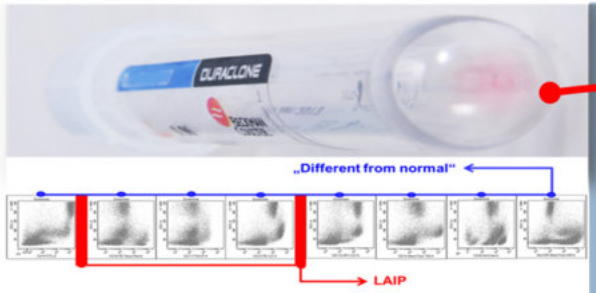
APC	APC-A700	APC-A750	PB	KrO
CD-APC	CD14	CD11b	CD45	HLA-DR
CD-APC	CD123	CD45RA	CD45	HLA-DR

CD-PE = CD2,7,19,56,64,99

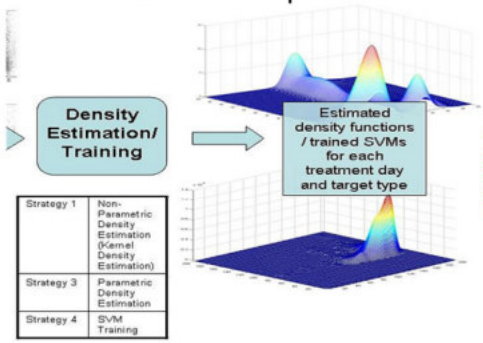
CD-APC = CD11a,13,CLL-1

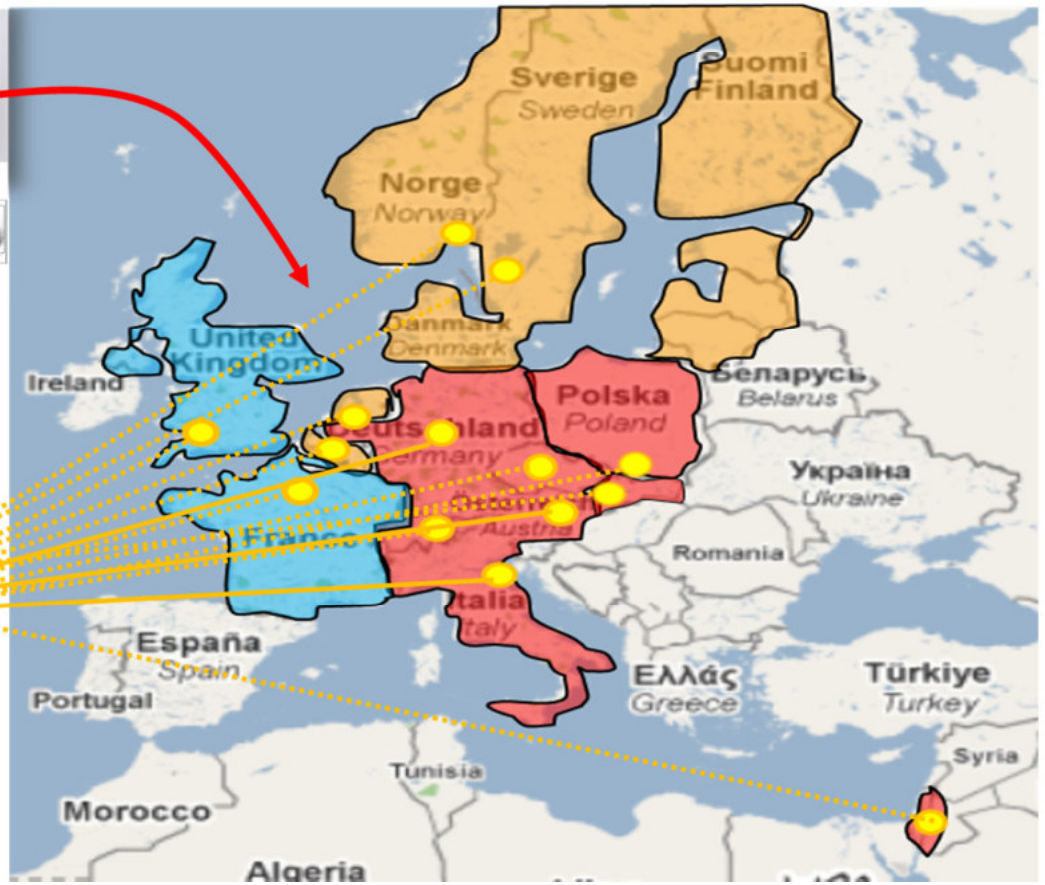


pedAML goes AutoFlow



iBFM/pan-EU pedAML-MRD
10-color panel





Auto Flow

wirtschaftsagentur wien
Ein Fonds der Stadt Wien

FlowCLUSTER

iBFM-FLOW-network
&
EU-AML FLOW-MRD study group

Standard Operating Procedure v1.1
for the joint project
flowCLUSTER

"Evaluation of a 10-color twin-tube panel for the immunophenotypic assessment of MRD in pediatric AML fortified by software development for an automated FLOW-data analysis supporting system"

Assoc. Prof. Dr. Michael N. Dworzak (CO, PI)
BFM-A, Vienna, AUSTRIA
Prof. Dr. Dirk Reinhardt (PI)
BFM-D, Essen, GERMANY
Universitätsklinikum Essen
Prof. Dr. Giuseppe Basso (PI)
AIEOP, Padova, ITALY

Advantages of the AutoFLOW approach

- Established trial-associated FLOW-lab community
- 20 years of joint experience in FLOW-MRD assessment
- High-end machine learning software created towards
  - Flexibility (LAIP or background-based MRD assessment)
  - No data reduction (advantage over *Principal Component Analysis*-based methods)
  - Rapid high throughput of very large data masses
  - Self-adaptiveness (software-based calibration)

Visit our demo video of the analysis software flowView at:
https://www.youtube.com/watch?v=fu0V76Cppa4&feature=youtu.be

Harmonization of leukemia immunophenotyping

AIEOP-BFM Consensus Guidelines 2016 for Flow Cytometric Immunophenotyping of Pediatric Acute Lymphoblastic Leukemia

Michael N. Dworzak,[1*] Barbara Buldini,[2] Giuseppe Gaipa,[3] Richard Ratei,[4] Ondrej Hrusak,[5] Drorit Luria,[6] Eti Rosenthal,[7] Jean-Pierre Bourquin,[8] Mary Sartor,[9] Angela Schumich,[1] Leonid Karawajew,[10] Ester Mejstrikova,[5] Oscar Maglia,[3] Georg Mann,[1] Wolf-Dieter Ludwig,[4] Andrea Biondi,[3] Martin Schrappe,[11] and Giuseppe Basso,[2] on behalf of the International-BFM-FLOW-network

Cytometry B Clin Cytometry, 2017

Immunophenotyping by flow cytometry (FCM) is a worldwide mainstay in leukemia diagnostics. For concordant multicentric application, however, a gap exists between available classification systems, technologic standardization, and clinical needs. The AIEOP-BFM consortium induced an extensive standardization and validation effort between its nine national reference laboratories collaborating in immunophenotyping of pediatric acute lymphoblastic leukemia (ALL). We elaborated common guidelines which take advantage of the possibilities of multi-color FCM: marker panel requirements, immunological blast gating, in-sample controls, tri-partite antigen expression rating (negative vs. weak or strong positive) with capturing of blast cell heterogeneities and subclone formation, refined ALL subclassification, and a dominant lineage assignment algorithm able to distinguish "simple" from bilineal/"complex" mixed phenotype acute leukemia (MPAL) cases, which is essential for choice of treatment. These guidelines are a first step toward necessary inter-laboratory standardization of pediatric leukemia immunophenotyping for a concordant multicentric application.

- multi-color (≥ 6)
- single panel recommended for all ALs
- immunological gate: „Bermude"-area of CD45 plus Lin marker
- in-sample cross-lineage negative controls
- semi-quantitative expression rating
- blast heterogeneities – subclone resolution
- dominant lineage assignement
- MPAL distinction
- refined ALL subclassification

Consensus antibody panel

- Extensive single-platform panel for acute leukemia in children

Mandatory and optional markers (each combined with CD45)	
Intracellular@#	iCD3, iCD22, iCD79a, iIgM (μ-chain), iLysozyme, iMPO
Surface@	CD2§, CD3, CD5, CD7; CD10, CD19, CD20; CD11c, CD11b, CD13, CD14, CD15, CD33, CD64, CD65&, CD117; CD34, (CD45), CD56, HLA-DR if T-ALL: CD1a, CD4, CD8, TCRαβ, TCRγδ if B-IV suspected: κ-chain, λ-chain (surface staining after pre-washing or intracellular)
Optional / Recommended	all cases: NG2§, CD371§% if BCP-ALL: CD11a§, CD22, CD24, CD38, CD44, CD58, CD66c, CD123§, CRLF2§* if T-ALL: CD99, iTdT if BAL according to general panel: CD24, iTdT

@ mandatory markers for WHO, EGIL, ETP classifications
# prefix "i" stands for intracellular staining
§ phycoerythrin-conjugate (PE) recommended
& available only labelled with fluorescein isothiocyanate (FITC)
$ clone 7.1
% clone 50C1
* clone 1D3

Compatible with:

- WHO 2008/2016
- EGIL score
- „New" ALL subtypes
  - ETP
  - Switch ALL
  - CRLF2+ ALL

New entity: ETP

Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia

Elaine Coustan-Smith, Charles G Mullighan, Mihaela Onciu, Frederick G Behm, Susana C Raimondi, Deqing Pei, Cheng Cheng, Xiaoping Su, Jeffrey E Rubnitz, Giuseppe Basso, Andrea Biondi, Ching-Hon Pui, James R Downing, Dario Campana

Summary

Background About a fifth of children with acute T-lymphoblastic leukaemia (T-ALL) succumb to the disease, suggesting an unrecognised biological heterogeneity that might contribute to drug resistance. We postulated that T-ALL originating from early T-cell precursors (ETPs), a recently defined subset of thymocytes that retain stem-cell-like features, would respond poorly to lymphoid-cell-directed therapy. We studied leukaemic cells, collected at diagnosis, to identify cases with ETP features and determine their clinical outcome.

Lancet Oncol 2009; 10: 147–56

Findings 30 patients (12·6%) had leukaemic lymphoblasts with an ETP-related gene-expression signature or its associated distinctive immunophenotype (CD1a$^-$, CD8$^-$, CD5^{weak} with stem-cell or myeloid markers). Cases of ETP-ALL showed increased genomic instability, in terms of number and size of gene lesions, compared with those with typical T-ALL. Patients with this form of leukaemia had high risk of remission failure or haematological relapse (72% [95% CI 40–100] at 10 years *vs* 10% [4–16] at 10 years for patients with typical T-ALL treated at St Jude Children's Research Hospital; and 57% [25–89] at 2 years *vs* 14% [6–22] at 2 years for patients treated in the AIEOP trial).

Interpretation ETP-ALL is a distinct, previously unrecognised, pathobiological entity that confers a poor prognosis with use of standard intensive chemotherapy. Its early recognition, by use of the gene expression and immunophenotypic criteria outlined here, is essential for the development of an effective clinical management strategy.

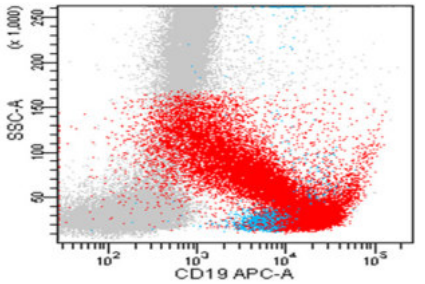
New entity: „switch“-ALL – CD371+

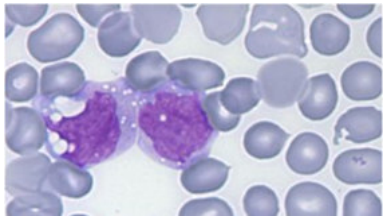
ORIGINAL ARTICLE

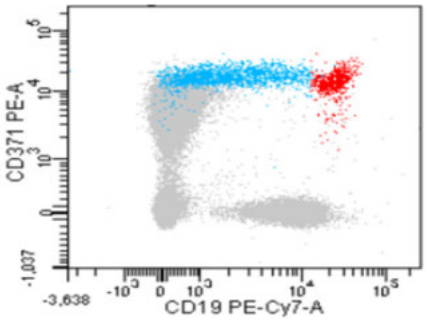
CD2-positive B-cell precursor acute lymphoblastic leukemia with an early switch to the monocytic lineage

L Slamova[1], J Starkova[1], E Fronkova[1], M Zaliova[1], L Reznickova[1], FW van Delft[2], E Vodickova[3], J Volejnikova[1], Z Zemanova[4], K Polgarova[1], G Cario[5], M Figueroa[6], T Kalina[1], K Fiser[1], JP Bourquin[7], B Bornhauser[7], M Dworzak[8], J Zuna[1], J Trka[1], J Stary[1], O Hrusak[1] and E Mejstrikova[1]

Switches from the lymphoid to myeloid lineage during B-cell precursor acute lymphoblastic leukemia (BCP-ALL) treatment are considered rare and thus far have been detected in MLL-rearranged leukemia. Here, we describe a novel BCP-ALL subset, switching BCP-ALL or swALL, which demonstrated monocytosis early during treatment. Despite their monocytic phenotype, 'monocytoids' share immunoreceptor gene rearrangements with leukemic B lymphoblasts. All swALLs demonstrated BCP-ALL with CD2 positivity and no MLL alterations, and the proportion of swALLs cases among BCP-ALLs was unexpectedly high (4%). The upregulation of CEBPα and demethylation of the CEBPA gene were significant in blasts at diagnosis, prior to the time when most of the switching occurs. Intermediate stages between $CD14^{neg}CD19^{pos}CD34^{pos}$ B lymphoblasts and $CD14^{pos}CD19^{neg}CD34^{neg}$ 'monocytoids' were detected, and changes in the expression of PAX5, PU1, M-CSFR, GM-CSFR and other genes accompanied the switch. Alterations in the Ikaros and ERG genes were more frequent in swALL patients; however, both were altered in only a minority of swALLs. Moreover, switching could be recapitulated *in vitro* and in mouse xenografts. Although children with swALL respond slowly to initial therapy, risk-based ALL therapy appears the treatment of choice for swALL. SwALL shows that transdifferentiating into monocytic lineage is specifically associated with CEBPα changes and CD2 expression.







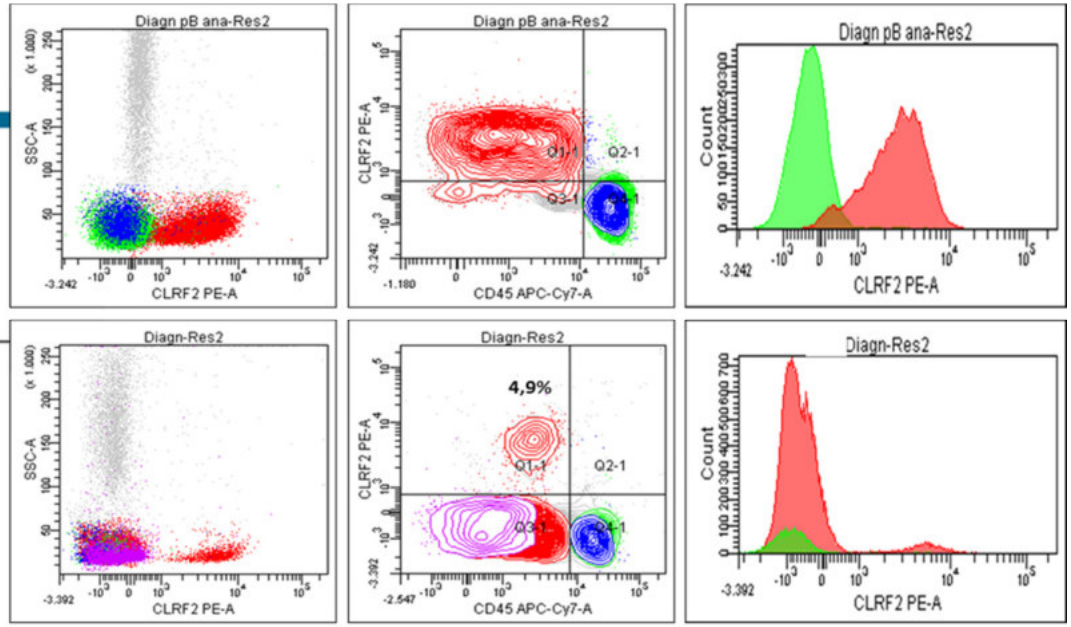
Slamova, Leukemia 2014

New entity: CRLF2r-ALL – CRLF2+

haematologica 2015; 100:e

Fine tuning of surface CRLF2 expression and its associated signaling profile in childhood B-cell precursor acute lymphoblastic leukemia

Cristina Bugarin,[1] Jolanda Sarno,[1] Chiara Palmi,[1] Angela Maria Savino,[1] Geertruy te Kronnie,[2] Michael Dworzak,[3] Angela Slumich,[3] Barbara Buldini,[2] Oscar Maglia,[1] Simona Sala,[1] Ilaria Bronzini,[2] Jean-Pierre Bourquin,[4] Ester Mejstrikova,[5] Ondrej Hrusak,[5] Drorit Luria,[6] Giuseppe Basso,[2] Shai Izraeli,[6] Andrea Biondi,[1,7] Giovanni Cazzaniga[1] and Giuseppe Gaipa;[1] on behalf of the I-BFM study group



P2RY8-CRLF2+, *IGH@-CRLF2+*

Cytometry Part B (Clinical Cytometry) 72B:S14–S22 (2007)

2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry: Optimal Reagents and Reporting for the Flow Cytometric Diagnosis of Hematopoietic Neoplasia

Brent L. Wood,[1*] Maria Arroz,[2] David Barnett,[3] Joseph DiGiuseppe,[4] Bruce Greig,[5] Steven J. Kussick,[6] Teri Oldaker,[7] Mark Shenkin,[8] Elizabeth Stone,[9] and Paul Wallace[10]

[1]Departme…shington

[3]Department of Haema…tal, Sheffield, England

[4]Depar…cticut

[5]De…ee

[6]Flow Cytomet…of Washington

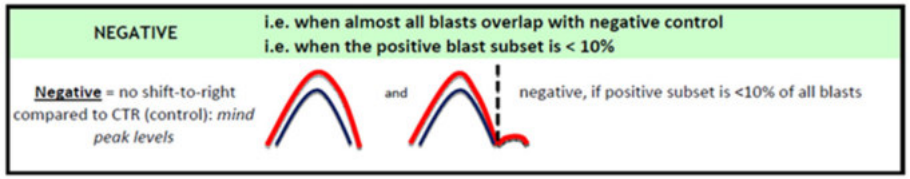
[7]I…

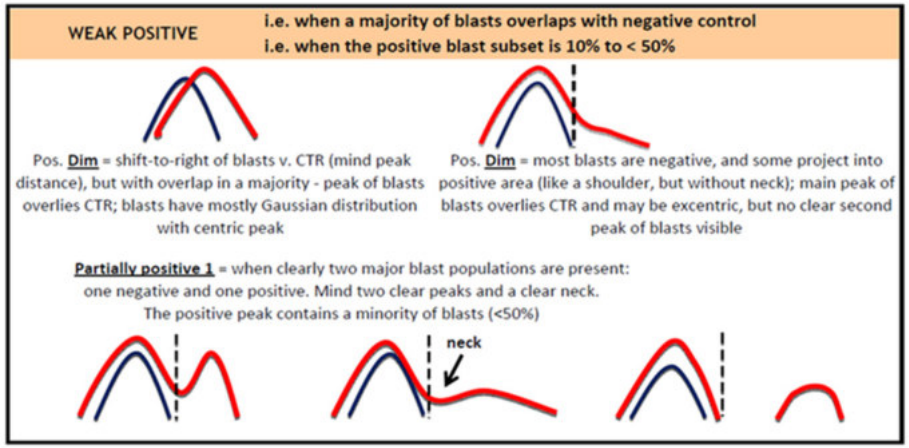
[10]Roswell Park C…atory Medicine, Buffalo, New York

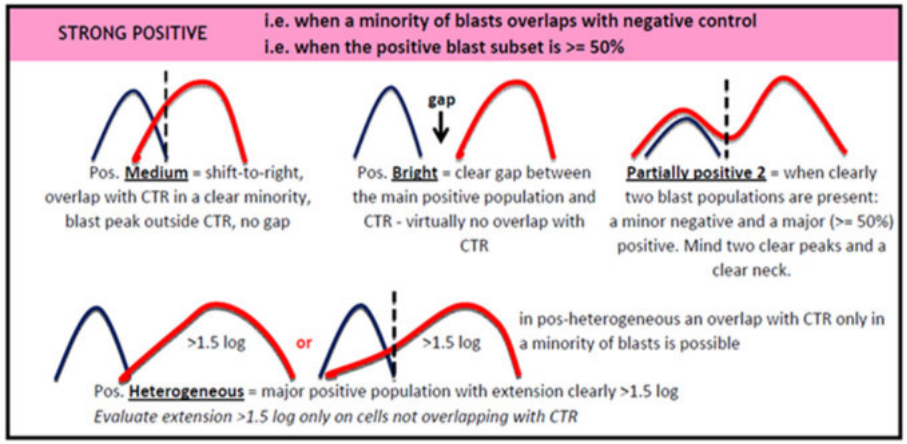
The group strongly affirmed the conclusion from the 1997 consensus conference that the reporting of numerical values for each antibody in a simple tabular form is generally unsatisfactory to indicate the presence of abnormal cells, cannot describe their phenotype in sufficient detail, and limits the ability of the recipient of the report to interpret results. Reporting of results in this manner is to be strongly discouraged.

Expression rating: neg / weak / strong

- In-sample cross-lineage negative control populations







Dominant lineage concept

TABLE 2. THE AIEOP-BFM DOMINANT LINEAGE ASSIGNMENT[@]

Lineage	Criteria	Antigens
BCP-ALL	≥2 positive of:	[§] CD19; CD10, (i)CD22, iCD79a
T-ALL	all 3 of:	[#] (i)CD3^{pos}, CD7^{pos}; iMPO$^{negative\ or\ weak}$
AML	≥2 positive of: and:	iMPO, CD13, CD33, CD64, CD65, CD117 BCP-/T-ALL criteria not met

[@] Of note, these markers are relevant for dominant lineage assignment, but are insufficient for a thorough description of leukemic immunophenotypes.

[§] BCP-ALL needs strong positivity in ≥2 of the four antigens – in the rare case of CD19-negativity, specifically CD10 must be strong positive. Mind that rare cases of MLL-rearranged BCP-ALL may drop out of this scheme due to biology-inherent lack of CD10, as well as weak (i)CD22 and iCD79a expression (CD19 is then usually strong positive).

[#] For T-ALL, iCD3 positivity must be either strong, or if rated weak, CD2 and/or CD5 should be any positive in addition. Surface CD3 expression needs to be tested in addition.

Process	Output
Sample **preparation** and **acquisition**	
Gating on blast population (CD45/LIN/SSC)	**Identify** blast population(s) and determine **blast percentage** among total NC
Complete immunophenotype assessment: **Antigen expression, distribution and intensity** according to *WHO & adapted Bethesda style*	**Inter-lineage** blast clone heterogeneity
Dominant lineage assignement according to *AIEOP-BFM criteria* (negative/weak/strong): based on iMPO, (i)CD3, CD7; CD10, CD19, (i)CD22, iCD79a	**ALL or not**
Further ALL blast characterization	**Intra-lineage** blast clone heterogeneity Immunological **subtype** (BI-BIV; TI-TIV; ETP) **MPAL status** (WHO); BAL status (EGIL)
Immunophenotyping report	**Indication for inclusion into AIEOP-BFM ALL treatment trial**

$P = 0{\cdot}027$). Our data suggest that an intensive therapy regimen including stem cell transplantation may be favourable for bilineal or lineage switch cases, whereas patients with *ETV6/RUNX1* fusion, lymphoid morphology and patients with expression of cyCD22 and cyCD79a should be treated with an ALL-directed therapy.

Gerr et al., BJH 2010

Therapy: BFM

MPAL in pediatrics

primarily shows that cytochemical MPO expression in childhood acute leukemia revealing typical lymphoblastic morphology and phenotype does rarely exist. Although a small number of patients studied, cytochemical MPO expression in acute leukemia does not seem to require myeloid leukemia treatment in case of otherwise lymphoblastic cytomorphology and phenotype.

Steiner et al., JPHO 2010

Therapy: BFM

Simple immunophenotypic criteria are useful for therapy decisions in MPAL. In B lineage leukemia, MPAL confers poorer prognosis. However, our data do not justify a preferential use of current acute myeloid leukemia-based therapy in MPAL.

Mejstrikova et al., Haematologica 2010

Therapy: BFM

An acute lymphocytic leukemia type of induction therapy, using agents that are active against lymphoid and myeloid leukemias, appears to be more effective in achieving and maintaining complete remissions regardless of whether the patients are classified according to EGIL criteria or the new WHO criteria. Hematopoietic stem cell transplantation may not be necessary for all patients in first complete remission.

Al-Seraihy et al., Haematologica 2009

Therapy: TTX 13

MPAL – trial inclusion & treatment

Dominant Lineage Assignment

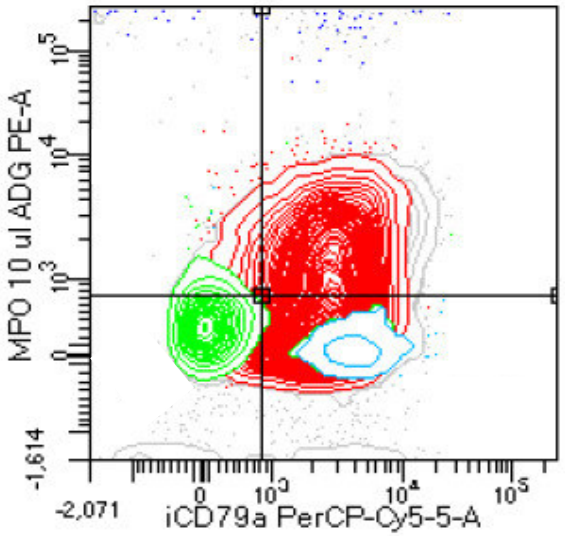
Inclusion into AIEOP-BFM ALL treatment trials is based on morphologically and genetically defined lymphoblastic leukemia with a dominant ALL-immunophenotype according to the AIEOP-BFM lineage assignment criteria (Table 2) adapted from Mejstrikova et al. (20). This is irrespective of whether or not a case fulfils also criteria of MPAL (WHO 2008/2016, Refs. 3,4, and 16; Supporting Information Table 1) or BAL (EGIL, Refs. 9 and 10; Supporting Information Table 2)—even in case of MPO positivity—in a single blast population. Nevertheless, apart from assigning the dominant lineage of a single or simply branched leukemic clone (e.g., by partial positivity with MPO), a finding of fulfilled MPAL/BAL-criteria needs to be reported as secondary detail. Thus, dominant lineage assignment overrules French-American-British (FAB)-classification as well as MPAL/BAL designations for clinical decision making in AIEOP-BFM with the—important—exception of cases with more than one (separated or complex) blast population including a non-lymphoblastic component (see Supporting Information Fig. 6). In such case, dominant lineage assignment is not applicable as far as both separate components comprise each ≥10% of cells of the sample. In

AIEOP-BFM Consensus Guidelines 2016 for Flow Cytometric Immunophenotyping of Pediatric Acute Lymphoblastic Leukemia

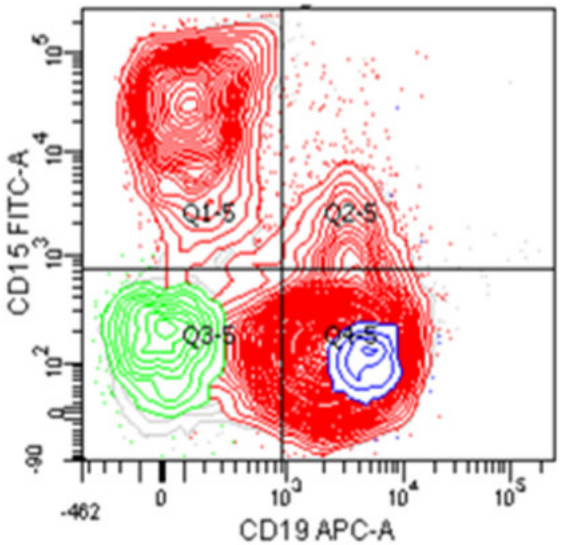
Cytometry Part B (Clinical Cytometry)

MPAL – trial inclusion & treatment

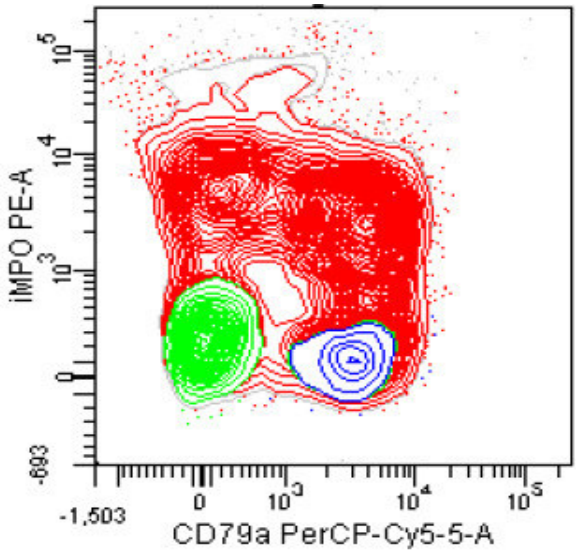
Types of MPAL…



simple



bilineal



complex

WHO 2008 Criteria for MPAL Definition

Myeloid lineage:
Myeloperoxidase (flow cytometry, immunohistochemistry, or cytochemistry)
or
Monocytic differentiation (NSE, CD11c, CD14, CD64, or lysozyme)

T-lineage:
Cytoplasmic CD3 (flow cytometry with antibodies to CD3 epsilon chain; immunohistochemistry using polyclonal anti- CD3 antibody may detect CD3 zeta chain, which is not T-cell specific)
or
Surface CD3 (rare in mixed phenotype leukaemias)

B-lineage (multiple antigens required):
Strong CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10
or
Weak CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10

Note: Monocytic differentiation requires **positivity of ≥2** of these antigens;

The **T-cell component** is recognized by **bright expression of iCD3**, either on the entire blast population **or on a separate subpopulation of leukemic cells** … should be as bright or nearly as bright as that of normal residual T cells present in the sample.

Cytometry Part B (Clinical Cytometry) 86B:152–153 (2014)

Editorial

Mixed Phenotype Acute Leukemia

Michael J. Borowitz*
Professor of Pathology and Oncology,
Johns Hopkins Medical Institutions,
Baltimore, Maryland

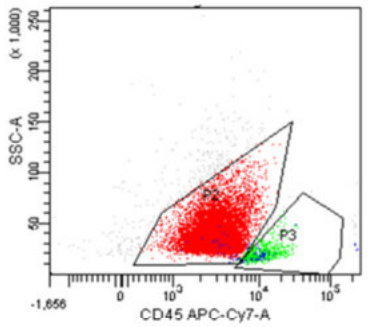
criteria for T/Myeloid MPAL (mixed phenotype acute leukemia) can be met in one of two ways. The criterion most are familiar with requires the expression of the most specific markers for each lineage—in this case cytoplasmic CD3 and myeloperoxidase.

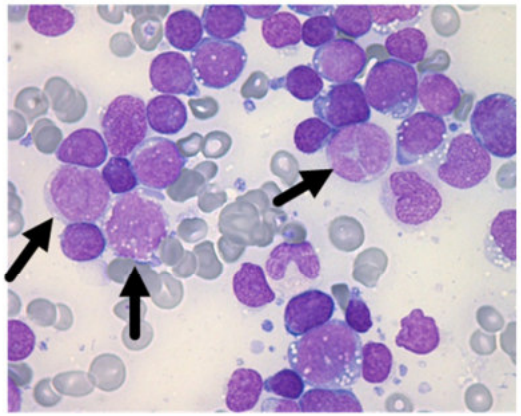
However, less frequently recognized is the fact that expression of these specific markers only applies to the situation in which there is a single population of blasts; criteria for identifying a myeloid component are also met "...when there are two or more distinct populations of leukaemic cells, one of which would meet immunophenotypic criteria for acute myeloid leukaemia (with the exception that this population need not comprise 20% of all nucleated cells)..."

Simple co-expressing MPAL

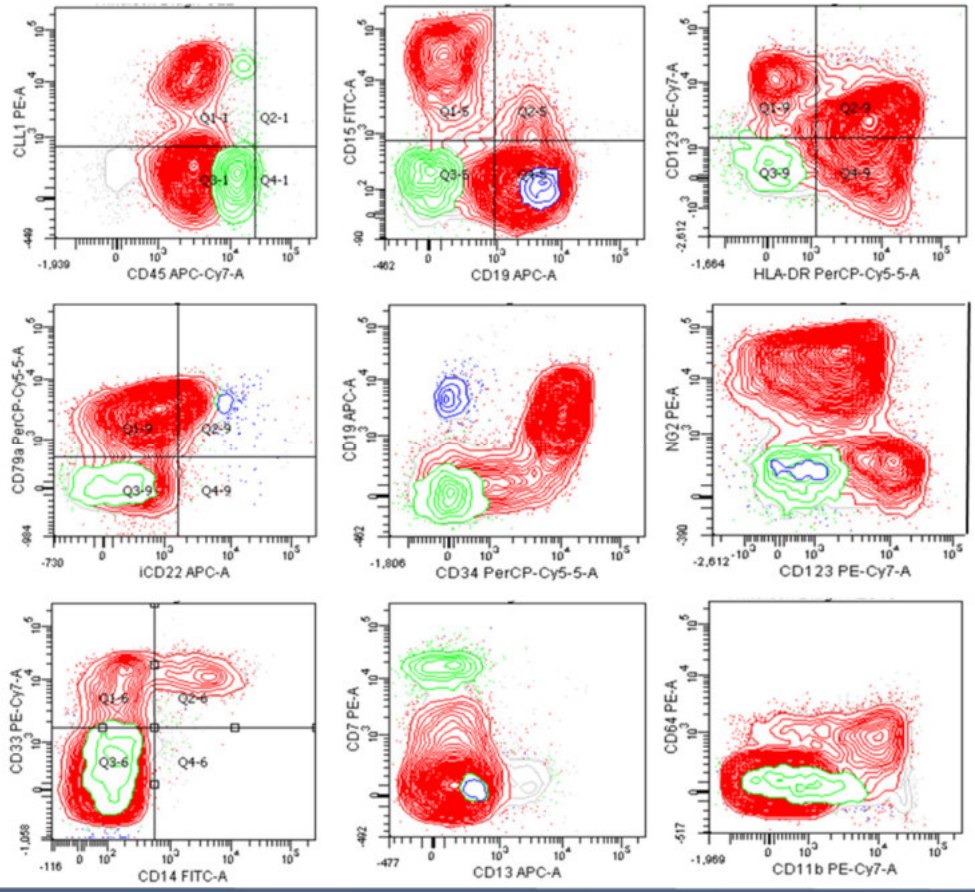
Bi-lineal MPAL

Heterogeneous blasts: MPAL - bilineal



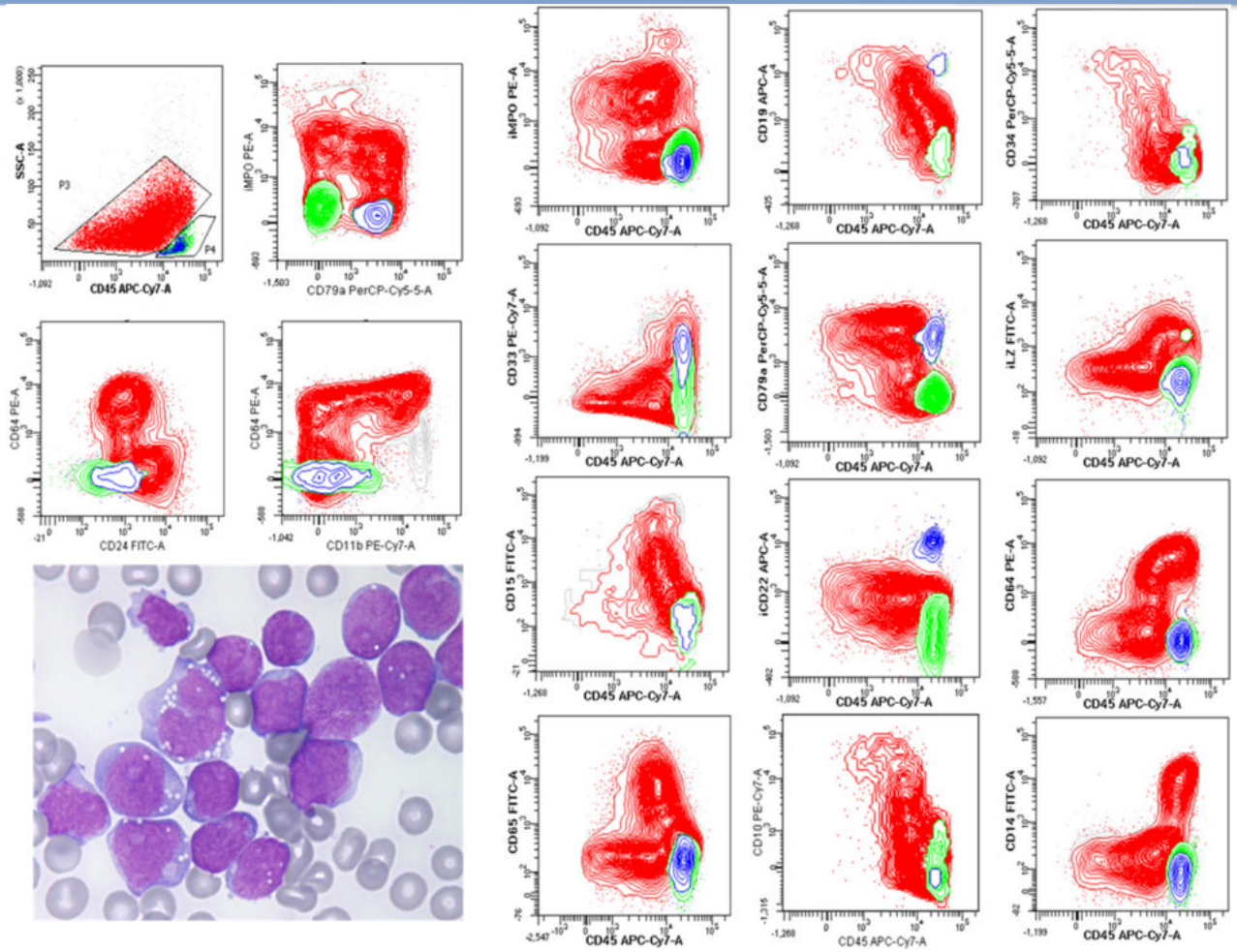


Genetics: 46XY; t(4;11)



- Diagnosis: MPAL B/M
- Complex immunophenotype
- Separate blast subsets with differentiation drift into opposing lineage directions
- No common antigenic denominator of lineage
- Dominant lineage cannot be determined
- No inclusion into AIEOP-BFM trial

Heterogeneous blasts: MPAL - complex



- Diagnosis: MPAL B/M
- Complex immunophenotype
- Branched, interconnected blast subsets with differentiation drift into opposing lineage directions
- No common antigenic denominator of lineage
- Dominant lineage cannot be determined
- No inclusion into AIEOP-BFM trial

Genetics: MLLr & BCR/ABL negative

New entity: ETP

Thus, in the broadest sense, ETP ALL is a kind of "T/myeloid" leukemia. From a definitional perspective, however, MPO expression excludes ETP ALL, while the great majority of cases of MPAL are MPO positive. In addition, the T cell component of T/myeloid leukemia frequently would meet criteria for ETP ALL. Thus, these two leukemias appear more alike than different, although because of the central importance of MPO to labeling something as myeloid, and the way leukemia treatment protocols are structured, they are typically treated differently. Unfortunately, this may make it difficult ever to understand whether these do in fact constitute different leukemic entities. It will be interesting to see how this situation will be treated in the next iteration of the WHO classification.

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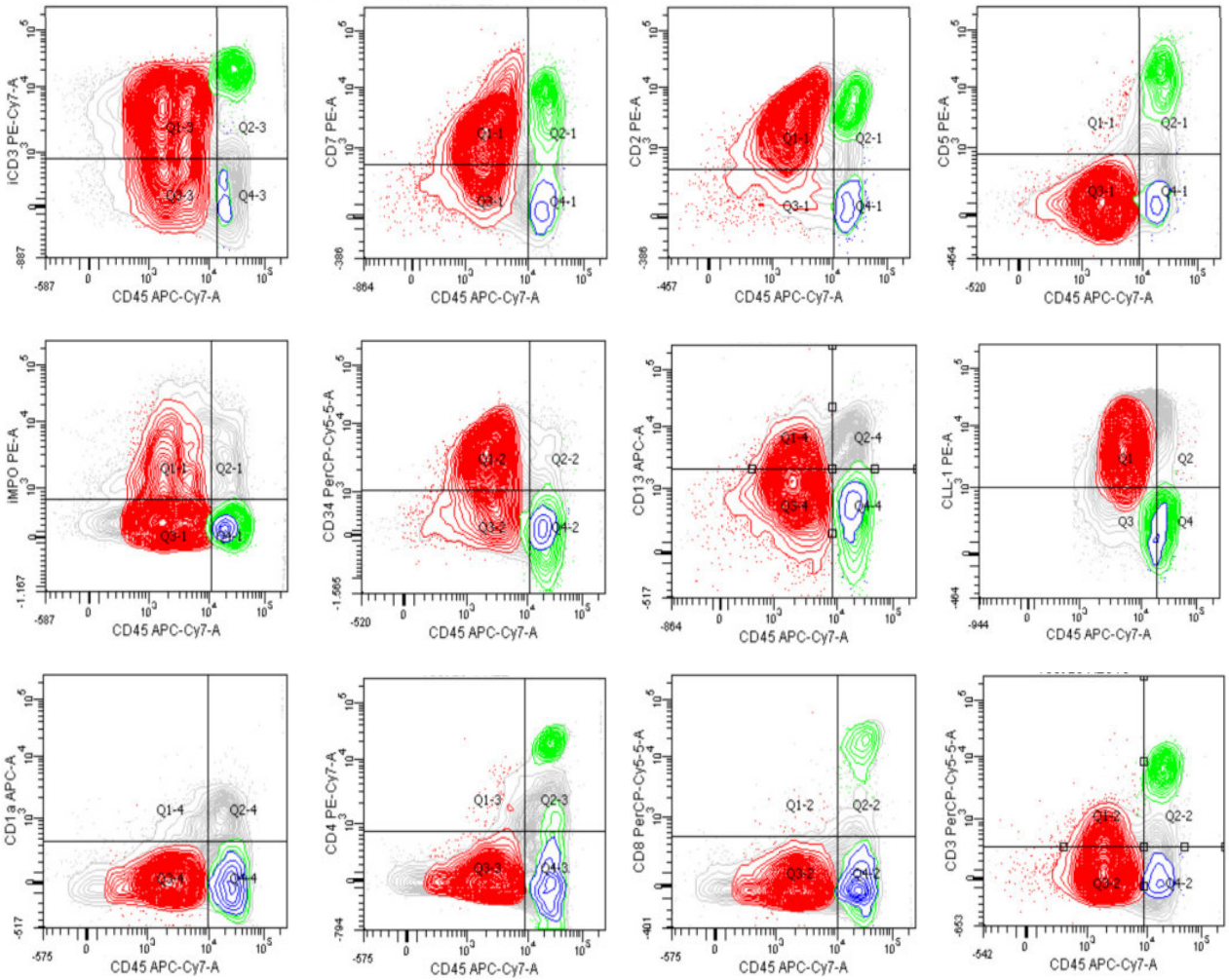
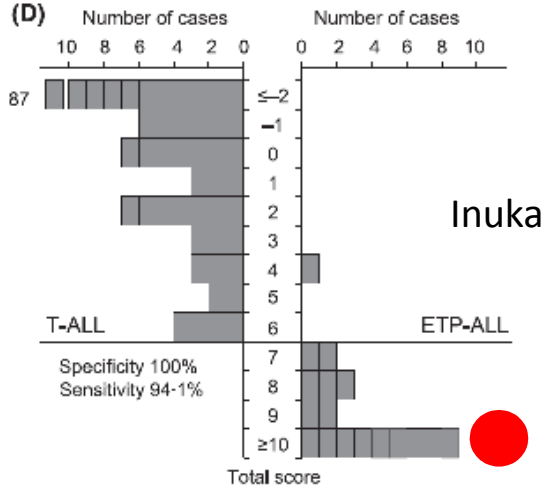
Subtype	Discriminators	Remarks
ETP (only additive to T-I or T-II)	CD1a^{neg}, CD8^{neg} usually CD5$^{neg\ or\ weak\ pos}$ and ≥1^{pos} of HLADR, CD11b,13,33,34,65,117	if CD5$^{strong\ pos}$: ≥2^{pos} of HLADR, CD11b,13,33,34,65,117; sCD3$^{weak\ pos}$ may occur*



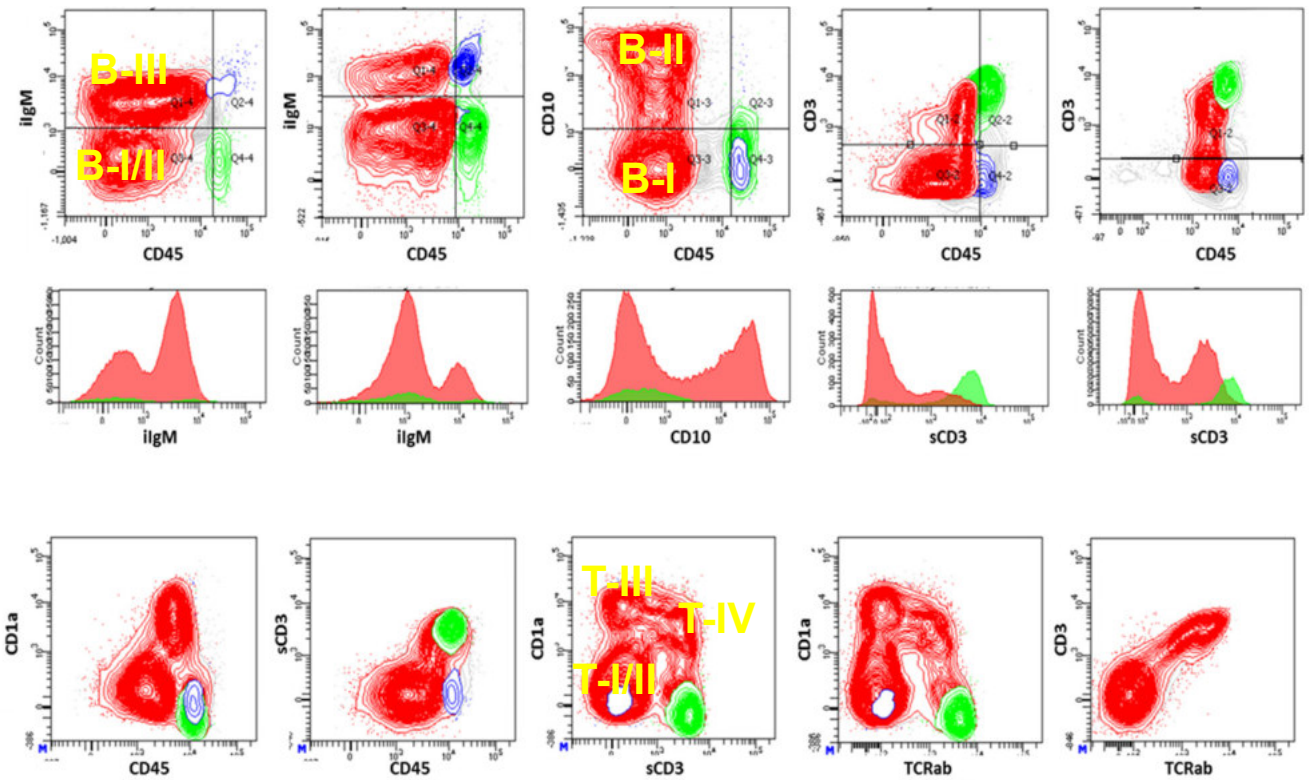
Dgn.: T-ALL
EGIL: T-II
ETP: yes
MPAL: yes
BAL: yes
Blast clone heterogeneity: yes



Inukai-Score

Process	Output
Sample **preparation** and **acquisition**	
Gating on blast population (CD45/LIN/SSC)	**Identify** blast population(s) and determine **blast percentage** among total NC
Complete immunophenotype assessment: **Antigen expression, distribution and intensity** according to *WHO & adapted Bethesda style*	**Inter-lineage** blast clone heterogeneity
Dominant lineage assignement according to *AIEOP-BFM criteria* (negative/weak/strong): based on iMPO, (i)CD3, CD7; CD10, CD19, (i)CD22, iCD79a	**ALL or not**
Further ALL blast characterization	**Intra-lineage** blast clone heterogeneity Immunological **subtype** (BI-BIV; TI-TIV; ETP) **MPAL status** (WHO); BAL status (EGIL)
Immunophenotyping report	**Indication for inclusion into AIEOP-BFM ALL treatment trial**

Heterogeneous blasts: ALL subtype drift



"heterogeneous blast populations"

- immunophenotypic subclone formation
- may lead to divergent interpretation and reporting
- characterized by partial expression of certain markers:
  - B-ALL: cIgM (in 40% of cases), CD10, K/λ-chain
  - T-ALL: sCD3 (in 50% of cases), CD1a, TCR
- differentiation may even drift in a single case from
  - B-I till B-IV
  - T-I/II till T-IV
- cumulative designation adapted from EGIL: e.g. TII/III/IV

TABLE 4: THE AIEOP-BFM SUBCLASSIFICATION OF ALL[#]

Subtype	Discriminators	Remarks
B-I (pro-B)	CD10^{neg}	BCP-ALL lineage criteria fulfilled
B-II (common)	CD10^{pos}	/
B-III (pre-B)	iIgM^{pos}	CD10$^{neg\ or\ weak\ pos}$ may occur[§]
B-IV (mature B)	κ- or λ-chain^{pos}	may occur with FAB L1/L2 morphology[&]
T-I (pro-T)[$]	only iCD3^{pos} and CD7^{pos}	T-ALL lineage criteria fulfilled
T-II (pre-T)	≥1 of CD2^{pos}, CD5^{pos}, CD8^{pos}	surface (s) CD3$^{weak\ pos}$ allowed*
T-III (cortical T)	CD1a^{pos}	sCD3^{weak} may occur*
T-IV (mature T)	CD1a^{neg} and sCD3^{pos}*	sCD3^{strong}, or sCD3$^{weak\ pos}$ with TCR^{pos}
ETP (only additive to T-I or T-II)	CD1a^{neg}, CD8^{neg} usually CD5$^{neg\ or\ weak\ pos}$ and ≥1^{pos} of HLADR, CD11b,13,33,34,65,117	if CD5$^{strong\ pos}$: ≥2^{pos} of HLADR, CD11b,13,33,34,65,117; sCD3$^{weak\ pos}$ may occur*

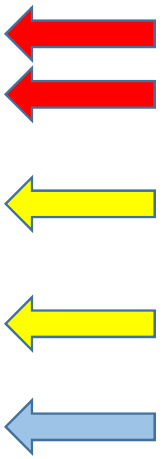
[#] adapted from refs. 8 & 9.

[§] CD10$^{neg/weak}$ B-III is frequently associated with MLL-rearrangements (12).

[&] light-chain^{pos} cases without FAB L3-morphology and without *MYC*-translocation are eligible for conventional ALL treatment, and thus must be separated from Burkitt-type mature B-ALL (40-43).

[$] T-I is very rare and can be reported together with T-II (as T-I/II)

* Dim or even more frequently partial surface positivity with CD3 (e.g. in a minor blast subpopulation) occurs when sensitive methodology is used and should not mislead to diagnose mature T-ALL in the absence of TCR expression.



Mixed Lineage Leukemia **– Rearranged Childhood Pro-B and CD10-Negative Pre-B Acute Lymphoblastic Leukemia Constitute a Distinct Clinical Entity**

Andishe Attarbaschi,[1,2] Georg Mann,[1] Margit König,[2] Manuel Steiner,[1] Sabine Strehl,[2] Anita Schreiberhuber,[2] Björn Schneider,[4] Claus Meyer,[4] Rolf Marschalek,[4] Arndt Borkhardt,[5] Winfried F. Pickl,[3] Thomas Lion,[1] Helmut Gadner,[1,2] Oskar A. Haas,[2] and Michael N. Dworzak[1,2] on behalf of the Austrian Berlin-Frankfurt-Münster Cooperative Study Group

Clin Cancer Res 2006;12(10) May 15, 2006

Refined subclassification: prec-B-IV (non-Burkitt)

Precursor B Lymphoblastic Leukemia With Surface Light Chain Immunoglobulin Restriction

A Report of 15 Patients

Rina Kansal, MD,[1] George Deeb, MD,[1] Maurice Barcos, MD, PhD,[2] Meir Wetzler, MD,[3] Martin L. Brecher, MD,[4] AnneMarie W. Block, PhD,[5] and Carleton C. Stewart, PhD[6]

Key Words: Precursor B cell; Acute lymphoblastic leukemia; Surface immunoglobulin–positive acute leukemia; Flow cytometry; WHO classification; Immunophenotyping

Am J Clin Pathol 2004;121:512-525

Abstract

We describe 15 patients (9 children) with precursor B-cell (pB) acute lymphoblastic leukemia (ALL) with surface immunoglobulin (sIg) light chain restriction revealed by flow cytometric immunophenotyping (FCI). The same sIg+ immunophenotype was present at diagnosis and in 3 relapses in 1 patient. In 15 patients, blasts were CD19+CD10+ (bright coexpression) in 14, CD34+ in 12, surface κ+ in 12, surface λ+ in 3; in 8 of 8, terminal deoxyribonucleotidyl transferase (TdT)+; and in 4, surface IgD+ in 2 and surface IgM+ in 1. The 3 CD34– cases included 1 TdT+ case, 1 with t(1;19)(q23;p13), and 1 infant with 70% marrow blasts. One adult had CD10–CD19+CD20–CD22+CD34+ TdT+sIg+ blasts with t(2;11)(p21;q23). Blasts were L1 or L2 in all cases (French-American-British classification). Karyotypic analysis in 12 of 12 analyzable cases was negative for 8q24 (myc) translocation. Karyotypic abnormalities, confirmed by fluorescence in situ hybridization in 6 cases, included hyperdiploidy, t(1;19)(q23;p13), t(12;21)(p13;q22), t(9;22)(q34;q11), t(2;11)(p21;q23), and trisomy 12. The sIg light chain restriction in pB ALL might be present in neoplasms arising from the early, intermediate, and late stages of precursor B-cell maturation; sIg light chain restriction revealed by FCI does not necessarily indicate a mature B-cell phenotype, further emphasizing the importance of a multidisciplinary approach to diagnosing B-lymphoid neoplasms.

ACKNOWLEDGMENTS

- iBFM-FLOW:
- AutoFlow:
- industry:

Demo video of the analysis software flowView at: https://www.youtube.com/watch?v=fu0V76Cppa4&feature=youtu.be