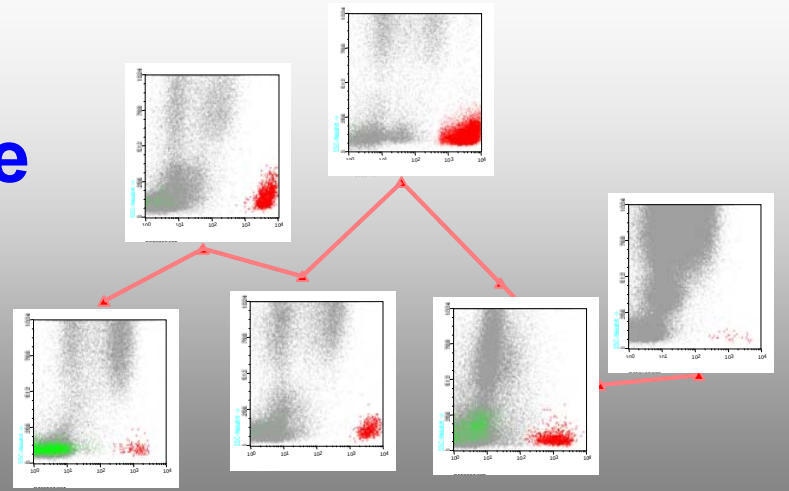
Flow Cytometric Minimal Residual Disease Monitoring in ALL:

Background, Pitfalls, and Values



M. N. Dworzak

on behalf of the I-BFM ALL FLOW-MRD-SG

XVI Congress of The Chilean Society of Hematology

Coquimbo, **Chile**, September 24-27, 2008

Flow Cytometry
for future stratifying clinical application
in multi-center trials of the I-BFM...

I-BFM *ALL FLOW MRD SG*

- **AIEOP-BFM (study 2008 upcoming)**

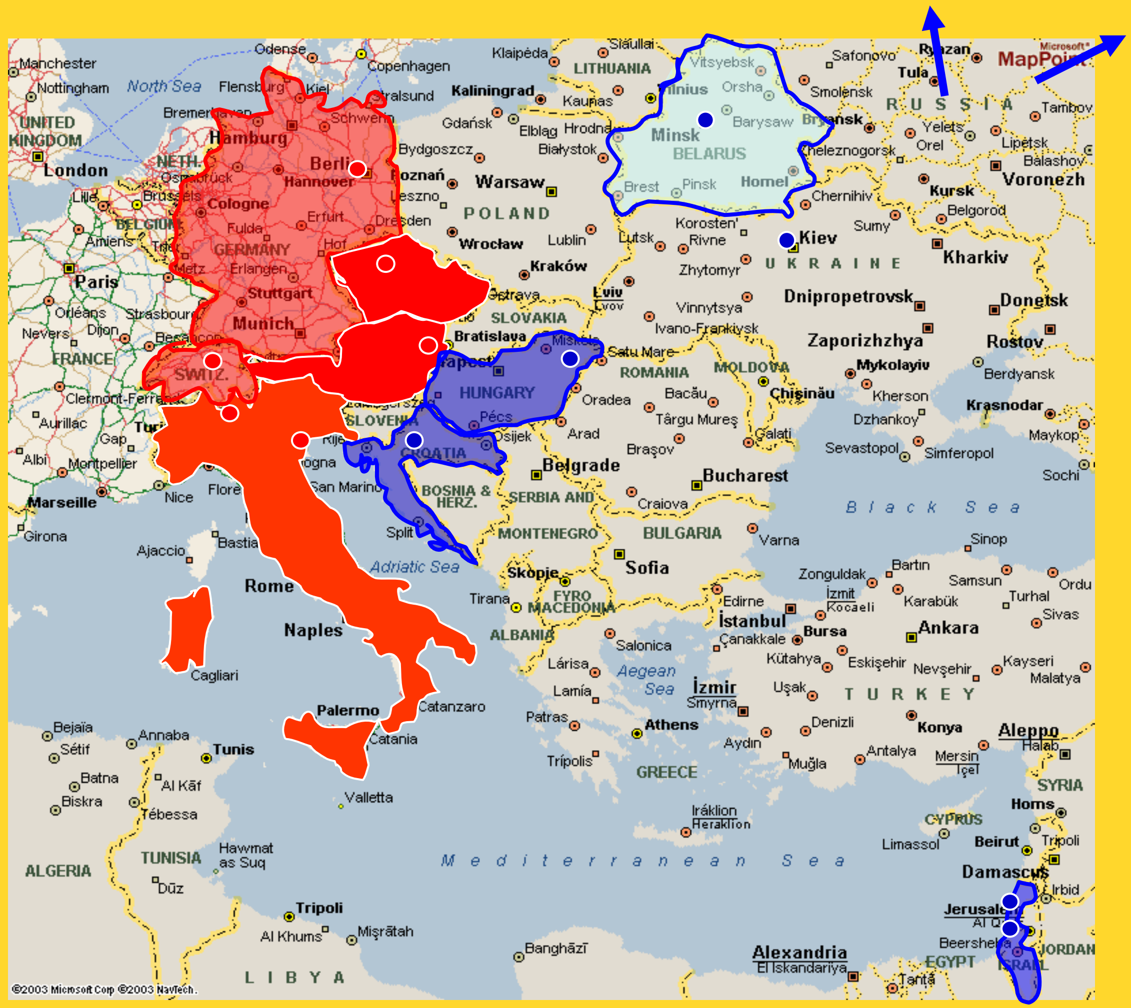
Berlin	Ratei/Ludwig
Monza	Gaipa/Biondi
Padova	Basso/Veltroni
Prague	Hrusak/Mejstrikova
Switzerland	Bourquin/Niggli
Vienna	**Dworzak**

- **ALL-IC BFM**

Debrecen	Kappelmayer/Kis
Zagreb	Batinic
Israel	Luria/Stark
Chile	Cabrera/Campbell
...	

- **Moscow-Berlin group**

Ekaterinburg	Popow/Fechina
Minsk	Belevtsev/Alainikova
Moscow	Boyakova/Roumantsev



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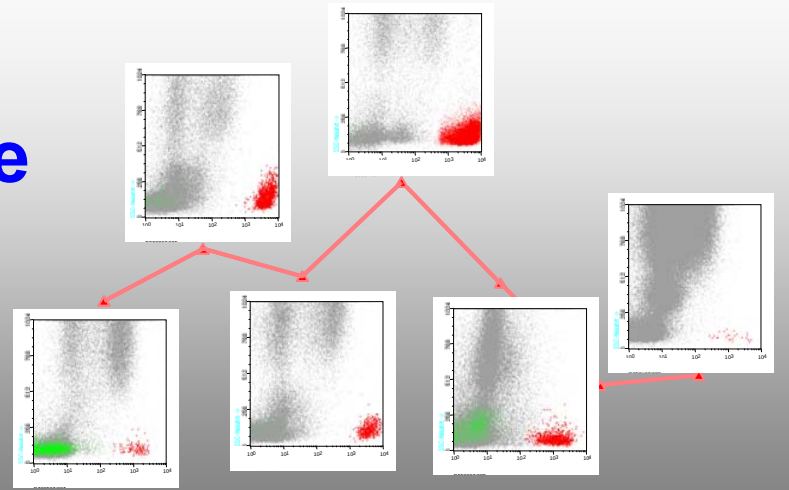


St. Anna Kinderkrebs Forschung

AIEOP-BFM-ALL 2000 FCM-MRD-SG

Berlin Ratei/Ludwig
Monza Gaipa/Biondi
Padova Basso/Veltroni
Vienna Dworzak

The AIEOP-BFM ALL 2000 FCM-MRD study

Part 1

MRD definition – old and new knowledge

Technical standardization in AIEOP-BFM

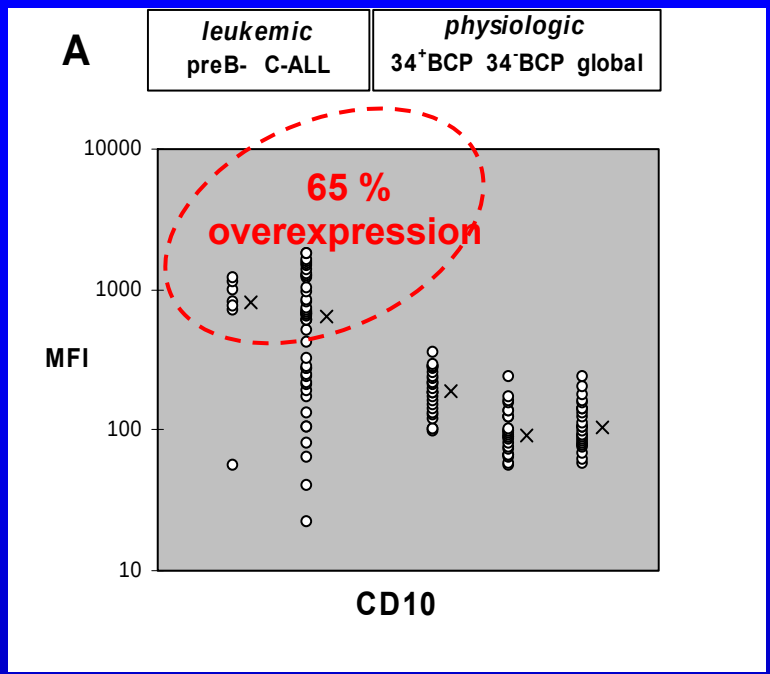
Part 2

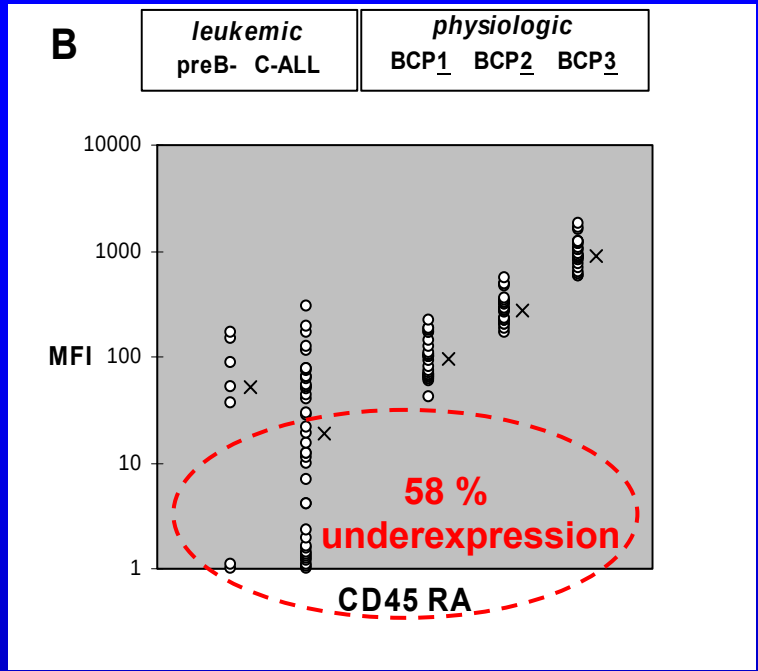
Correlation with PCR-MRD

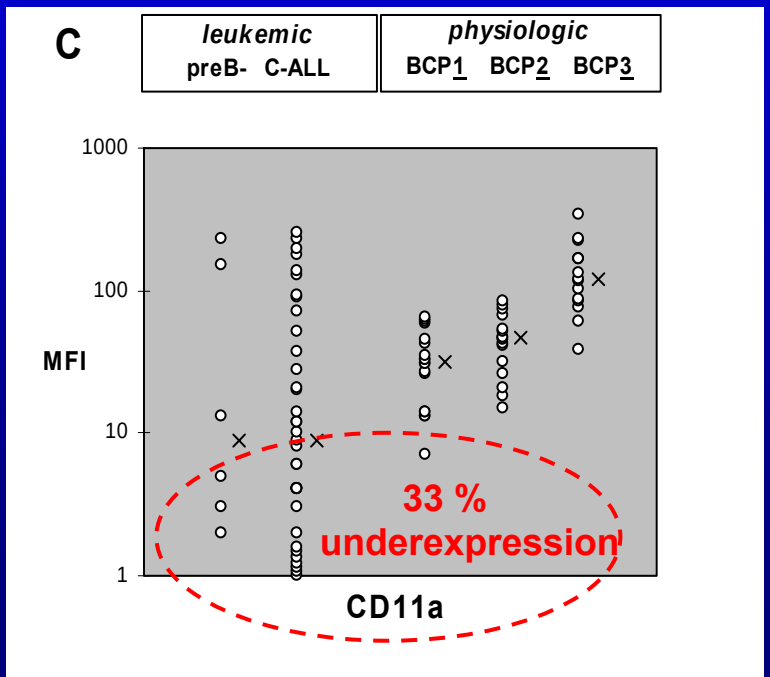
FCM vs. Outcome: interim results

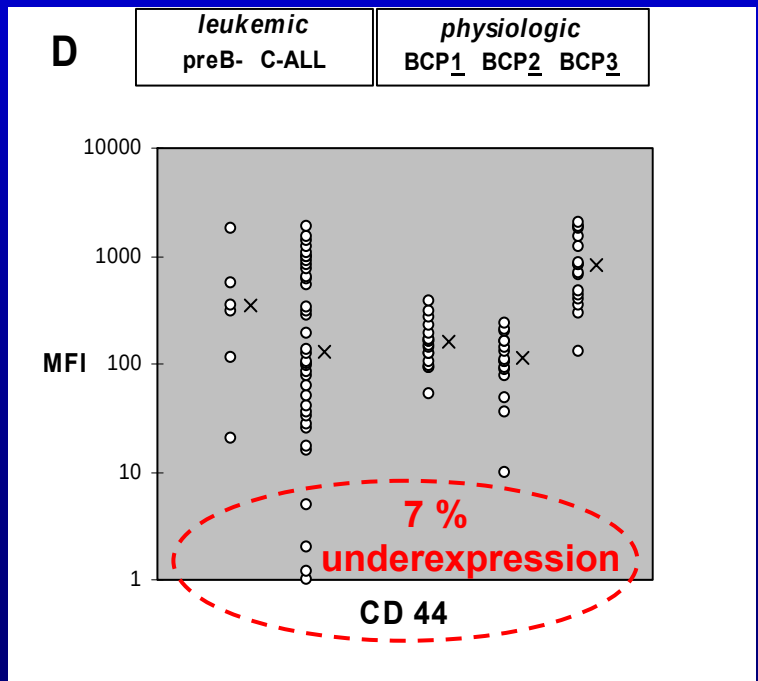
Deranged patterns of antigen expression in BCP-ALL

with respect to normal differentiation







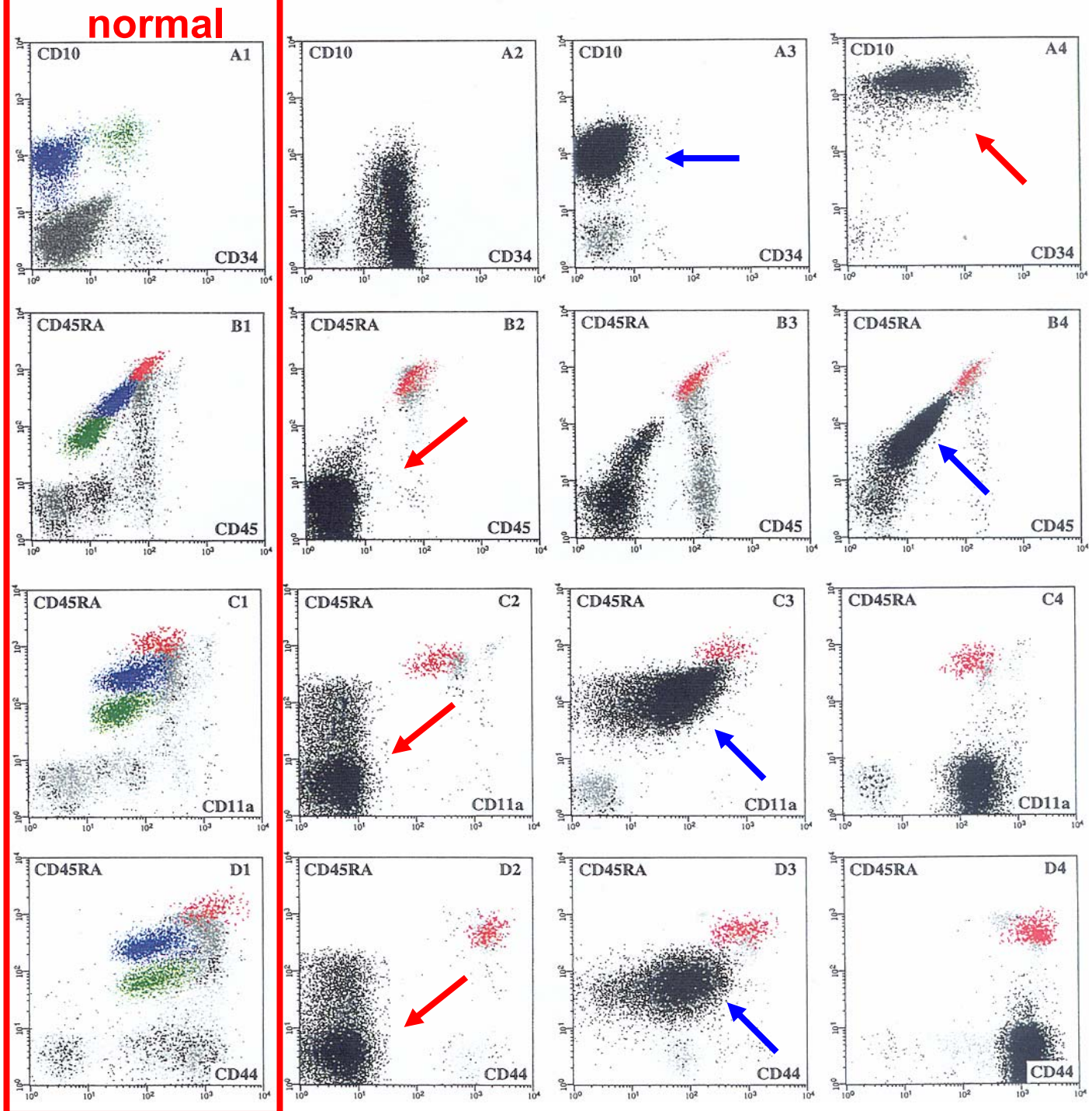


Examples of

deranged patterns of antigen expression in BCP-ALL with respect to normal differentiation

frequently involved antigens:

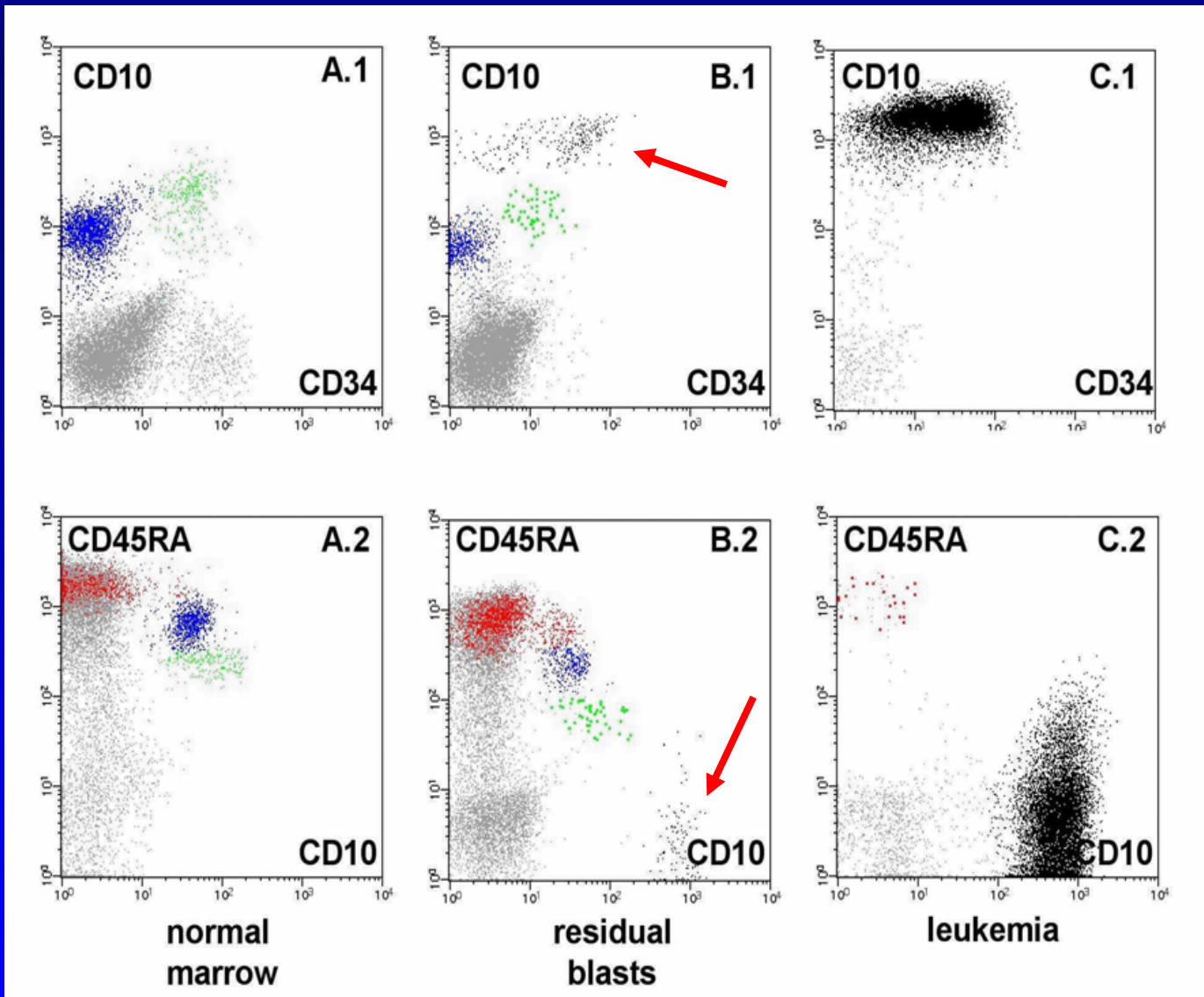
CD10, CD11a, CD19, CD20, CD34, CD38, CD44, CD45, CD58



Flow cytometric MRD assessment in ALL

examples of MRD

detected upon generic deranged patterns of antigen expression with respect to normal differentiation



Flow cytometric MRD assessment in ALL

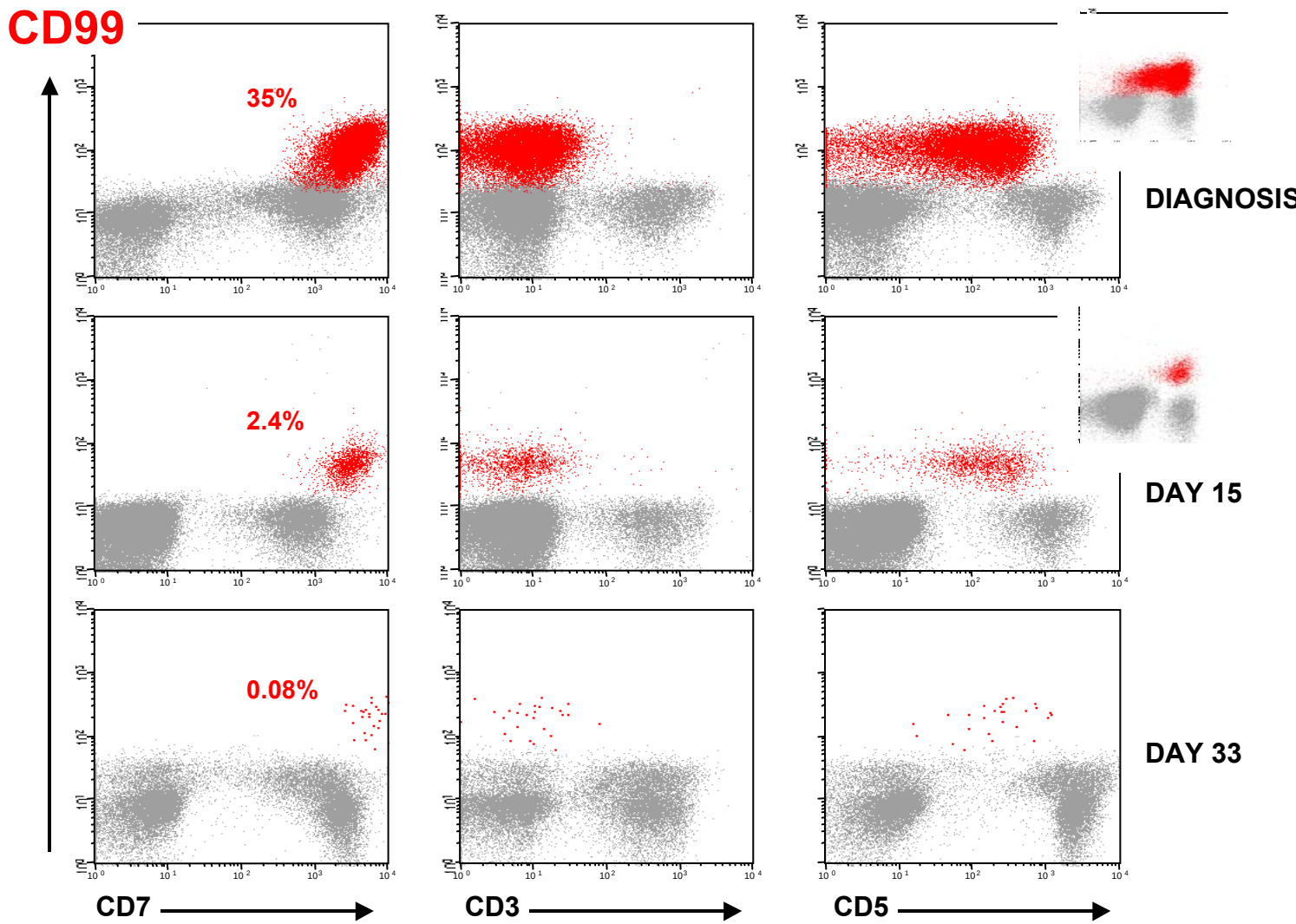
examples
of MRD

detected upon
deranged patterns
of antigen expression

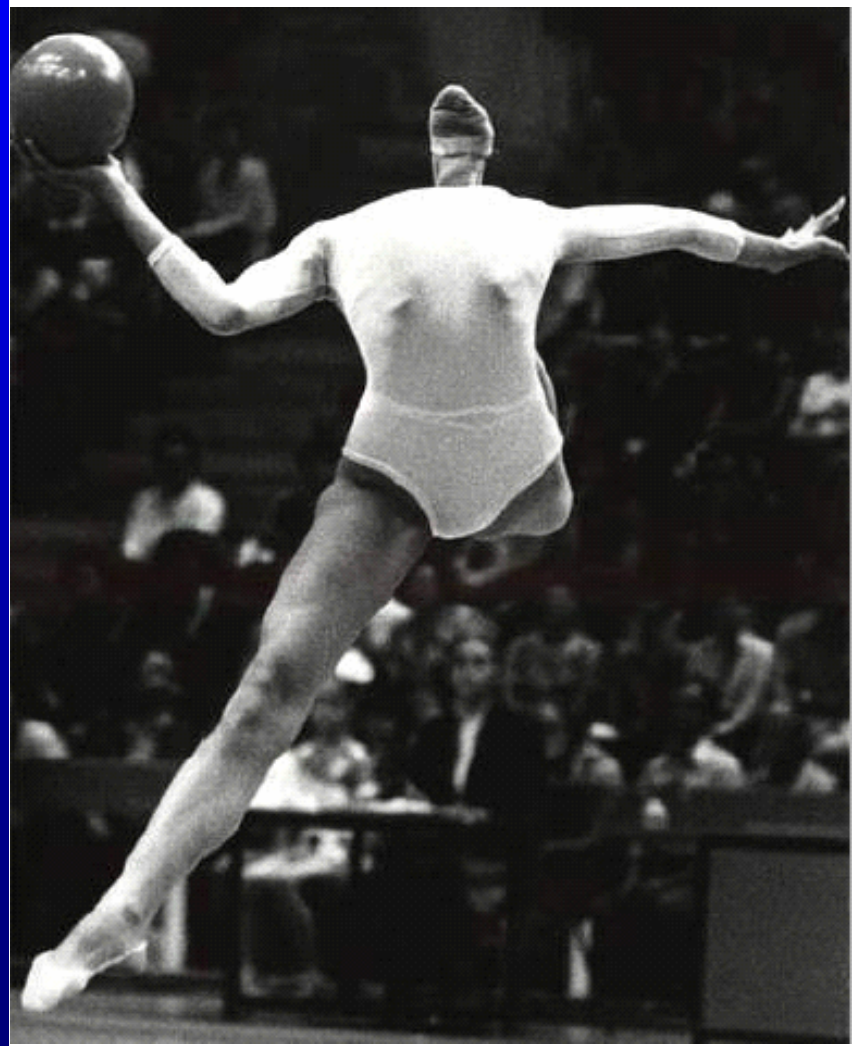
with respect to
location of occurrence

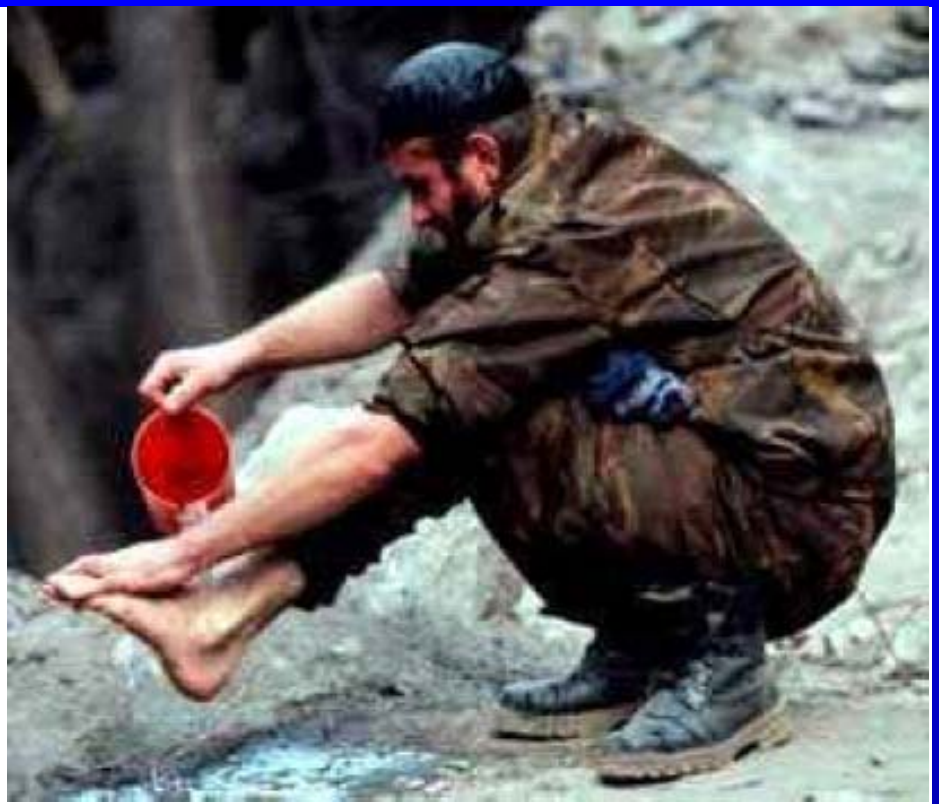
e.g. T-ALL

Antigens: CD99, TdT



Watch out for
weird phenotypes

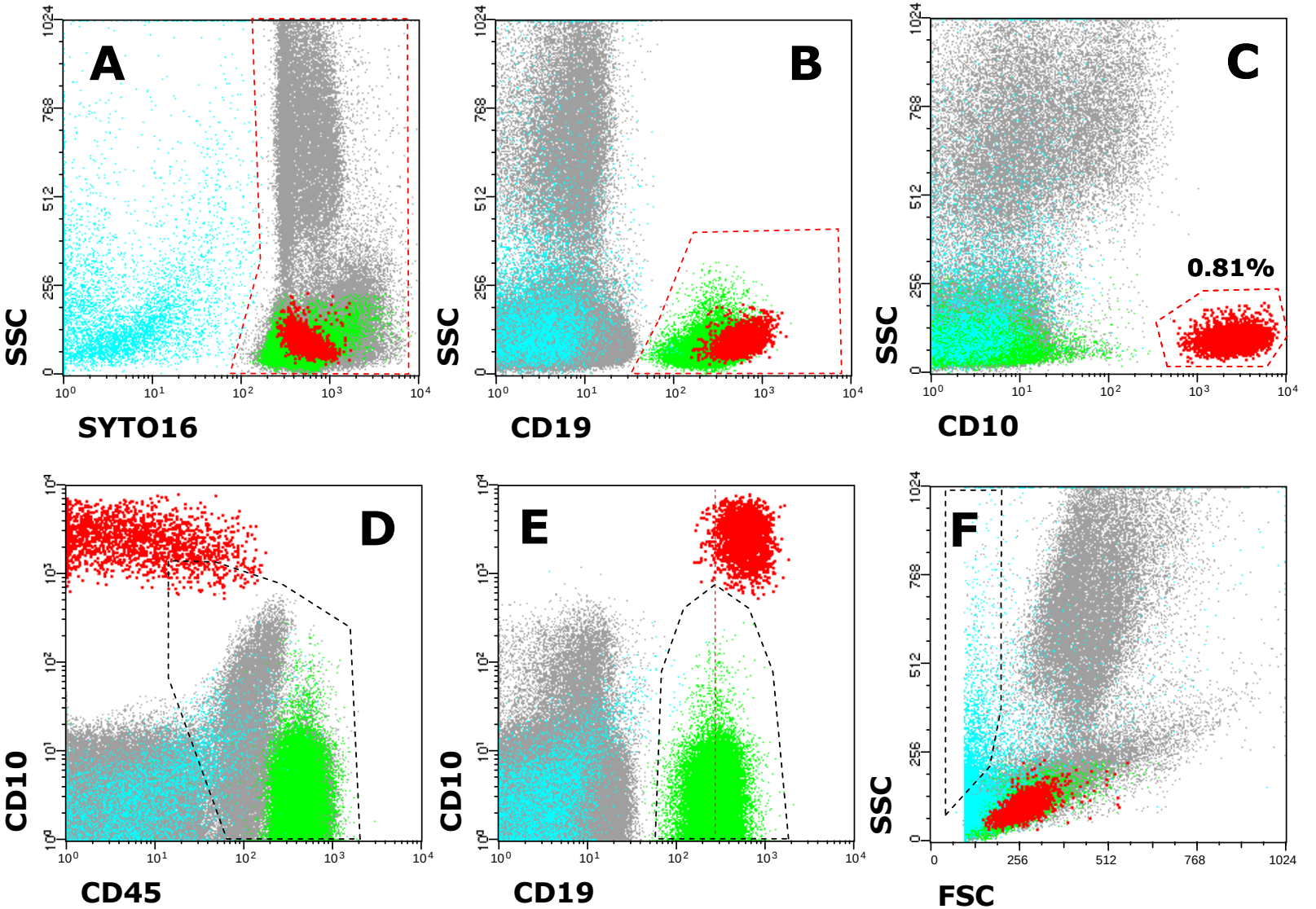




LAIPs
Leukemia -associated
immunophenotypes

AIEOP-BFM ALL 2000 FLOW-MRD:

The gating and analysis strategy



General AIEOP-BFM FCM-guidelines

for acquisition and MRD interpretation

- **acquisition of 300 000 nucleated events per tube**
- **MRD is sound population of ≥ 10 dots**
- **exclude dead cell area dots (low FSC/hiSSC)**
- **usually tube 1 for quantification (e.g. CD10PE)**
  other tubes for control purpose

Comparison of established MRD-MoAb panels

St. Jude CRH	AIEOP-BFM	Biomed
22 10 34 19	20 10 34 19	TdT 10 19
58 10 34 19	58 10 34 19	19 34 45
45 10 34 19	10 34 45 19	10 20 19
38 10 34 19	10 11a 45 19	34 22 45 (19)
13 10 34 19	(10 38 45 19)	34 38 19
33 10 34 19		10 13 19
15 10 34 19	10+20 38 34 19	33
65 10 34 19	15 34 45 19	15
66c 10 34 19	65 34 45 19	65
56 10 34 19		
7.1 10 34 19		
TdT 10 34 19		
Cμ 10 34 19		
WT1 10 34 19		

MyM →

cyM →

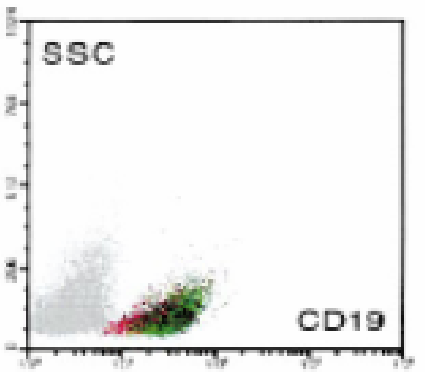
limited panel !!

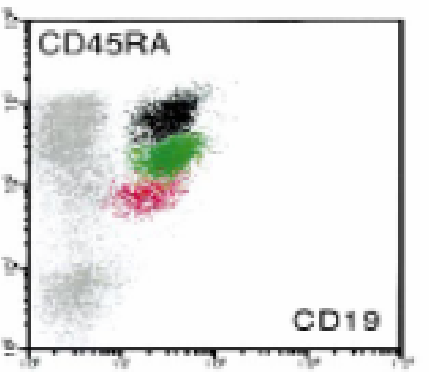
triple-backbone !

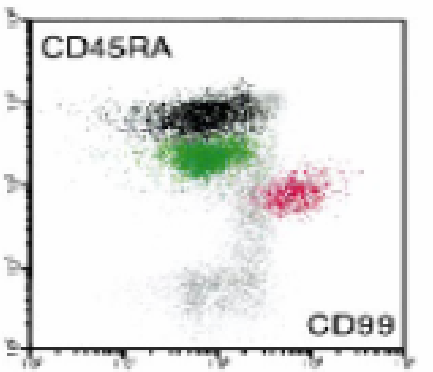
CD diversity: n= 17 n= 10 n= 12

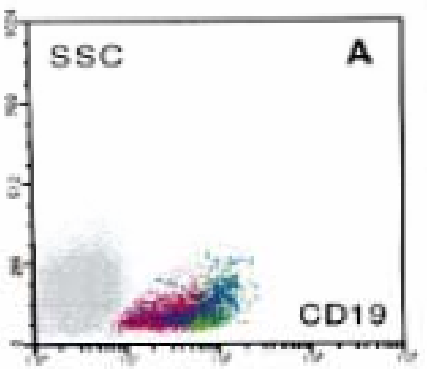
LAIP - limitations:

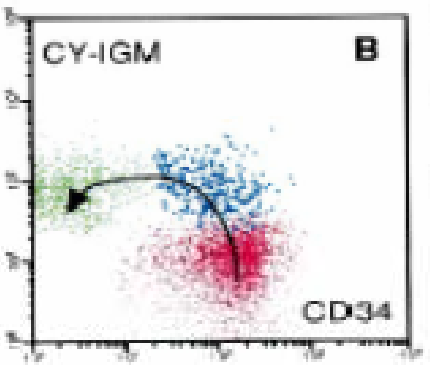
Immature normal BCP may express cyIgM along with CD34, TdT, CD10, and CD99

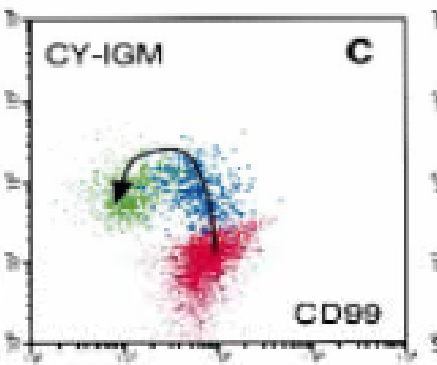


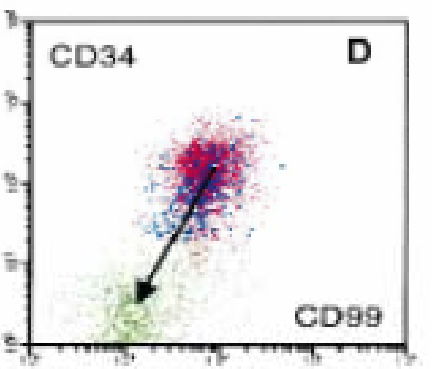










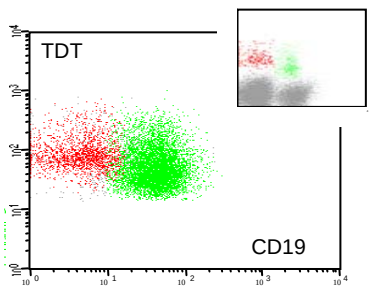


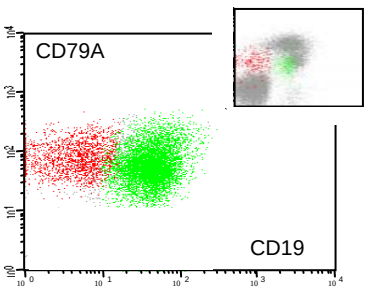
LAIP - limitations:

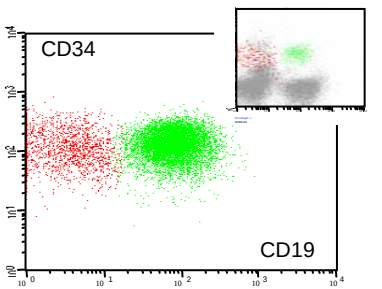
Very immature normal BCP may express CD33 and CD7 along with CD34, TdT, and CD10

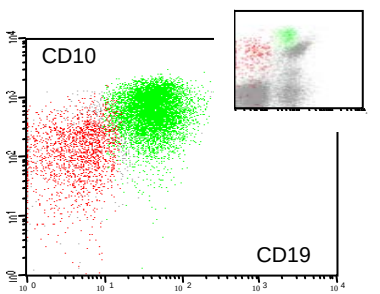
Table 2. Coexpression Patterns of CD79a⁺TdT⁺ Precursors

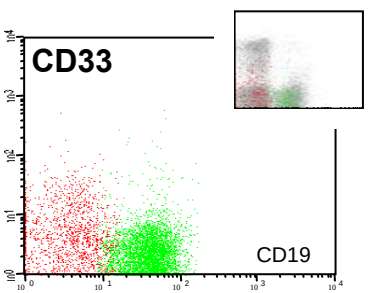
	Mean % (range; n = 9) of CD79a⁺TdT⁺ Cells Positive With Marker										
	CD19	CD34⁺	CD10	CD10^{lo}	CD10^{hi}	CD33	CD7	MPO⁺	cytoCD3⁺	CD14⁺	Control
Total	78 (61-92)	93 (88-97)	96 (93-99)	26 (17-33)	73 (67-83)	5 (1-8)	6 (2-10)	3 (2-6)	3 (1-6)	3 (1-8)	2 (1-4)
CD19⁺		ND	ND	14 (7-17)	87 (83-93)	3 (1-4)	4 (2-6)	3 (2-5)	3 (1-4)	2 (1-6)	2 (1-3)
CD19⁻		ND	ND	61 (30-79)	39 (21-70)	18 (2-29)	17 (5-37)	6 (3-12)	5 (2-14)	5 (2-13)	4 (1-8)

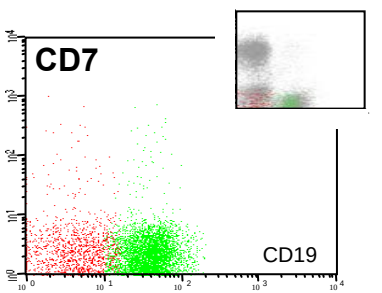




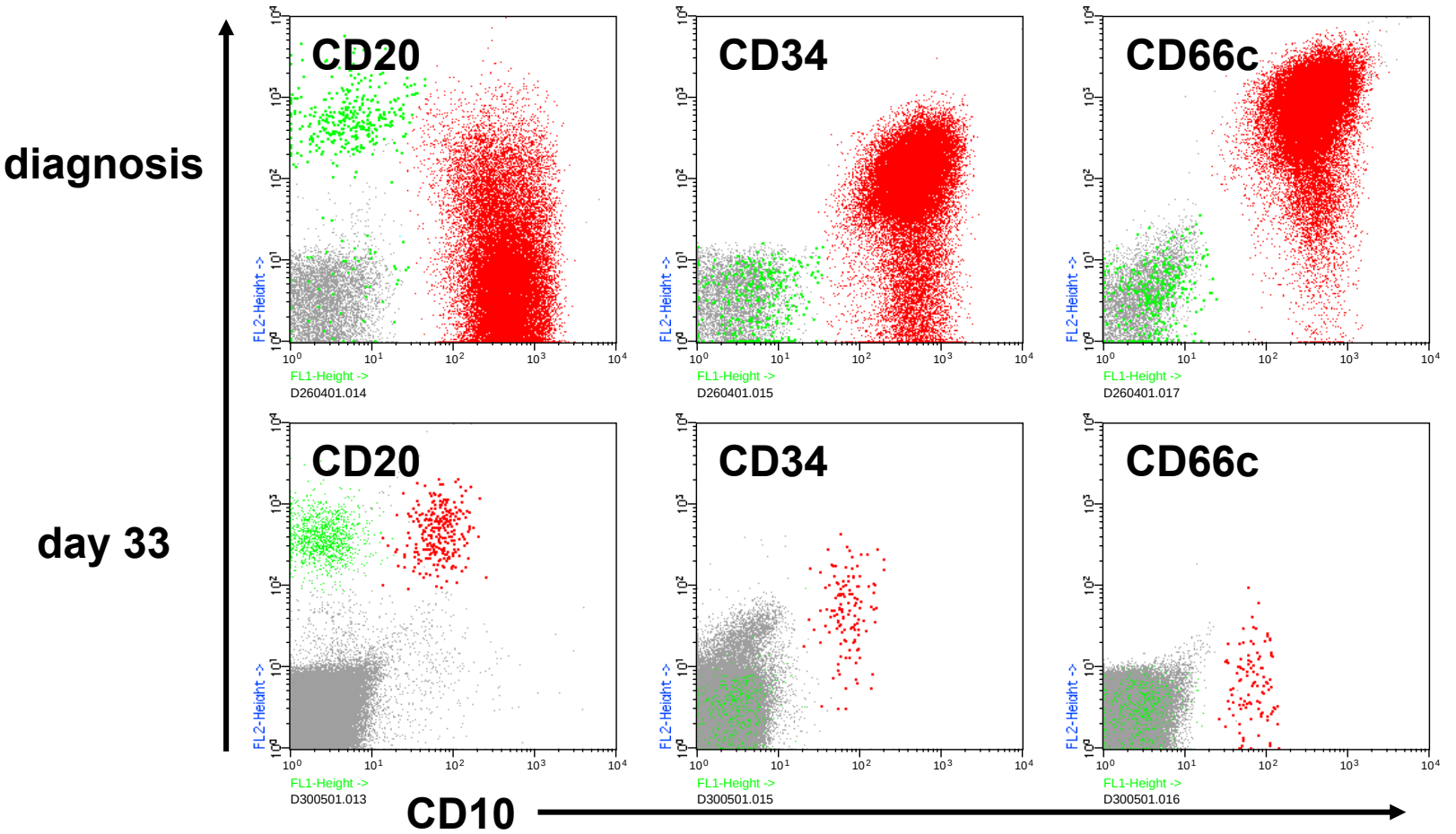






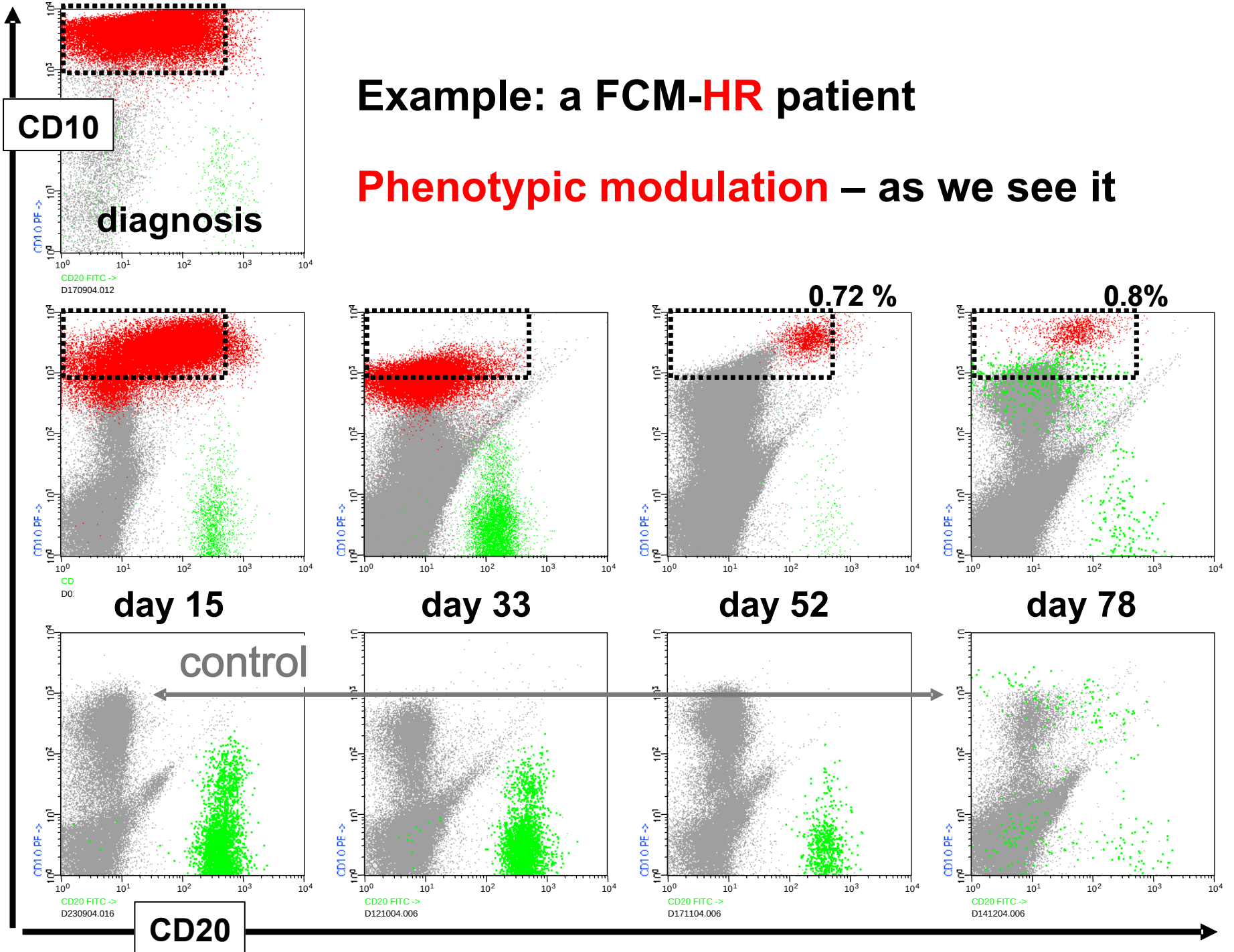


The choice of markers – consult the oracle at diagnosis ?



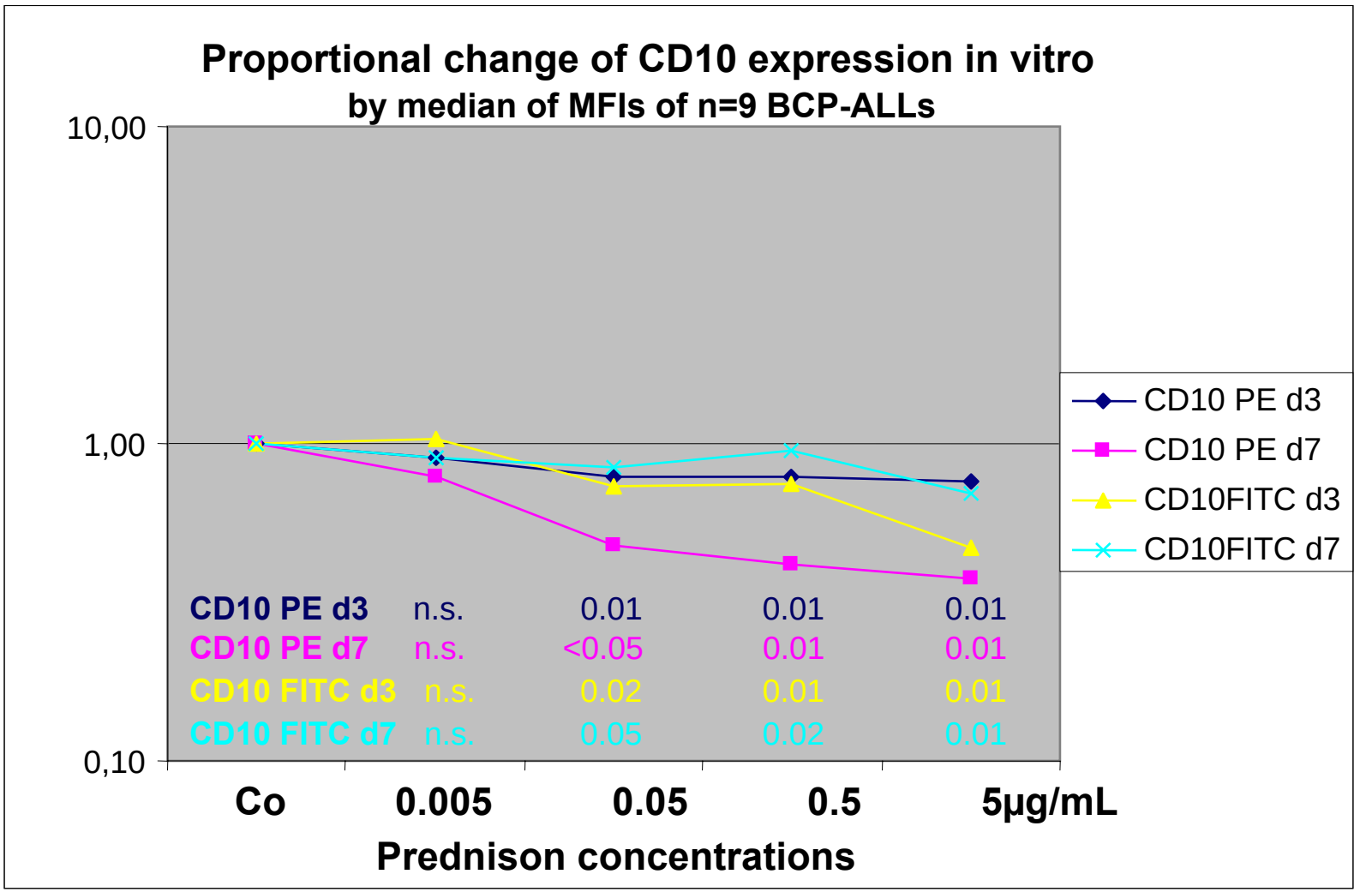
Example: a FCM-HR patient

Phenotypic modulation – as we see it



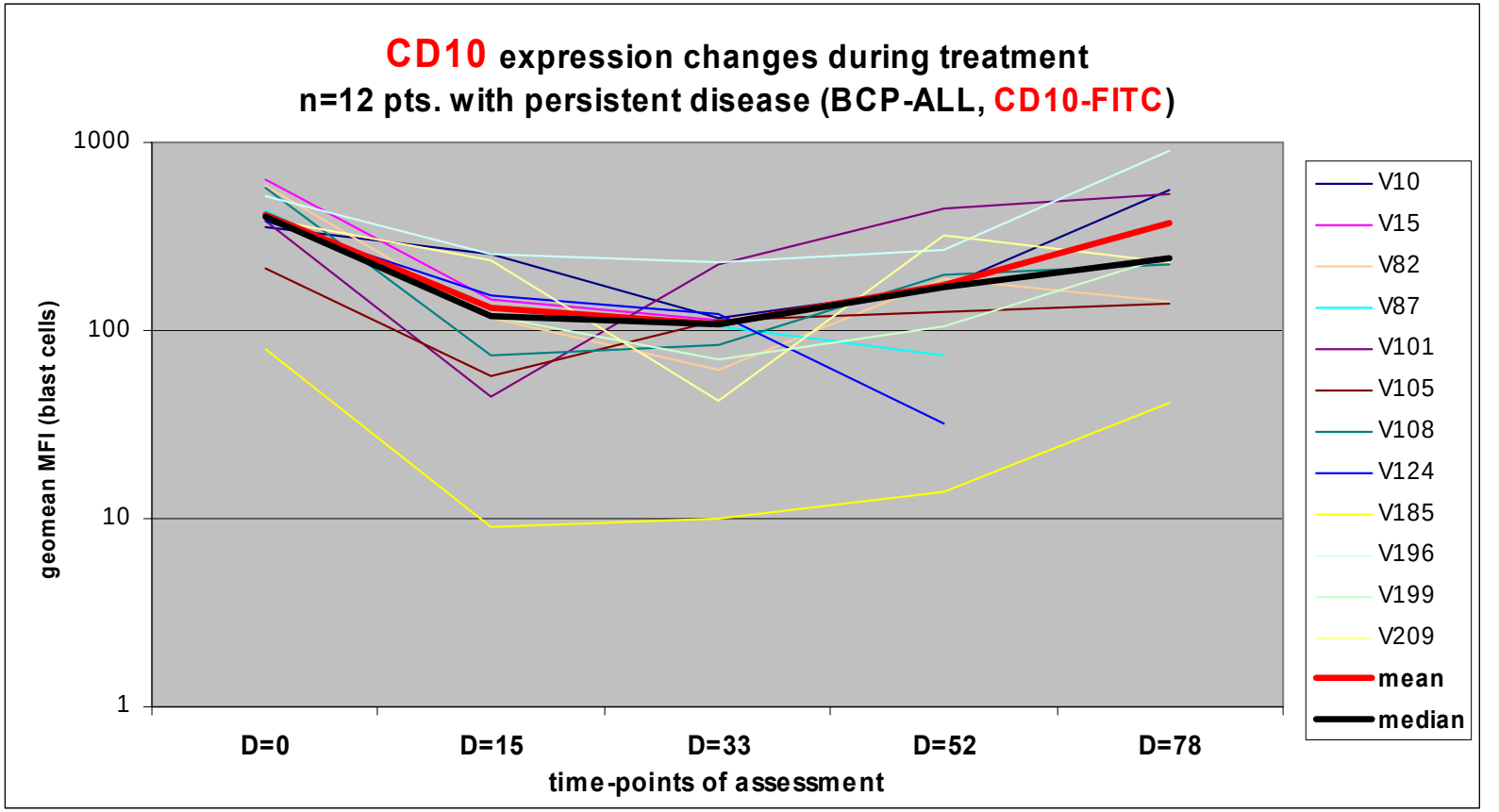
CD10 Modulation in vitro

stroma supported culture system



Wilcoxon signed rank test for paired data

Drug-induced and reversible gene expression modulation rather than selection…



< 0.01 ---------- < 0.01

< 0.01 ---------- < 0.01

< 0.01

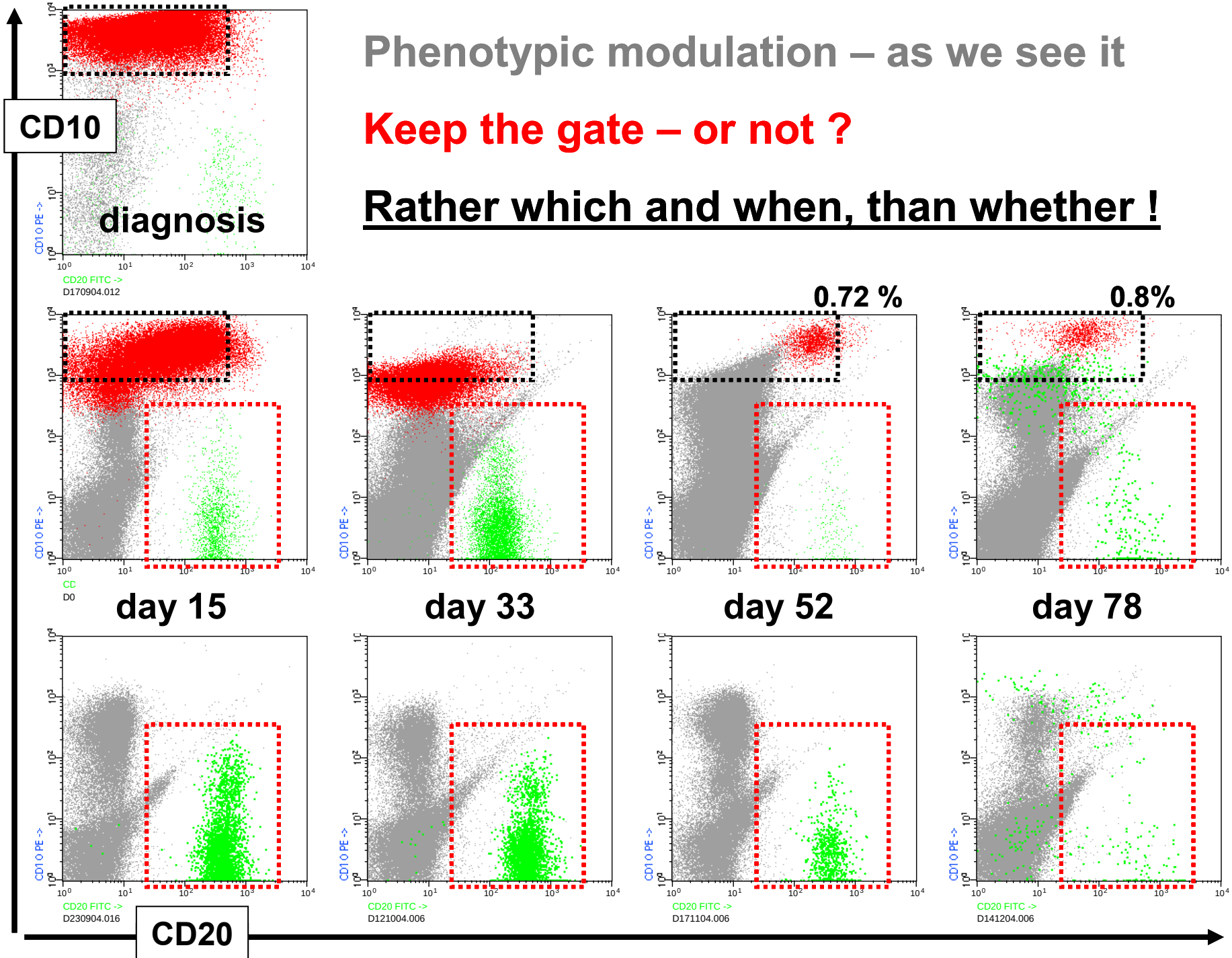
n.s.

Wilcoxon signed rank test for paired data

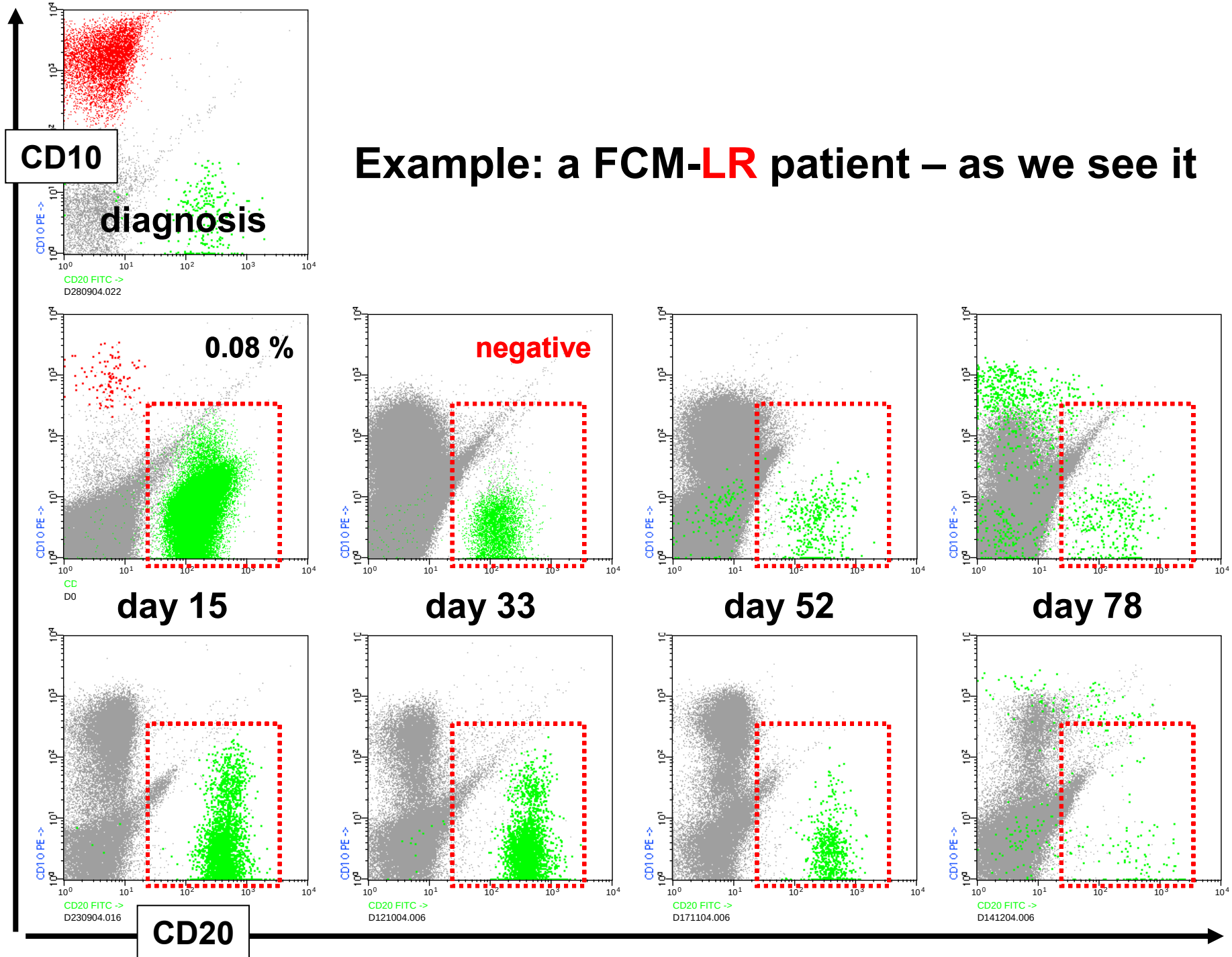
Phenotypic modulation – as we see it

Keep the gate – or not ?

Rather which and when, than whether !



Example: a FCM-LR patient – as we see it

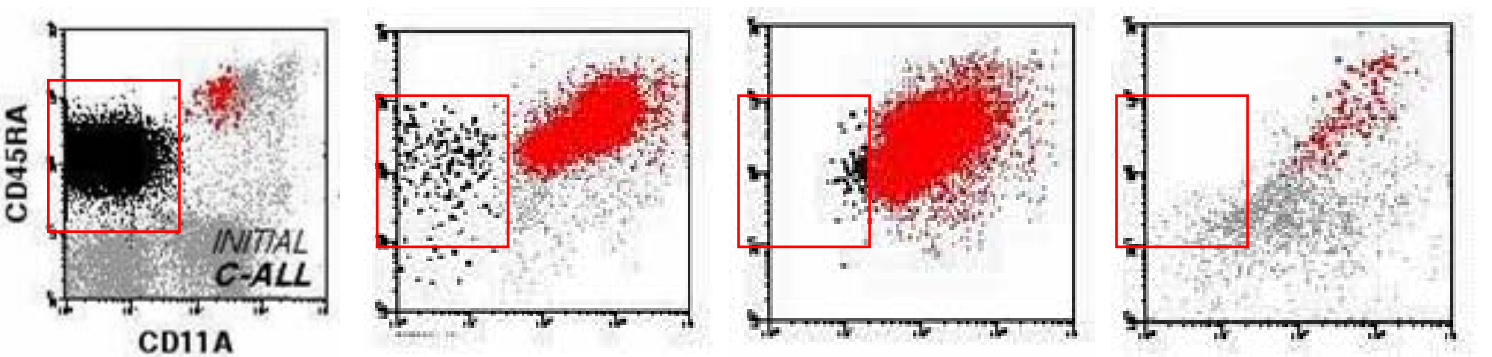


Flow Cytometry for MRD-assessment in ALL

what are the pitfalls ?

- **drug-induced phenotypic modulation** (steroid therapy)
- **flexible sensitivity range** (normal **regeneration dependent**)

  >> **multiple marker strategy**
  >> **knowledge on normal background** (time-point related)
  favorable during treatment due to lymphopoietic hypoplasia



BONE MARROW CHARACTERISTICS AT FOLLOW UP TIME-POINTS

PARAMETER	DAY 15	DAY 33	DAY 78	WEEK 23
Total NC Count [&]	4.3 (0.6 – 45)	7.3 (0.9 – 32)	15.7 (2 – 120)	24.2 (2.4 – 75)
BCP immature [#]	0.0 (0.0 – 0.0)	0.0 (0.0 – 0.0)	51.0 (0.0 – 99)	44.5 (0.0 – 95)
BCP intermed. [#]	0.0 (0.0 – 5.0)	0.0 (0.0 – 1.0)	7.3 (0.0 – 57)	25.7 (0.0 – 61)
BCP mature [#]	99.9 (95 – 100)	98.2 (97.6 – 100)	8.7 (0.0 – 82)	6.8 (0.0 – 48)
Threshold [$]	0.013 (0.001 – 0.32)	0.01 (0.001 – 0.14)	0.065 (0.002 – 2.7)	0.07 (0.002 – 2.6)

[&] **Total nucleated cells** (including erythroid precursors) x 10^9/L; median (range)

[#] **Proportion of B -cell precursor stage** among total $CD19^{pos}$ B-cells; median (range)

[$] **Threshold proportions** of NC at/above which samples were definitely MRD -negative, median (range); **characterizes the test -sensitivity** in MRD -negative BM samples

Flow Cytometry for MRD-assessment in ALL

what are the technical advantages ?

- **generic aberrant pattern recognition (LAIPs)**

  ⟶ application **even in absence of initial material**

  ⟶ application **in absence of full-blown leukemia**
  (extramedullary, smoldering, or lymphomatous presentation)

- **speed**

  ⟶ turn-around time **1 day** from sampling

- **low cellular input**

  ⟶ 0.75 x 10^6 cells x 2-4 (n= tubes) per sample
  if using limited panel diagnostics

- **low prime costs** (if using limited panel diagnostics)

  ⟶ **< 300 €** per patient = risk assessment
  includes BM analysis 4 time-points (dx, days15, 33, 78)

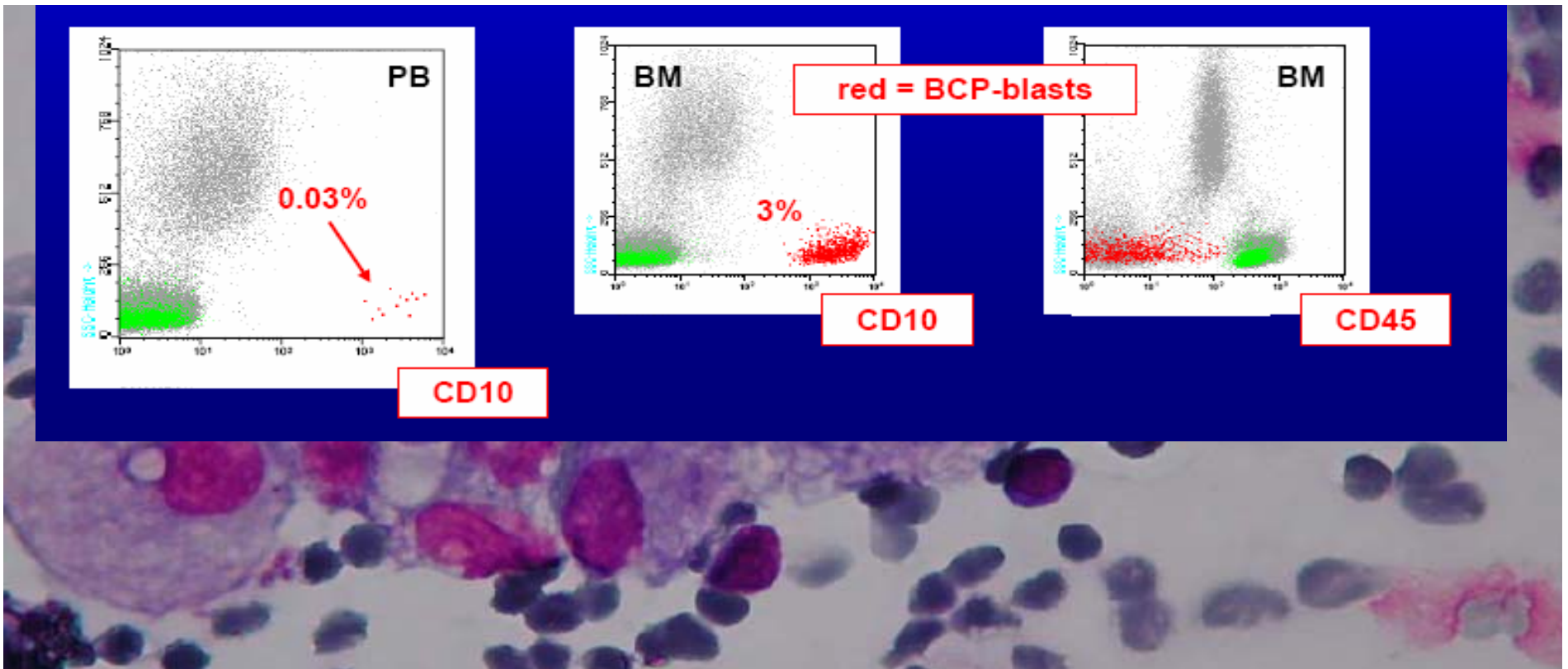
Case report:

girl, 13y, severe PB tri-lineage cytopenia, bone pain

BM aspirate: no definite blasts, many histiocytic cells

Trephine: severe myelofibrosis,
megakaryocytic hyper/dysplasia – AMF u.o.

FLOW: "ALL"



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Part 1

- MRD definition – old and new knowledge
- Technical standardization in AIEOP-BFM

Part 2

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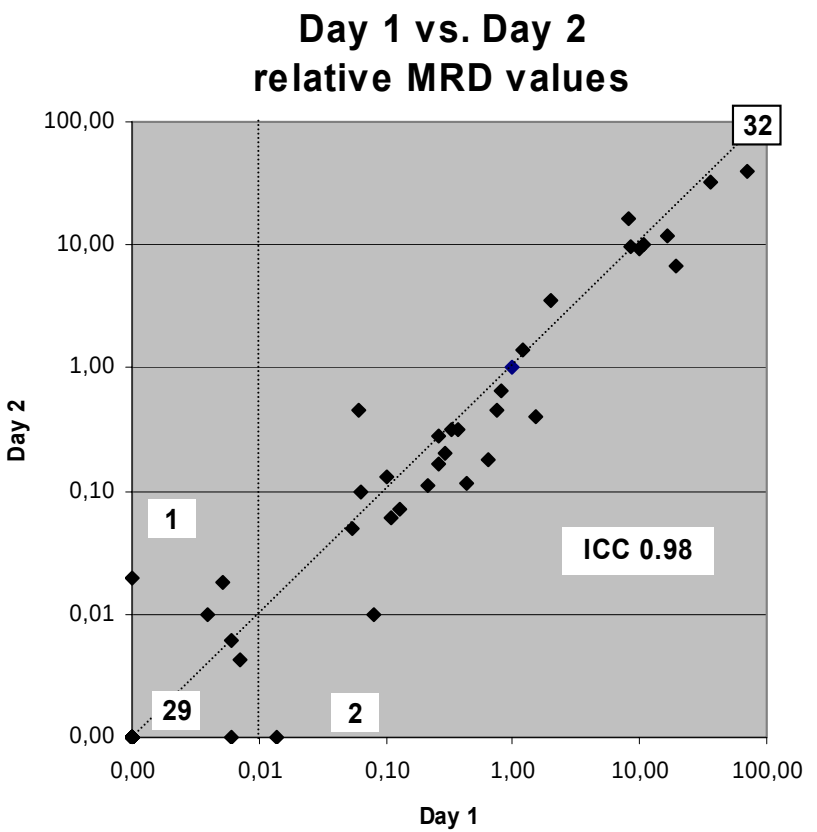
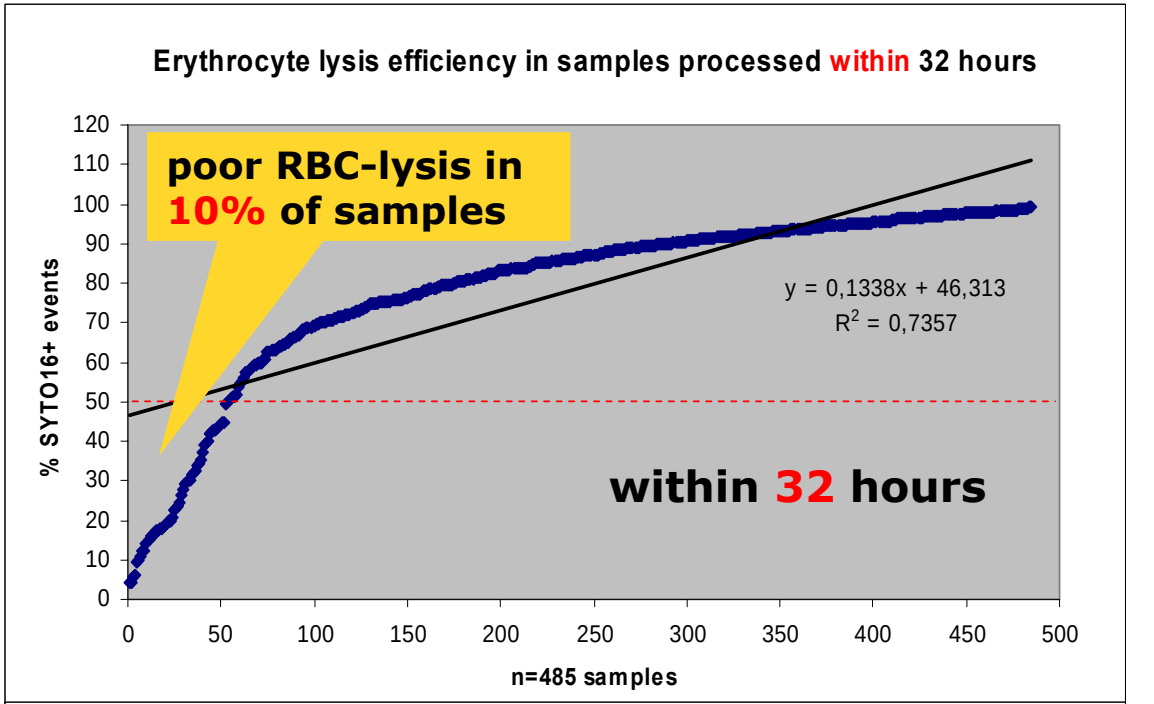
- FCM vs. Outcome: interim results

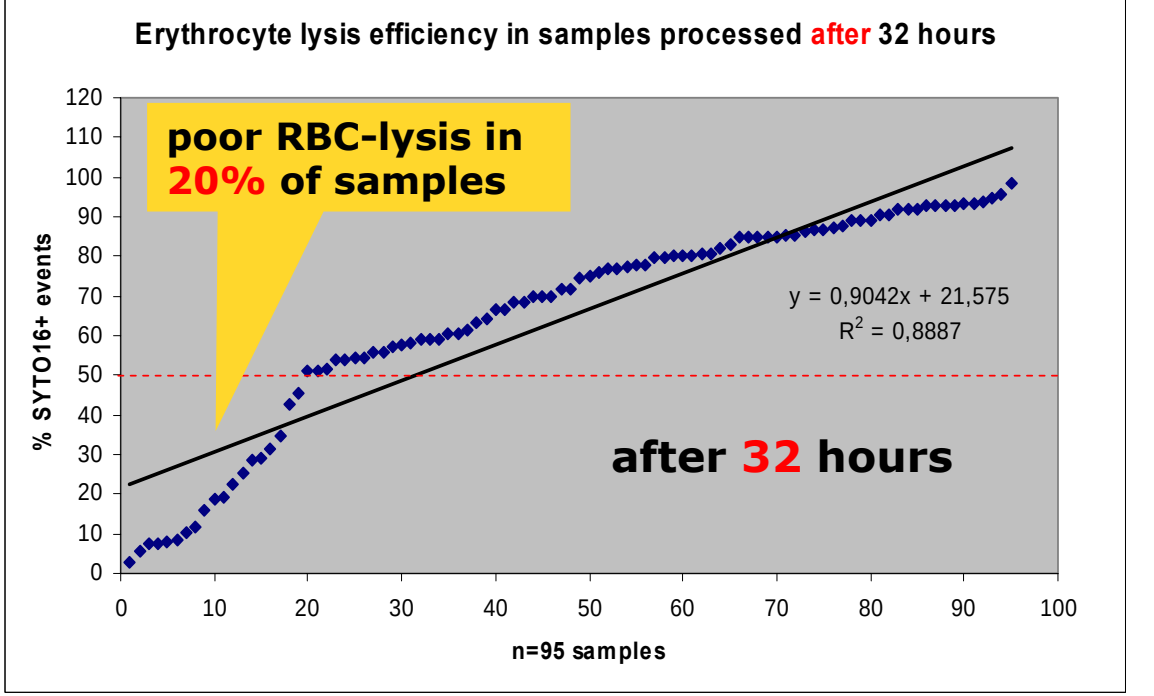
Standardization and QC of Flow Cytometry

for MRD Assessment in ALL

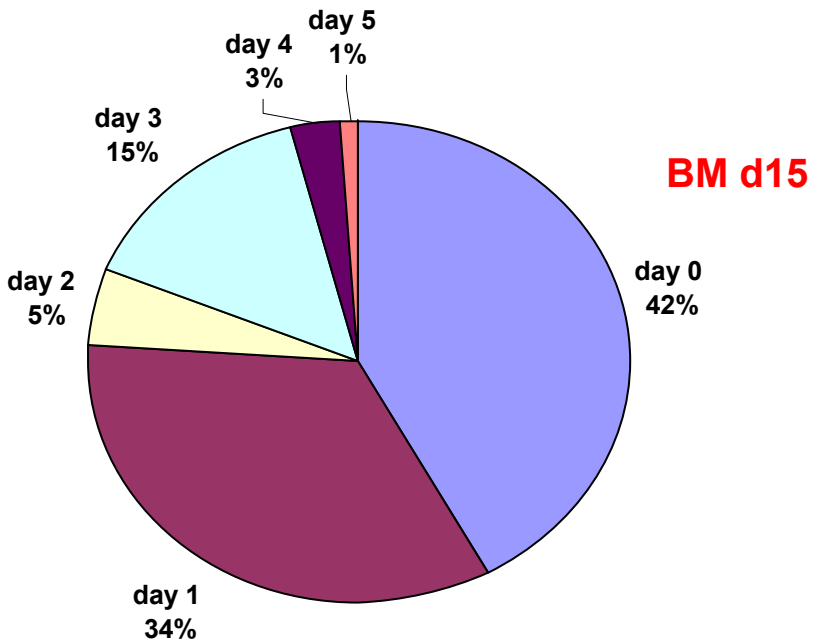
Influence of time delay from sampling to processing on quality of preparation



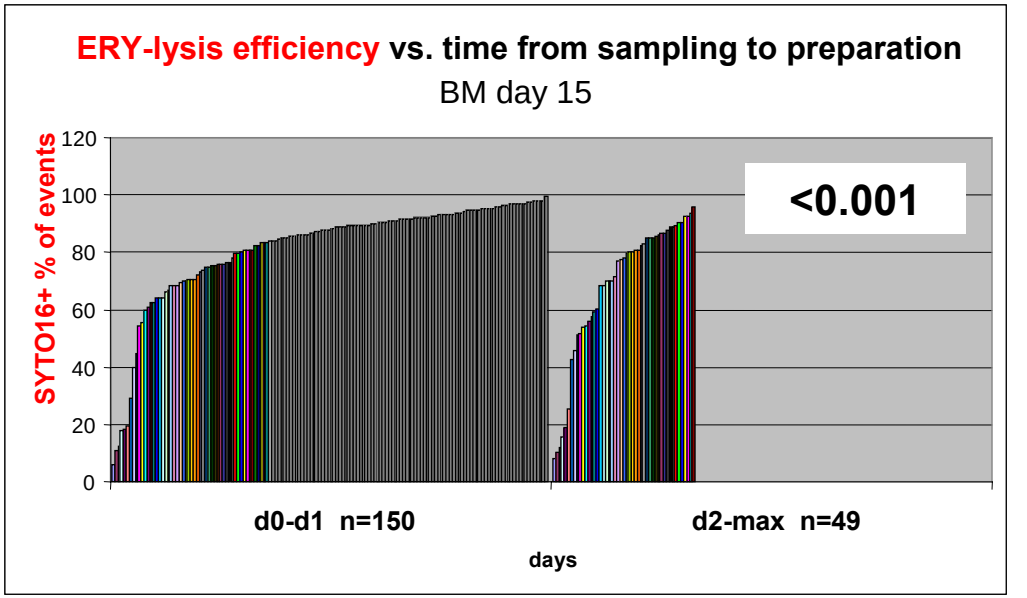


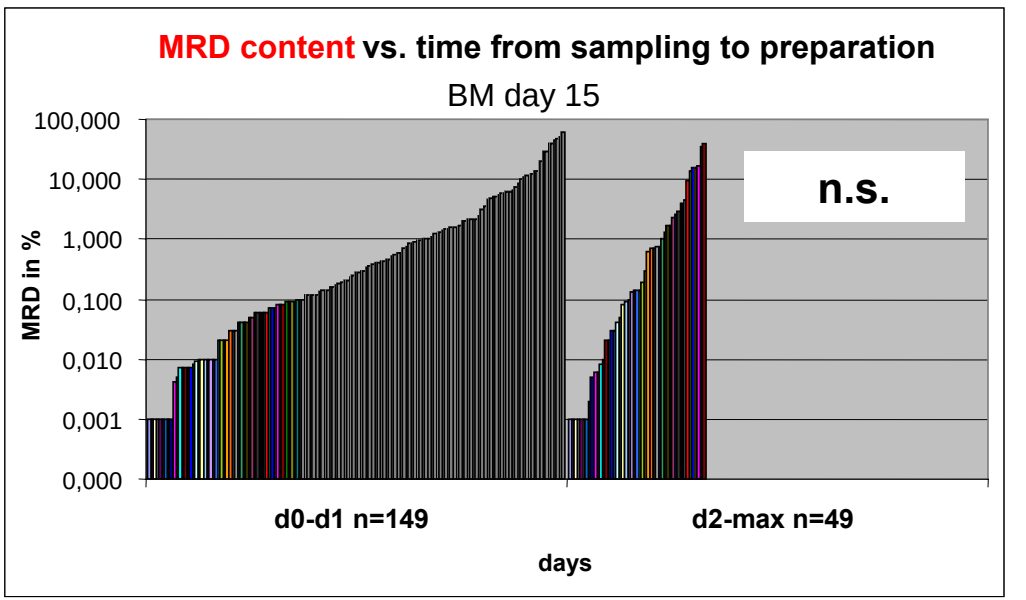


Influence of time delay from sampling to processing on quality of preparation



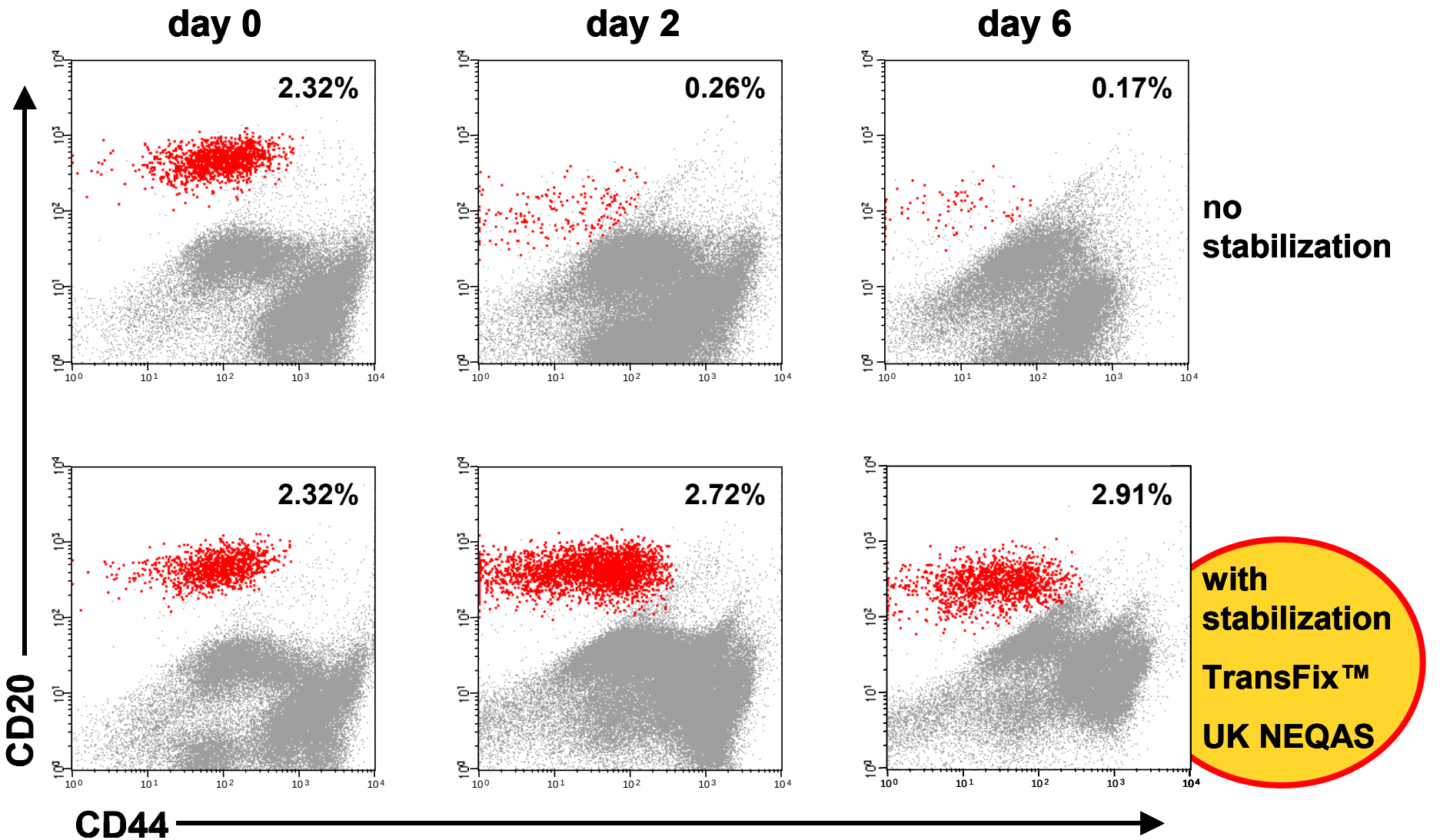
BM d15



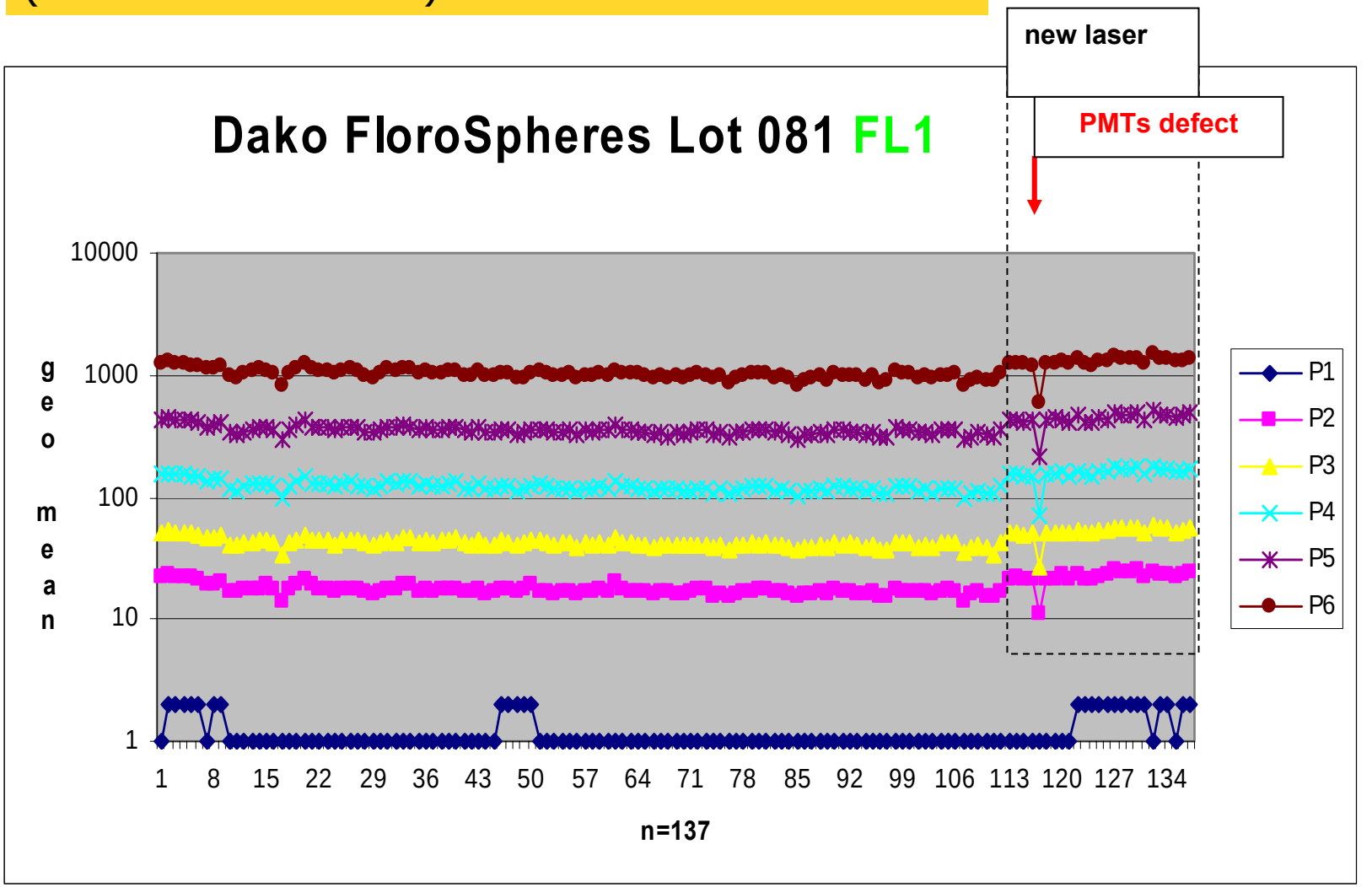


Burkitt's lymphoma - **minimal disseminated disease**

Stabilization for transport needed (avoid apoptotic cell loss)



flow-cytometer performance monitoring
(IIIa **beads** - MESF)



Steps of standardization

- post-acquisition standardization

  how standardize the human factor ??

  → education by personnel exchange and discussion

  → review rounds (incl. online accessibility – ftp server)

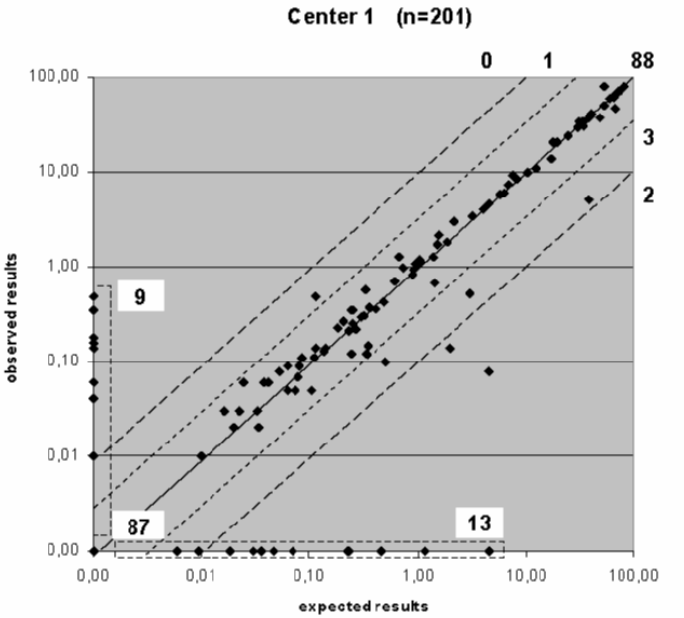
  → „human“ concordance development program

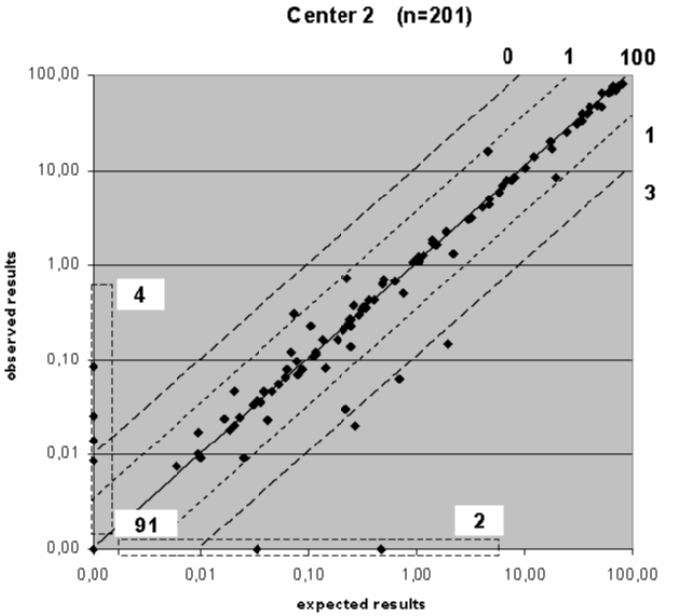
    LMD file exchange ring trials

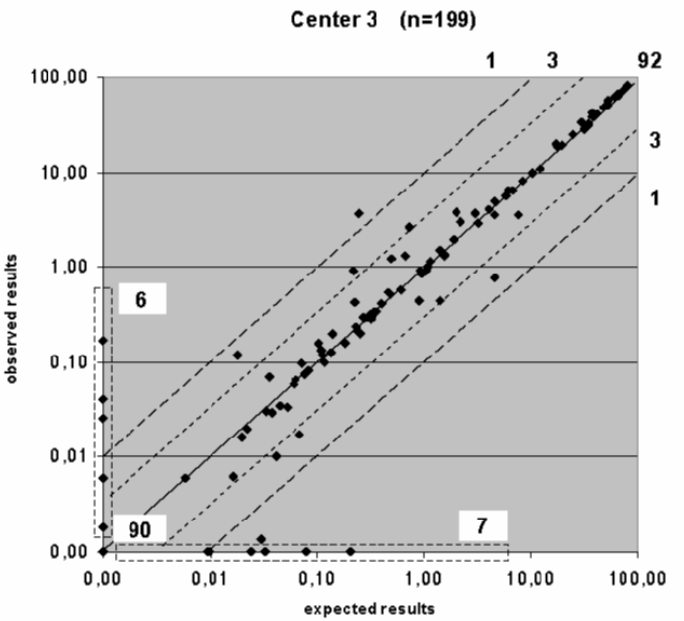
    sample exchange ring trials (twice yearly: spiked a/o „real“)

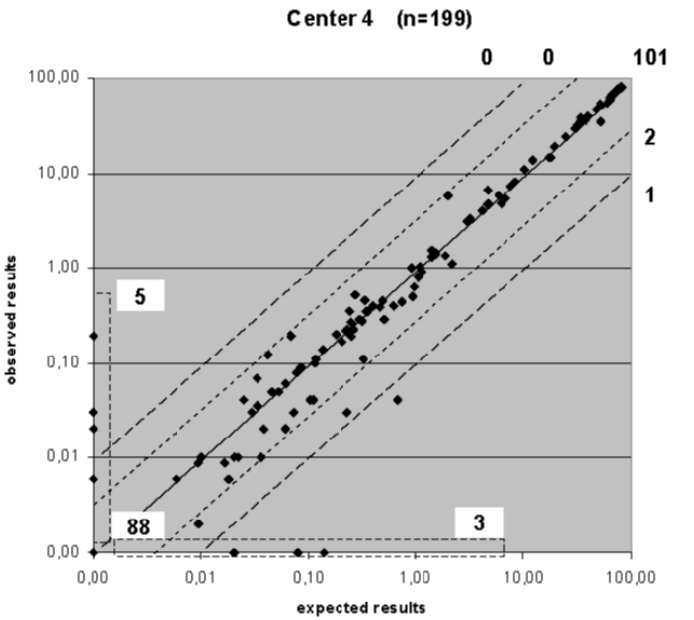
    independent data concordance comparisons

LMD file interpretation ring trials





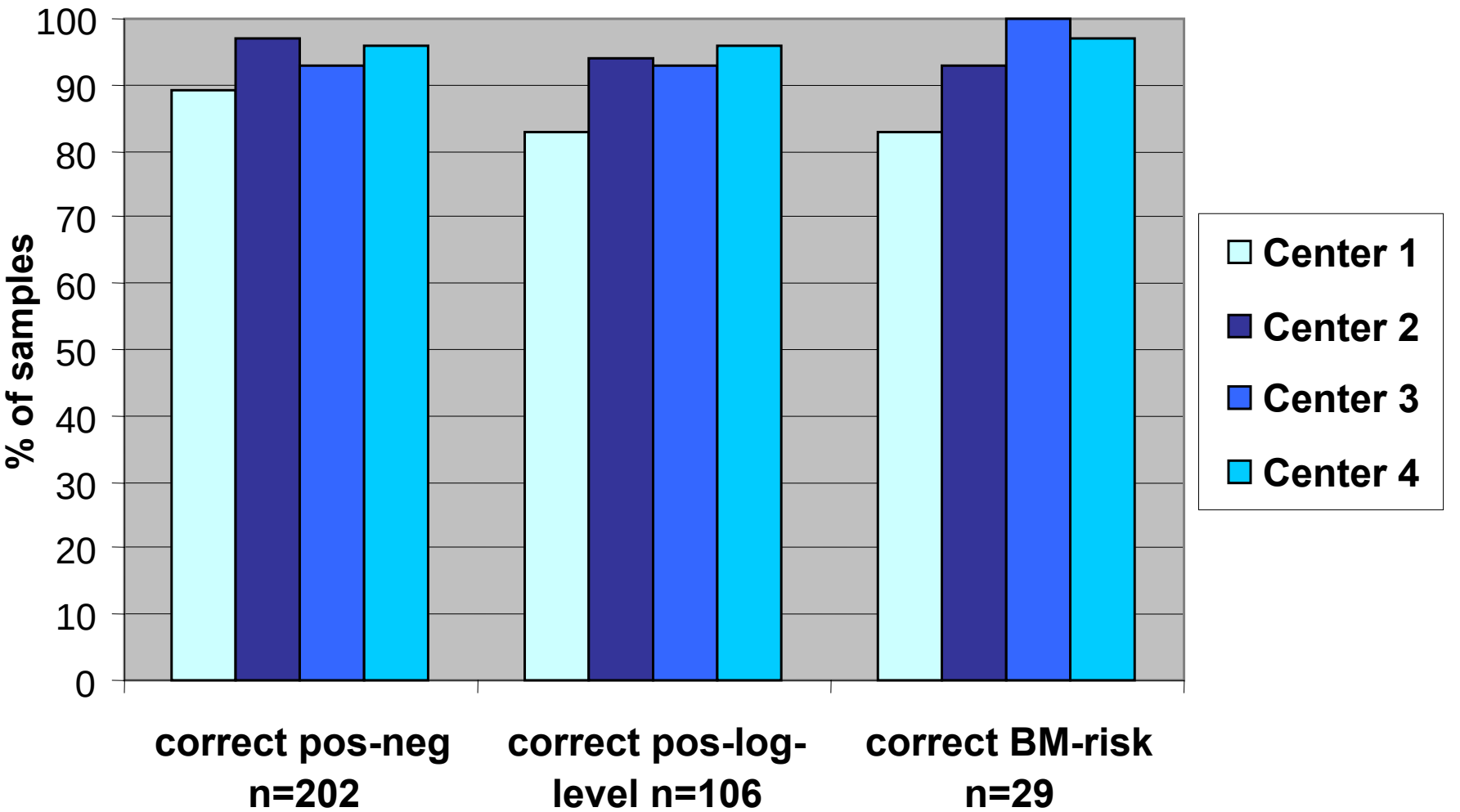




Dworzak et al.,
Clin. Cytometry 2008

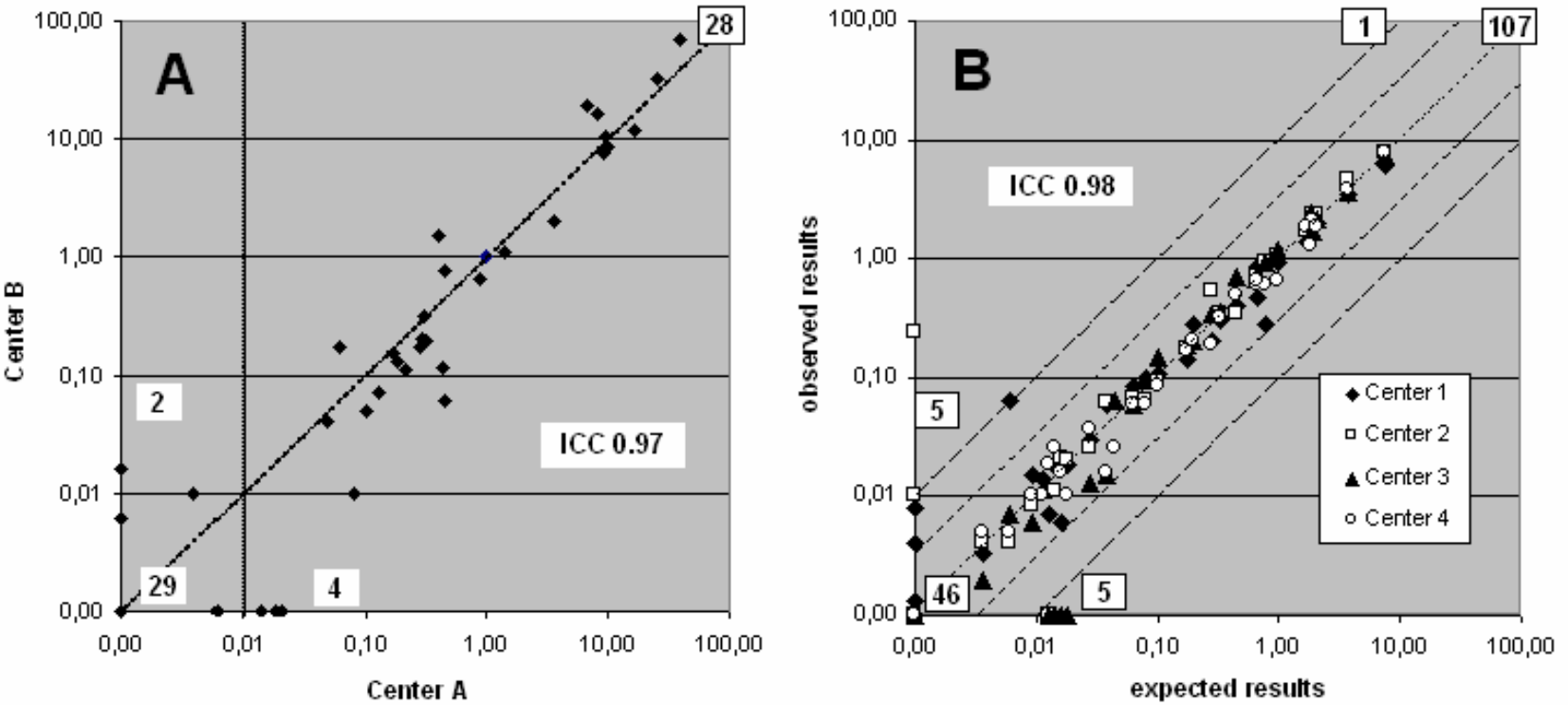
Interpretation concordance

Center-concordance: LMD file exchange



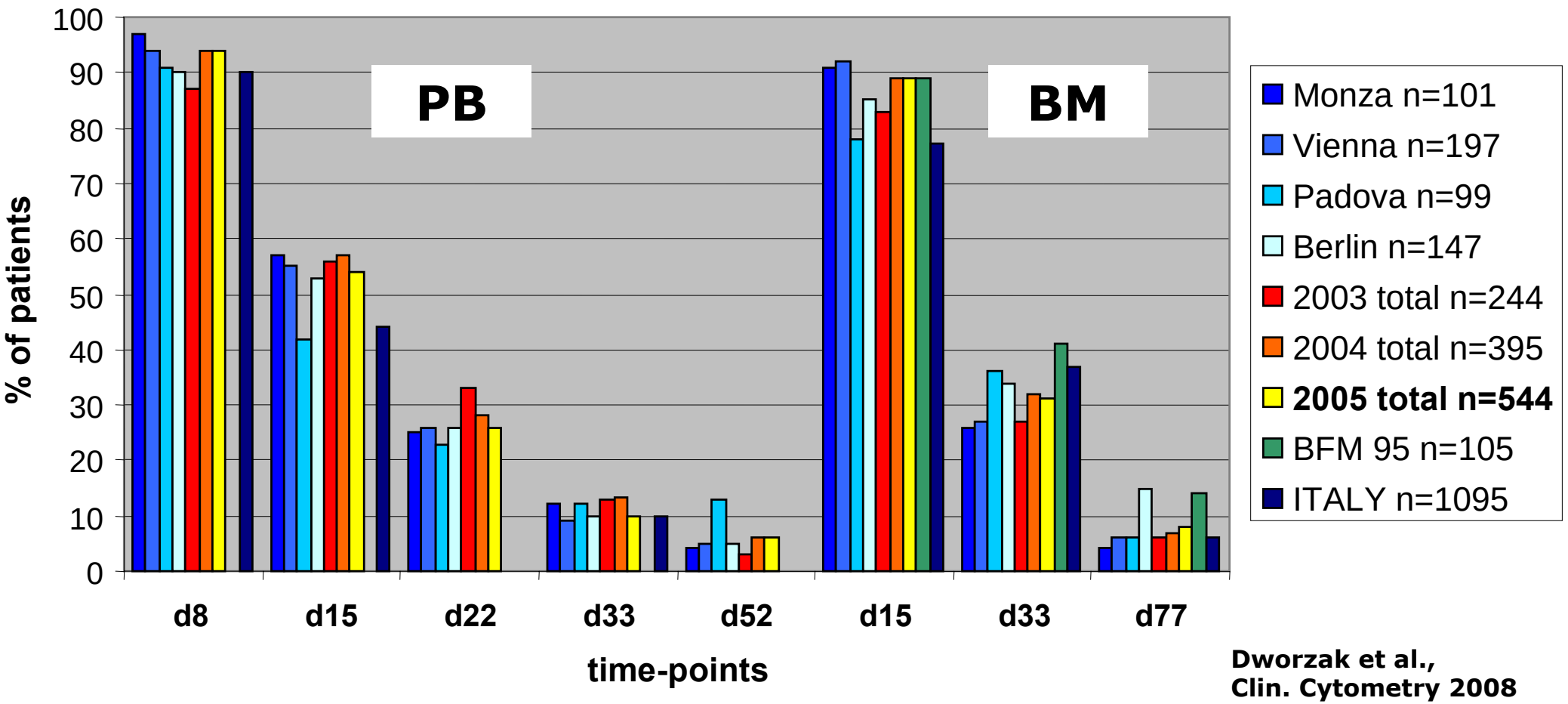
Sample exchange program:

"**real**" follow-up samples **spiked** MRD-samples



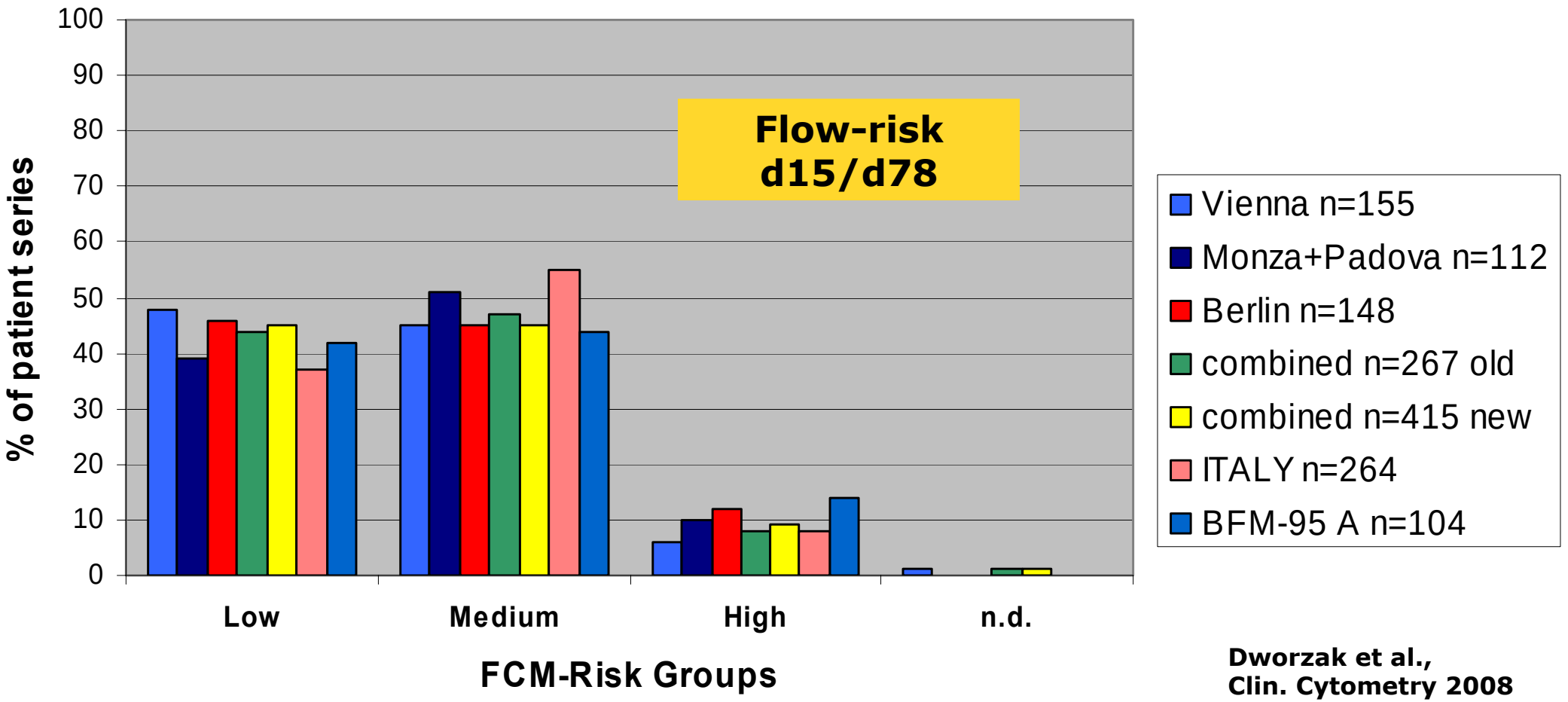
Dworzak et al.,
Clin. Cytometry 2008

Independent data concordance



Dworzak et al.,
Clin. Cytometry 2008

Independent data concordance



Dworzak et al.,
Clin. Cytometry 2008

LMD file exchange
ring trial 2008

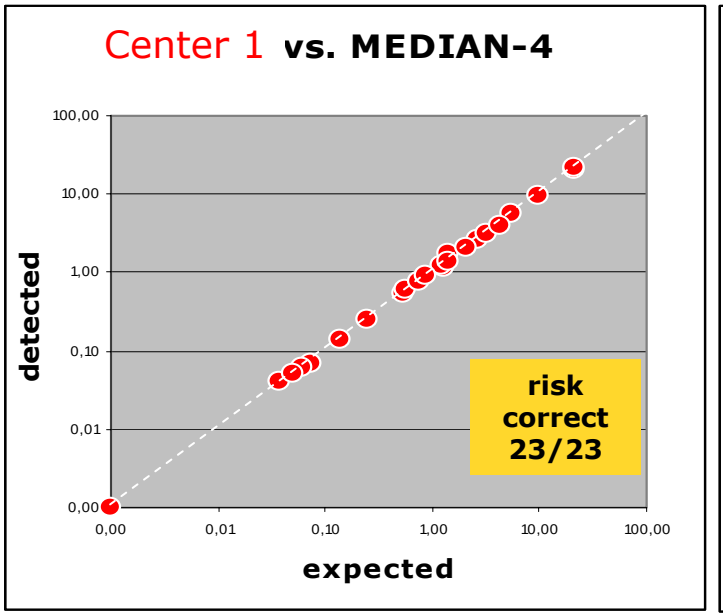
BCP-ALL
n=23 d15

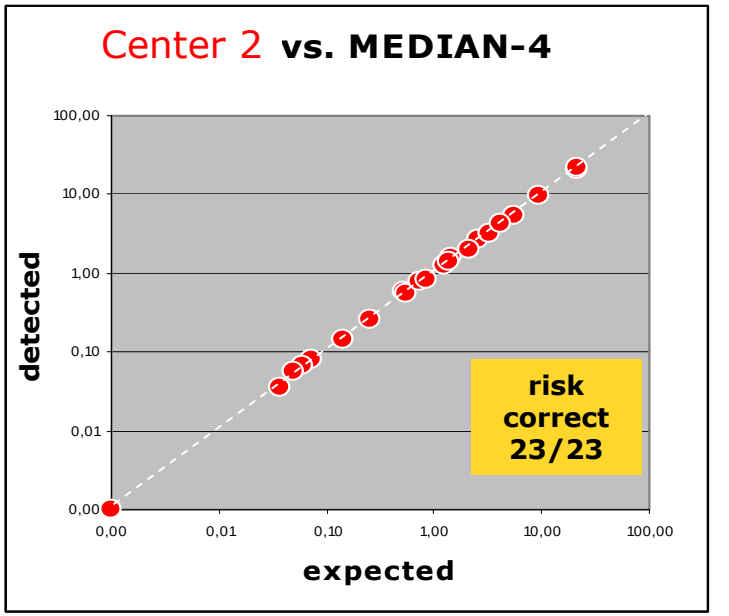
expected stratification:

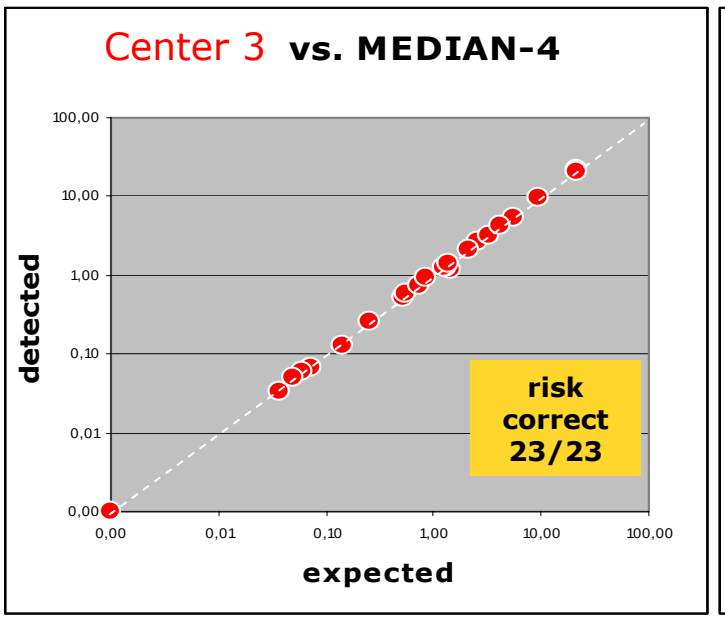
FLR=5

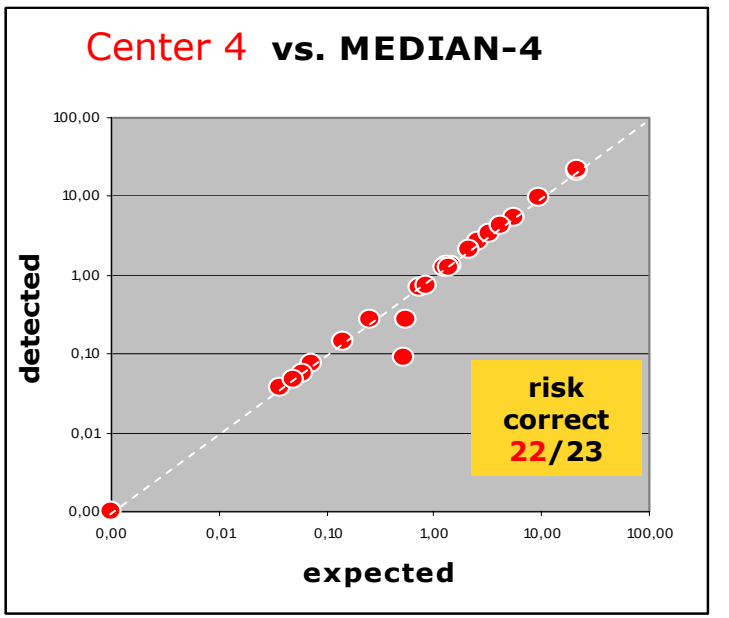
FMR=16

FHR=2

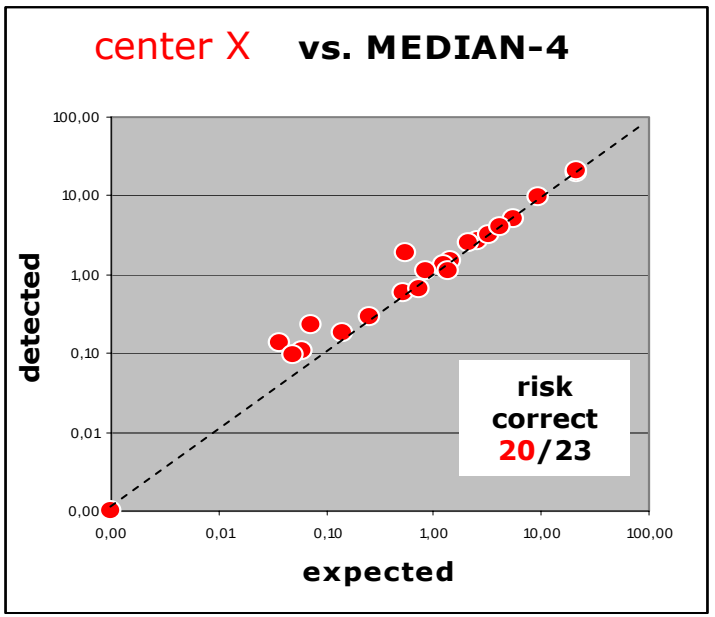


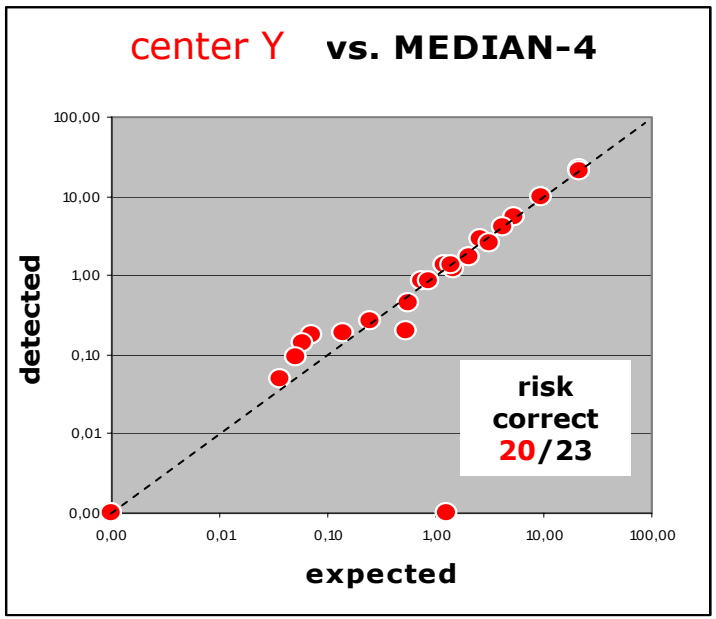


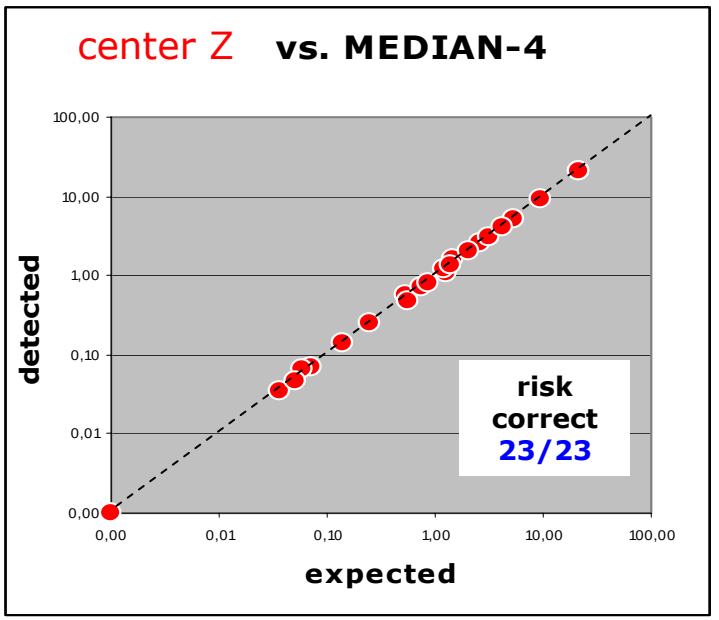




the values of cluster-gating !





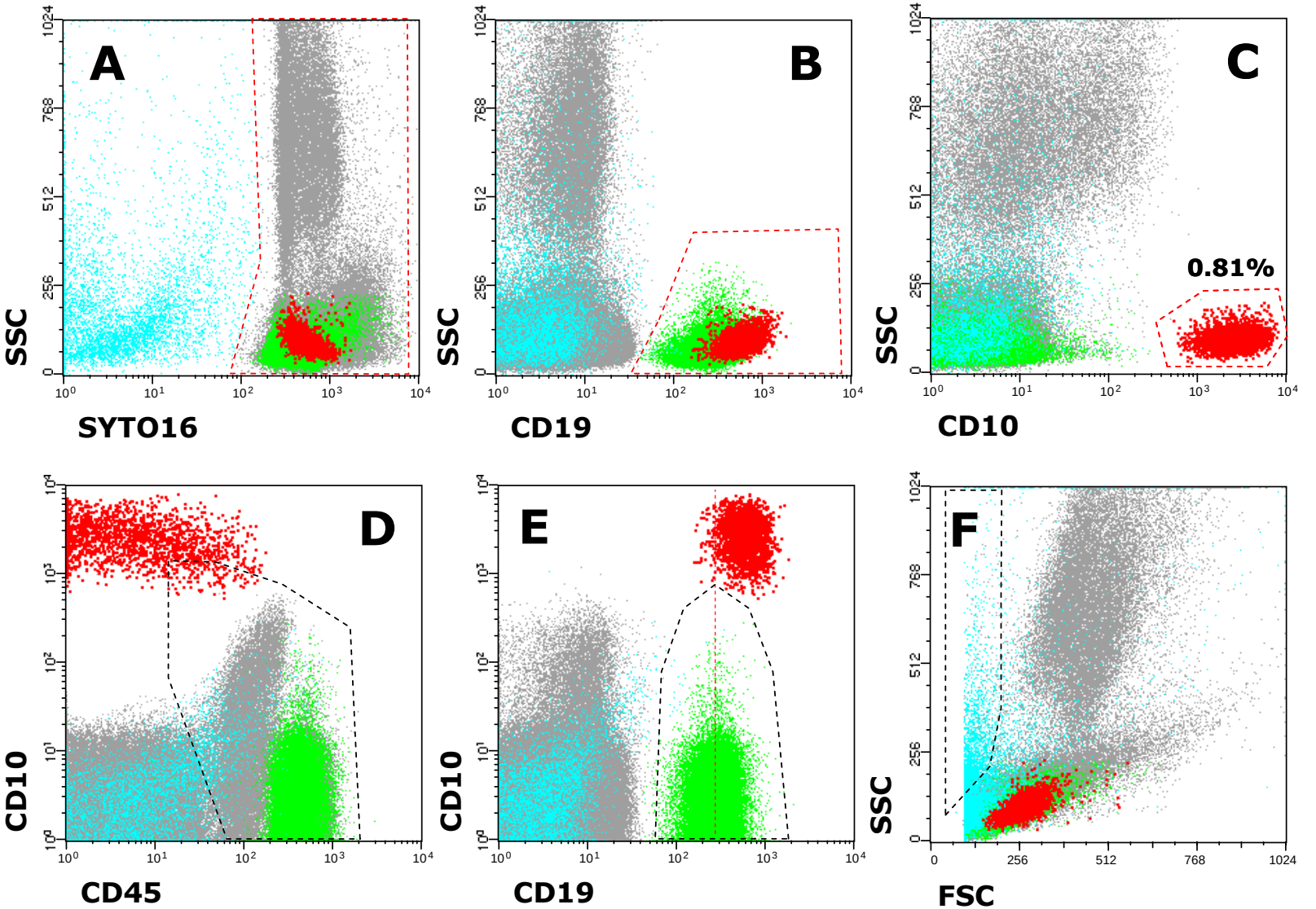


Missed:

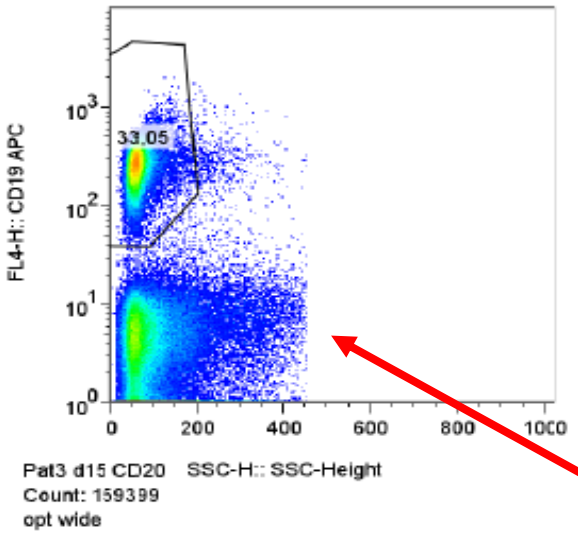
FLR >> FMR	**2x**	**2x**
FMR >> FLR	**1x**	**1x**

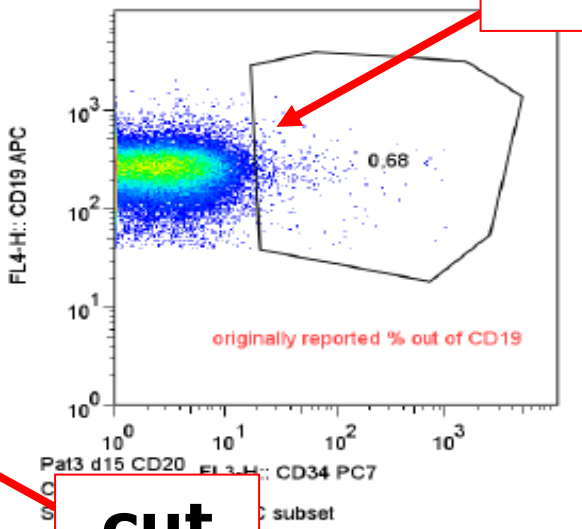
AIEOP-BFM ALL 2000 FLOW-MRD:

The gating and analysis strategy

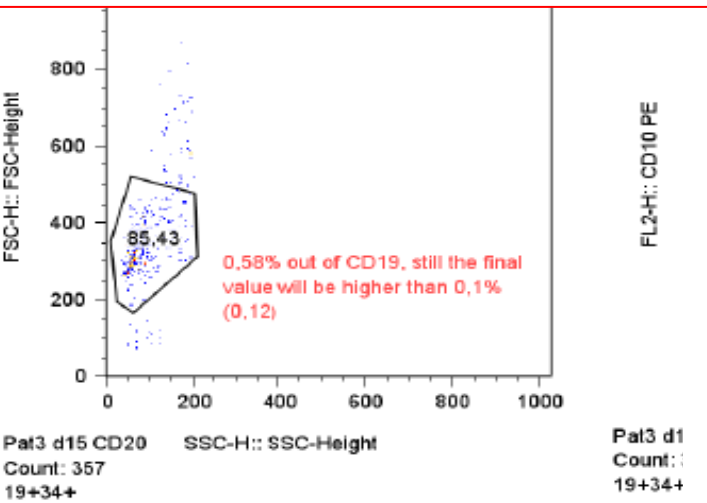


patient 3





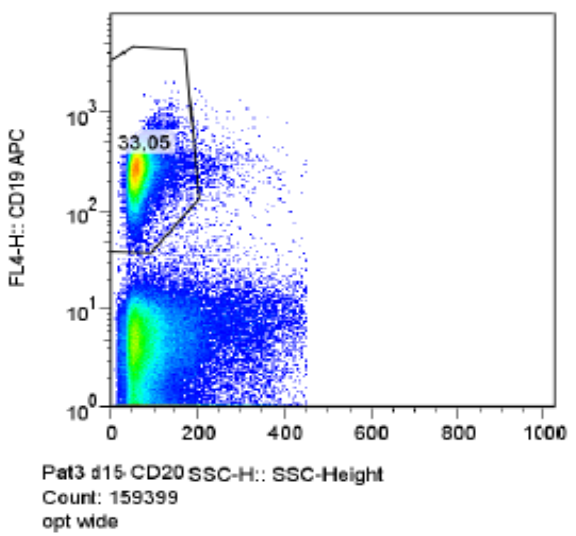
„cutting" is dangerous

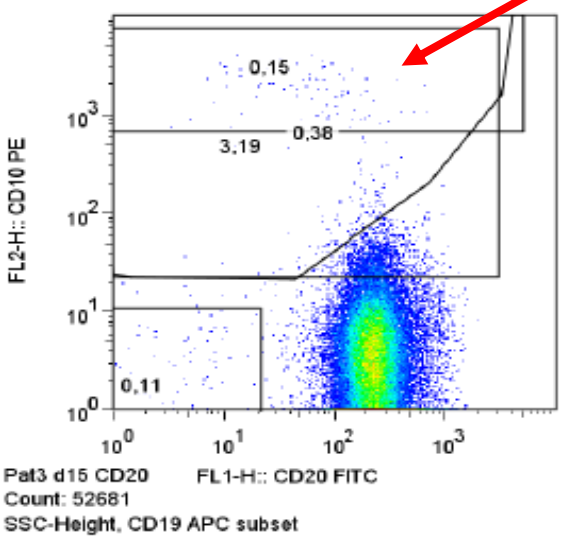


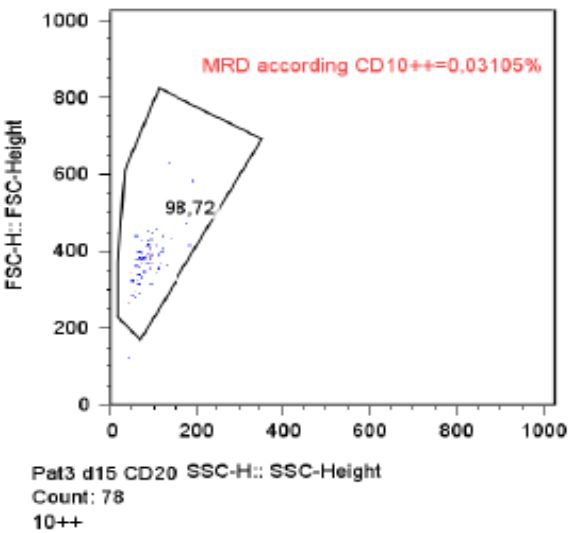
cut

The value originally reported was CD19+34. In the first round I did all the analyses from the wide lymphomono gate and no final gating according optical properties to evaluate cluster characterisitics were done (this was similar to minimini). This is something we want to add tc all your data originally analysed also in Minimini. When applied backgrcund value this value is below cut off - this was mistake not to do that

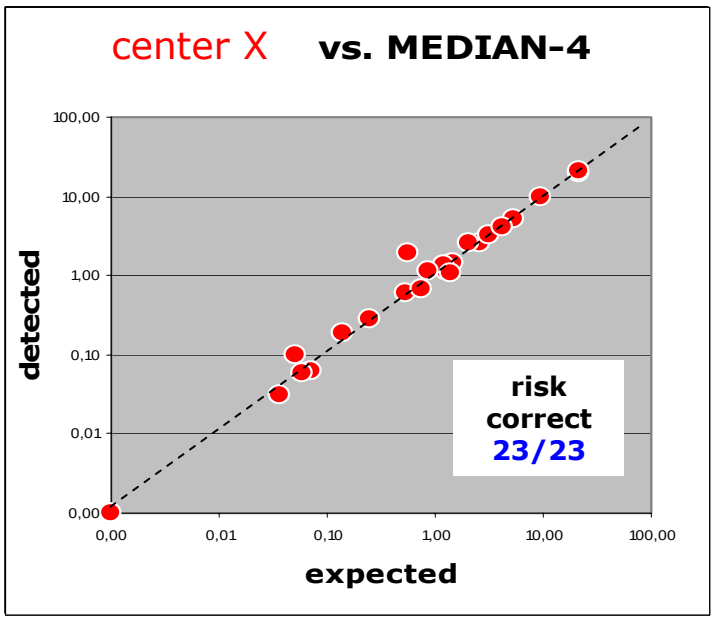
„cluster"-gating is better

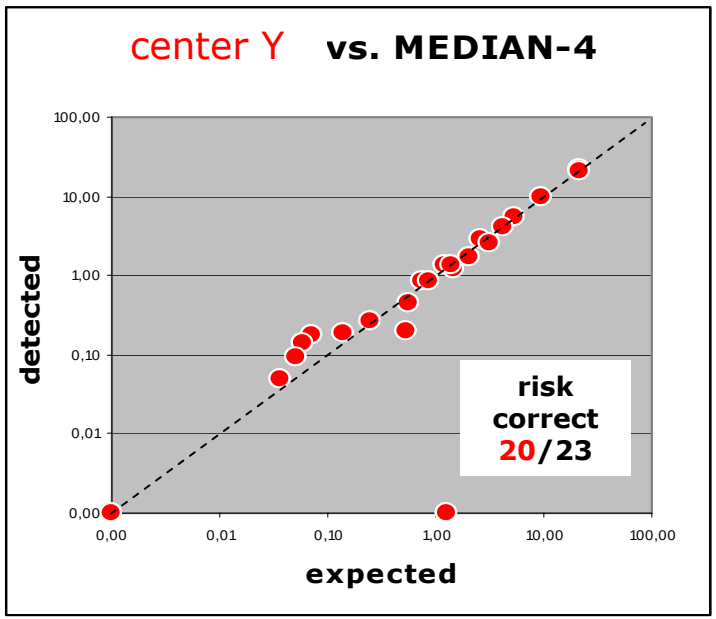


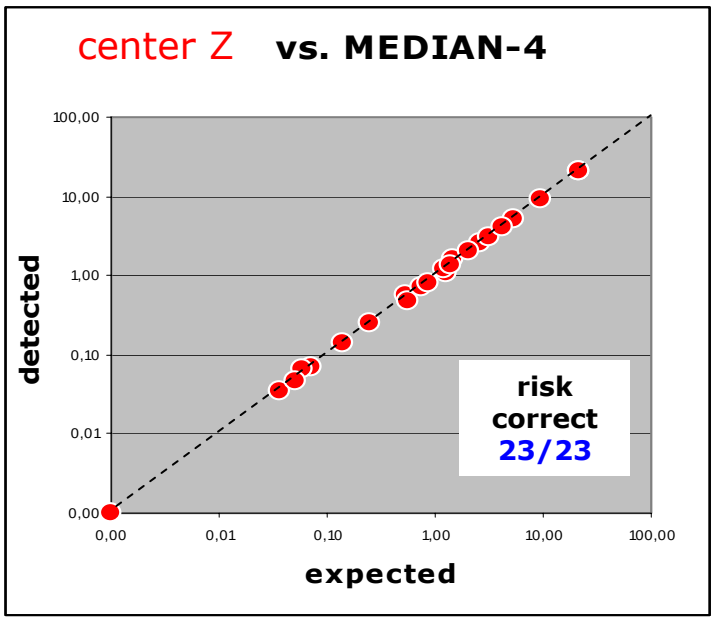




the values of cluster-gating !



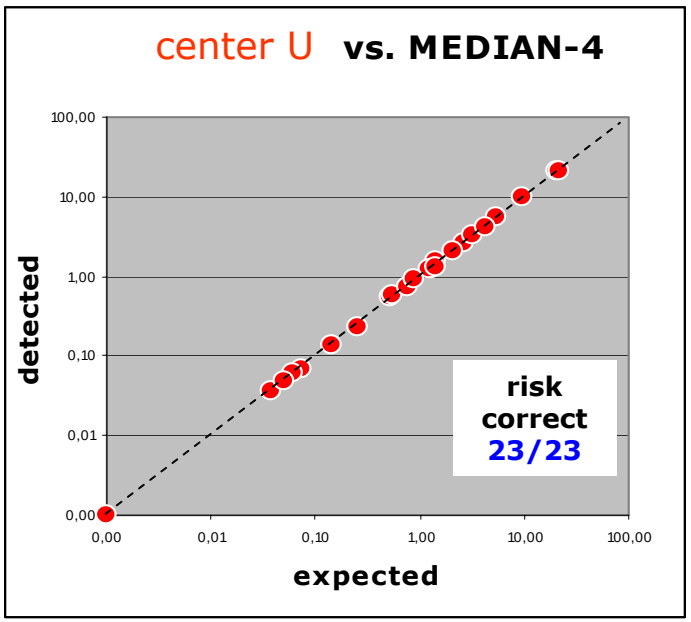


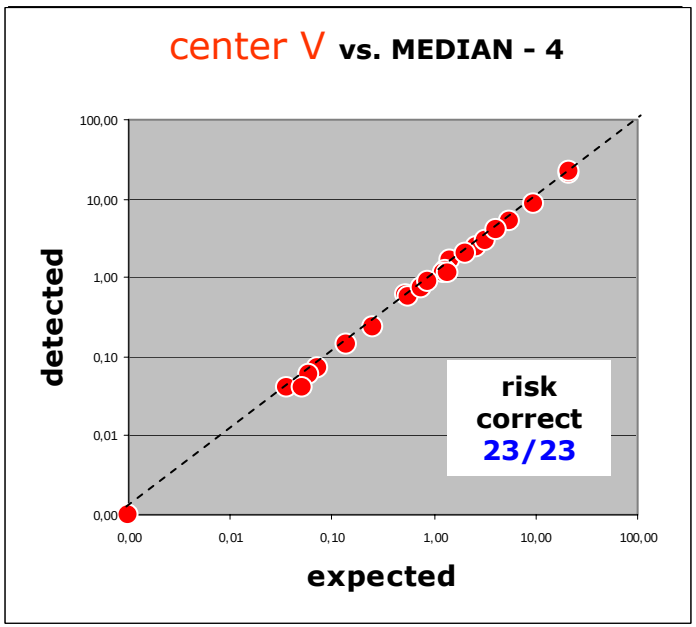


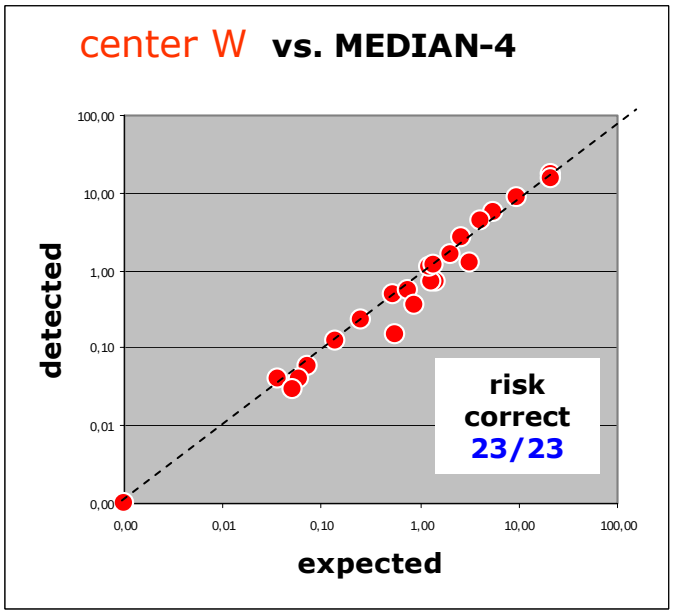
Missed:

FLR >> FMR	**2x**
FMR >> FLR	**1x**

effect of training and experience !







The AIEOP-BFM ALL 2000 FCM-MRD study

Part 1

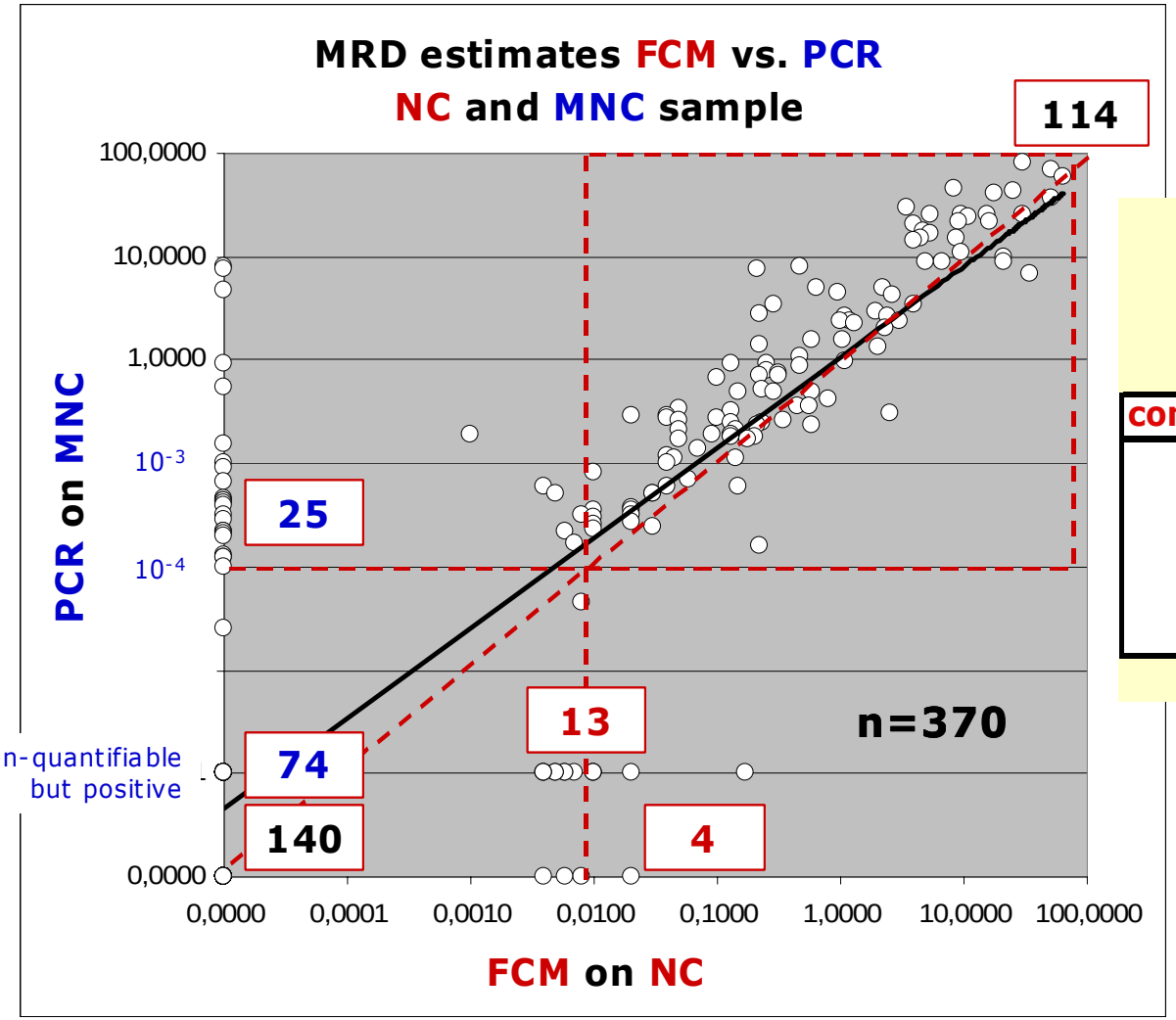
MRD definition – old and new knowledge

Technical standardization in AIEOP-BFM

Part 2

Correlation with PCR-MRD

FCM vs. Outcome: interim results



pos-neg concordance

concordance	all	quantifiable
d15	89,0	93,0
d33	56,3	77,3
d52	71,8	88,7
d78	72,4	98,6
overall	72,2	89,8

concordance

total	FCMpos	FCMneg		
PCRpos	127	99	226	56,2%
PCRneg	4	140	144	97,2%
	131	239	370	
	96,9%	58,6%		72,2%

only quantifiable	FCMpos	FCMneg		
PCRpos	114	25	139	82,0%
PCRneg	4	140	144	97,2%
	118	165	283	
	96,6%	84,8%		89,8%

PCR vs. FCM:

high concordance in the quantifiable range (≥0.01%)

more positive results of AIEOP-BFM-type RQ-PCR
in the non-quantifiable range as compared to FCM

AIEOP-BFM-type PCR-based risk stratification
relying on mere positive/negative results
can not be mimicked by current FCM methodology

PCR vs. FCM comparisons are characterized by methodological differences (methods as used by the FCM-SG and the MRD-TF).

A major factor to explain why **FCM is frequently negative when PCR is positive in the low range**, is the very **different input of cells** for a test.

PCR is based on replicate (**3x**) tests of $0.75x10^5$ **MNC** (3x 500ng DNA) and thus is able to detect **1 : 225.000** (positive if 1 of 3 tubes results positive).

FCM input (**1x**) $3x10^5$ **NC** enabling detection of about **1 : 30.000** at maximum due to the ≥10 dots requirement for positivity.

Theoretically, AIEOP-BFM 2000-**PCR is therefore ~1 log (7.5 - 10x) more sensitive** than AIEOP-BFM 2000-**FCM**.

Sensitivity (neg=really neg) is therefore poorer with *this type* of FCM analysis, but specificity (pos=pos) is fine with FCM.

adapted from: Proceedings of the 11th AIEOP-BFM-ALL-FCM-MRD-SG Meeting; *Vienna; April29-30, 2005*

Not everything that counts is countable,
not everything that is countable counts.

A. Einstein

Technical FCM-MRD research issues....

1) **Increase sensitivity by increasing cellular input**

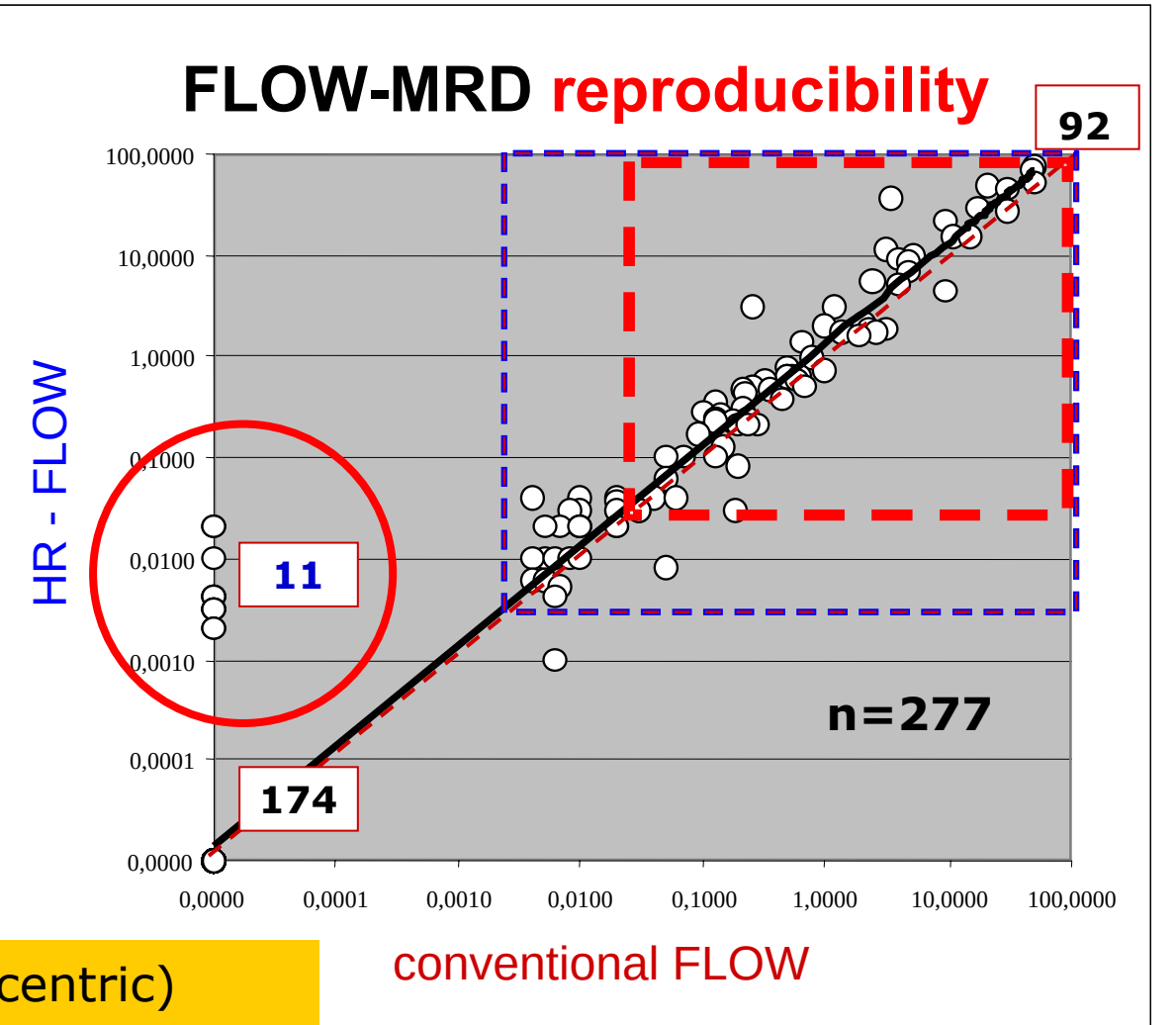
   via ≥1 x10^6 per tube instead of 3 x10^5
   (new sensitivity 1: ≥ 100.000)

2) **Reduce variance and costs by reducing tubes**

   via ≥6-color technology instead of 3-4
   (increased specificity ?!)

new
high-resolution
FLOW-MRD

- **7- or 8-colors**
- **higher cell input/tube**
- **fewer tubes**



Good reproducibility (multi-centric)

Conventional FLOW-MRD: ≥ 0.05%

HiRes-FLOW-MRD: ≥ 0.005%

input 3 x10^5 (4 colors)

input 0.75 - 1 x10^6 (8 colors)

Vienna panel for high-resolution FLOW-MRD 7-/8-colors on BD LSR II

status 08-2008

mAb-combination for BCP-ALL (B-I, B-II, B-III):

Tube 1 (compulsory):
CD20FITC/CD10PE/CD45PerCP/CD34PECy7/CD19APC/CD38Ax700/SYTO41

Tube 2 (compulsory):
CD58FITC/CD11aPE/CD45PerCP/CD10PECy7/CD19APC/CD20APCCy7/CD38Ax700/SYTO41

mAb-combination for T-ALL:

Tube 1 (compulsory in immature T-ALL but additional in mature T-ALL):
TdTFITC/CD99PE/sCD3PE-TR/CD45PerCP/CD5PC7/CD7APC/cyCD3Ax700/SYTO41

Tube 2 (compulsory in mature T-ALL, additional in immature T-ALL):
CD4FITC/CD99PE/sCD3PE-TR/CD45PerCP/CD5PC7/CD7APC/CD8APC-Cy7/SYTO41

The AIEOP-BFM ALL 2000 FCM-MRD study

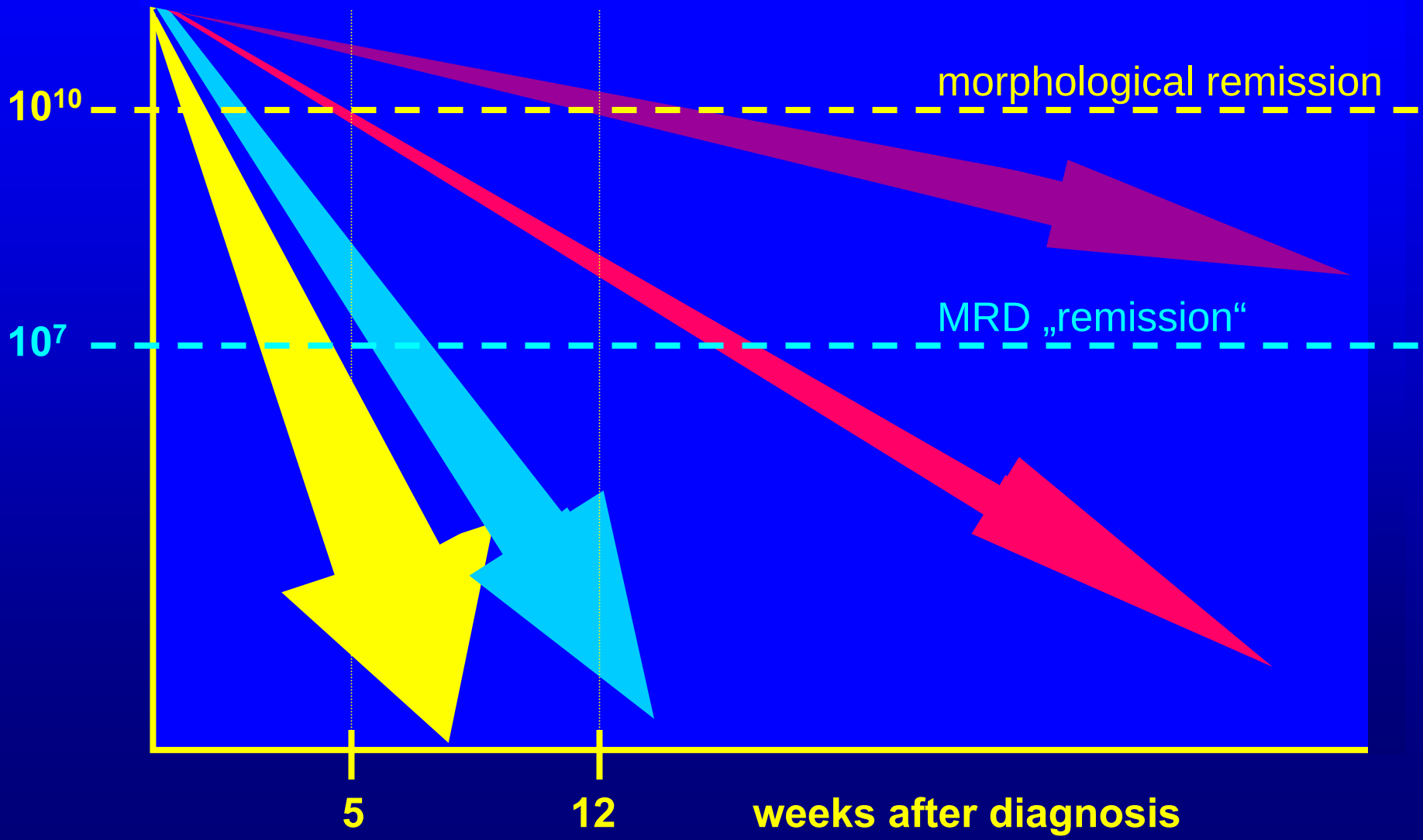
Part 1

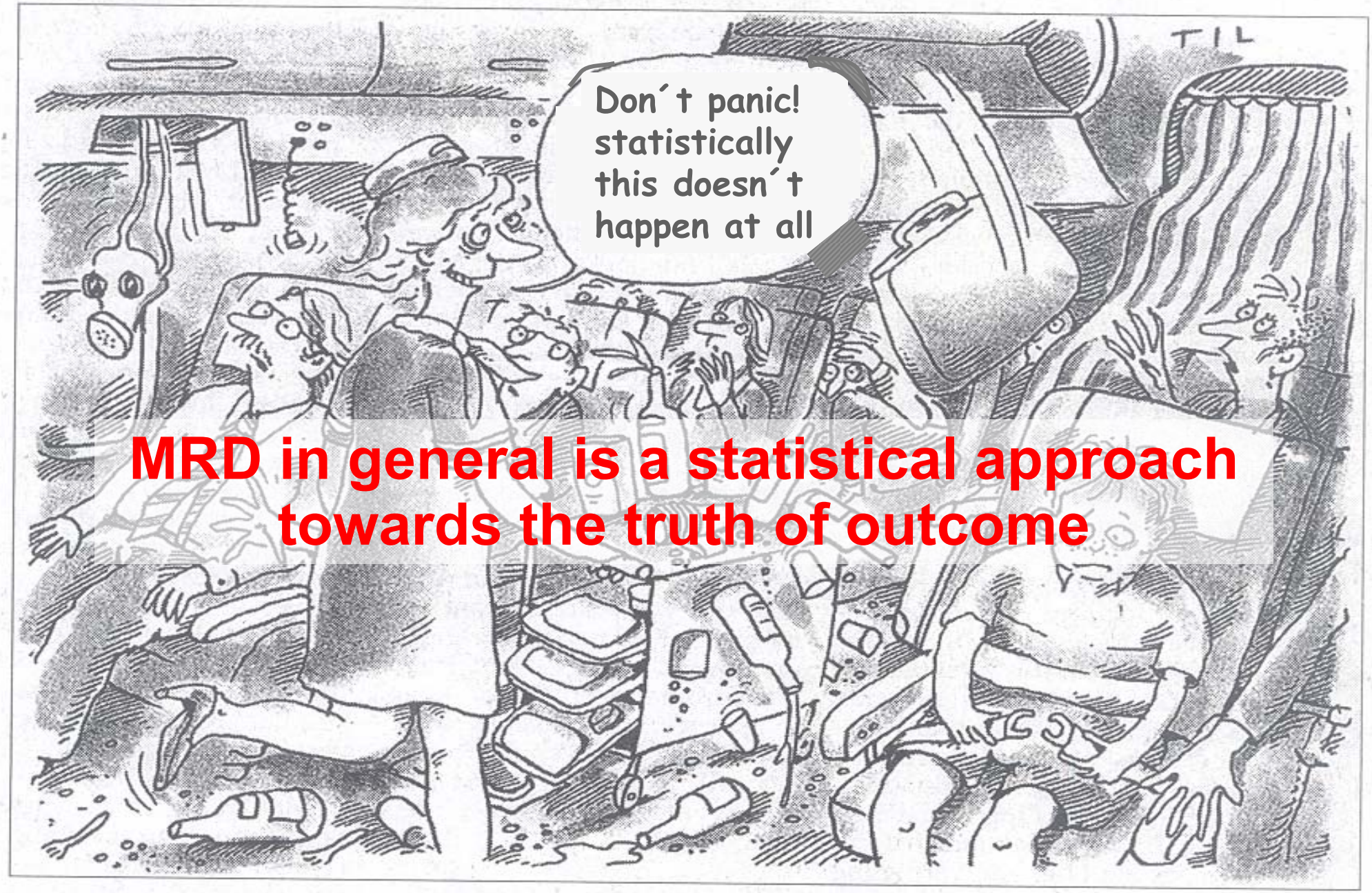
- MRD definition – old and new knowledge
- Technical standardization in AIEOP-BFM

Part 2

- Correlation with PCR-MRD
- **FCM vs. Outcome: interim results**

MRD aims: intensify or reduce treatment

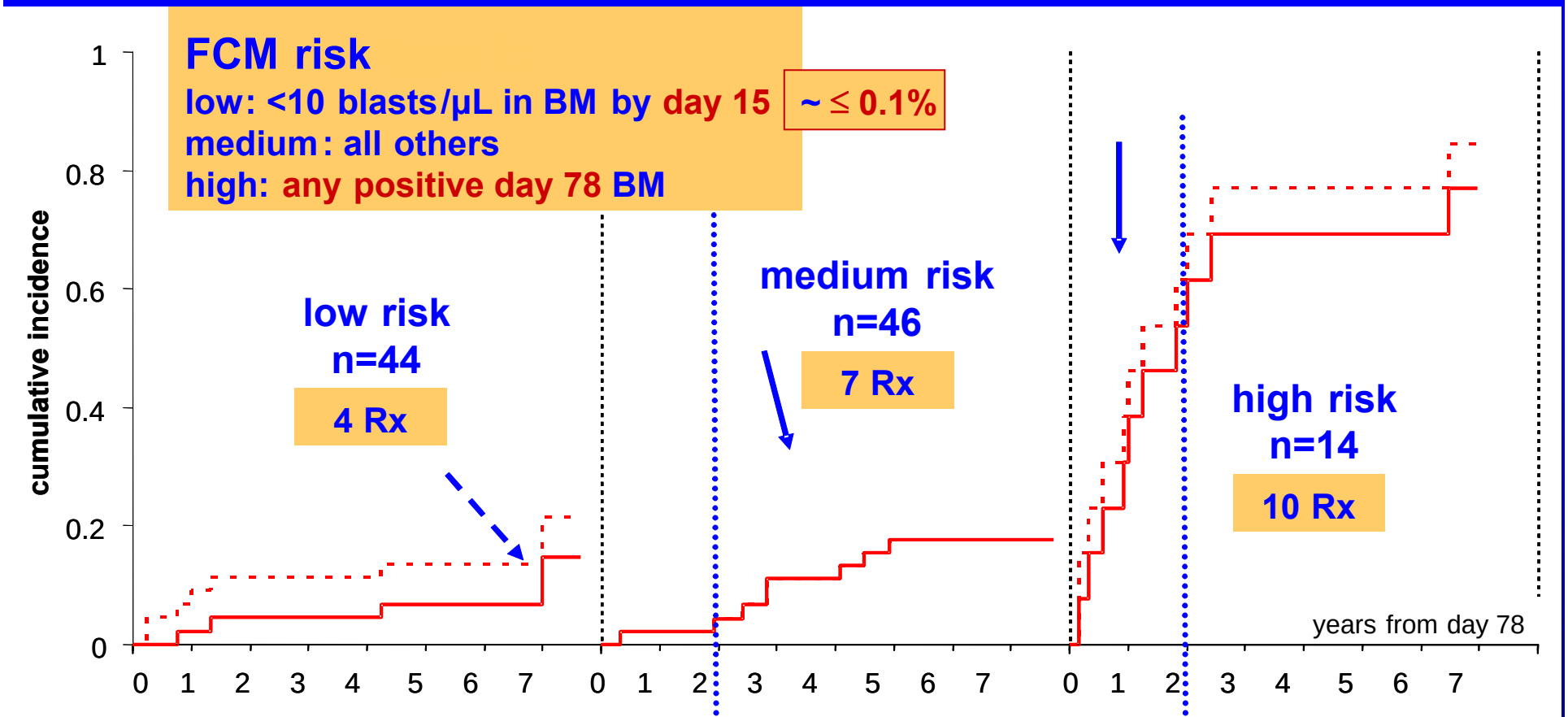




MRD in general is a statistical approach towards the truth of outcome

Austrian ALL-BFM 95 study: update results on FCM-MRD

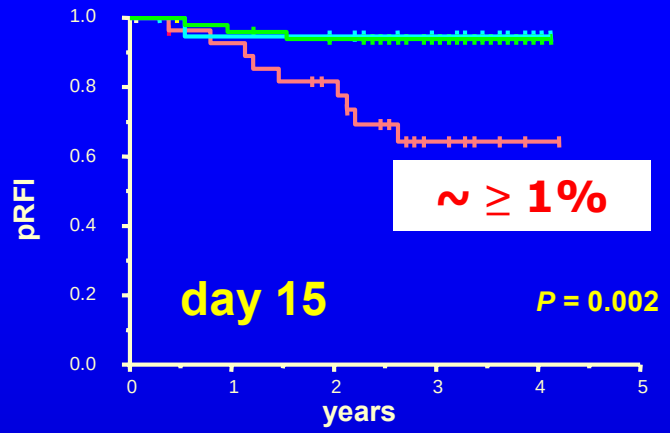
Dworzak et al., Blood 2002
updated in Leuk Lymph 2003



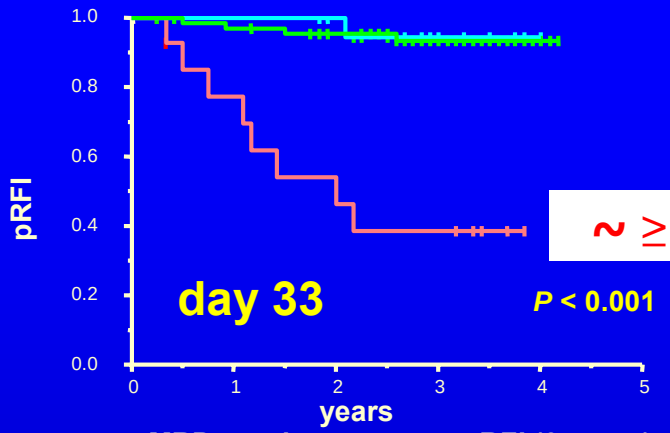
	5-yrs. CI±SE		5-yrs. CI±SE		5-yrs. CI±SE
Relapse	**0.07±0.04**	n.s.	**0.18±0.06**	p<0.001	**0.69±0.13**
Death in CR	0.07±0.04		0		0.08±0.07
Event	0.14±0.05		0.18±0.06		0.77±0.12

Austrian ALL-BFM 1995 data

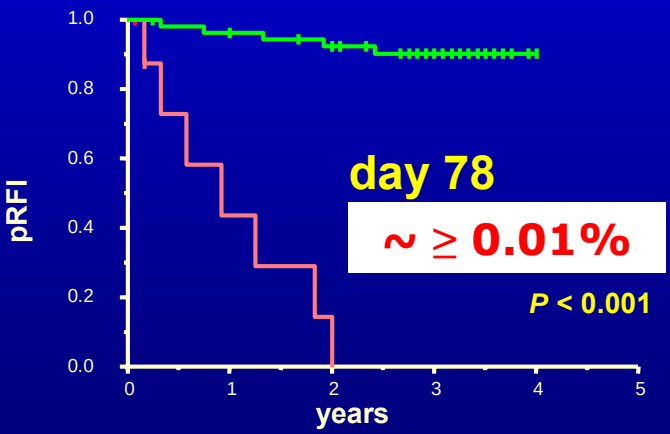
Dworzak et al., Blood 2002



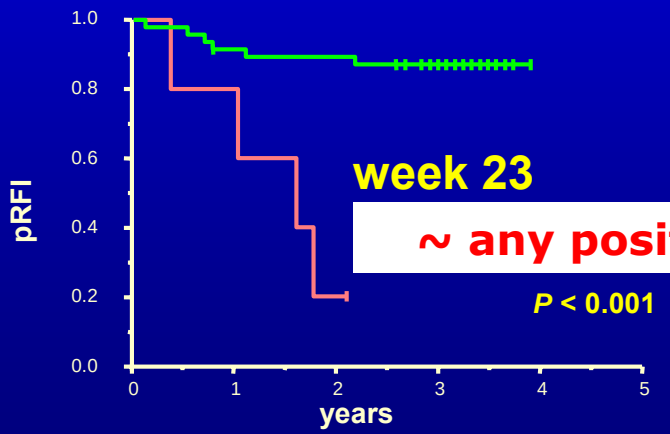
MRD-result	n=	pRFI (3-years)
<10 /µL	52	0.94 ±0.03
<100 - 10 /µL	19	0.95 ±0.05
≥100 /µL	30	0.64 ±0.1



MRD-result	n=	pRFI (3-years)
<1 /µL	69	0.93 ±0.03
<10 - 1 /µL	21	0.94 ±0.05
≥10 /µL	15	0.39 ±0.14

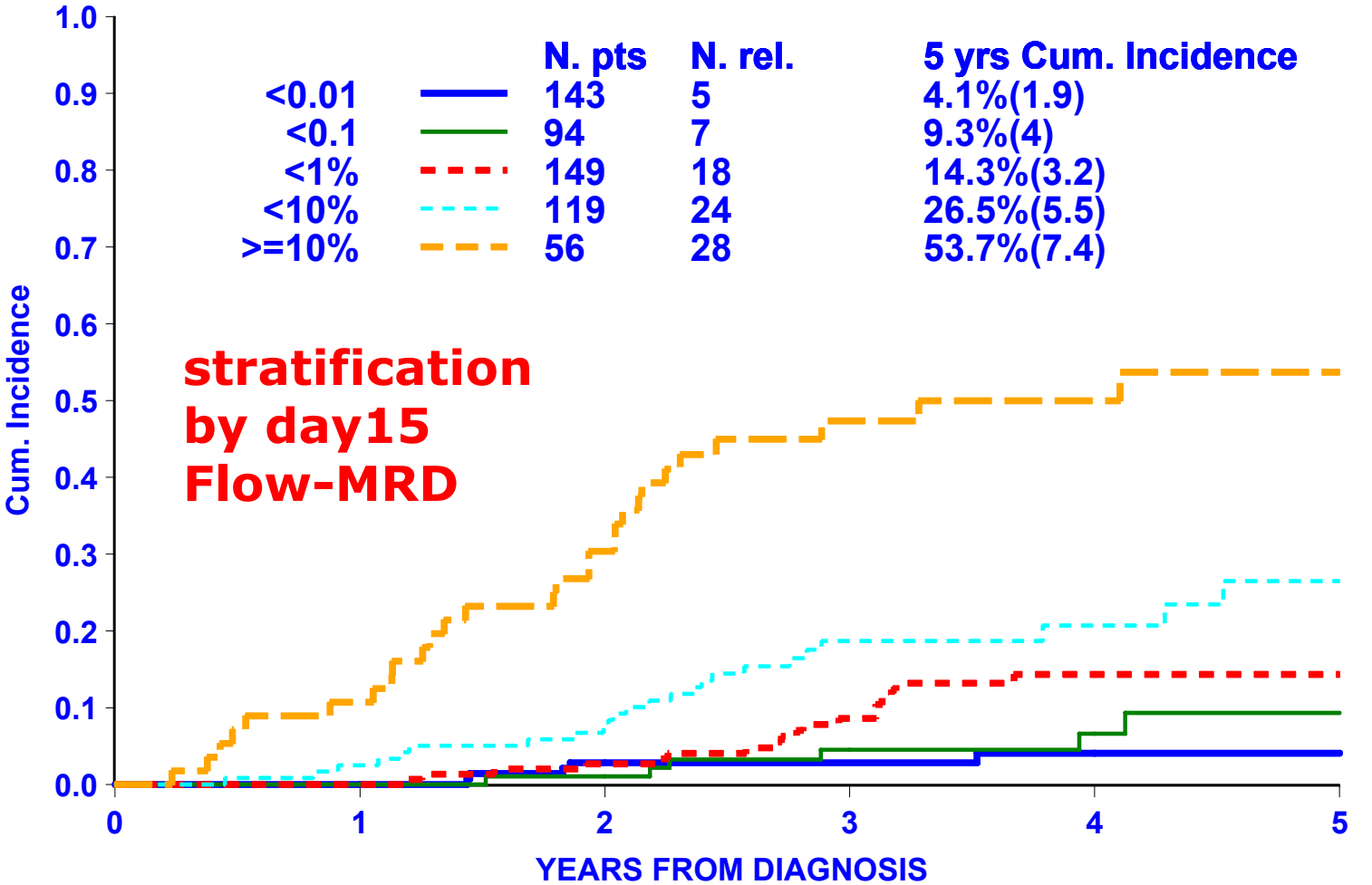


MRD-result	n=	pRFI (3-years)
< 1 /µL	55	0.90 ±0.04
≥ 1 /µL	9	0.0



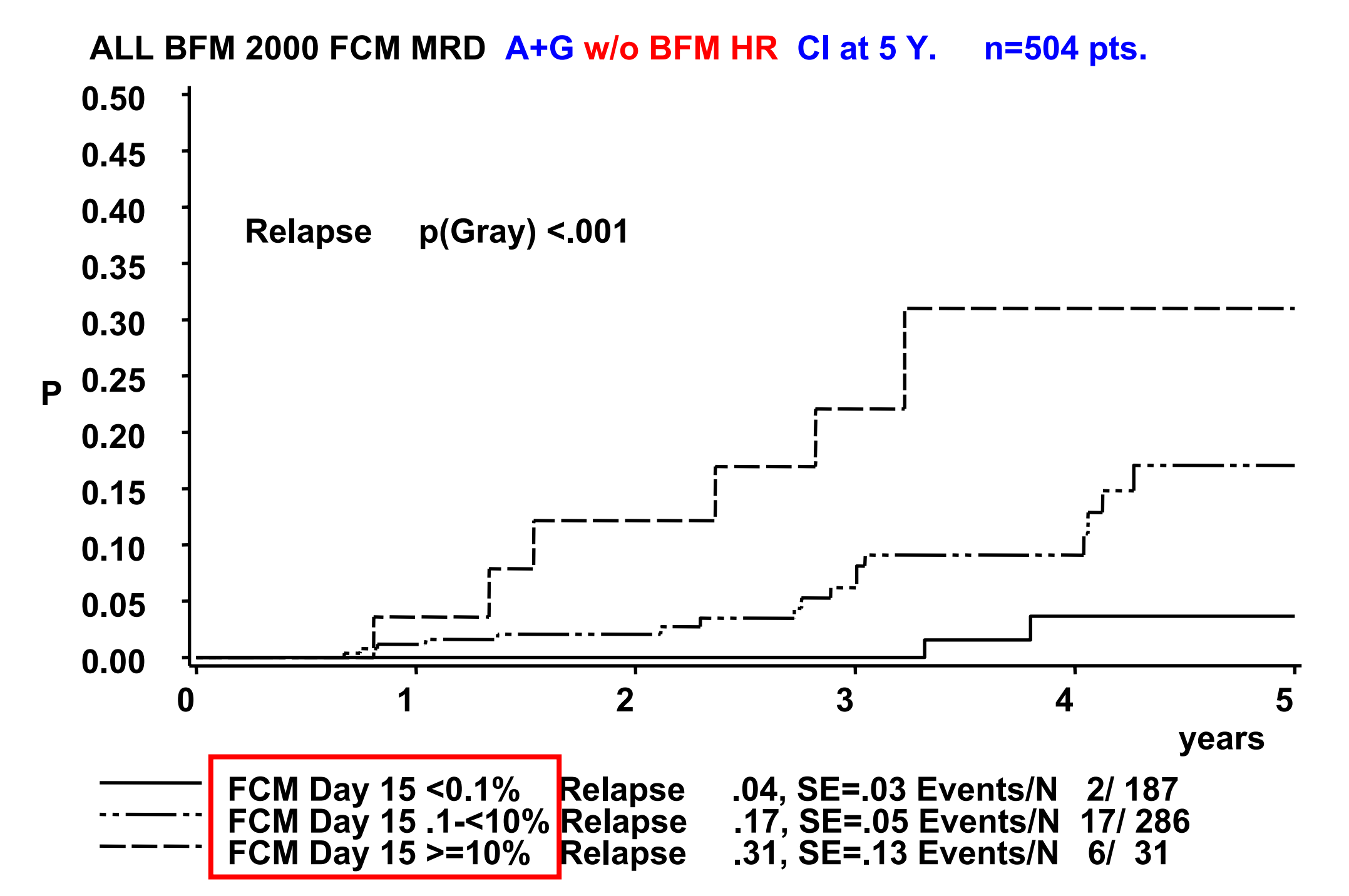
MRD-result	n=	pRFI (3-years)
negative	47	0.87 ±0.05
positive	5	0.2

AIEOP ALL 2000 - FCM/PCR
by FCM at day +15
561 patients

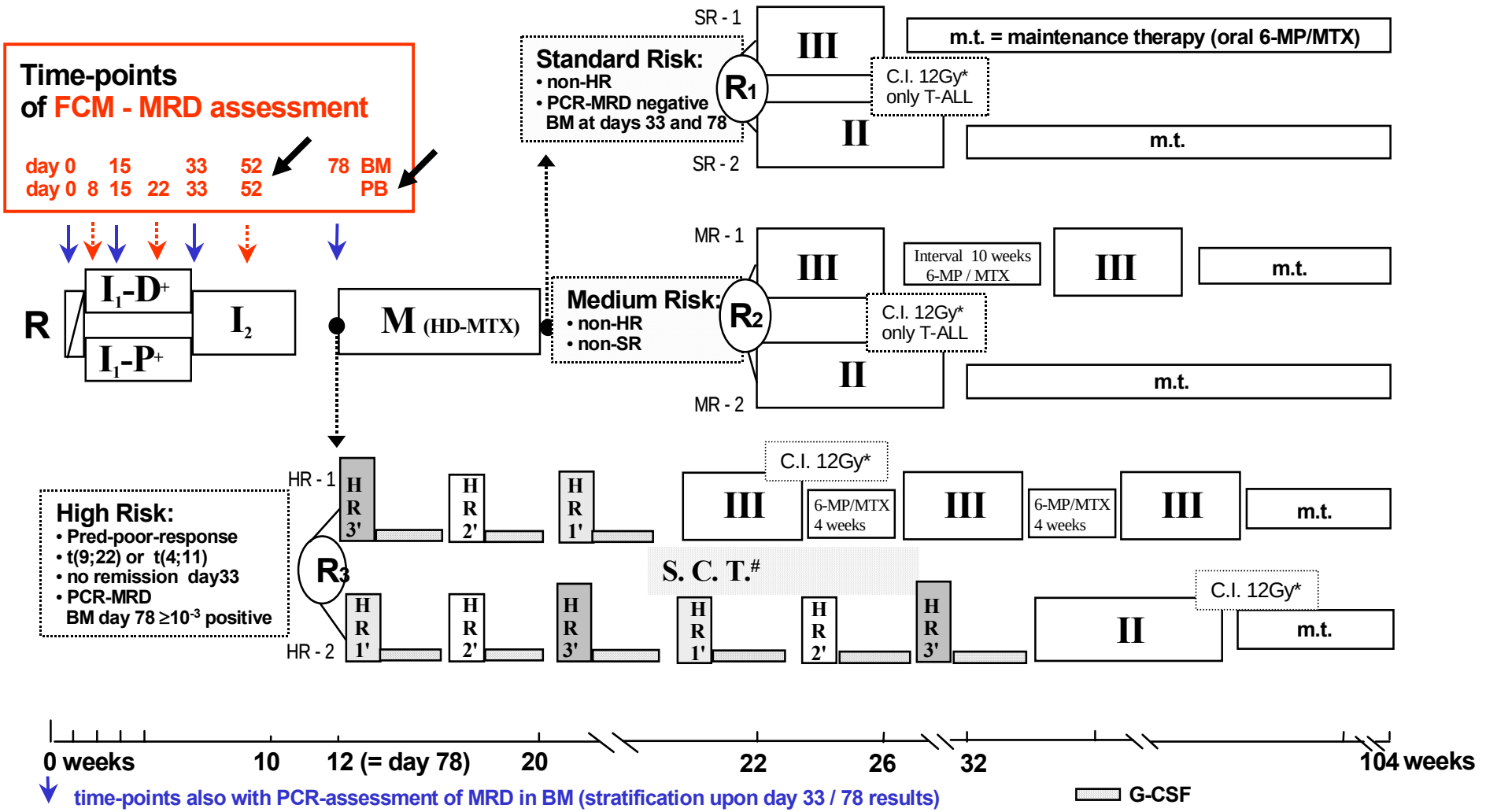


by courtesy of G.Basso/AEIOP

CORS - April 2007


ALL BFM 2000 FCM MRD A+G w/o BFM HR CI at 5 Y. n=504 pts.
Relapse p(Gray) <.001
P
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
1
2
3
4
5
years
FCM Day 15 <0.1% Relapse .04, SE=.03 Events/N 2/ 187
FCM Day 15 .1-<10% Relapse .17, SE=.05 Events/N 17/ 286
FCM Day 15 >=10% Relapse .31, SE=.13 Events/N 6/ 31

Treatment protocol AIEOP-BFM-ALL 2000



+ **I_1-D+:** „Protokoll I, Phase 1“ w dexamethasone
I_1-P+: „Protokoll I, Phase 1“ w prednisone

* **C.I.**, i.e. cranial irradiation (12 Gy for prophylaxis; 18Gy therapy for C.N.S.-positive pts.)
\# **S.C.T.**, i.e. allogeneic stem cell transplantation in selected cases

BM

Possible **time-point related** **FCM-thresholds**

for **HR**-description (BM): **BFM-A cohort** data (n=297/**17**Rx)

		pts.	(%of all)	**relapses**	(med.obs. 3.83y)
D15	≥10%	**36**	(12.1)	**5**	(FCM4R3)
D33	≥0.1%	**37**	(12.5)	**5**	
D52	positive	**42**	(14.1)	**7**	
D52	≥0.01%	**30**	(10.1)	**5**	
D78	positive	**18**	(6.1)	**5**	(FCM1R)
D78	≥0.01%	**11**	(3.7)	**1**	
Comb. D15+D33		**46**	(15.5)	**7**	(FCM4R33)
Comb. D15+D52		**55**	(20.2)	**10**	(FCM4R52)

update 03-2008

Which time-point characterizes which type of relapse?

HR is:
D15 ≥10%
D33 ≥0.1%
D52 ≥0.01% (or any positive)
D78 ≥0.01% (or any positive)

	HR per time-point
	D52orD78 positive <0.01%
	LR per D15 <0.1%
	not HR/LR
	n.a.

UPN	D15	D33	D52	D78	PCR	BFM
15					IR	IR
31					IR	IR
32					LR	LR
35					IR	IR
64					IR	IR
67					na	IR
72					IR	IR
91					na	IR
101					IR	IR
105					HR	HR
111					IR	IR
125					IR	IR
129					IR	IR
185					HR	HR
229					IR	IR
240					IR	IR
253					IR	IR
266					IR	HR
288					IR	IR

all Austrian patients relapsing on study AIEOP-BFM ALL 2000

AIEOP-BFM 2000 based data:

Recommendations for new multi-center co-operations

Towards the "best" risk algorithm by FCM-MRD

for BFM-type protocols

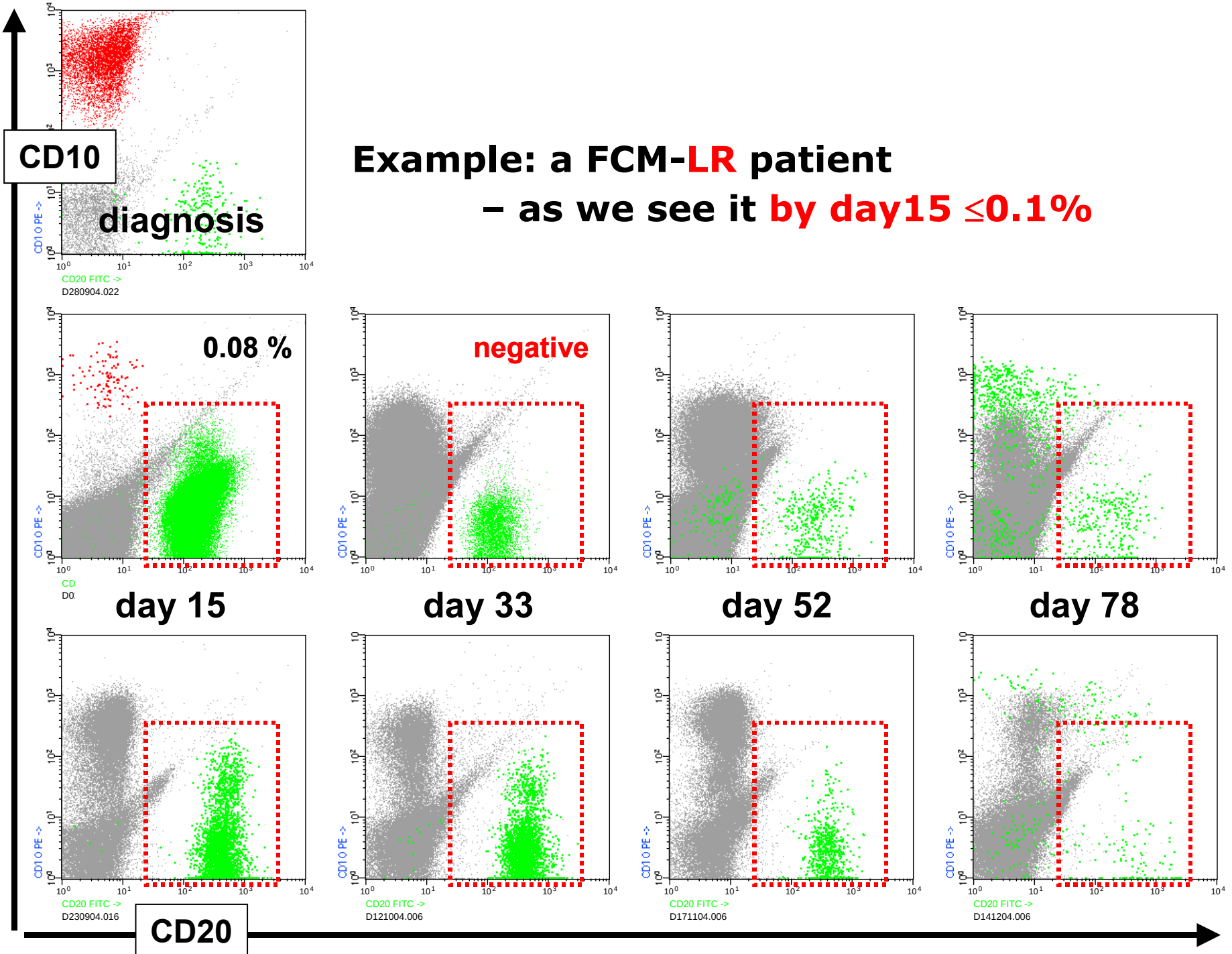
→ **Low risk of relapse:** **BM D15 ≤0.1%**

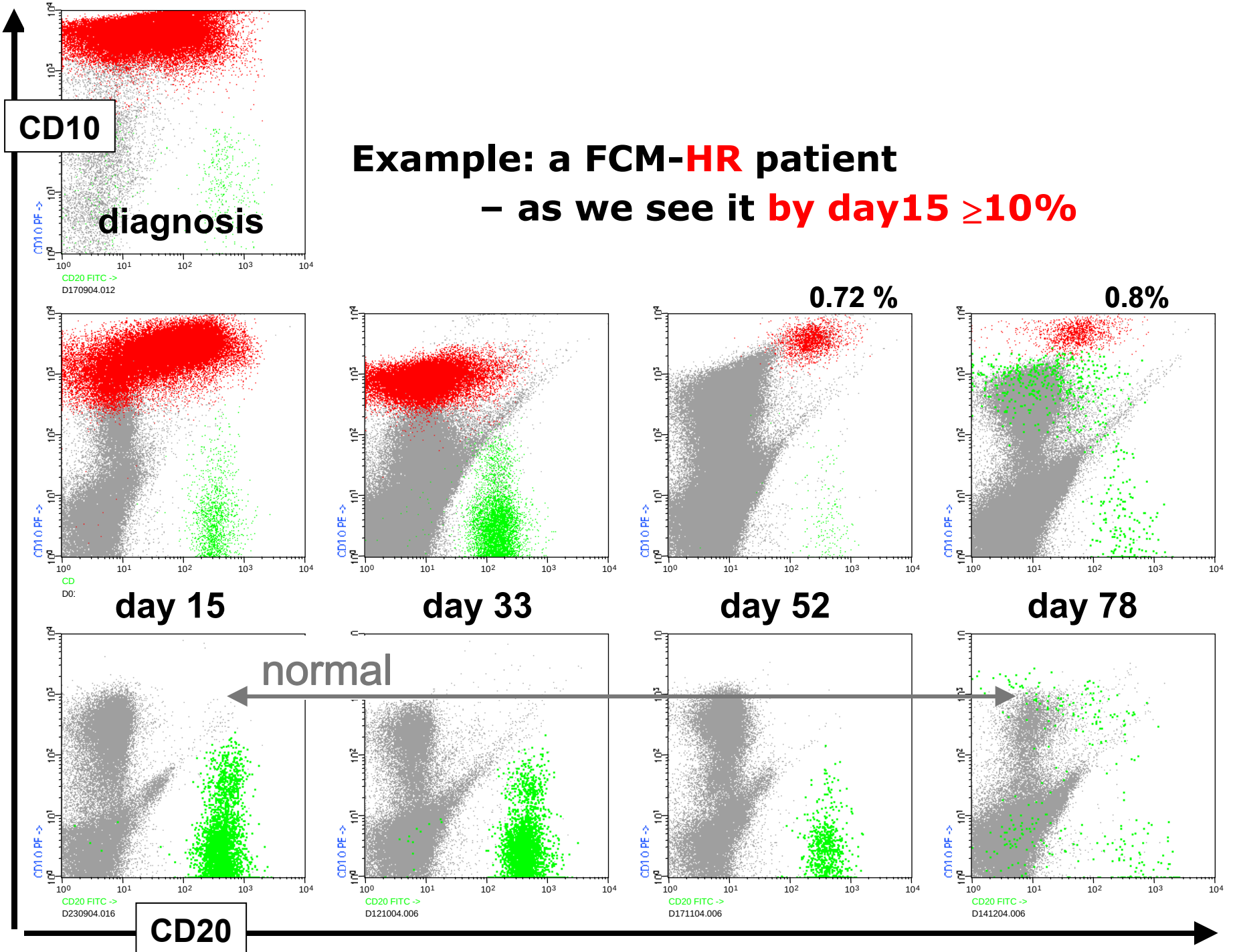
High risk of relapse: **various possibilities !!**

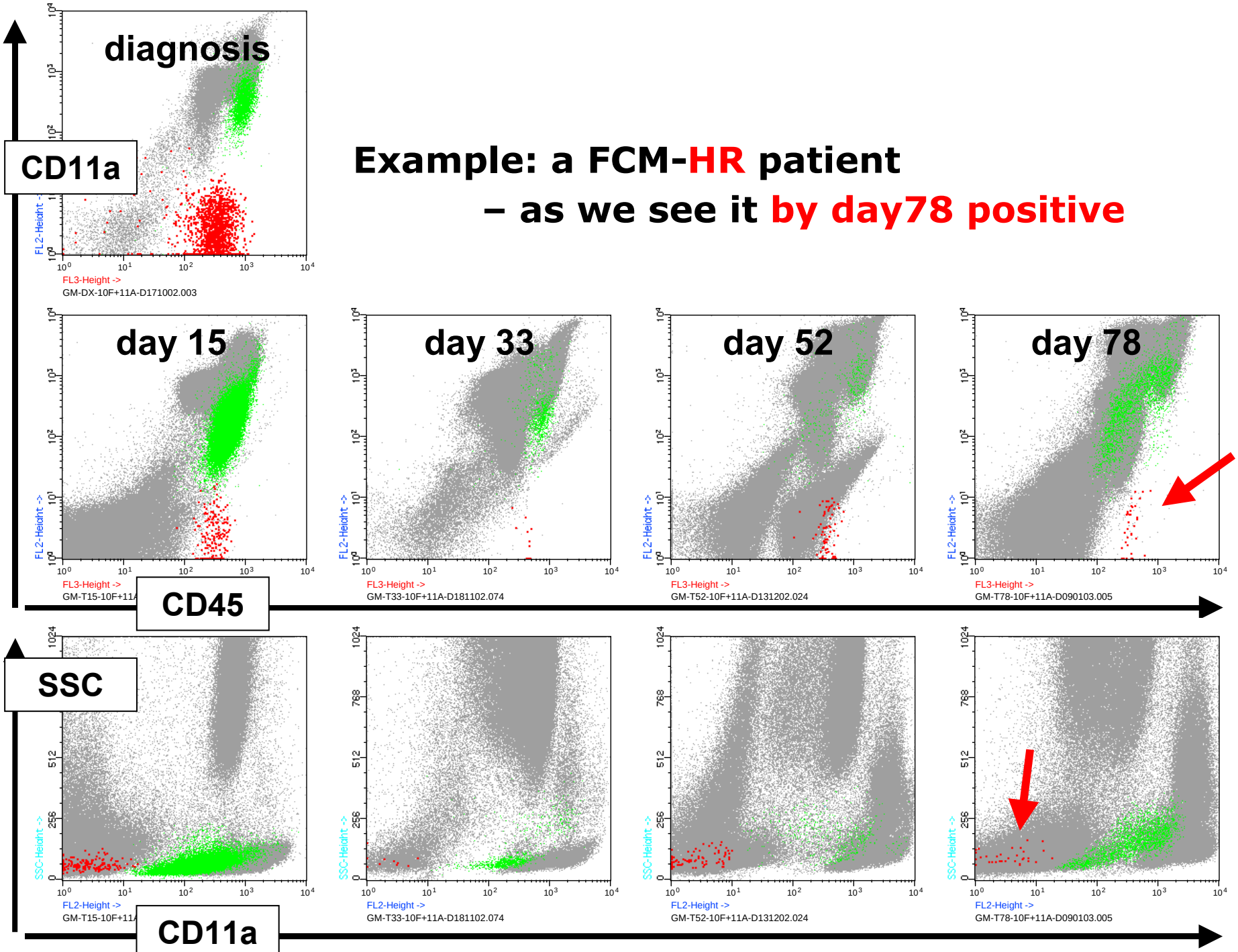
→ **D15≥ *10 %*** **very easy, target group 2xn per d78**

D78*positive* **most stringent, most complicate**

Example: a FCM-LR patient
– as we see it by day15 ≤0.1%



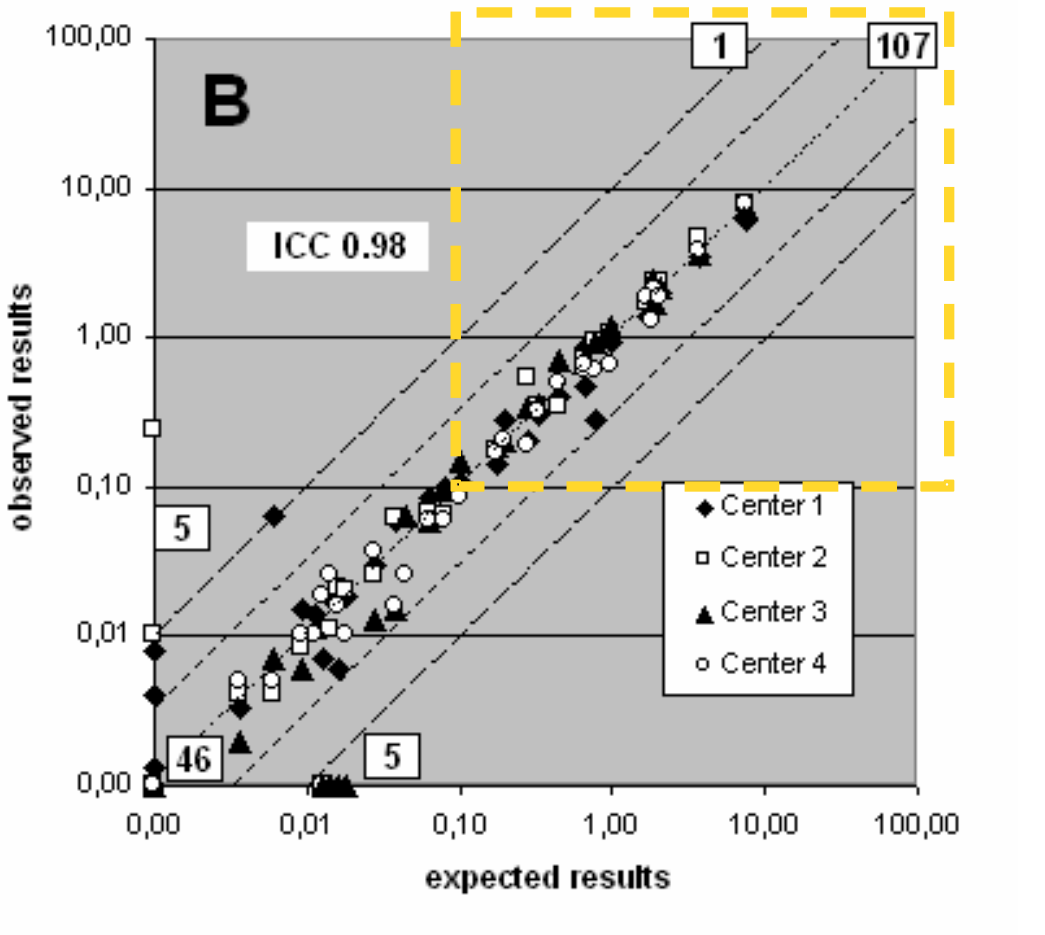

CD10
diagnosis
Example: a FCM-HR patient
– as we see it by day15 ≥10%
0.72 %
0.8%
day 15
day 33
day 52
day 78
normal
CD20


diagnosis
CD11a
FL2-Height ->
FL3-Height ->
GM-DX-10F+11A-D171002.003
Example: a FCM-HR patient
– as we see it by day78 positive
day 15
day 33
day 52
day 78
CD45
FL3-Height ->
GM-T15-10F+11A
GM-T33-10F+11A-D181102.074
GM-T52-10F+11A-D131202.024
GM-T78-10F+11A-D090103.005
SSC
CD11a
SSC-Height ->
FL2-Height ->
GM-T15-10F+11A
GM-T33-10F+11A-D181102.074
GM-T52-10F+11A-D131202.024
GM-T78-10F+11A-D090103.005

The ≥0.1% threshold is easily reproducible
in a multi-center setting

No matter, whether done

- by different FCmeters
- with different analysis soft wares
- ***in different labs***



AIEOP-BFM 2000 the international cohort data

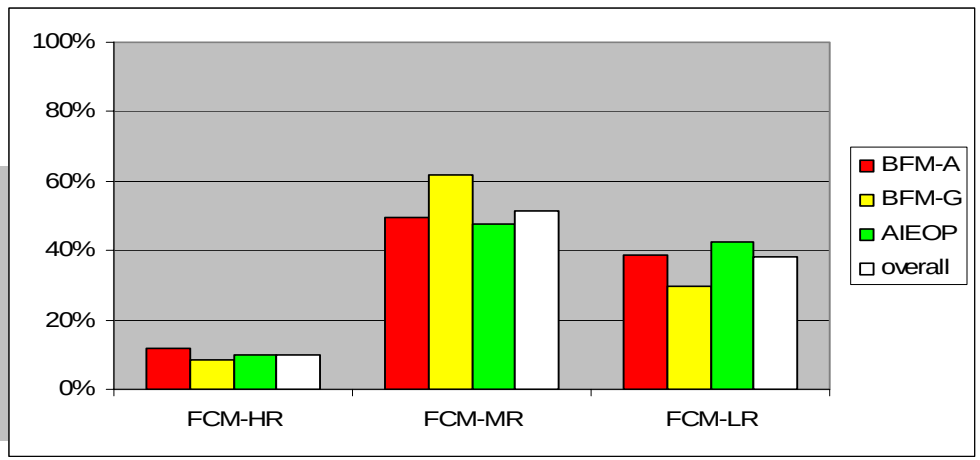
preliminary outcome correlations

FCM on day15 vs. relapse incidence

FCM4R3	BFM-A	%of all	Rx	%Rx	BFM-G	%of all	Rx	%Rx	AIEOP	%of all	Rx	%Rx	total	%of all	Rx	%Rx
	291		17	5,8%	265		16	6,0%	561		88	15,7%	1117		121	10,8%
FCM d15 ≥10%	34	11,7%	5	14,7%	22	8,3%	5	22,7%	56	10,0%	31	55,4%	112	10,0%	41	36,6%
all other	144	49,5%	11	7,6%	164	61,9%	10	6,1%	268	47,8%	43	16,0%	576	51,6%	64	11,1%
FCM d15 ≤0.1%	113	38,8%	1	0,9%	79	29,8%	1	1,3%	237	42,2%	14	5,9%	429	38,4%	16	3,7%

Size of risk groups:

	BFM-A	BFM-G	AIEOP	overall
	291	265	561	1096
FCM-HR	11,7%	8,3%	10,0%	10,0%
FCM-MR	49,5%	61,9%	47,8%	51,6%
FCM-LR	38,8%	29,8%	42,2%	38,4%

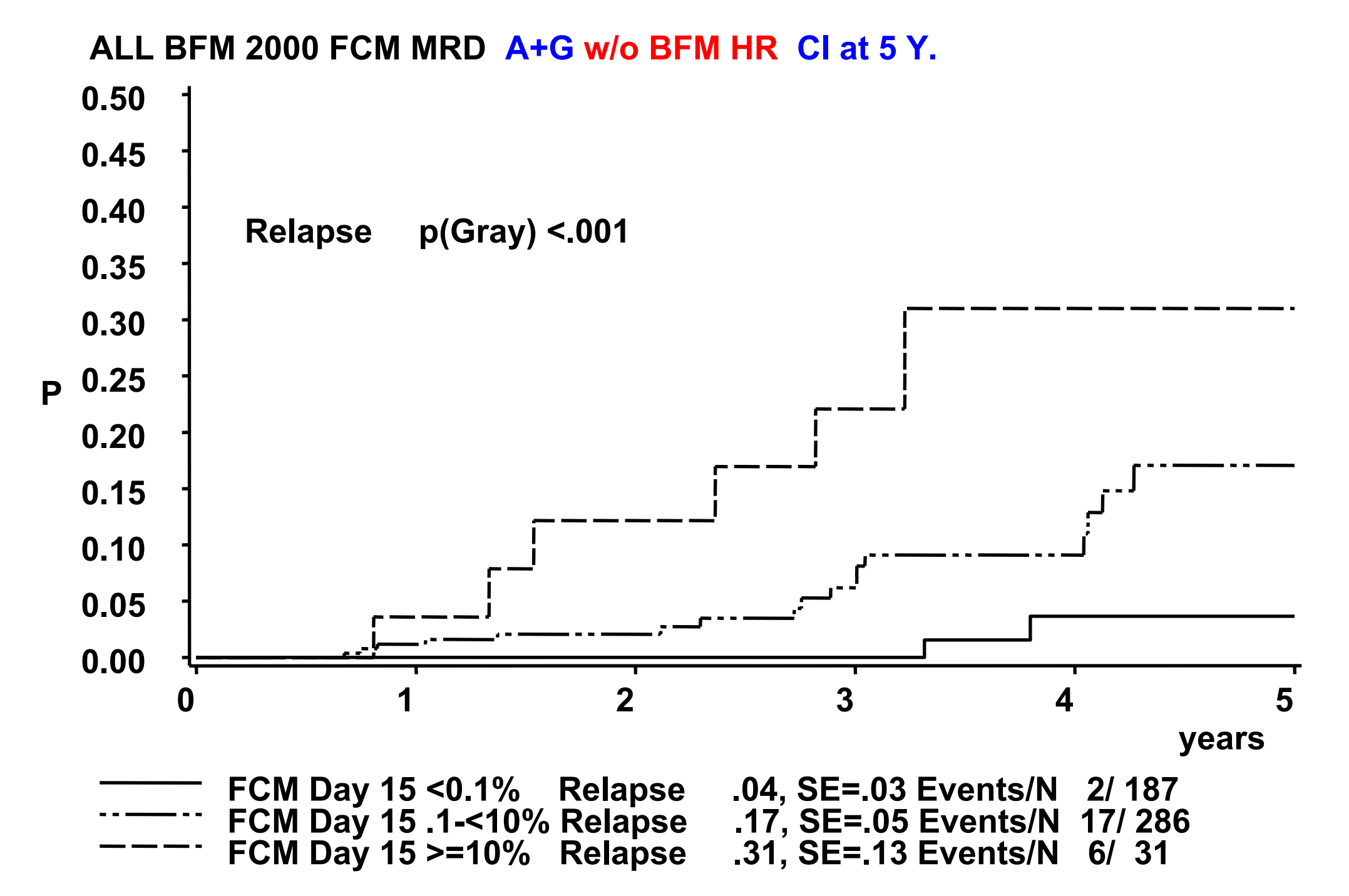


AIEOP-BFM 2000 the **BFM A+G** cohort data (n=559)

preliminary outcome correlations

FCM vs. clin. non-HR

FCM4R3	BFM-HR	Rx	BFM-nonHR	Rx	total	Rx	%Rx	nonHR	Rx	%Rx
BFM-A+G	55	6	504	29	559	35	6,26%	504	29	5,75%
FCM d15 ≥10%	26	3	31	8	57	11	19,30%	31	8	25,81%
all other	28	2	286	19	314	21	6,69%	286	19	6,64%
FCM d15 ≤0.1%	1	0	187	2	188	2	1,06%	187	2	1,07%


ALL BFM 2000 FCM MRD A+G w/o BFM HR CI at 5 Y.
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
P
Relapse p(Gray) <.001
0
1
2
3
4
5
years
FCM Day 15 <0.1% Relapse .04, SE=.03 Events/N 2/ 187
FCM Day 15 .1-<10% Relapse .17, SE=.05 Events/N 17/ 286
FCM Day 15 >=10% Relapse .31, SE=.13 Events/N 6/ 31

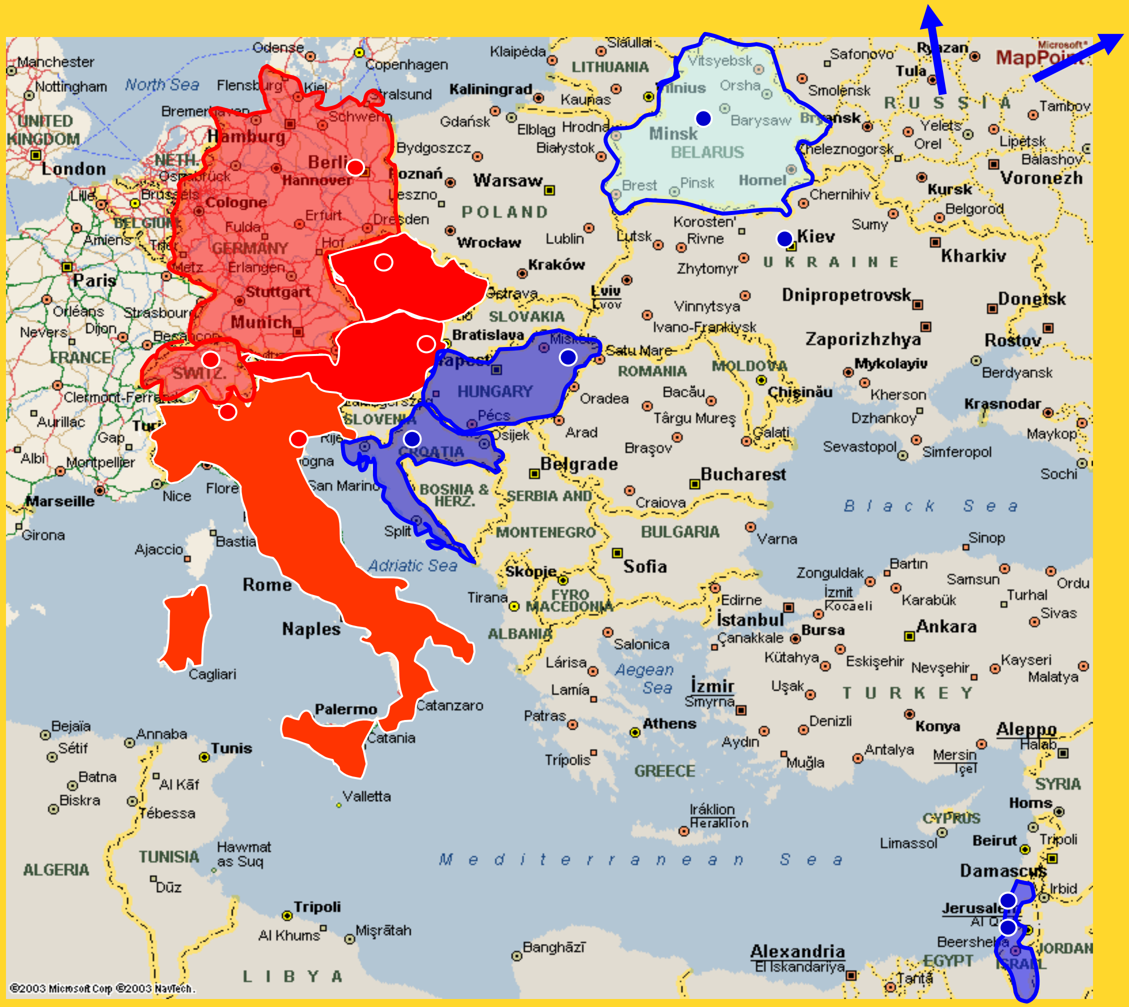
Flow Cytometry
for future stratifying clinical application in multi-center trials of the I-BFM...

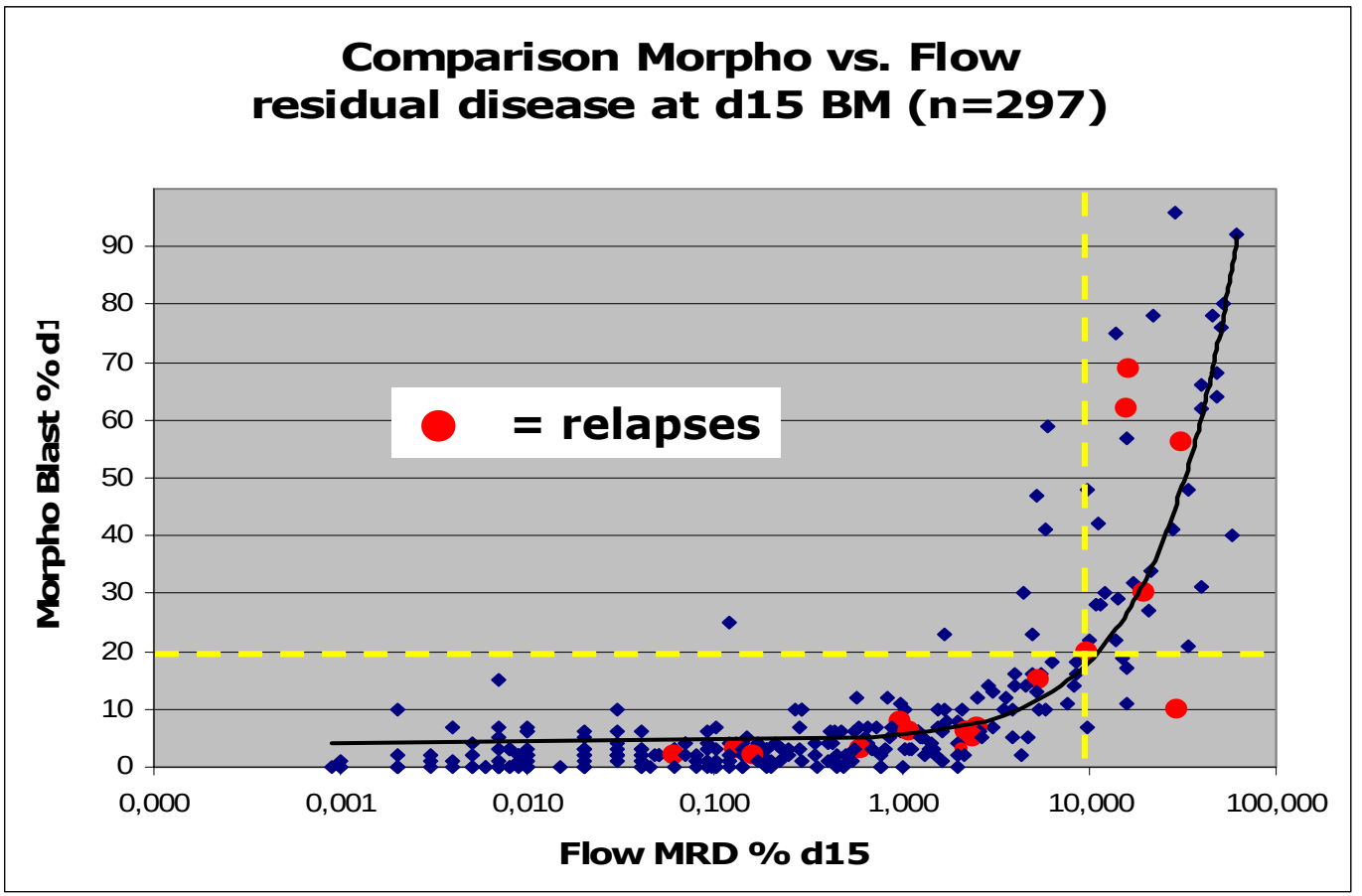
I-BFM *ALL FLOW MRD SG*

- **AIEOP-BFM (study 2008 upcoming)**
  - **Berlin** Ratei/Ludwig
  - **Monza** Gaipa/Biondi
  - **Padova** Basso/Veltroni
  - **Prague** Hrusak/Mejstrikova
  - **Switzerland** Bourquin/Niggli
  - **Vienna** **Dworzak**

- **ALL-IC BFM**
  - **Debrecen** Kappelmayer/Kis
  - **Zagreb** Batinic
  - **Israel** Luria/Stark
  - **Chile** Cabrera/Campbell
  - ...

- **Moscow-Berlin group**
  - **Ekaterinburg** Popow/Fechina
  - **Minsk** Belevtsev/Alainikova
  - **Moscow** Boyakova/Roumantsev





Morphology: <u>NO</u> striking „extra"-information over FHR

FlowMRD: High risk <u>AND</u> low risk definition possible

FLOW-MRD vs. ALL IC criteria (based on BFM95)

	FLR	FMR	FHR	all	
ALL IC SR	56 / 1 R	51 / 4 R	3 / 1 R	110 / 6 R	5.5 %
ALL IC MR	57 / 0 R	80 / 8 R	12 / 3 R	149 / 11 R	7.4 %
ALL IC HR	1 / 0 R	18 / 1 R	21 / 1 R	40 / 2 R	5.0 %
	114 / 1 R	149 / 13 R	36 / 5 R	299 / 19 R	
	0.9 %	8.7 %	13.9 %		6.4 %

FLOW–MRD – future applications

- in **PB** diagnostics
- in **relapsed** ALL*****
- for individualized **treatment tailoring***

* will be described in my afternoon-talk!

PB

Possible **time-point related** **FCM-thresholds**

for **Risk**-description (**PB**): **BFM-A cohort** data (n=276/17Rx)

PB D15	≥0.01% (Rx; %)		<0.01% (Rx; %)	
all patients	**126**	**14** (11.1%)	**150**	**3** (2.0%)

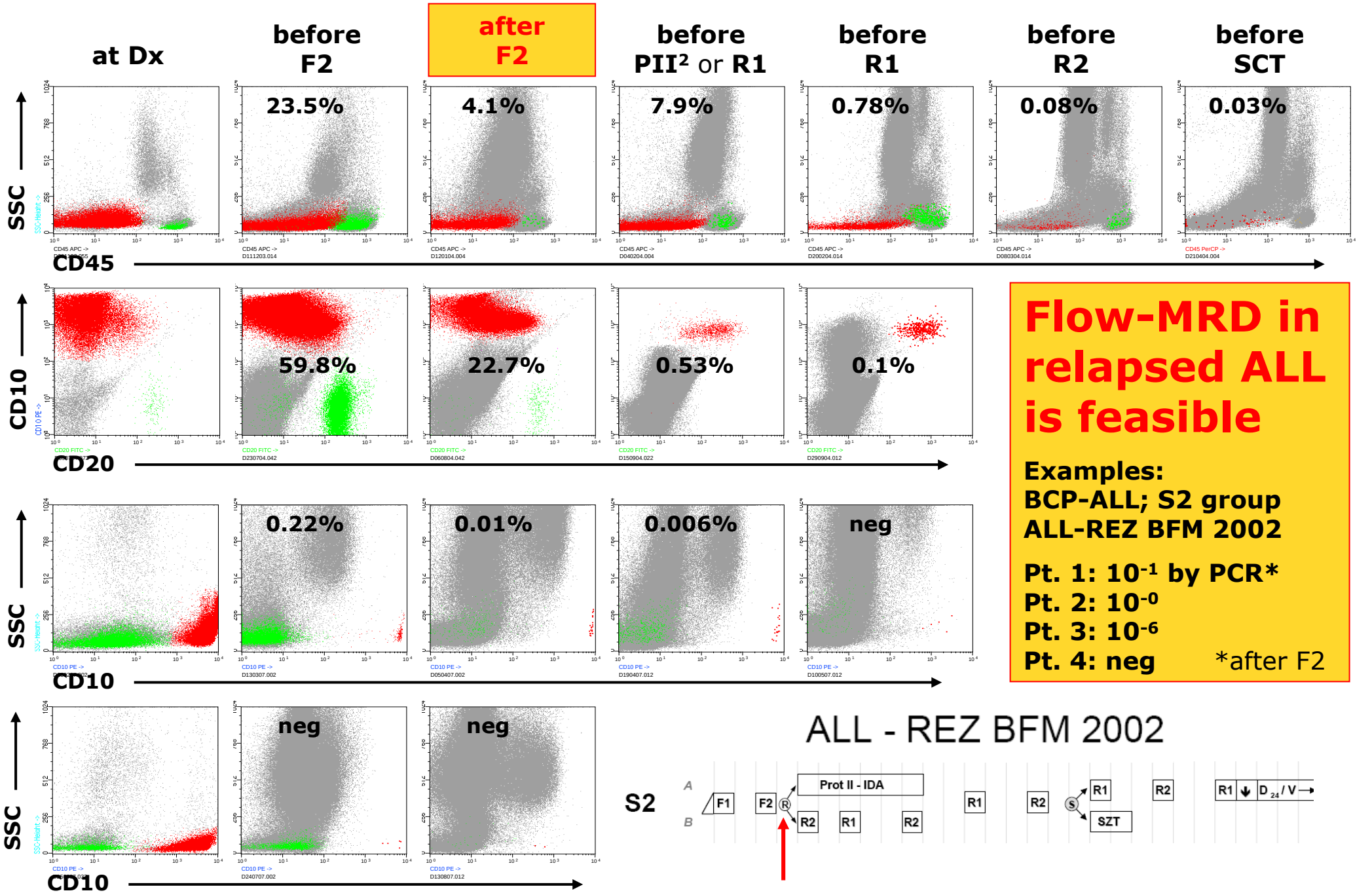
PB vs BM

MRD vs relapses BFM-A all pts.		FHR Rx in	FHR ≥ 10%	FHR %R	FMR Rx in	FMR ≥ 0.1%	FMR %R	FLR Rx in	FLR < 0.1%	FLR %R	by BM d15 Rx in	by BM d15 total	by BM d15 %R
PB	≥ 0.01%	4	33	12,1	10	87	11,5	0	6	0,0	14	126	11,1
d15	< 0.01%	0	0		2	56	3,6	1	94	1,1	3	150	2,0
	total	4	33	12,1	12	143	8,4	1	100	1,0	17	276	6,2

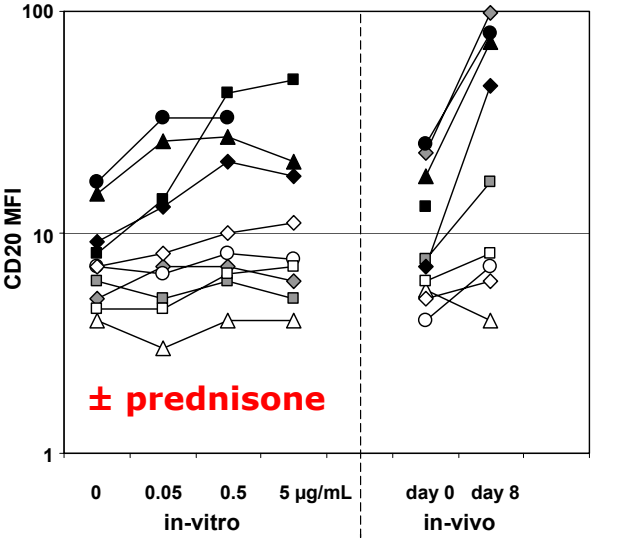
BFM-A BCP only		Rx in	≥ 10%	%R	Rx in	≥ 0.1%	%R	Rx in	< 0.1%	%R	Rx in	total	%R
PB	≥ 0.01%	3	27	11,1	8	68	11,8	0	4	0,0	11	99	11,1
d15	< 0.01%	0	0		2	54	3,7	1	87	1,1	3	141	2,1
	total	3	27	11,1	10	122	8,2	1	91	1,1	14	240	5,8

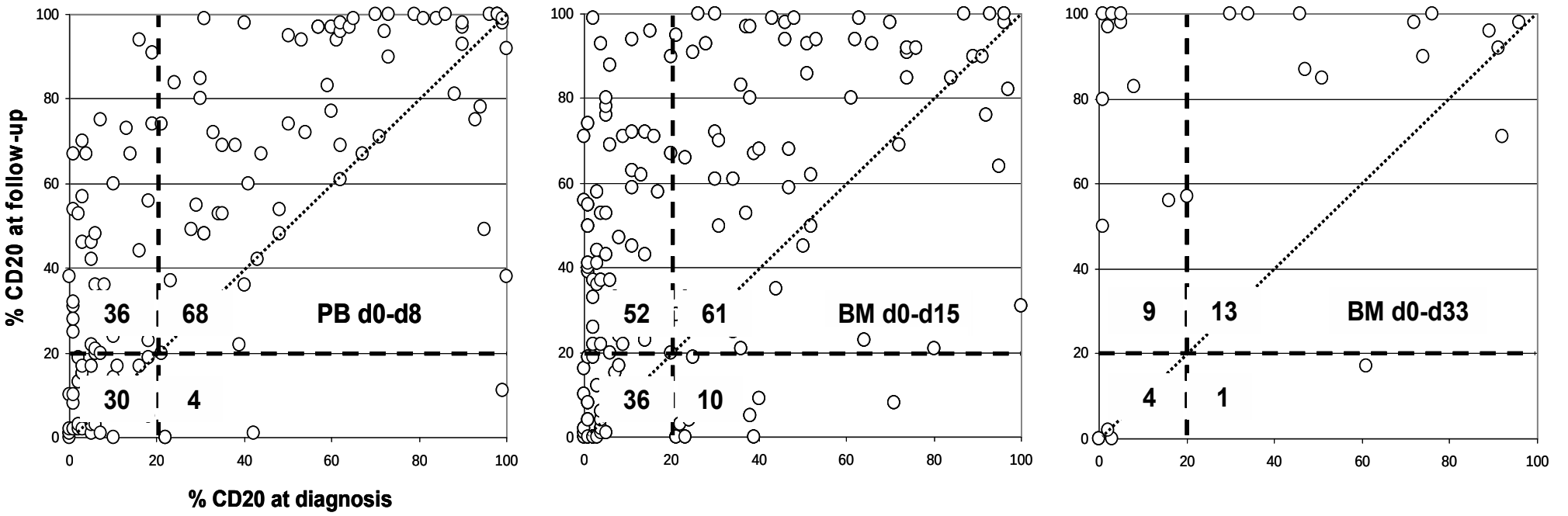
BFM-A BCP non-HR*		Rx in	≥ 10%	%R	Rx in	≥ 0.1%	%R	Rx in	< 0.1%	%R	Rx in	total	%R
PB	≥ 0.01%	2	13	15,4	7	59	11,9	0	4	0,0	9	76	11,8
d15	< 0.01%	0	0		2	50	4,0	1	87	1,1	3	137	2,2
*clin HR w/o PCR	total	2	13	15,4	9	109	8,3	1	91	1,1	12	213	5,6

→ **PB has additive value for FMR subdivision**


at Dx
before F2
after F2
before PII² or R1
before R1
before R2
before SCT
SSC
CD45
23.5%
4.1%
7.9%
0.78%
0.08%
0.03%
CD10
CD20
59.8%
22.7%
0.53%
0.1%
0.22%
0.01%
0.006%
neg
neg
neg
CD10
Flow-MRD in relapsed ALL is feasible
Examples:
BCP-ALL; S2 group
ALL-REZ BFM 2002
Pt. 1: 10-1 by PCR*
Pt. 2: 10-0
Pt. 3: 10-6
Pt. 4: neg
*after F2
ALL - REZ BFM 2002
S2
A
B
F1
F2
R
Prot II - IDA
R2
R1
R2
R1
R2
S
R1
SZT
R2
R1
D 24 / V

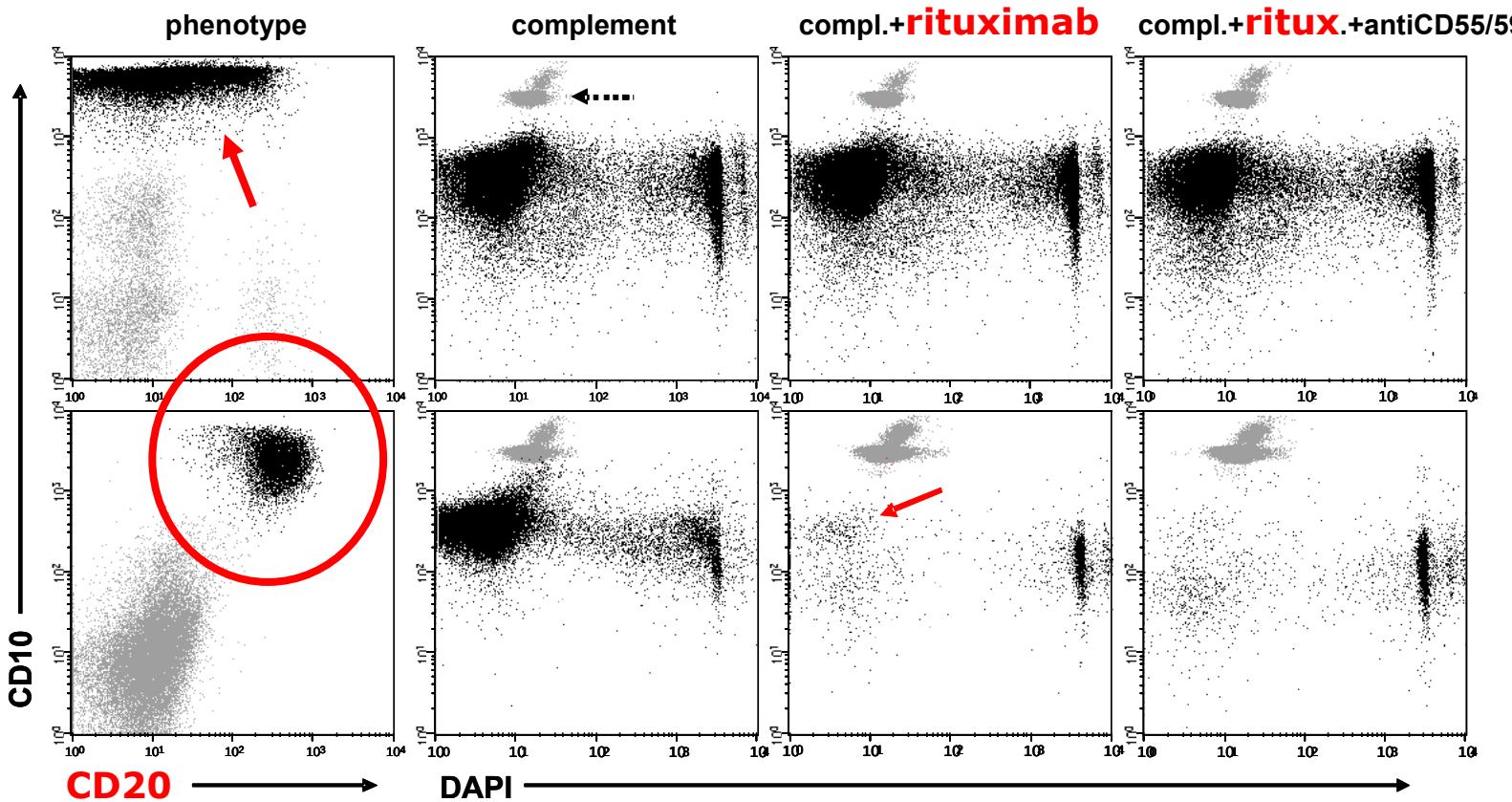
Steroid-induced CD20 up-regulation in-vivo





Dworzak et al., BLOOD
published online 2008

Steroid-induced CD20 up-regulation in-vivo translates into higher rituximab sensitivity



diagnosis

end- induction

Dworzak et al., BLOOD
published online 2008

Take-home message:

√ FLOW-MRD technically possible in ALL: yes

√ Can pitfalls be managed: yes

√ Assay standardization feasible: yes

√ Can method be disseminated: yes

√ Do results correlate with outcome: yes

√ Worth doing?: - Q to be solved for each protocol

Not everything that counts is countable,
not everything that is countable counts.

A. Einstein

¡GRACIAS!