More than Minimal: Laboratory Monitoring of Therapeutic Response in Acute Leukemia

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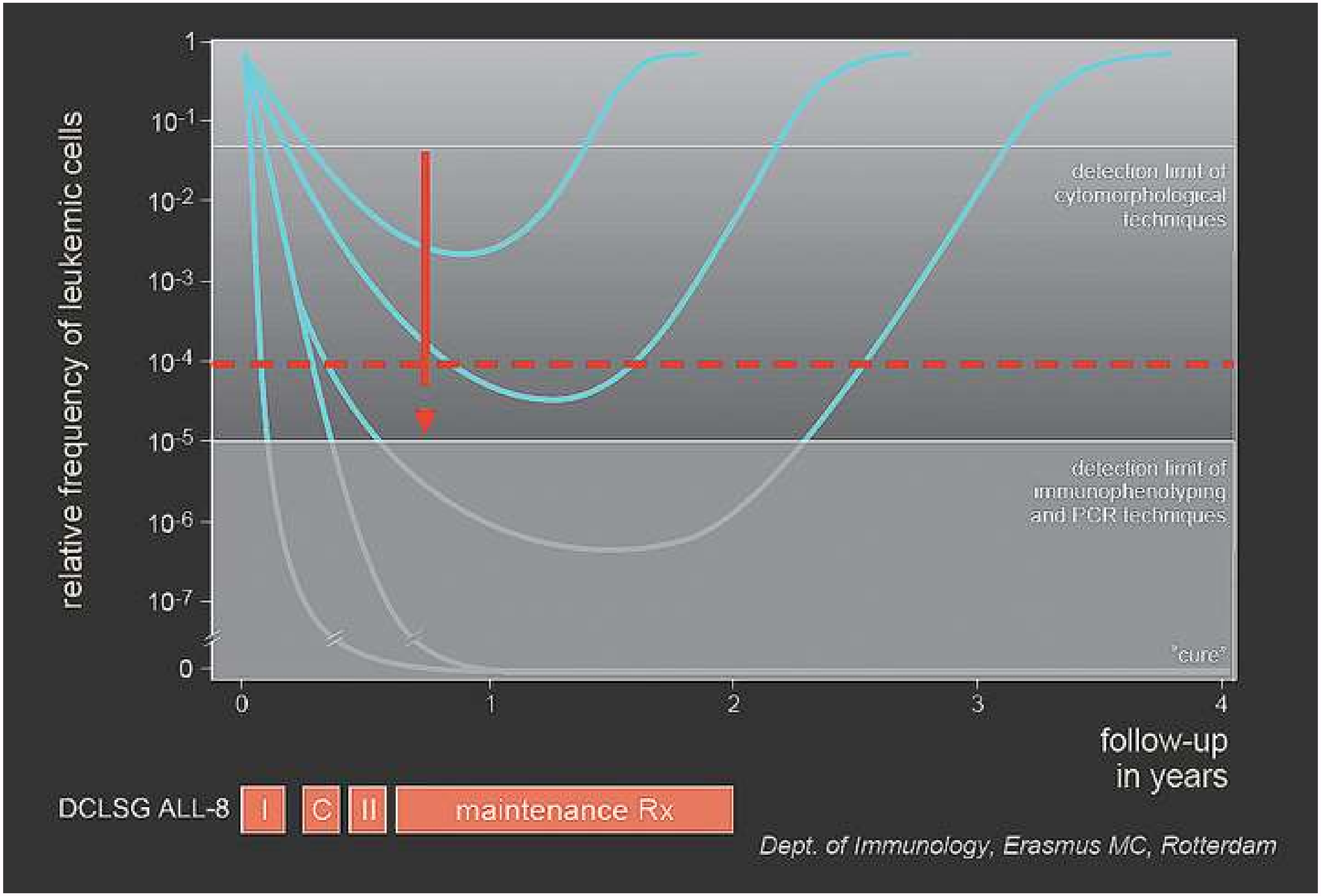
Response, MRD, and Relapse



J.J.M. van Dongen, FDA ALL MRD Workshop 4/2012

Methodology

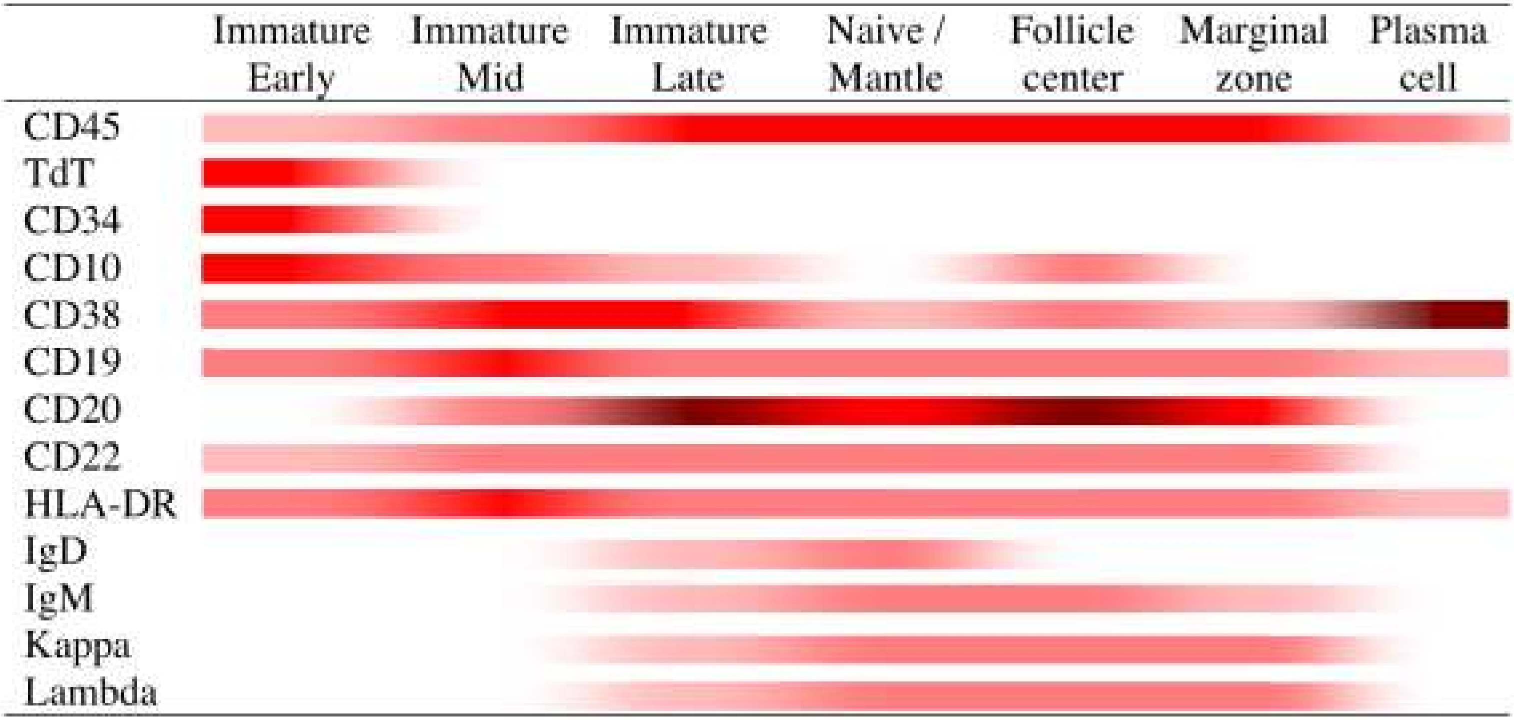
Methods for MRD Detection

- Antigenic abnormalities
  - Alterations in protein expression
  - Integrates effect of molecular abnormalities
  - Flow cytometry commonly used
- Molecular mutations
  - Specific molecular lesions
    - No single target
  - Clonality assessment (IgH, TCR)
  - PCR or Next generation sequencing

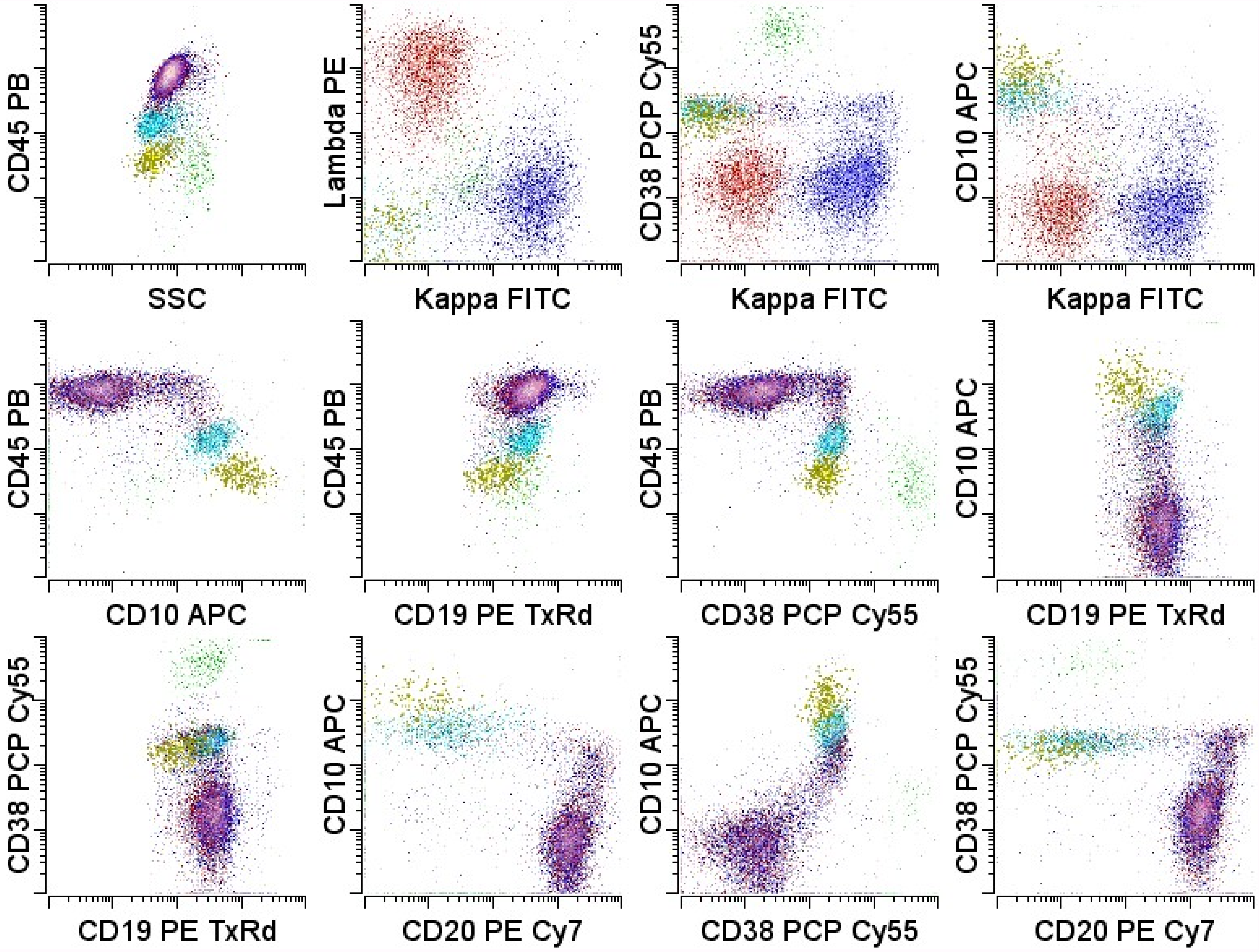
Abnormal population identification

- Normal
  - Antigens expressed in consistent and reproducible patterns with maturation
- Neoplastic
  - Increased or decreased normal antigens
  - Asynchronous maturational expression
  - Aberrant antigen expression
  - Homogeneous expression

Normal B cell Maturation



Wood and Borowitz (2006) Henry's Laboratory Medicine



Wood, Methods in Cell Biology, 2004

ALL Informative Antigens

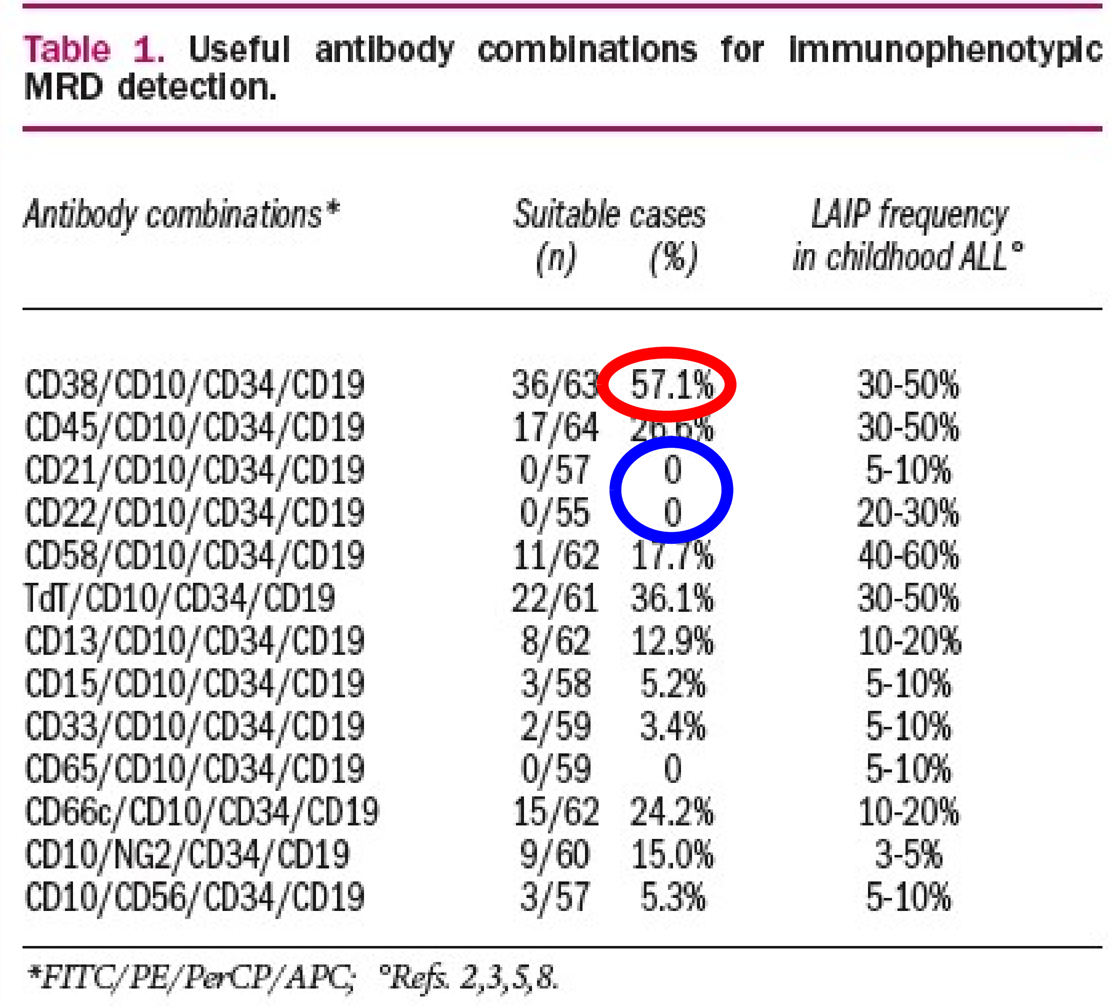


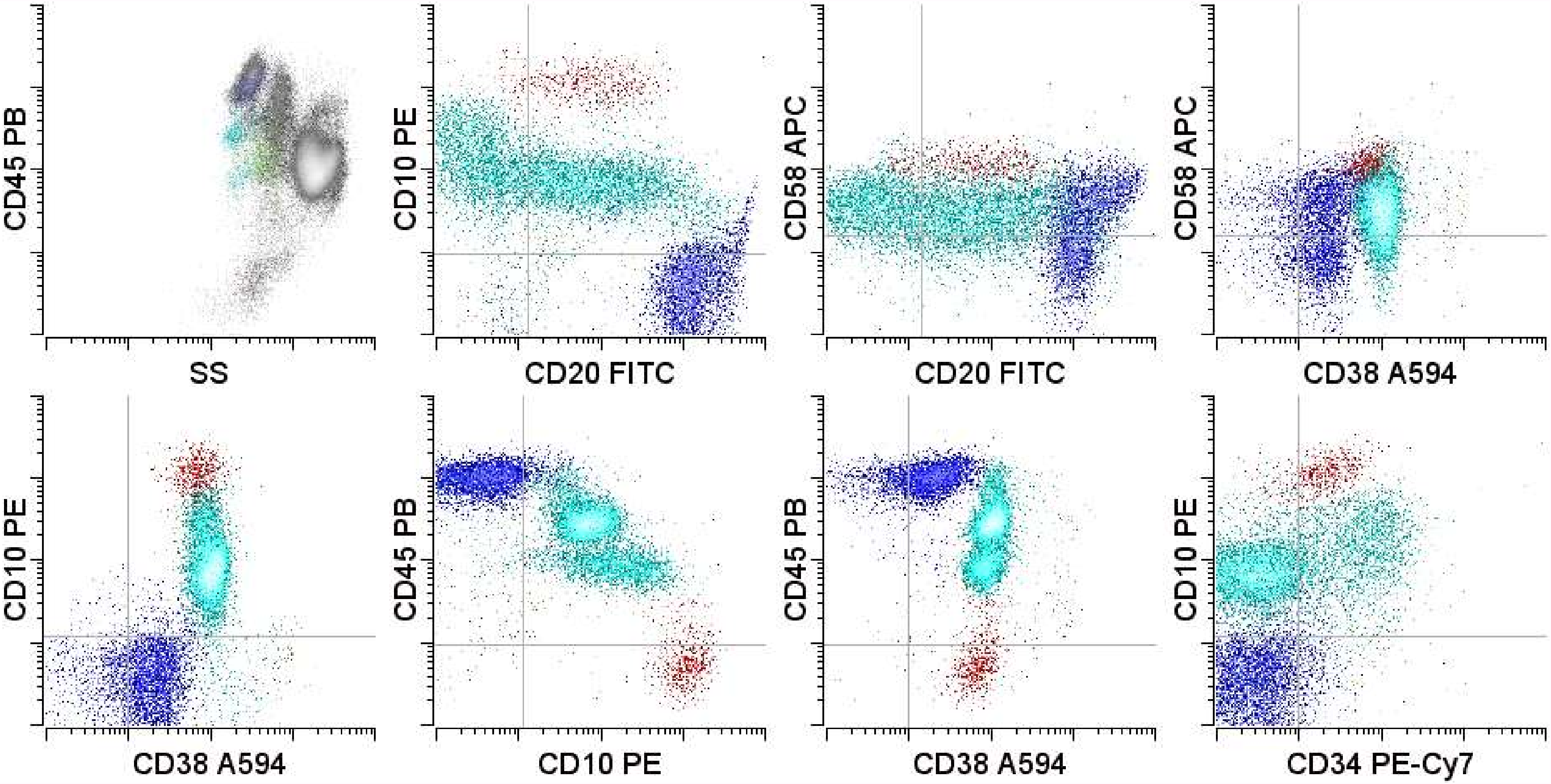
Table 1. Useful antibody combinations for immunophenotypic MRD detection.

*Antibody combinations**	*Suitable cases (n)*	*(%)*	*LAIP frequency in childhood ALL°*
CD38/CD10/CD34/CD19	36/63	57.1%	30-50%
CD45/CD10/CD34/CD19	17/64	26.6%	30-50%
CD21/CD10/CD34/CD19	0/57	0	5-10%
CD22/CD10/CD34/CD19	0/55	0	20-30%
CD58/CD10/CD34/CD19	11/62	17.7%	40-60%
TdT/CD10/CD34/CD19	22/61	36.1%	30-50%
CD13/CD10/CD34/CD19	8/62	12.9%	10-20%
CD15/CD10/CD34/CD19	3/58	5.2%	5-10%
CD33/CD10/CD34/CD19	2/59	3.4%	5-10%
CD65/CD10/CD34/CD19	0/59	0	5-10%
CD66c/CD10/CD34/CD19	15/62	24.2%	10-20%
CD10/NG2/CD34/CD19	9/60	15.0%	3-5%
CD10/CD56/CD34/CD19	3/57	5.3%	5-10%

**FITC/PE/PerCP/APC; °Refs. 2,3,5,8.*

From Krampera et al (2006) Haematologica 91:1109-1112

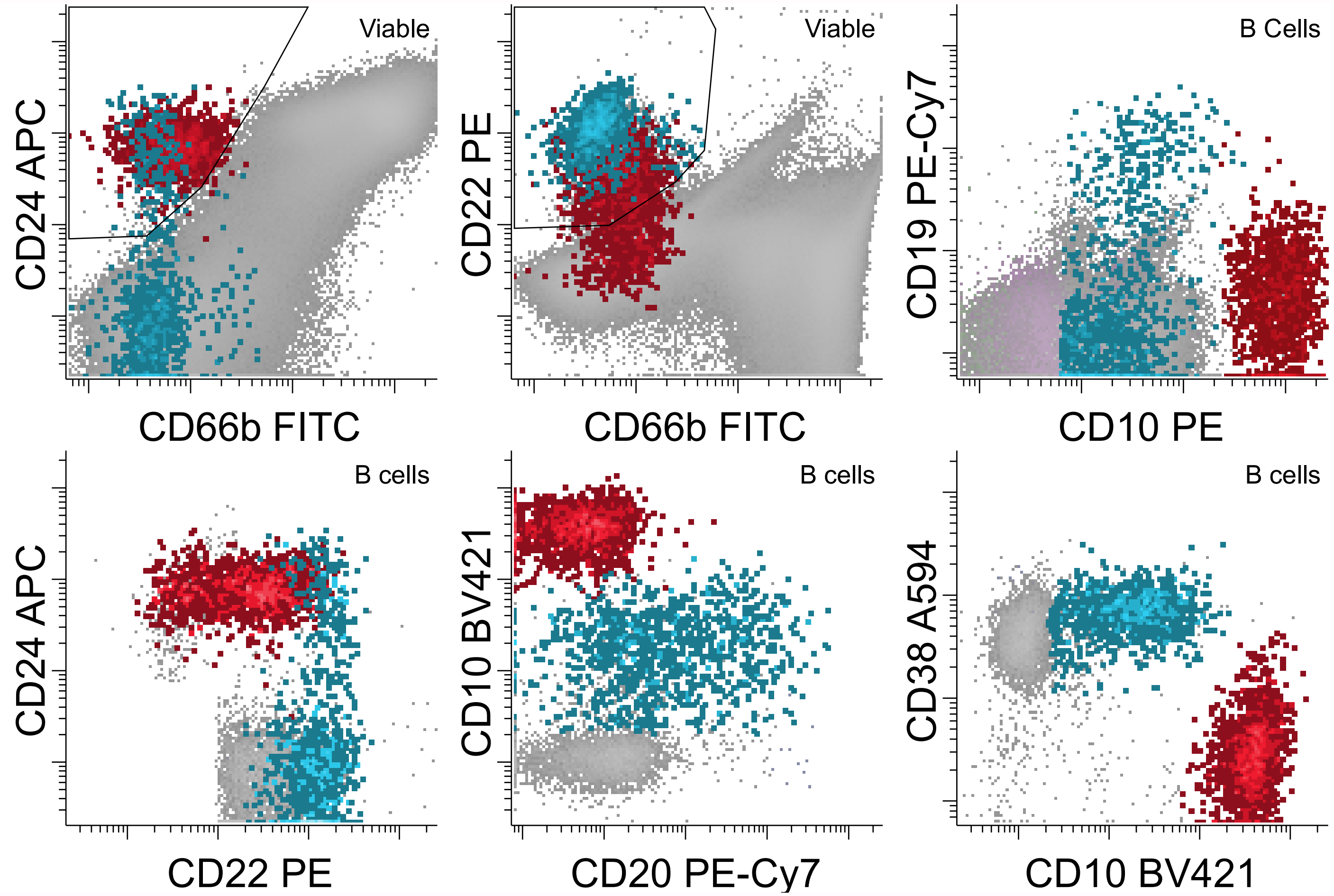
ALL MRD



0.1% abnormal immature B cells

Wood BL (2016) Cytometry B Clin Cytom. 90(1):47-53

Anti-CD19 Immunotherapy



CD19-negative MRD with background hematogones

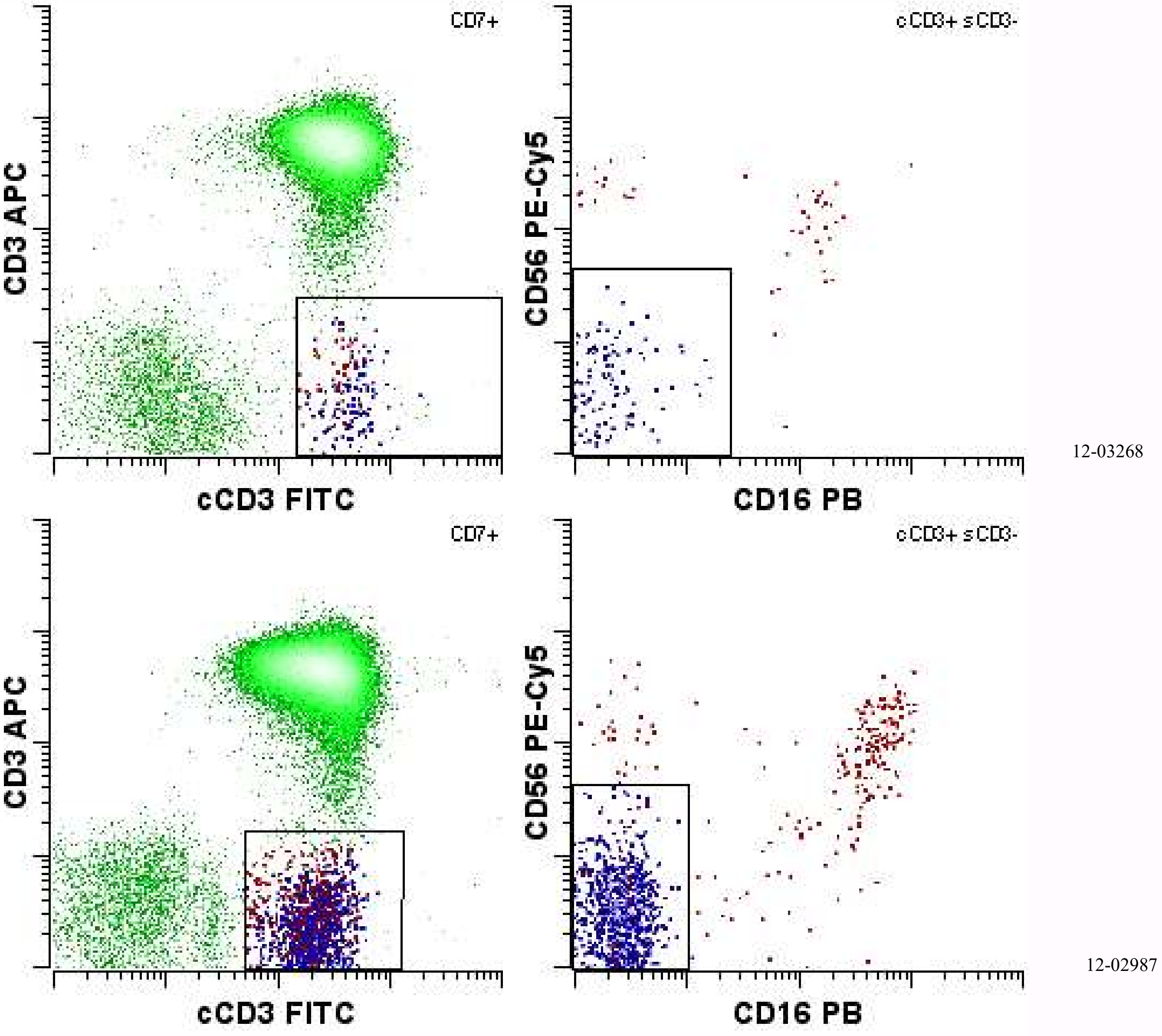
Cherian, et al (2016) Clin Cyto Part B

HP15-16377

T-ALL MRD

Case 1

Case 2



COG MRD Panels

- B lineage ALL

	FITC	**PE**	**PerCP-Cy5.5**	**PE-Cy7**	**APC**	**APC-H7**
Tube 1	CD20	CD10	CD38	CD19	CD58	CD45
Tube 2	CD9	CD13/33	CD34	CD19	CD10	CD45

Borowitz, Wood, et al (2015) Blood 126(8):964-71

- T lineage ALL

	PB	FITC	PE	PE-TR	PE-Cy5	PE-Cy7	A594	APC	APC-H7
Tube 1	CD16	cCD3	CD7		CD56	CD5	CD38	CD3	CD45
Tube 2	CD8 BV421	CD48	CD5	CD34	CD16+56	CD3	CD4	CD7	CD45

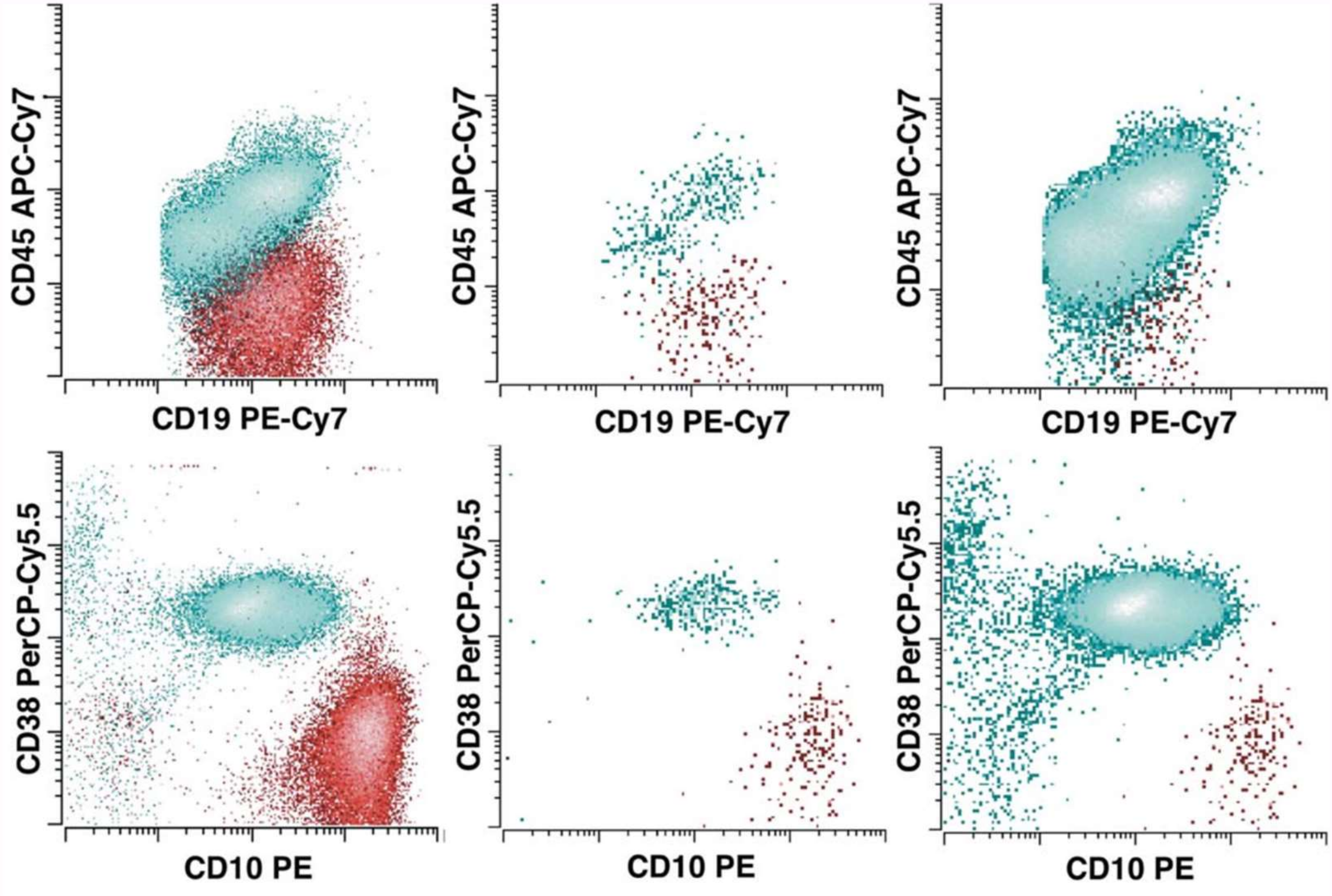
Wood BL (2013) Methods Mol Biol 999:123-136

Analytical Sensitivity

- Determinants
  - Degree of immunophenotypic aberrancy
  - Immunophenotype of background populations

- Identification vs. Enumeration
  - Number of events acquired
    - ~10 events required to identify a population
    - CV ~ Sqrt (N)/N
      - 100 events gives CV of 10%
    - Sensitivity of 0.01% requires 1,000,000 events
    - Noise can limit

Analytical Sensitivity



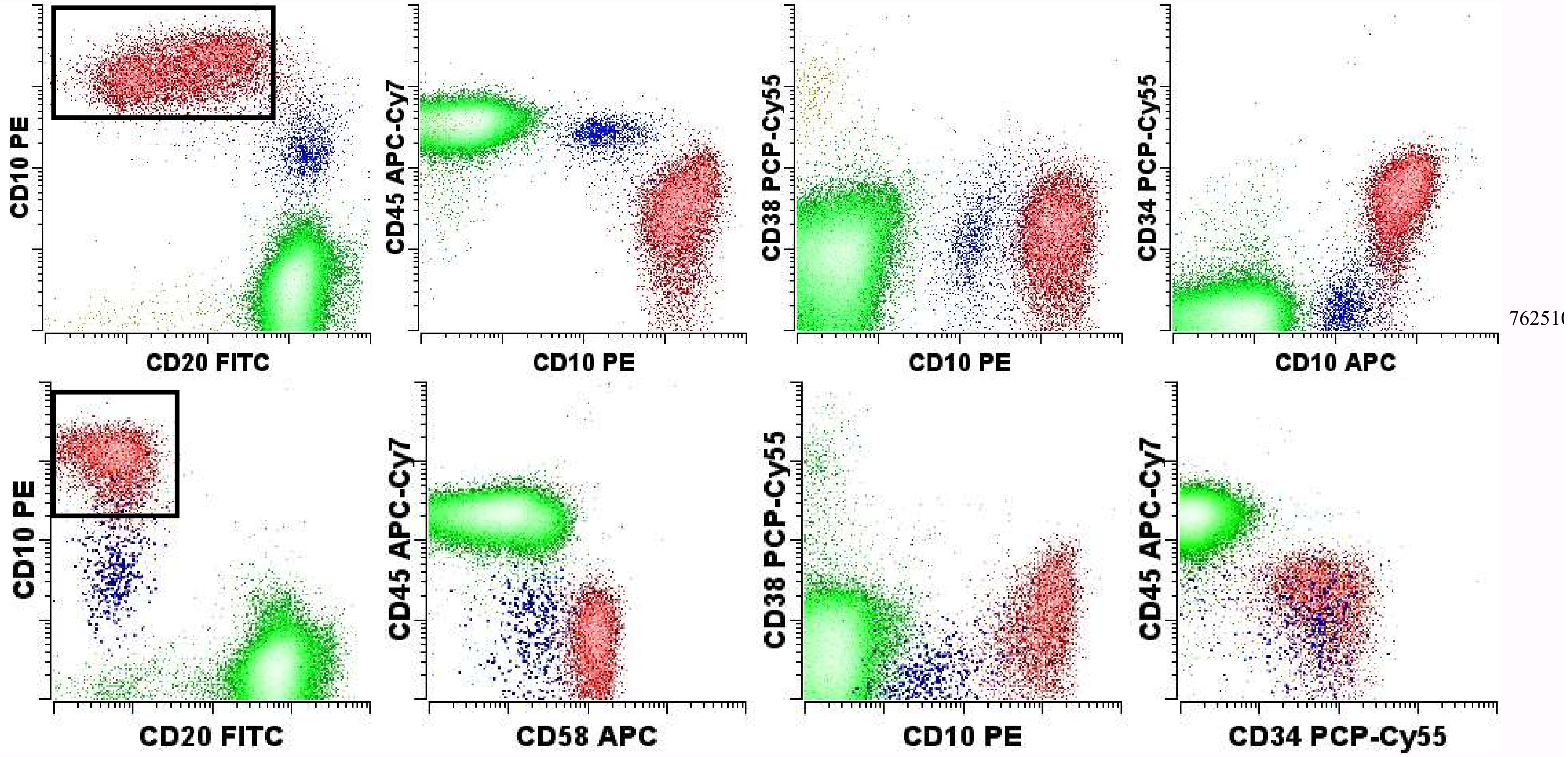
Wood BL (2016) Cytometry B Clin Cytom. 90(1):47-53

MRD Identification

- Pre-treatment
  - Identify abnormal immunophenotypic features
- Post-treatment
  - Identify population different from normal
    - “Difference from Normal”
      - Discrete population defined by multiparametric gating
      - Use pre-treatment immunophenotype as starting point
    - “Leukemia-Associated Immunophenotype (LAIP)”
      - Enumerate events in predefined gate determined pretreatment
      - Assumes stability of leukemia and background populations
      - Evaluate multiple LAIP when possible
  - Harmonize by allowing flexibility in gating

Immunophenotypic Stability

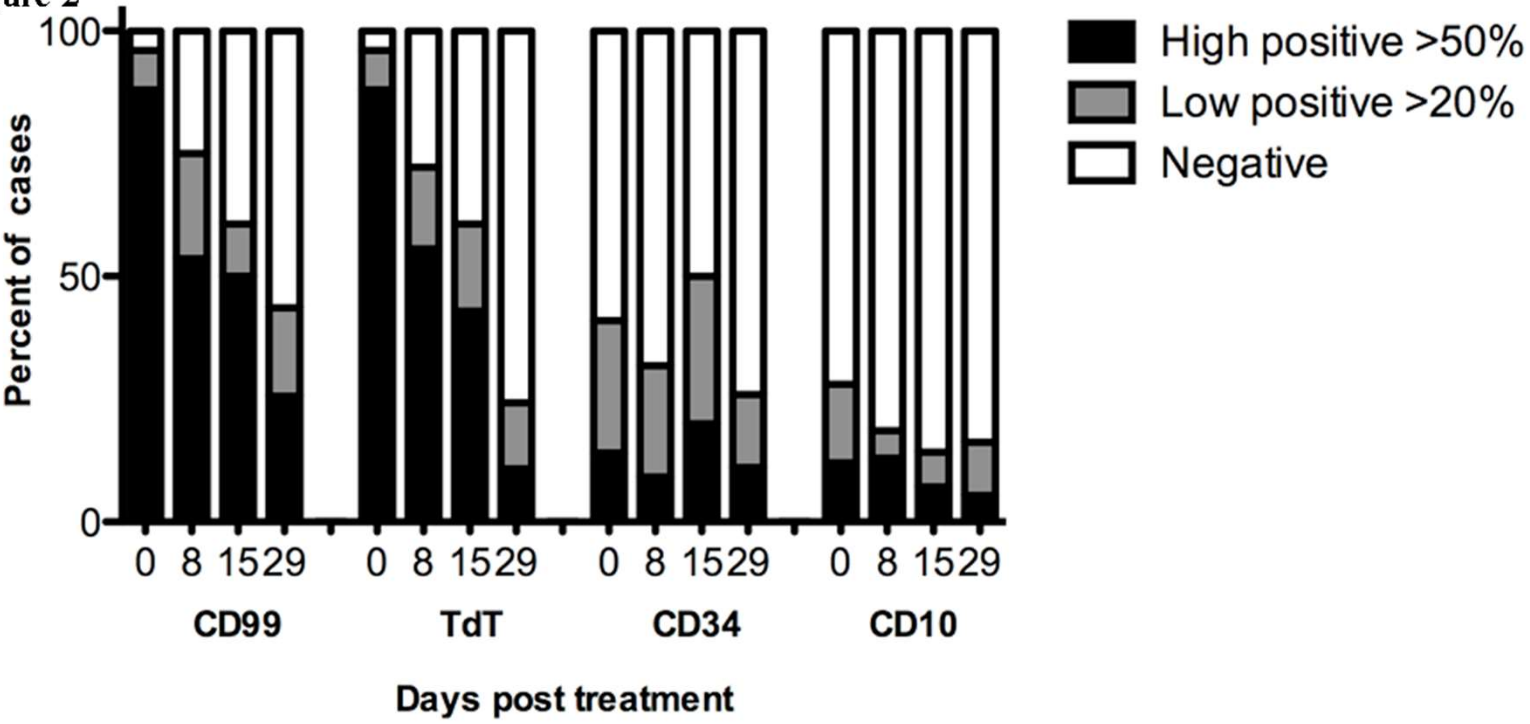
- ALL



Wood BL (2016) Cytometry B Clin Cytom. 90(1):47-53

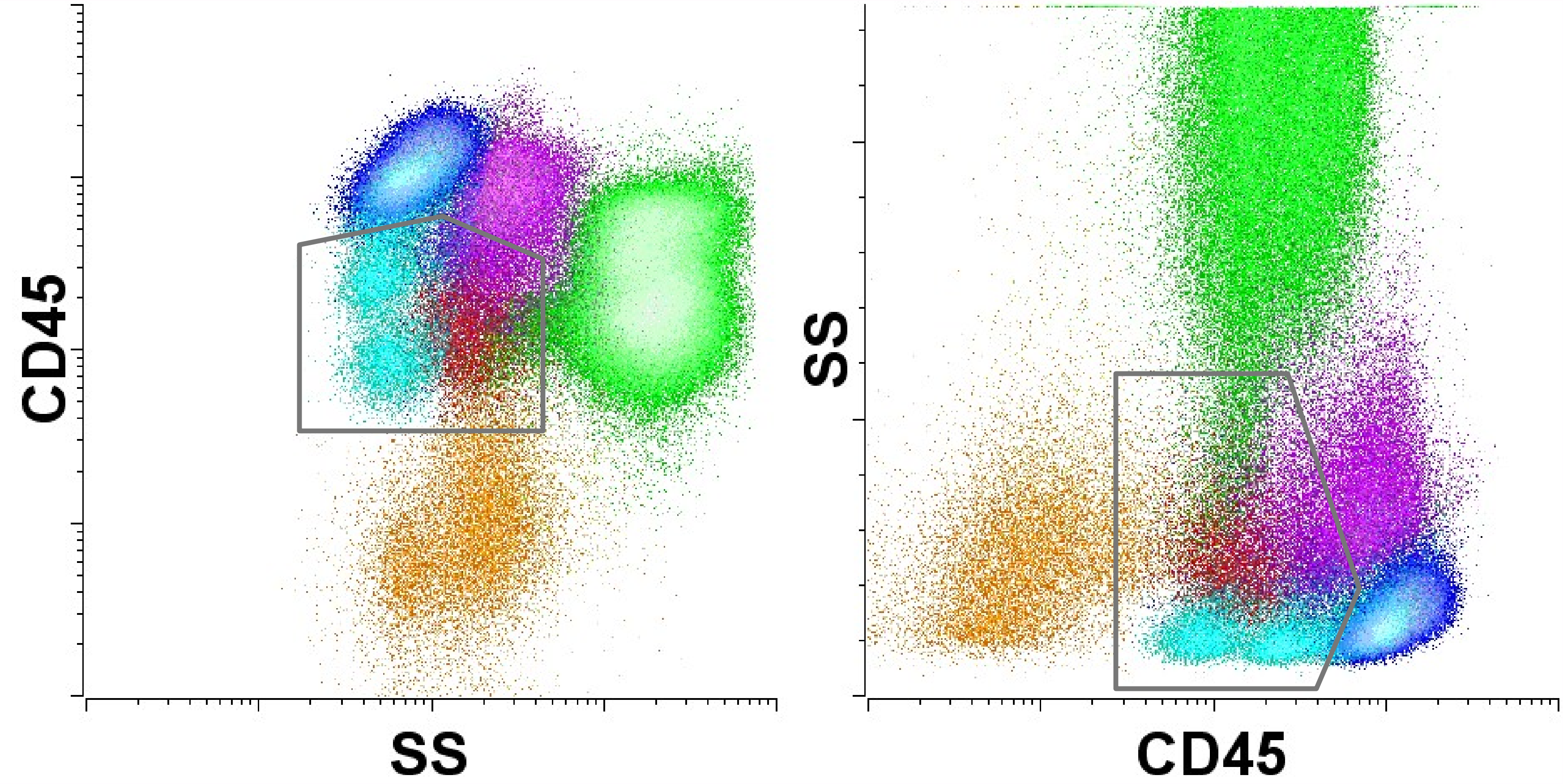
Immunophenotypic Stability
T-ALL

Figure 2



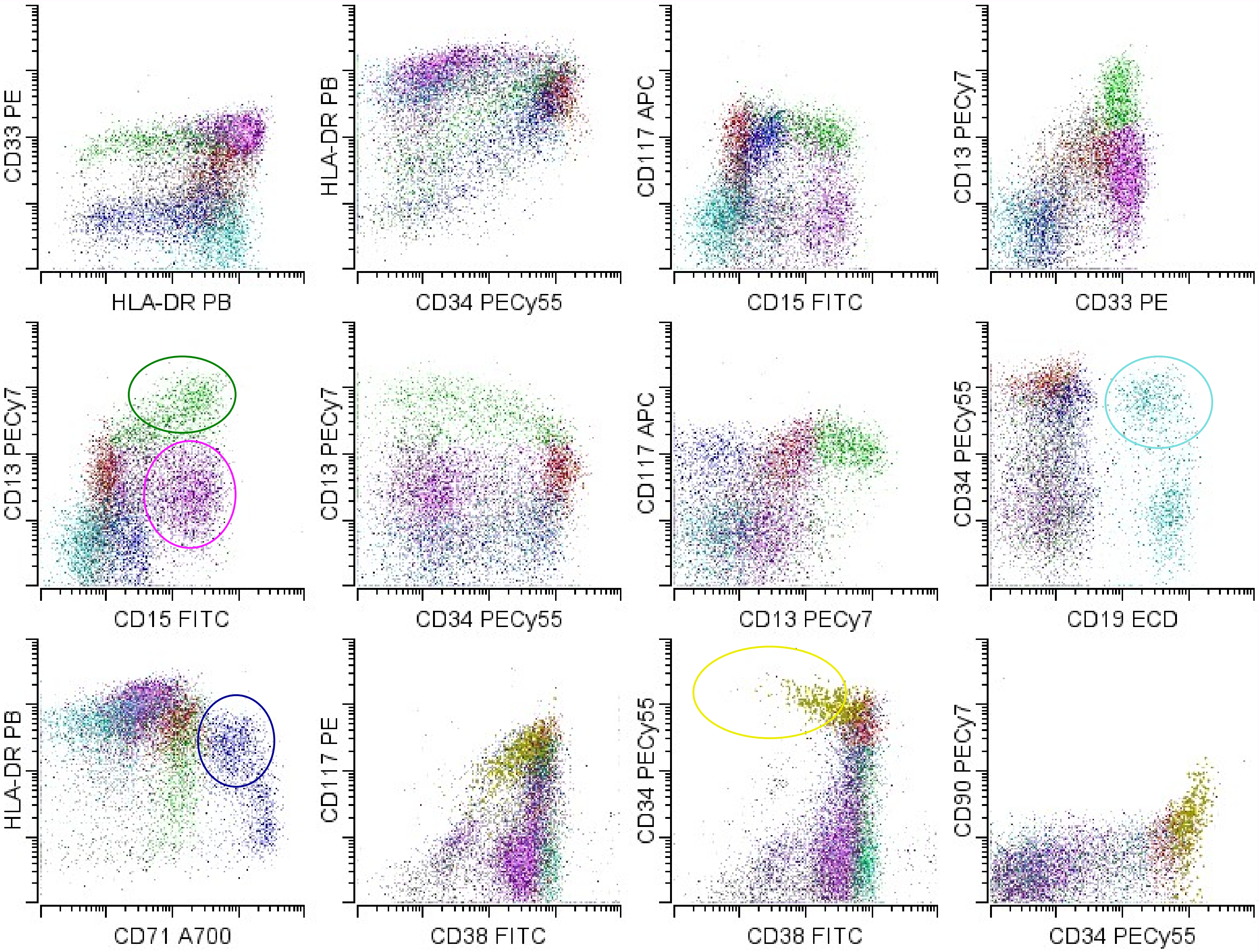
Roshal, et al (2010) Cytometry B 78:139-46

Cell Type Identification



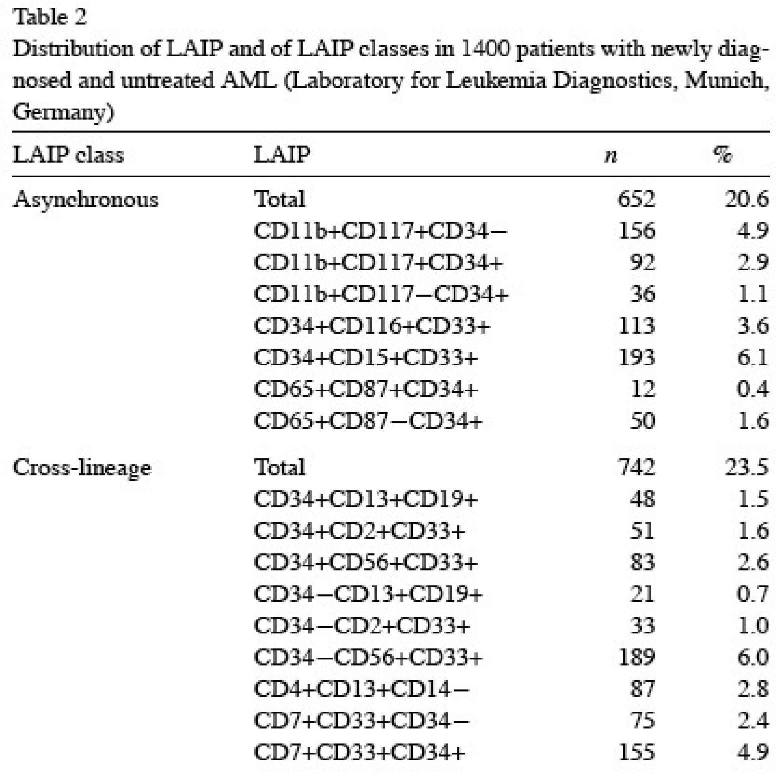
Borowitz et al (1993) AJCP 100:534-40.
Steltzer et al (1993) Ann NY Acad Sci 667:265-280

Normal Blast Maturation



Wood (2004) Methods Cell Biology 75:559-576

AML Informative Antigens



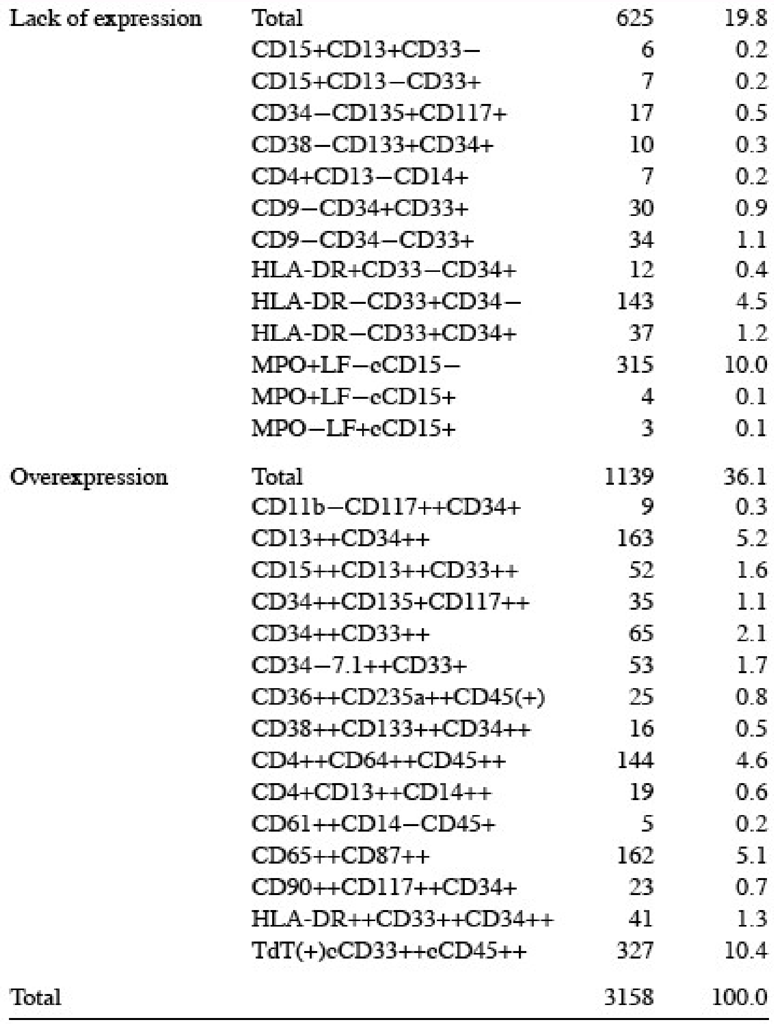


Table 2

Distribution of LAIP and of LAIP classes in 1400 patients with newly diagnosed and untreated AML (Laboratory for Leukemia Diagnostics, Munich, Germany)

LAIP class	LAIP	*n*	%
Asynchronous	Total	652	20.6
	CD11b+CD117+CD34−	156	4.9
	CD11b+CD117+CD34+	92	2.9
	CD11b+CD117−CD34+	36	1.1
	CD34+CD116+CD33+	113	3.6
	CD34+CD15+CD33+	193	6.1
	CD65+CD87+CD34+	12	0.4
	CD65+CD87−CD34+	50	1.6
Cross-lineage	Total	742	23.5
	CD34+CD13+CD19+	48	1.5
	CD34+CD2+CD33+	51	1.6
	CD34+CD56+CD33+	83	2.6
	CD34−CD13+CD19+	21	0.7
	CD34−CD2+CD33+	33	1.0
	CD34−CD56+CD33+	189	6.0
	CD4+CD13+CD14−	87	2.8
	CD7+CD33+CD34−	75	2.4
	CD7+CD33+CD34+	155	4.9
Lack of expression	Total	625	19.8
	CD15+CD13+CD33−	6	0.2
	CD15+CD13−CD33+	7	0.2
	CD34−CD135+CD117+	17	0.5
	CD38−CD133+CD34+	10	0.3
	CD4+CD13−CD14+	7	0.2
	CD9−CD34+CD33+	30	0.9
	CD9−CD34−CD33+	34	1.1
	HLA-DR+CD33−CD34+	12	0.4
	HLA-DR−CD33+CD34−	143	4.5
	HLA-DR−CD33+CD34+	37	1.2
	MPO+LF−cCD15−	315	10.0
	MPO+LF−cCD15+	4	0.1
	MPO−LF+cCD15+	3	0.1
Overexpression	Total	1139	36.1
	CD11b−CD117++CD34+	9	0.3
	CD13++CD34++	163	5.2
	CD15++CD13++CD33++	52	1.6
	CD34++CD135+CD117++	35	1.1
	CD34++CD33++	65	2.1
	CD34−7.1++CD33+	53	1.7
	CD36++CD235a++CD45(+)	25	0.8
	CD38++CD133++CD34++	16	0.5
	CD4++CD64++CD45++	144	4.6
	CD4+CD13++CD14++	19	0.6
	CD61++CD14−CD45+	5	0.2
	CD65++CD87++	162	5.1
	CD90++CD117++CD34+	23	0.7
	HLA-DR++CD33++CD34++	41	1.3
	TdT(+)cCD33++cCD45++	327	10.4
Total		3158	100.0

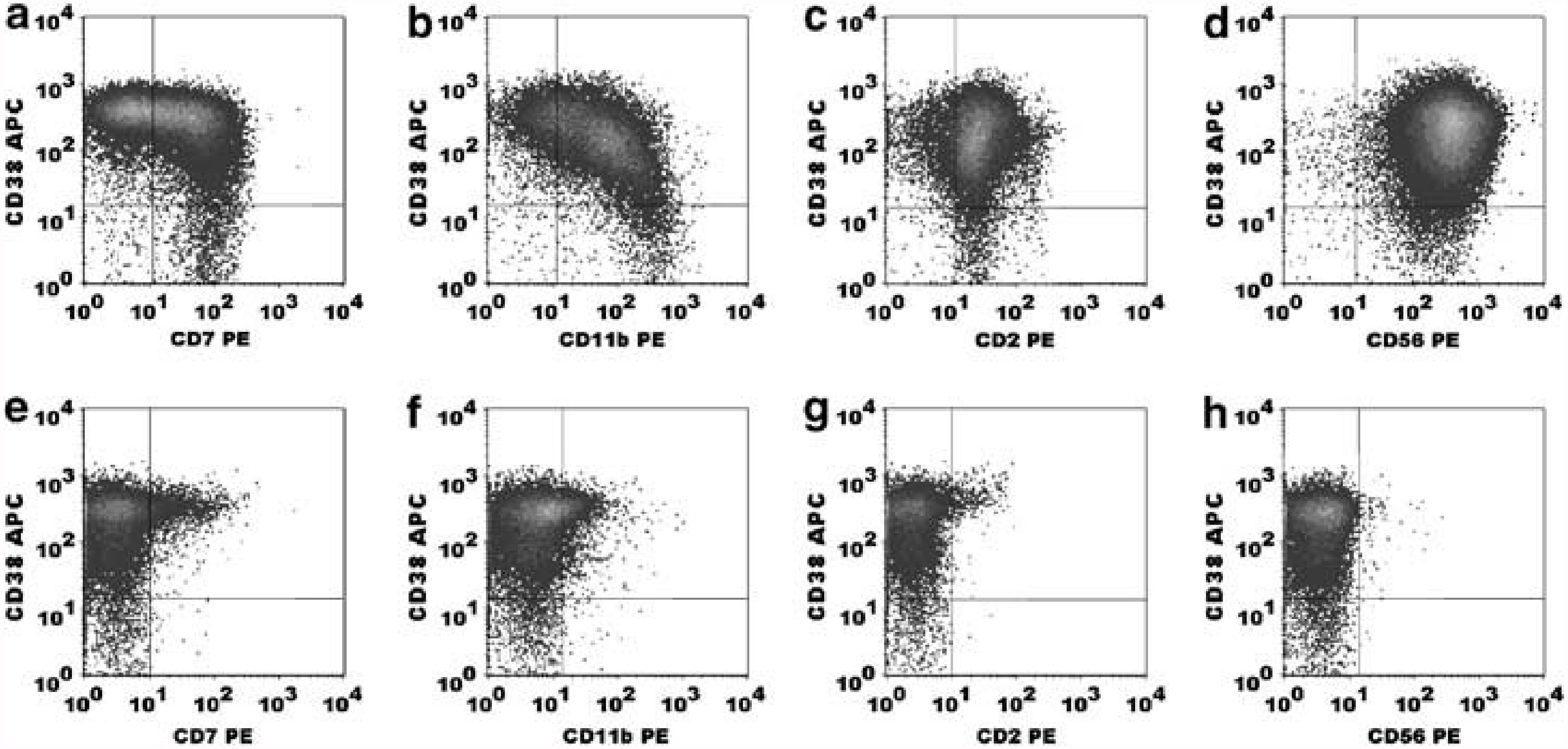
Average 2.3 LAIP per patient

From Kern et al (2005) CRC Rev Onc/Hem 56:283-309

AML MRD stem cells

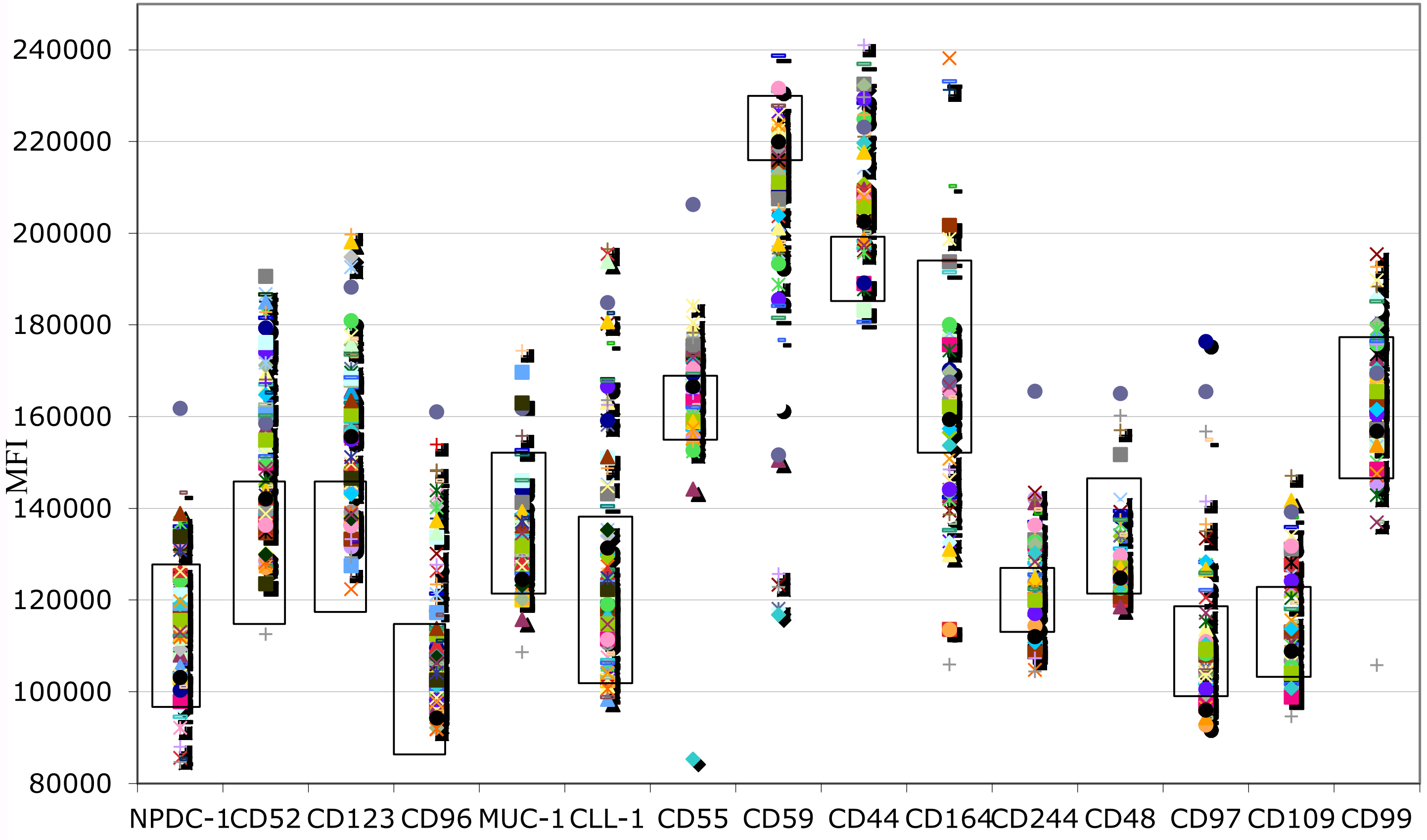
AML

Normal



van Rhenen, et al (2007) Leukemia 21:1700-7

Myeloid Stem Cell Disorders – CD34+ / CD38 Low



N = 87

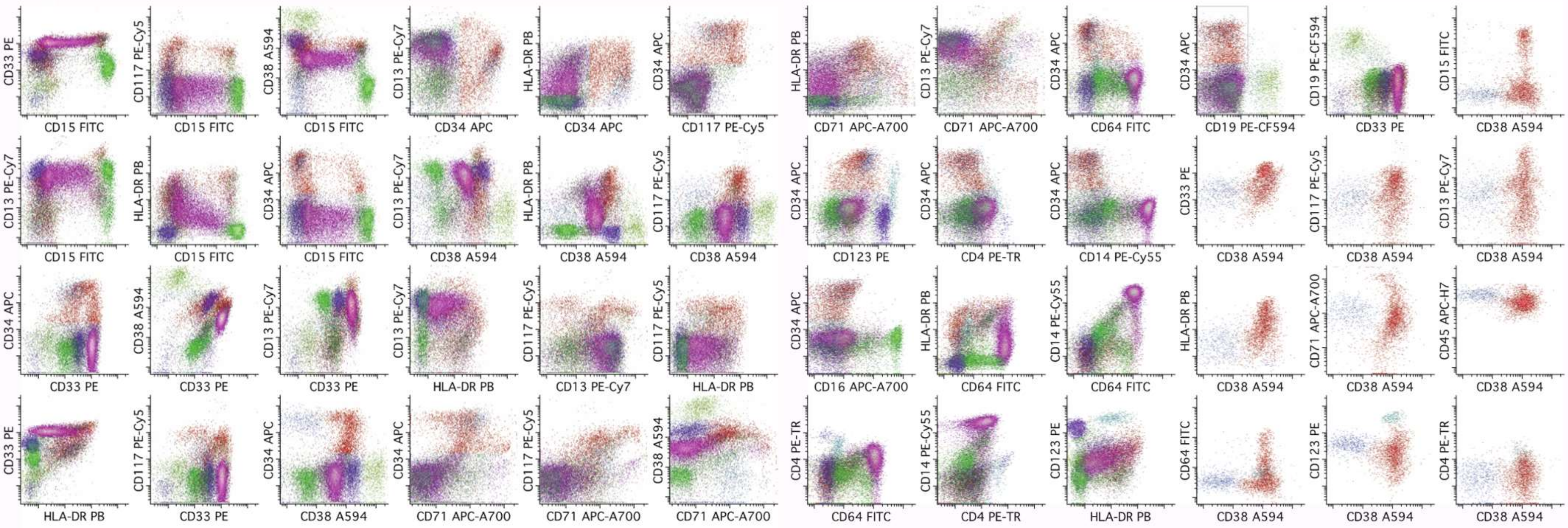
Wood, unpublished

AML MRD Panel

	PB	**FITC**	**PE**	**PE-TR**	**PE-CyX**	**PE-Cy7**	**A594**	**APC**	**APC-A700**	**APC-H7**
1	DR	15	33	19	117	13	38	34	71	45
2	DR	64	123	4	14	13	38	34	16	45
3	DR	56	7		5	33	38	34		45

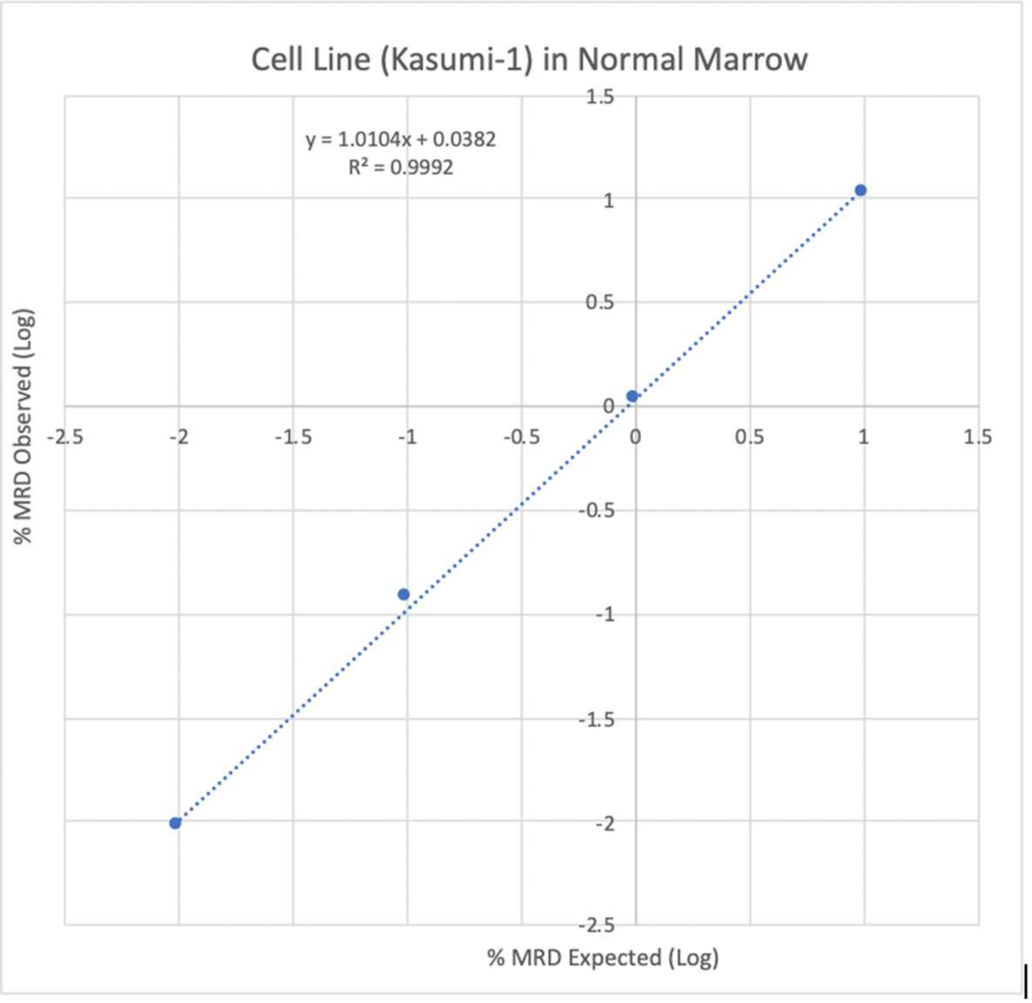
Wood BL (2013) Methods Mol Biol 999:123-136

AML MRD
Compare to Normal Maturation

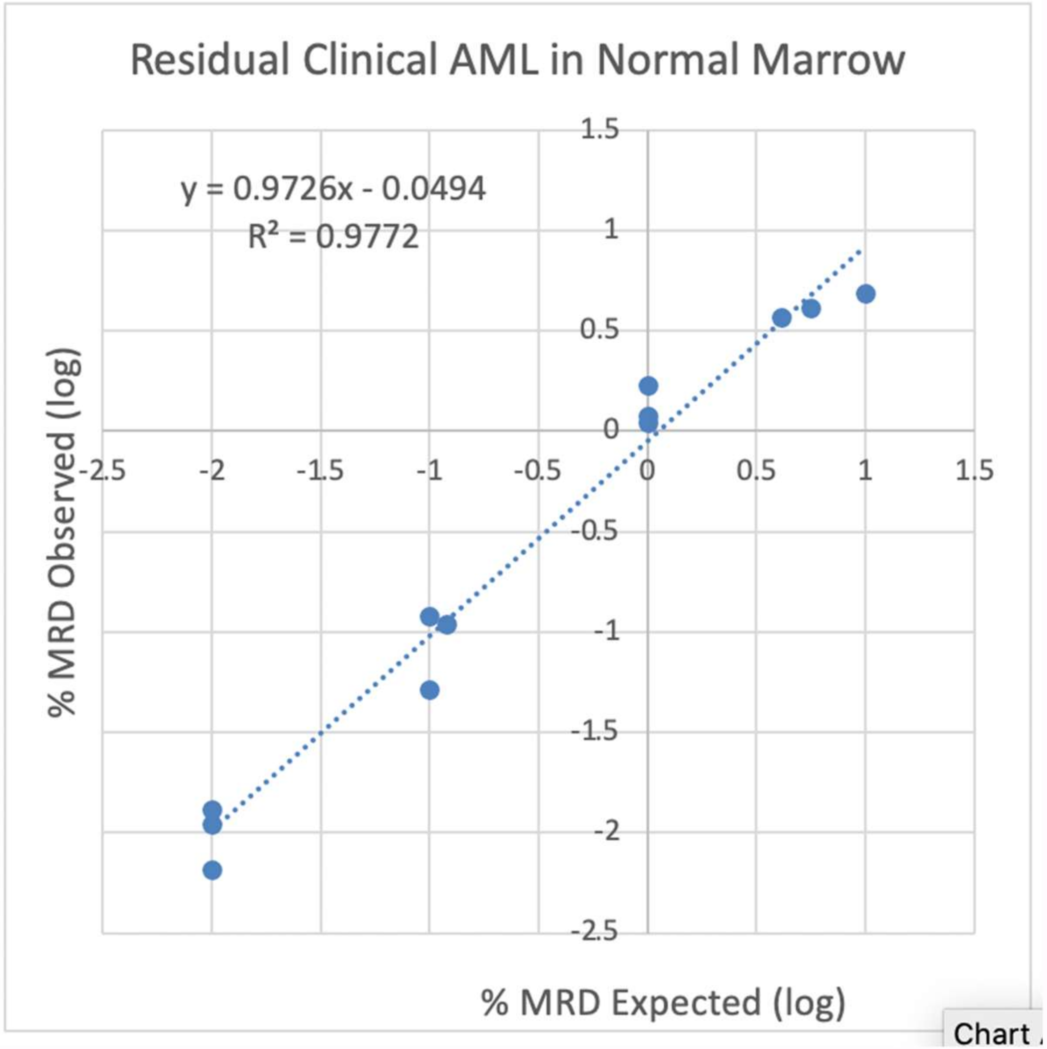


Wood BL (2020) Current Protocols in Cytometry, 93, e73.

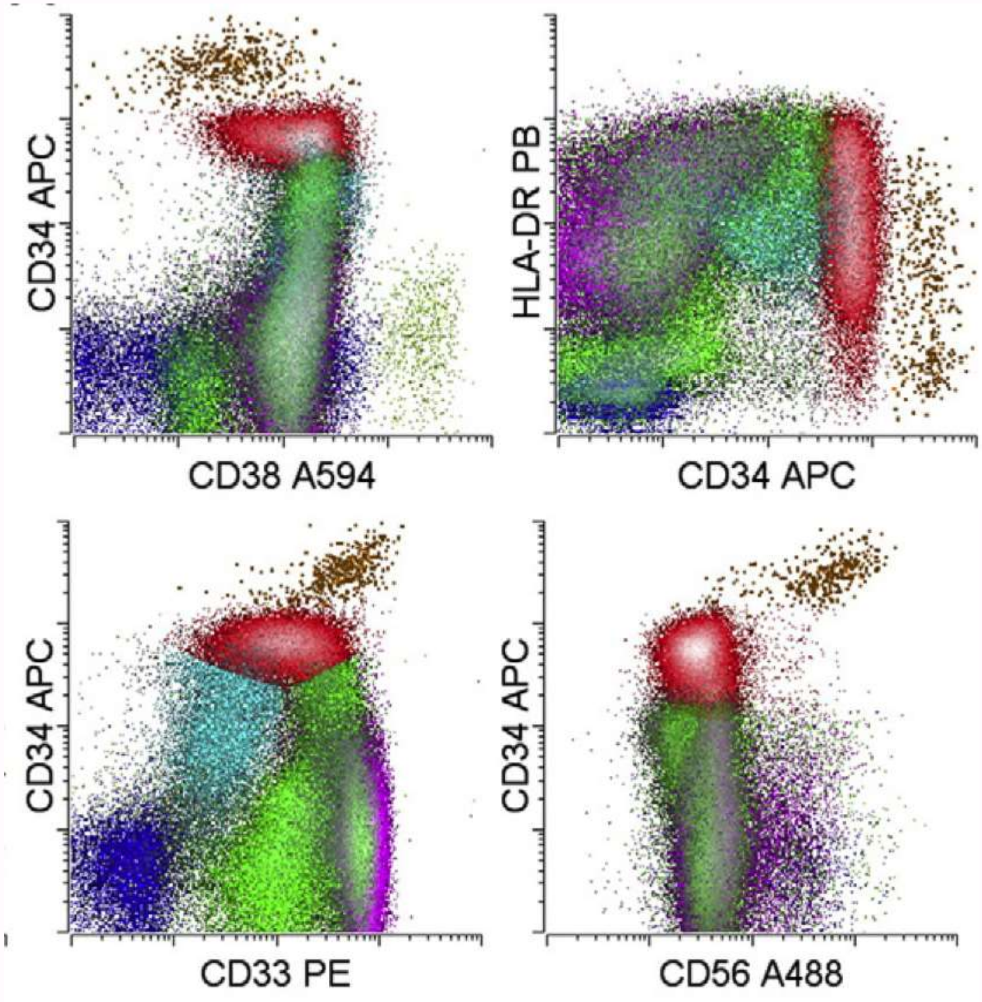
AML MRD Validation



AML MRD Validation

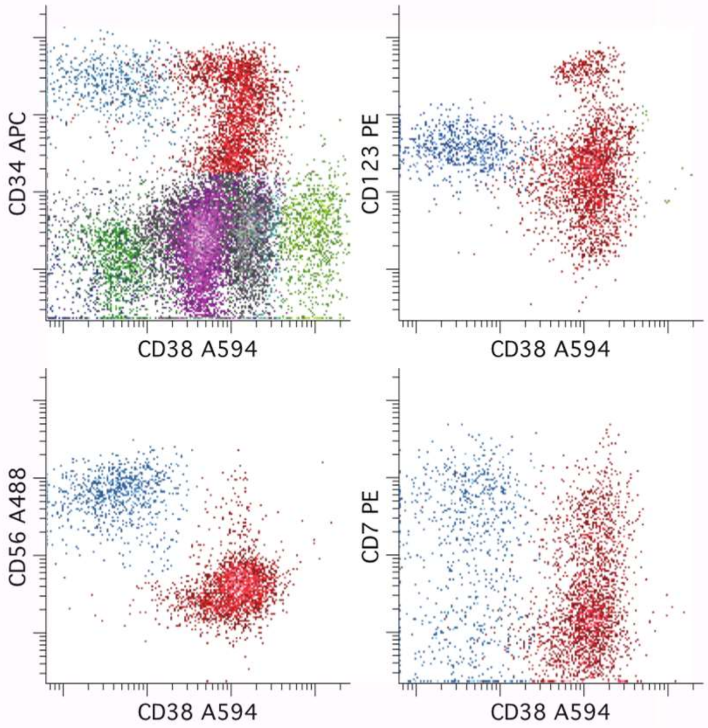


Residual Acute Myeloid Leukemia

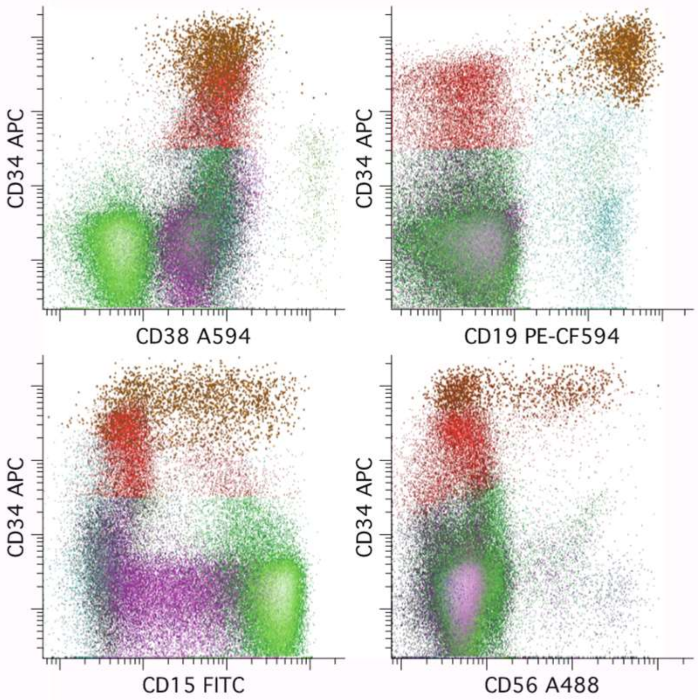


Chen & Wood (2016) Blood Reviews 31:63–75

AML MRD
Immunophenotypic Stereotypes



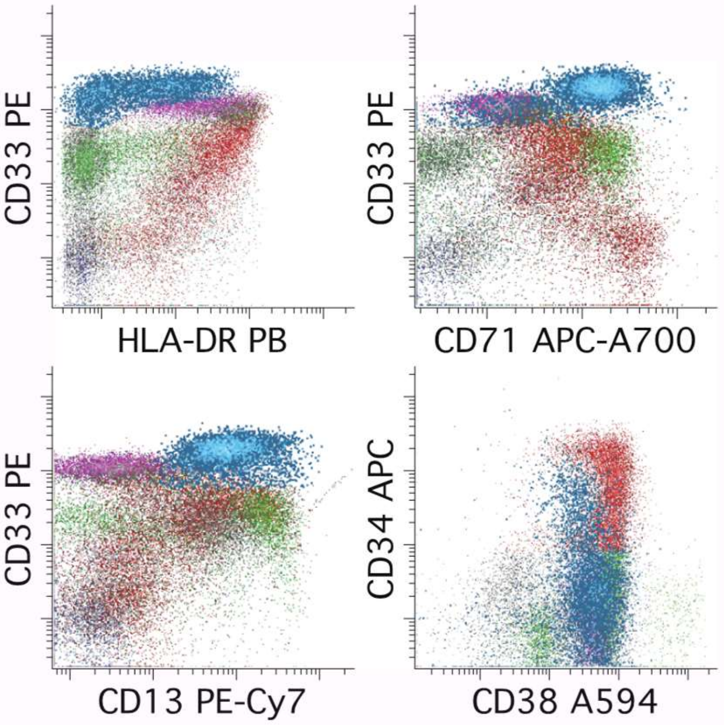
Stem cell



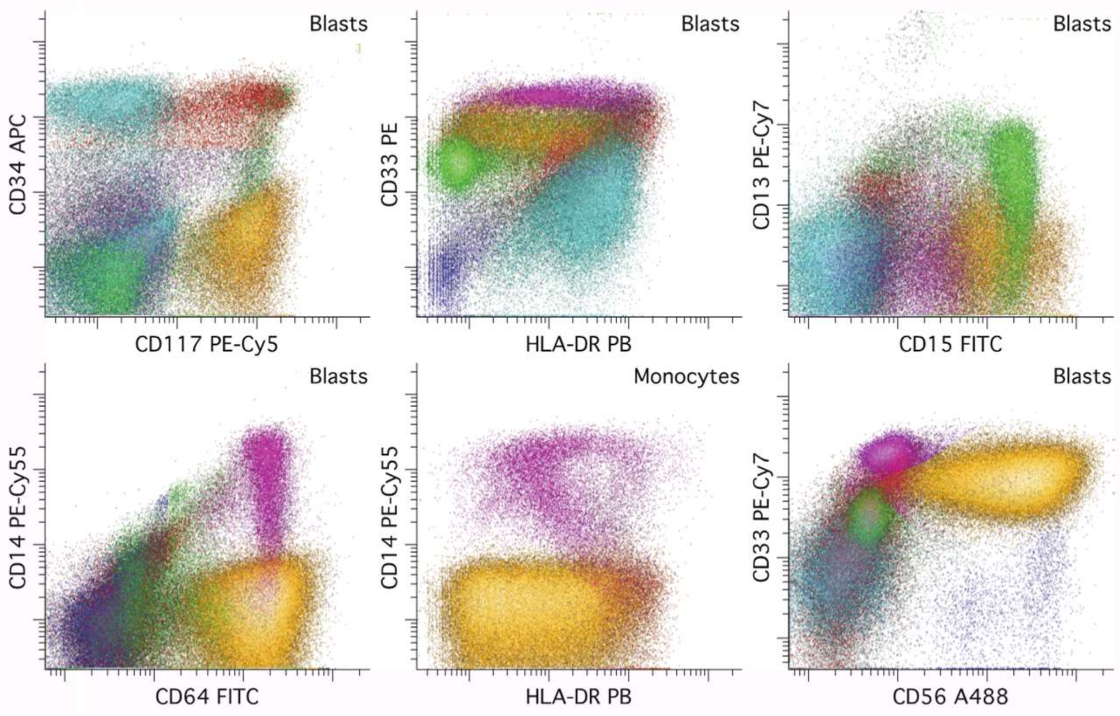
t(8;21)

Wood BL (2020) Current Protocols in Cytometry, 93, e73.

AML MRD
Immunophenotypic Stereotypes



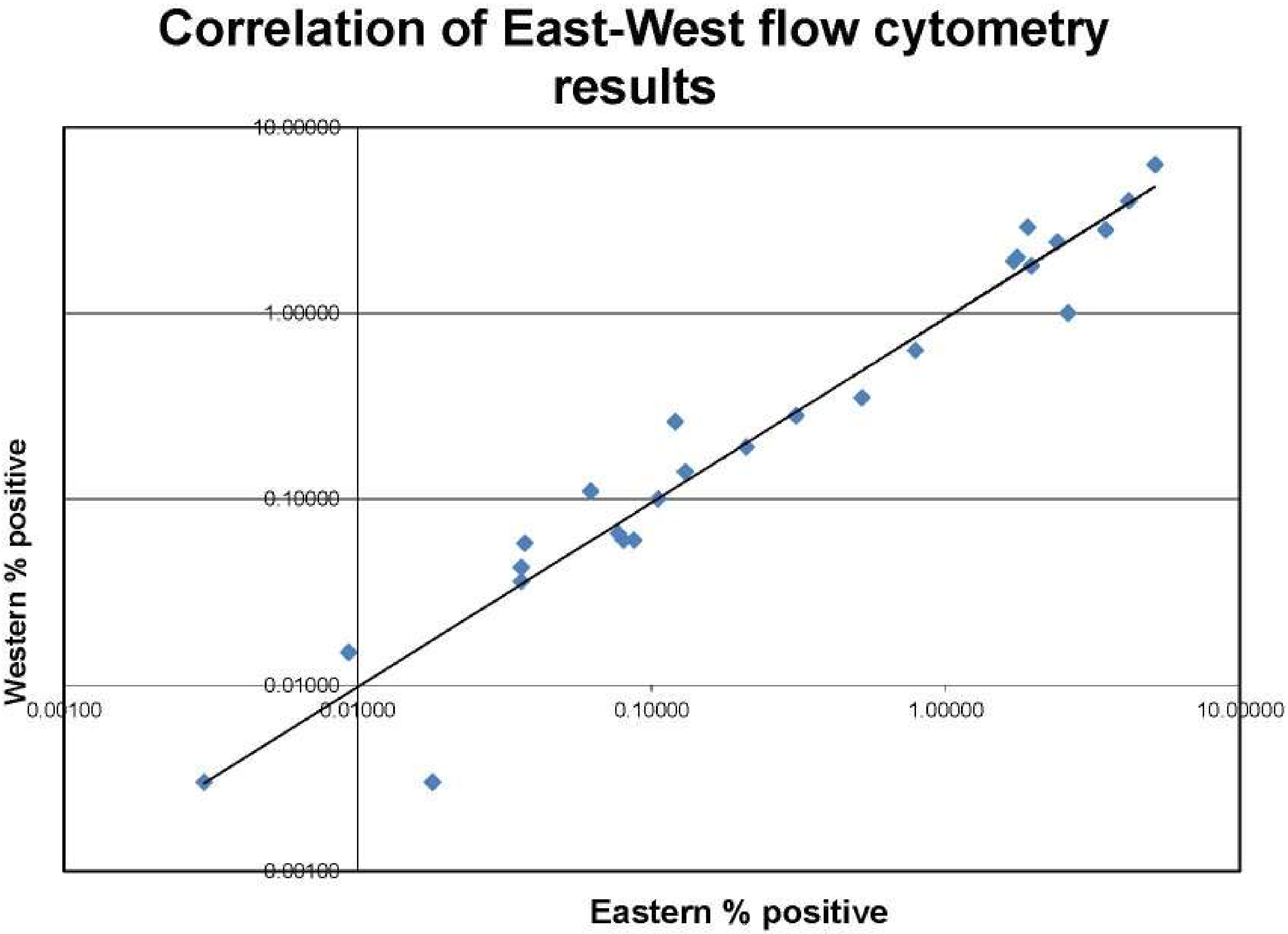
NPM1



Monocytic

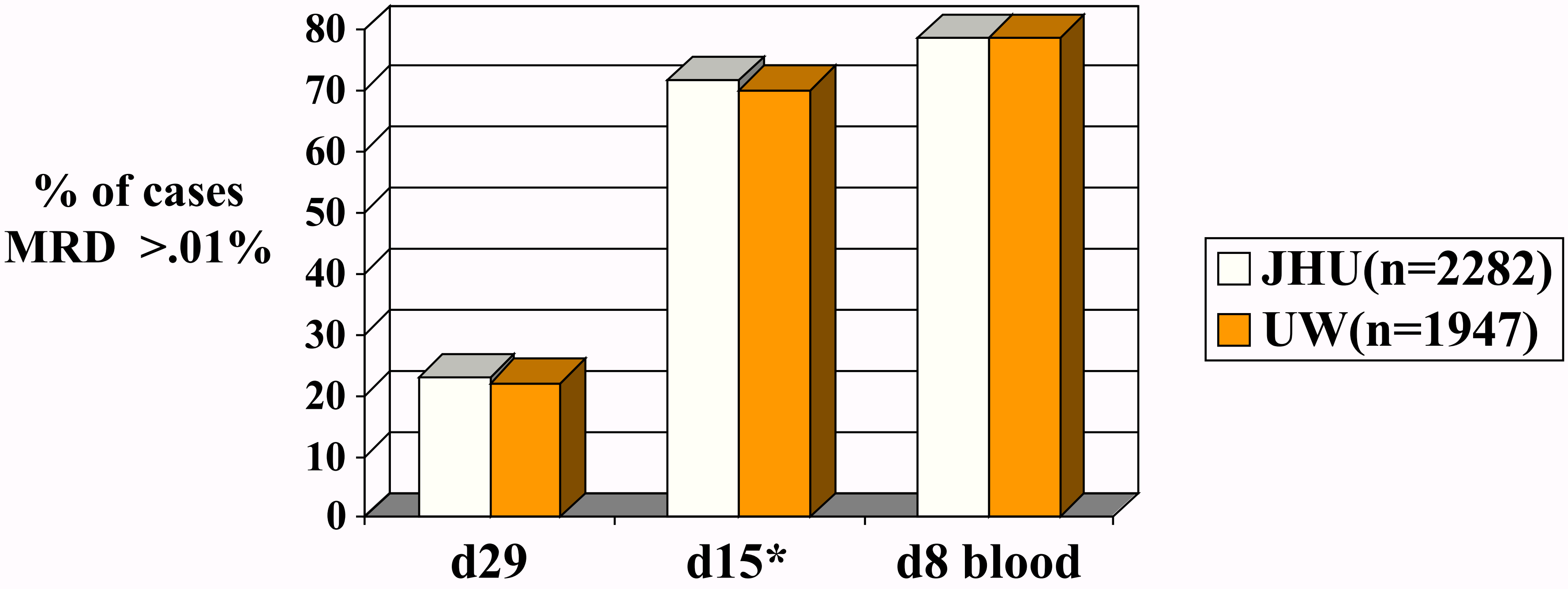
Wood BL (2020) Current Protocols in Cytometry, 93, e73.

Reproducibility



Borowitz, Wood, et al (2015) Blood 126(8):964-71

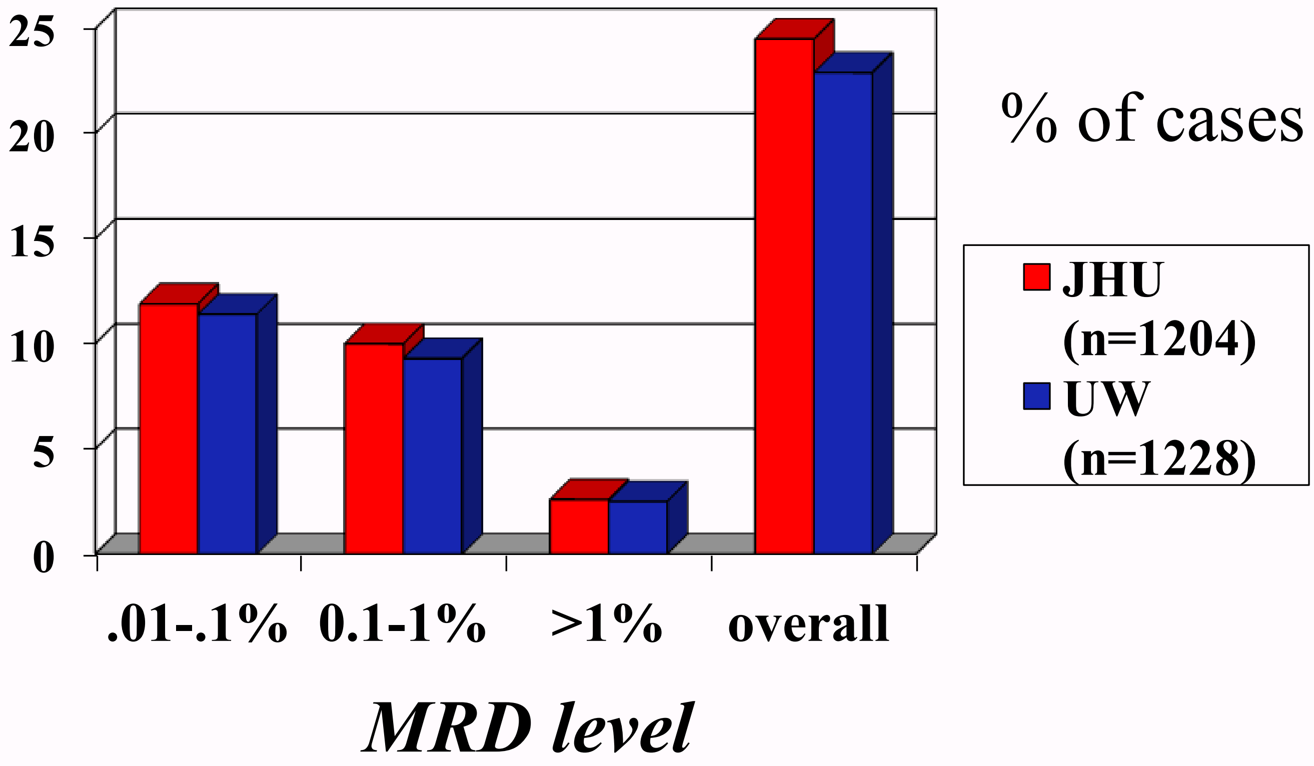
Flow MRD on AALL03B1



* day 8 M1 patients excluded

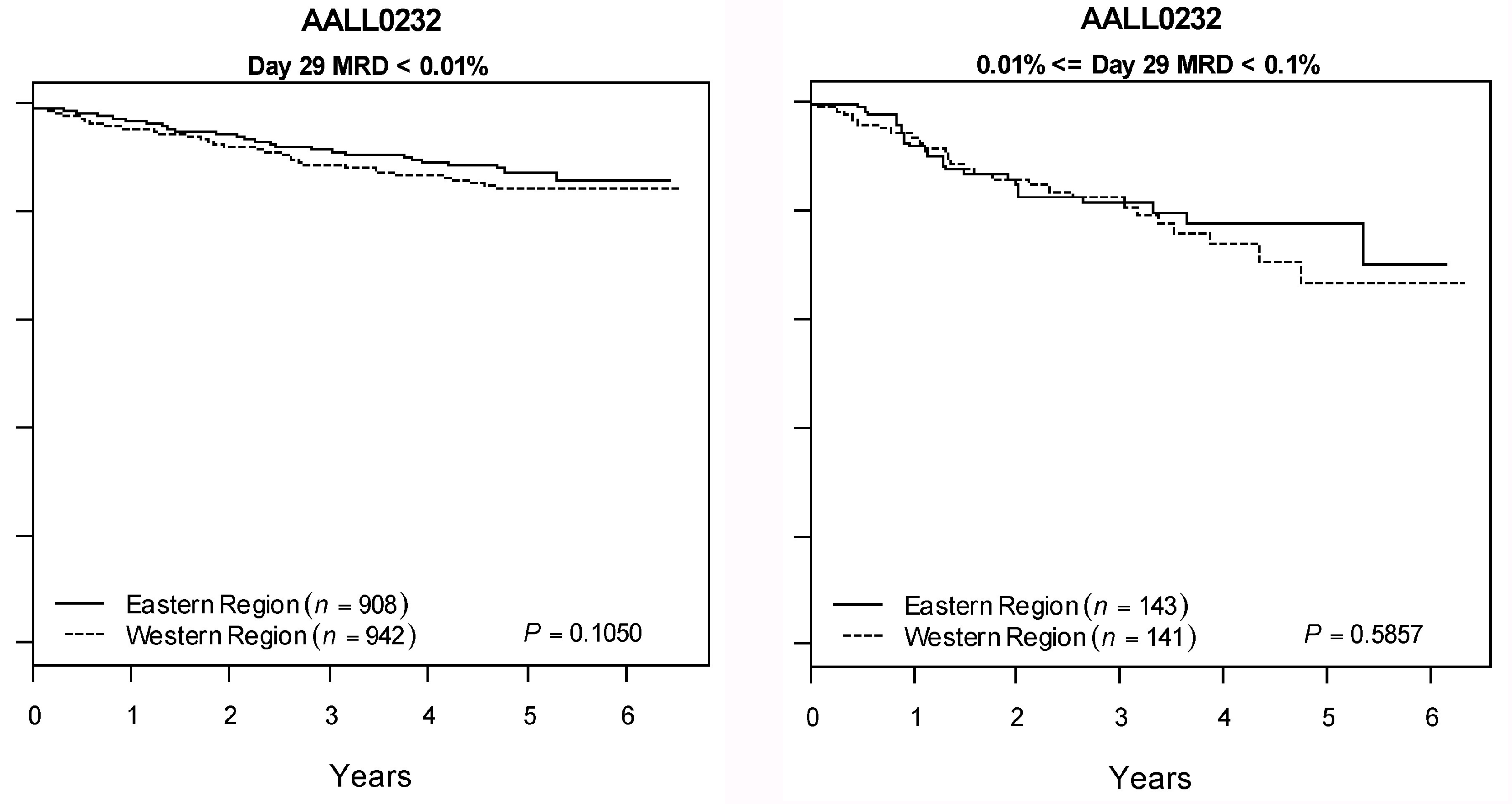
Unpublished data, courtesy Mike Borowitz

Day 29 Flow MRD on AALL0232



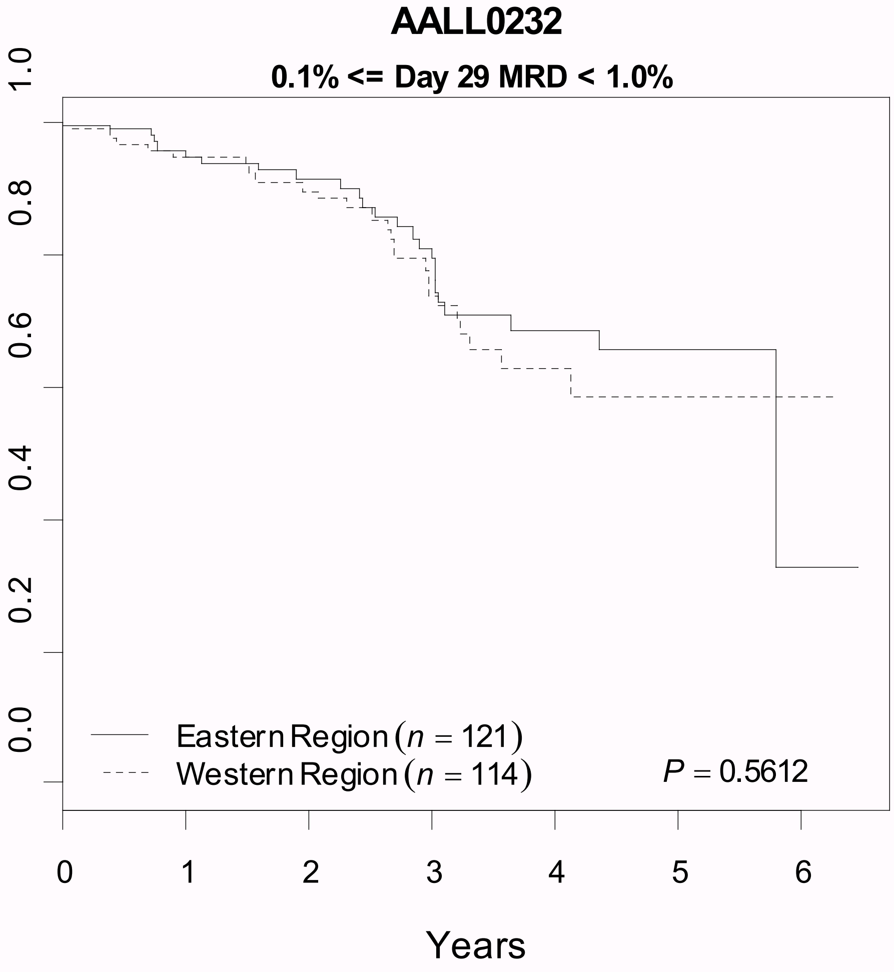
Borowitz, Wood, et al (2015) Blood 126(8):964-71

Correlation between labs



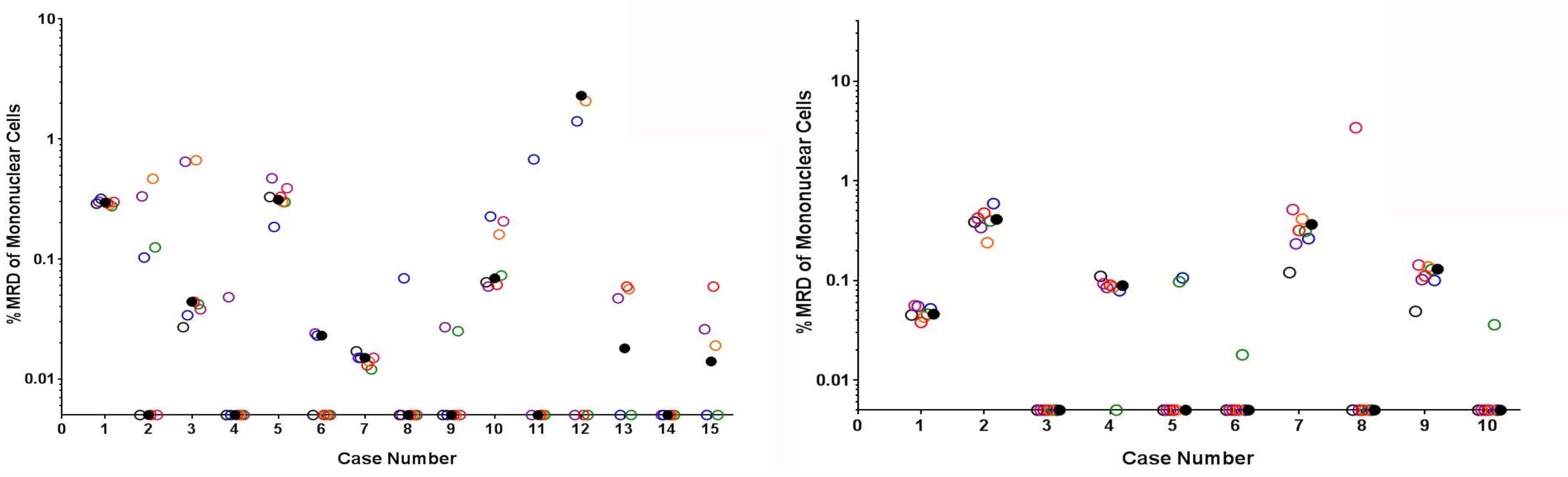
Borowitz, Wood, et al (2015) Blood 126(8):964-71

Correlation between labs



Borowitz, Wood, et al (2015) Blood 126(8):964-71

Interpretive Variability



First 3 rounds

Last 2 rounds

Experience with MRD Testing in B- ALL By Flow Cytometry Does Not Prevent Interpretative Discordance

Keeney, Wood et al. (2017) Cytometry B

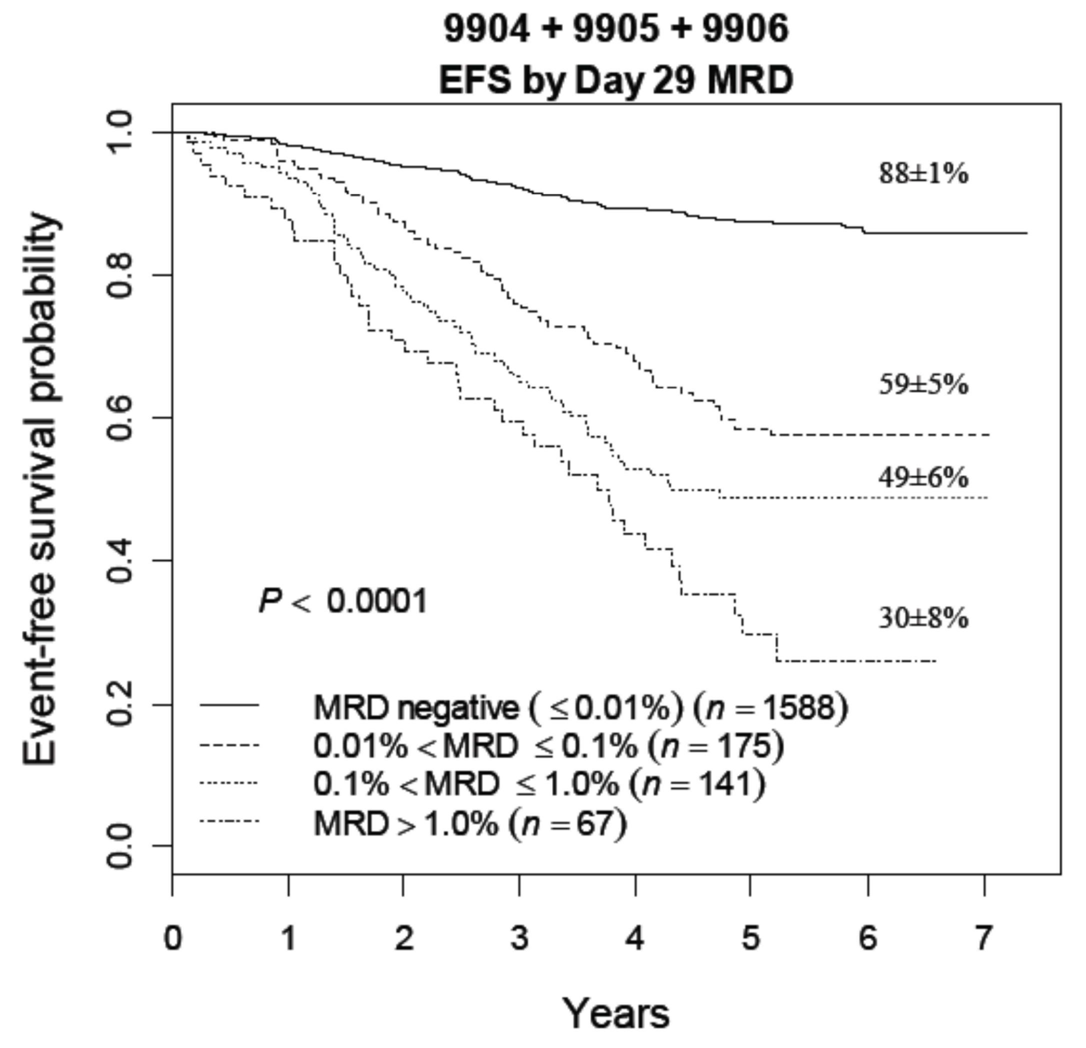
Reproducibility

Flow cytometry is capable of reproducible MRD detection and enumeration

Lack of standardization in implementation is the source of variability in current practice

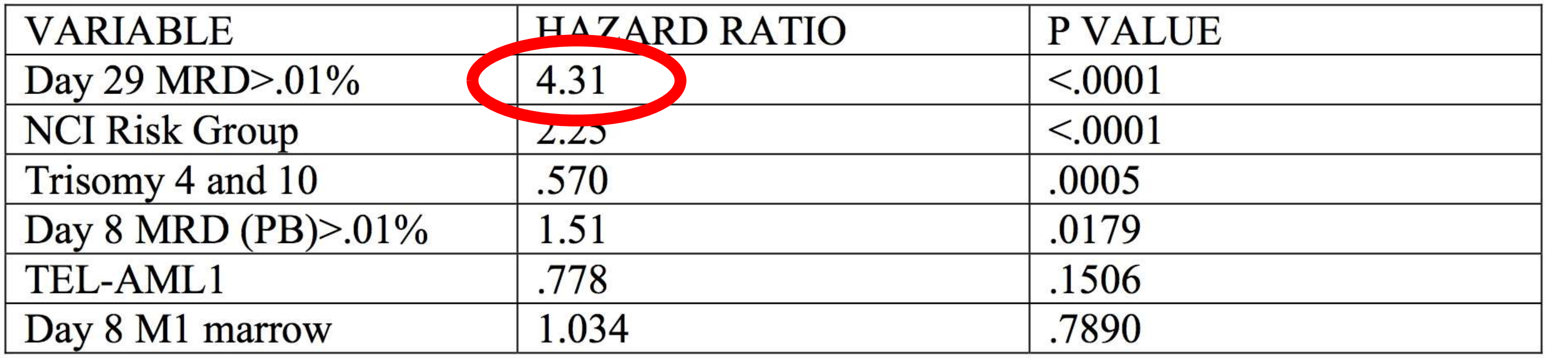
Clinical Outcome

MRD Prognosis in Pediatric B-ALL



Borowitz et al. Blood 111:5477-85 (2008)

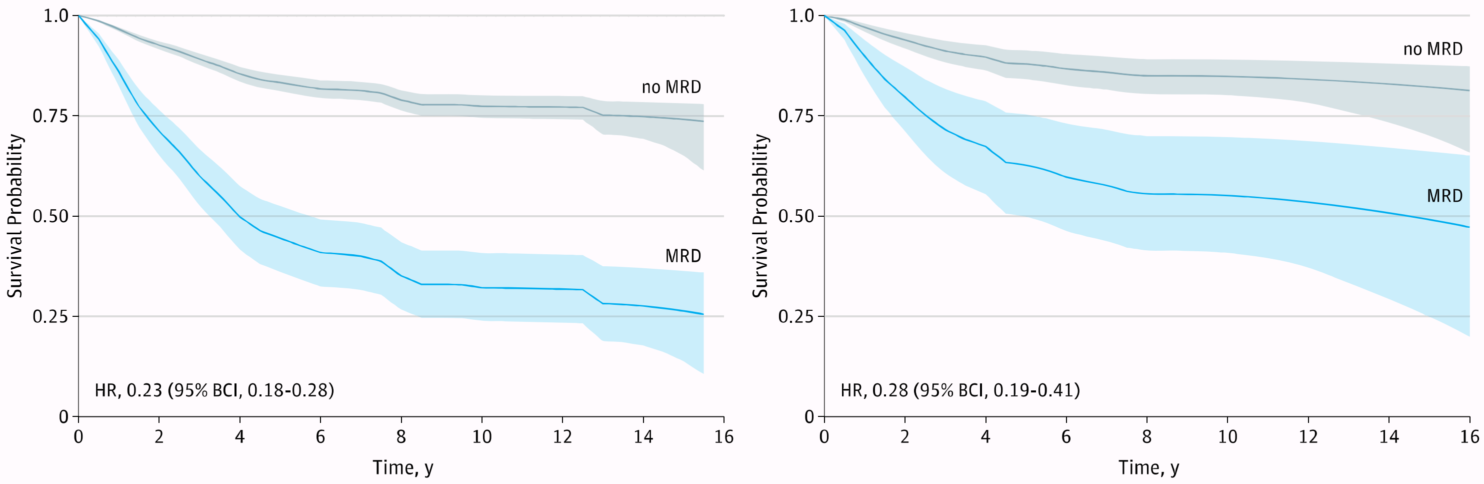
MRD Prognosis in Pediatric B-ALL



VARIABLE	HAZARD RATIO	P VALUE
Day 29 MRD>.01%	4.31	<.0001
NCI Risk Group	2.25	<.0001
Trisomy 4 and 10	.570	.0005
Day 8 MRD (PB)>.01%	1.51	.0179
TEL-AML1	.778	.1506
Day 8 M1 marrow	1.034	.7890

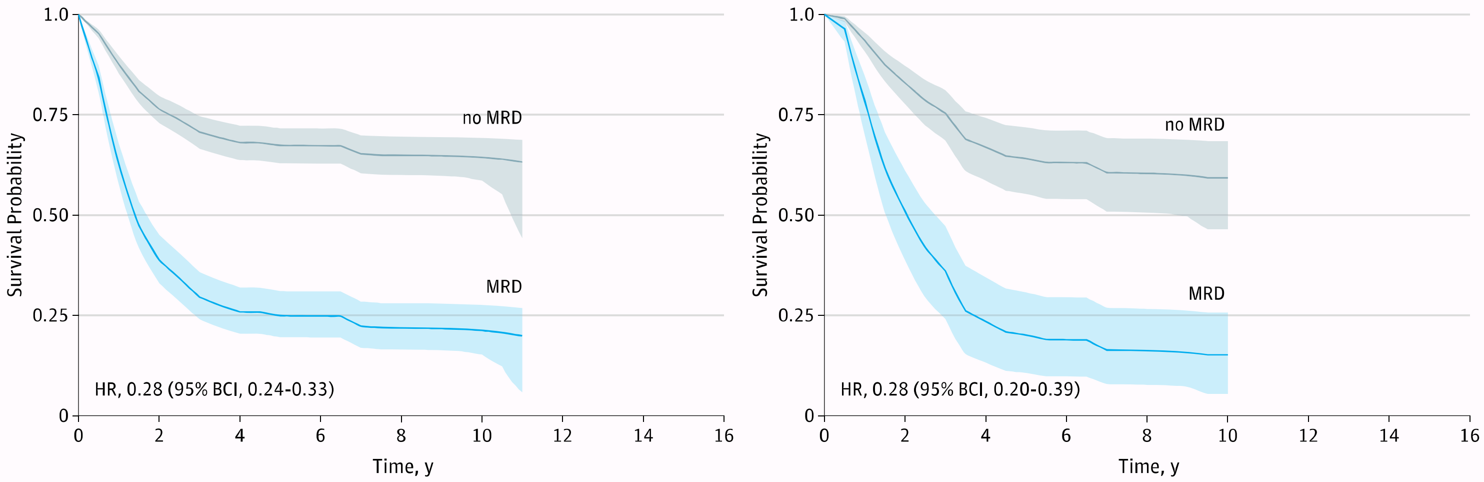
Borowitz et al. Blood 111:5477-85 (2008)

ALL MRD Meta-analysis



A EFS for pediatric ALL: 20 studies with 11 249 patients

B OS for pediatric ALL: 5 studies with 2876 patients



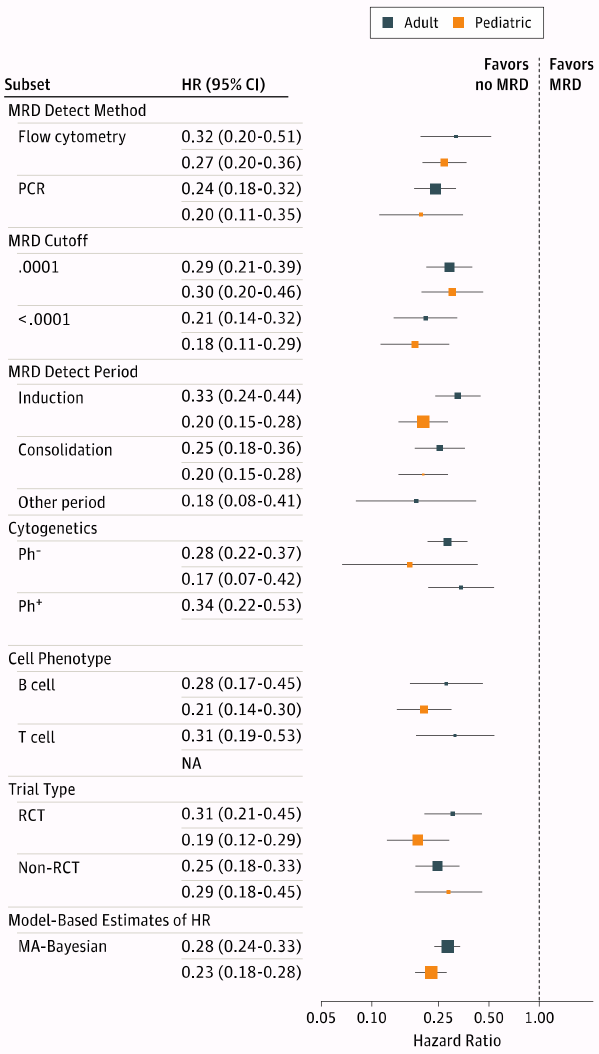
C EFS for adult ALL: 16 studies with 2065 patients

D OS for adult ALL: 5 studies with 806 patients

Berry D, et al. JAMA Oncology (2017) doi:10.1001/jamaoncol.2017.0580

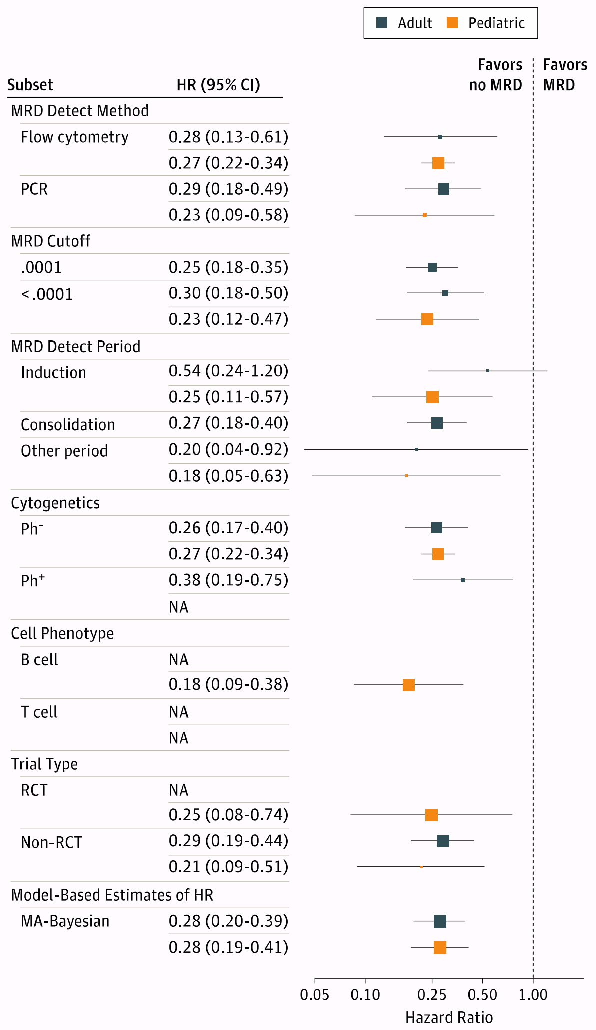
ALL MRD Meta-analysis

Figure 3. Forest Plot of EFS HRs for Pediatric and Adult ALL Subtypes



Subset	HR (95% CI)
MRD Detect Method	
Flow cytometry	0.32 (0.20-0.51)
	0.27 (0.20-0.36)
PCR	0.24 (0.18-0.32)
	0.20 (0.11-0.35)
MRD Cutoff	
.0001	0.29 (0.21-0.39)
	0.30 (0.20-0.46)
<.0001	0.21 (0.14-0.32)
	0.18 (0.11-0.29)
MRD Detect Period	
Induction	0.33 (0.24-0.44)
	0.20 (0.15-0.28)
Consolidation	0.25 (0.18-0.36)
	0.20 (0.15-0.28)
Other period	0.18 (0.08-0.41)
Cytogenetics	
Ph⁻	0.28 (0.22-0.37)
	0.17 (0.07-0.42)
Ph⁺	0.34 (0.22-0.53)
Cell Phenotype	
B cell	0.28 (0.17-0.45)
	0.21 (0.14-0.30)
T cell	0.31 (0.19-0.53)
	NA
Trial Type	
RCT	0.31 (0.21-0.45)
	0.19 (0.12-0.29)
Non-RCT	0.25 (0.18-0.33)
	0.29 (0.18-0.45)
Model-Based Estimates of HR	
MA-Bayesian	0.28 (0.24-0.33)
	0.23 (0.18-0.28)

Figure 4. Forest Plot of OS HRs for Pediatric and Adult ALL Subtypes

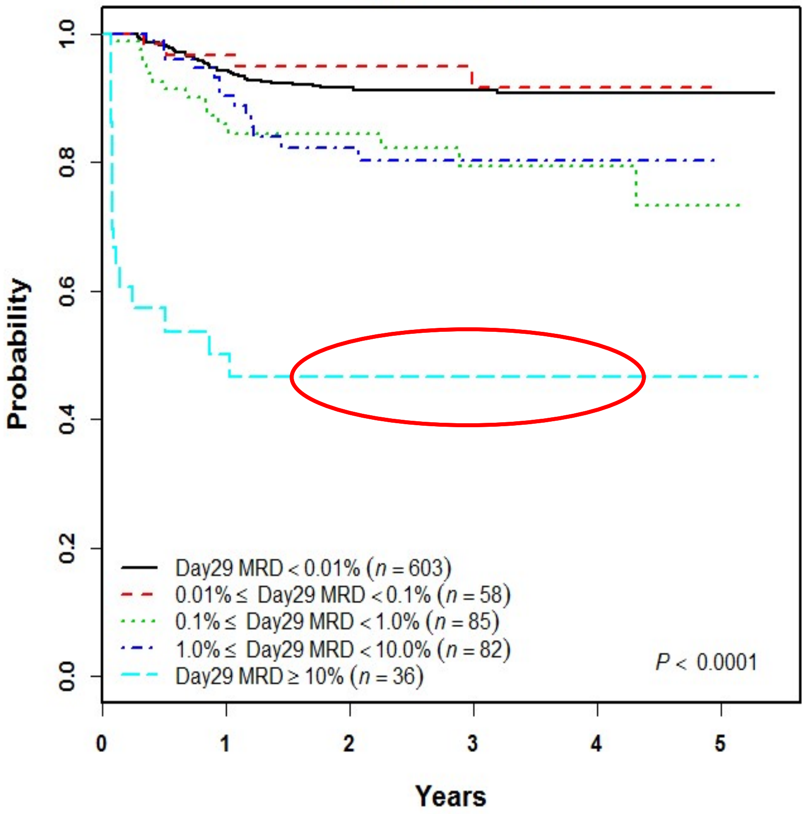


Subset	HR (95% CI)
MRD Detect Method	
Flow cytometry	0.28 (0.13-0.61)
	0.27 (0.22-0.34)
PCR	0.29 (0.18-0.49)
	0.23 (0.09-0.58)
MRD Cutoff	
.0001	0.25 (0.18-0.35)
<.0001	0.30 (0.18-0.50)
	0.23 (0.12-0.47)
MRD Detect Period	
Induction	0.54 (0.24-1.20)
	0.25 (0.11-0.57)
Consolidation	0.27 (0.18-0.40)
Other period	0.20 (0.04-0.92)
	0.18 (0.05-0.63)
Cytogenetics	
Ph⁻	0.26 (0.17-0.40)
	0.27 (0.22-0.34)
Ph⁺	0.38 (0.19-0.75)
	NA
Cell Phenotype	
B cell	NA
	0.18 (0.09-0.38)
T cell	NA
	NA
Trial Type	
RCT	NA
	0.25 (0.08-0.74)
Non-RCT	0.29 (0.19-0.44)
	0.21 (0.09-0.51)
Model-Based Estimates of HR	
MA-Bayesian	0.28 (0.20-0.39)
	0.28 (0.19-0.41)

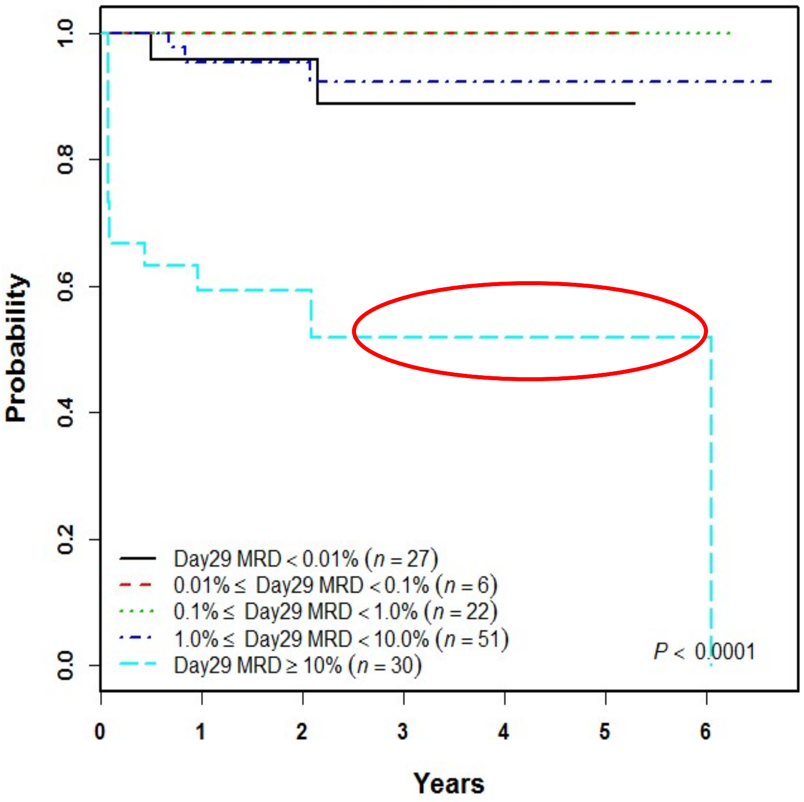
Berry D, et al. JAMA Oncology (2017) doi:10.1001/jamaoncol.2017.0580

AALL0434 T-ALL MRD

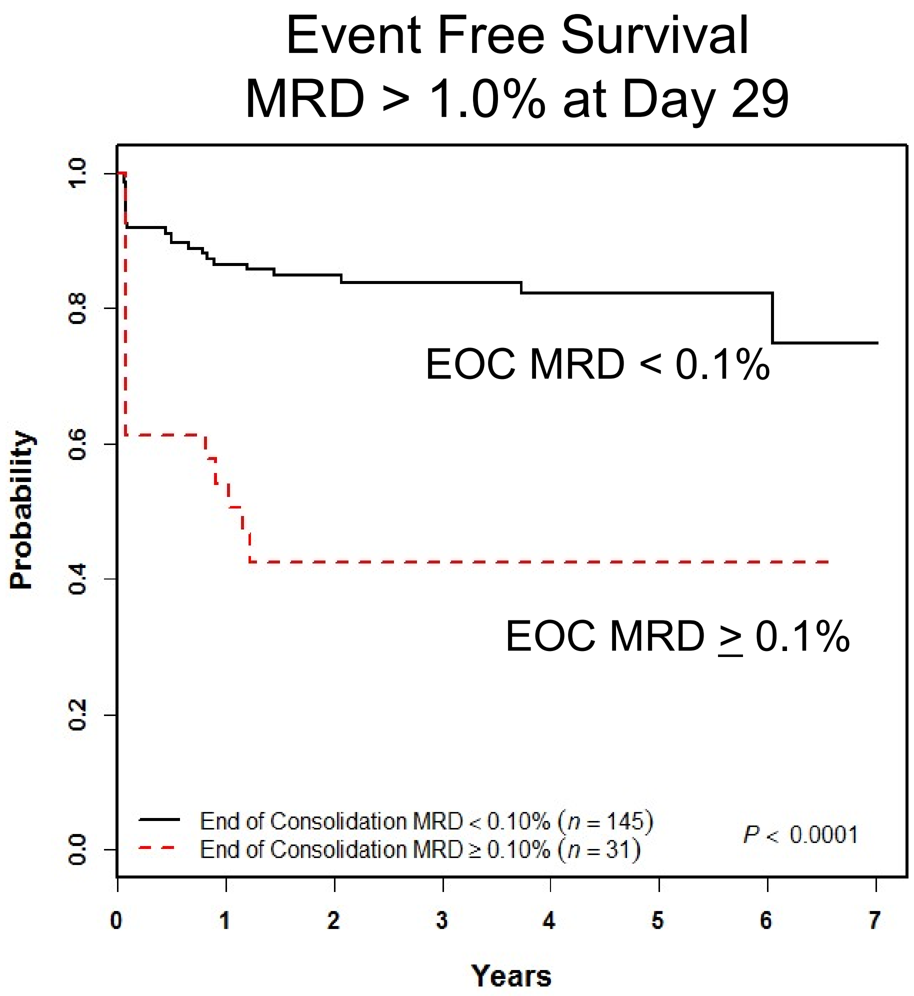
Event Free Survival
Not ETP patients



Event Free Survival
ETP patients

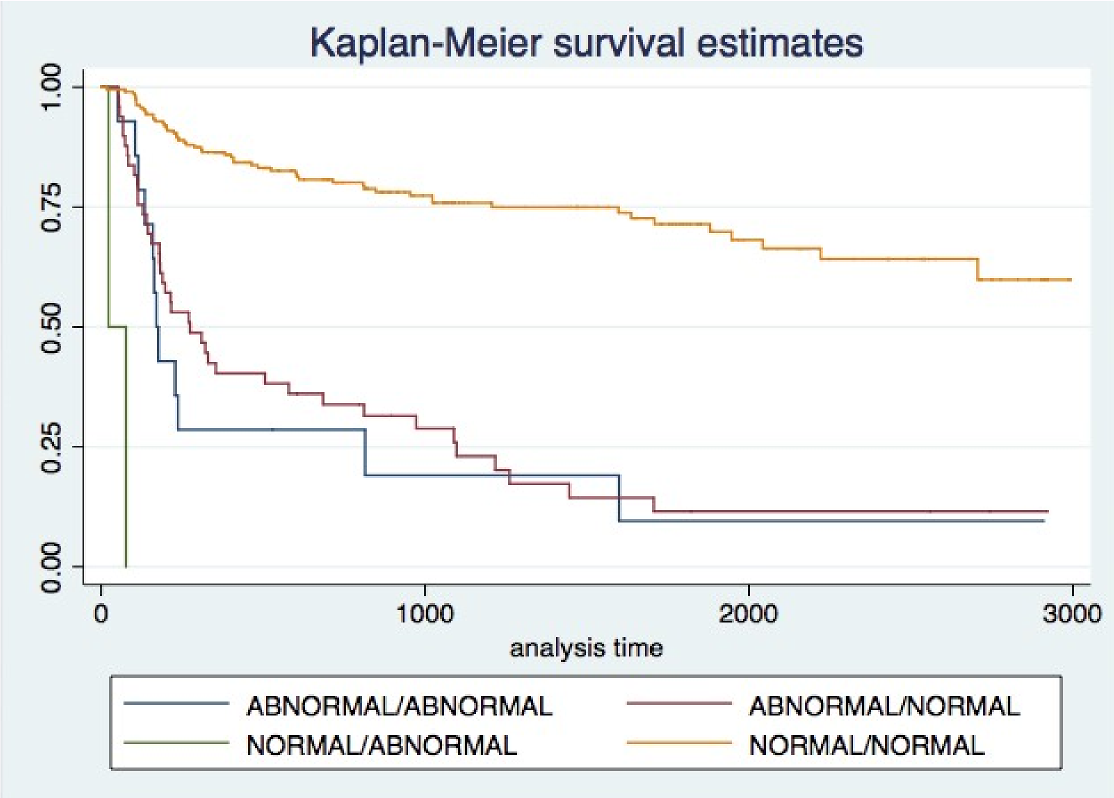


AALL0434 MRD – End of Consolidation



CHILDREN'S
ONCOLOGY
GROUP

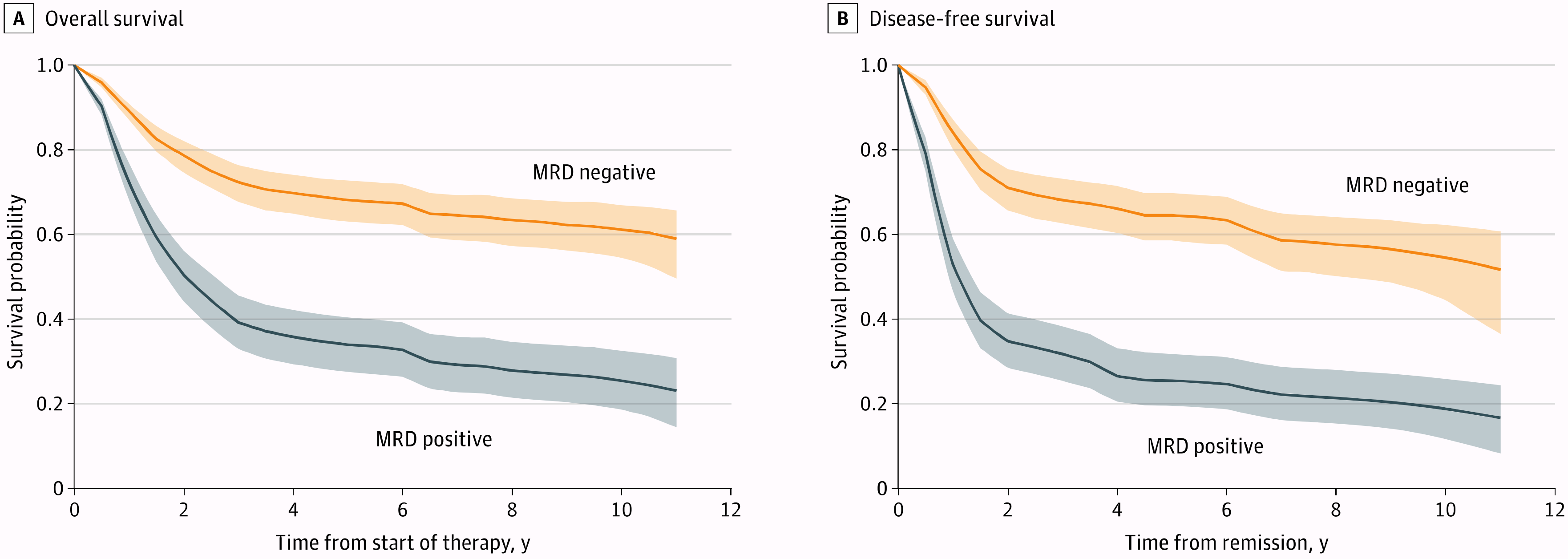
Pre / Post Transplant MRD



MRD by Flow Cytometry

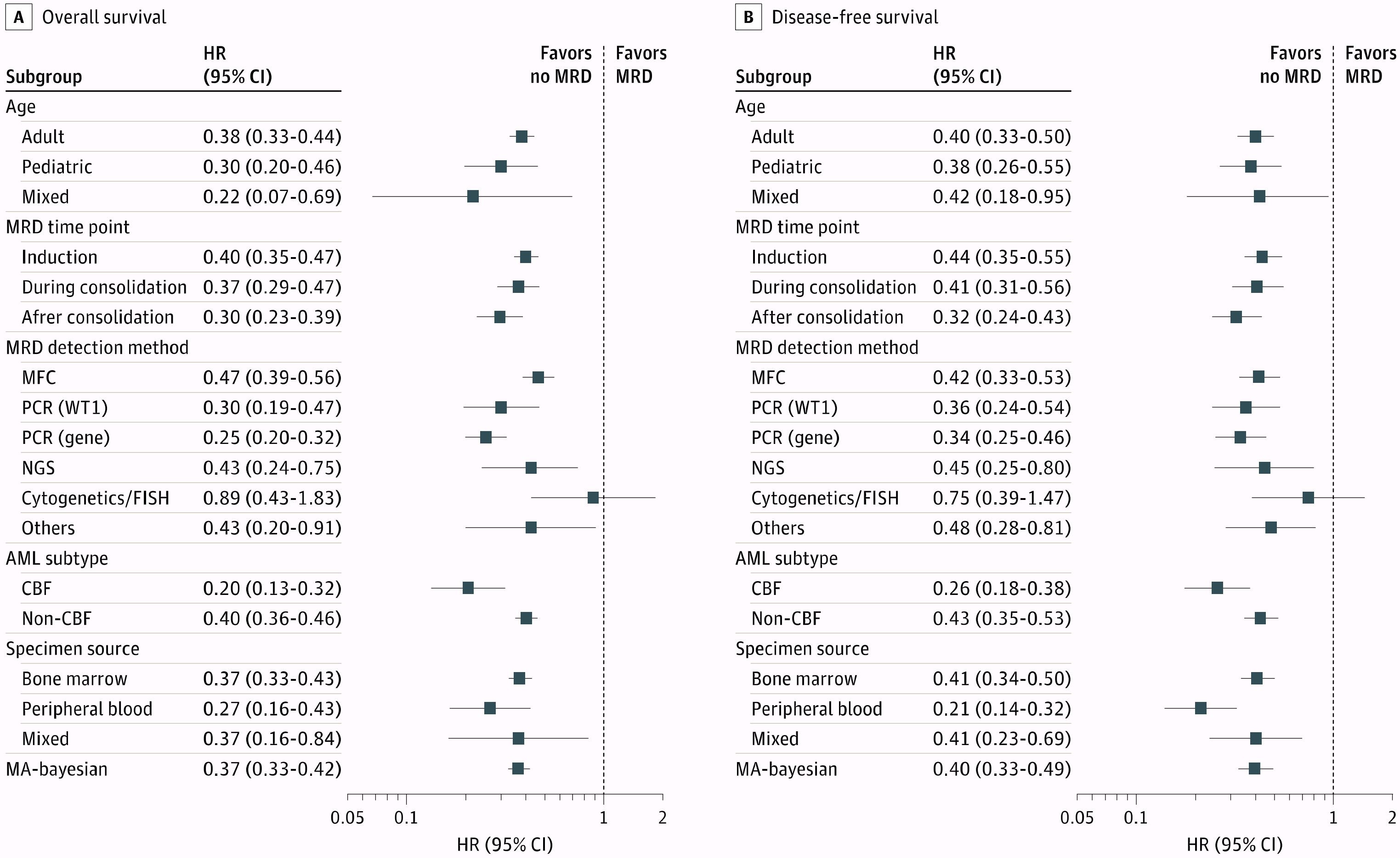
Zhou Y, et al. (2016) Leukemia 30(7):1456-64

AML MRD Meta-analysis



Short N, et al. JAMA Oncology (2020) 6(12):1890-1899

AML MRD Meta-analysis



A Overall survival

Subgroup	HR (95% CI)
Age	
Adult	0.38 (0.33-0.44)
Pediatric	0.30 (0.20-0.46)
Mixed	0.22 (0.07-0.69)
MRD time point	
Induction	0.40 (0.35-0.47)
During consolidation	0.37 (0.29-0.47)
Afrer consolidation	0.30 (0.23-0.39)
MRD detection method	
MFC	0.47 (0.39-0.56)
PCR (WT1)	0.30 (0.19-0.47)
PCR (gene)	0.25 (0.20-0.32)
NGS	0.43 (0.24-0.75)
Cytogenetics/FISH	0.89 (0.43-1.83)
Others	0.43 (0.20-0.91)
AML subtype	
CBF	0.20 (0.13-0.32)
Non-CBF	0.40 (0.36-0.46)
Specimen source	
Bone marrow	0.37 (0.33-0.43)
Peripheral blood	0.27 (0.16-0.43)
Mixed	0.37 (0.16-0.84)
MA-bayesian	0.37 (0.33-0.42)

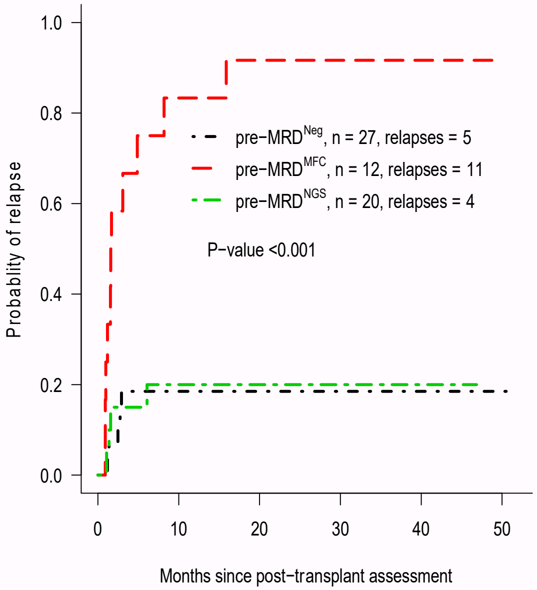
B Disease-free survival

Subgroup	HR (95% CI)
Age	
Adult	0.40 (0.33-0.50)
Pediatric	0.38 (0.26-0.55)
Mixed	0.42 (0.18-0.95)
MRD time point	
Induction	0.44 (0.35-0.55)
During consolidation	0.41 (0.31-0.56)
After consolidation	0.32 (0.24-0.43)
MRD detection method	
MFC	0.42 (0.33-0.53)
PCR (WT1)	0.36 (0.24-0.54)
PCR (gene)	0.34 (0.25-0.46)
NGS	0.45 (0.25-0.80)
Cytogenetics/FISH	0.75 (0.39-1.47)
Others	0.48 (0.28-0.81)
AML subtype	
CBF	0.26 (0.18-0.38)
Non-CBF	0.43 (0.35-0.53)
Specimen source	
Bone marrow	0.41 (0.34-0.50)
Peripheral blood	0.21 (0.14-0.32)
Mixed	0.41 (0.23-0.69)
MA-bayesian	0.40 (0.33-0.49)

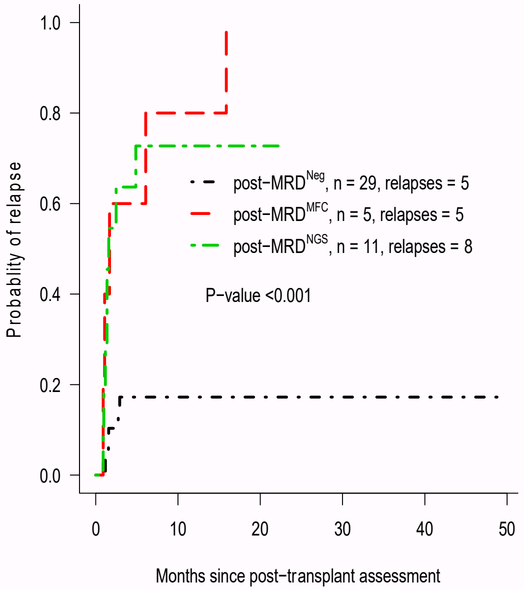
Short N, et al. JAMA Oncology (2020) 6(12):1890-1899

Significance of MRD is Context Dependent

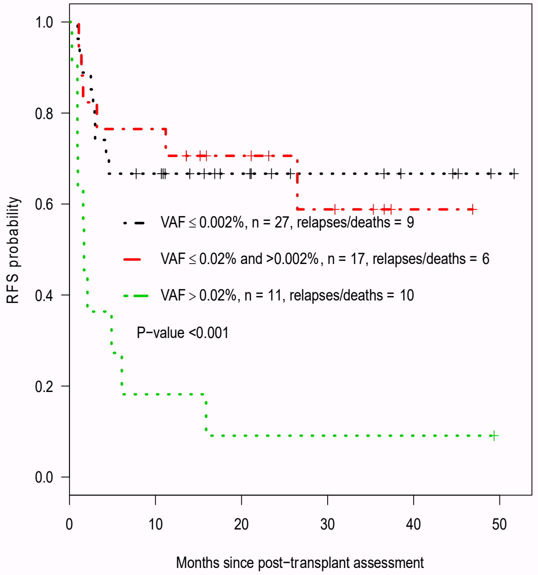
Pre-transplant



Post-transplant



Pre-transplant VAF 0.02%



NPM1+ AML

Zhou Y, et al (2018) BBMT 24(8):1615-1620.

Conclusions

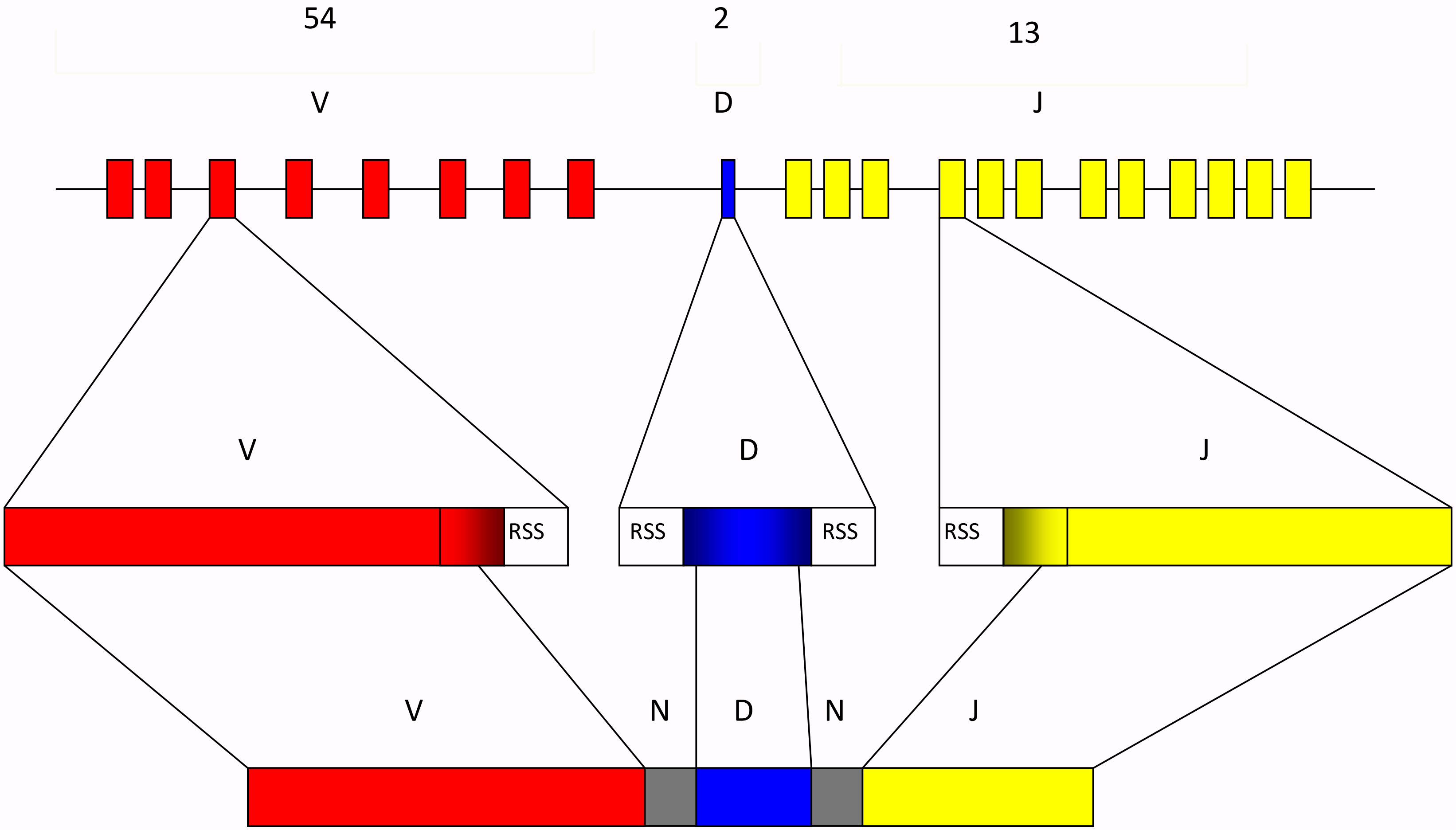
- Diagnosis is immunophenotypic, classification is genotypic
- MRD detection provides important unique prognostic information in pediatric ALL
  - Is becoming standard of care
- MRD by flow cytometry can be done reproducibly
- MRD by NGS is feasible for B-ALL, T-ALL and AML
- Single cell molecular methods are the next frontier

Acknowledgements

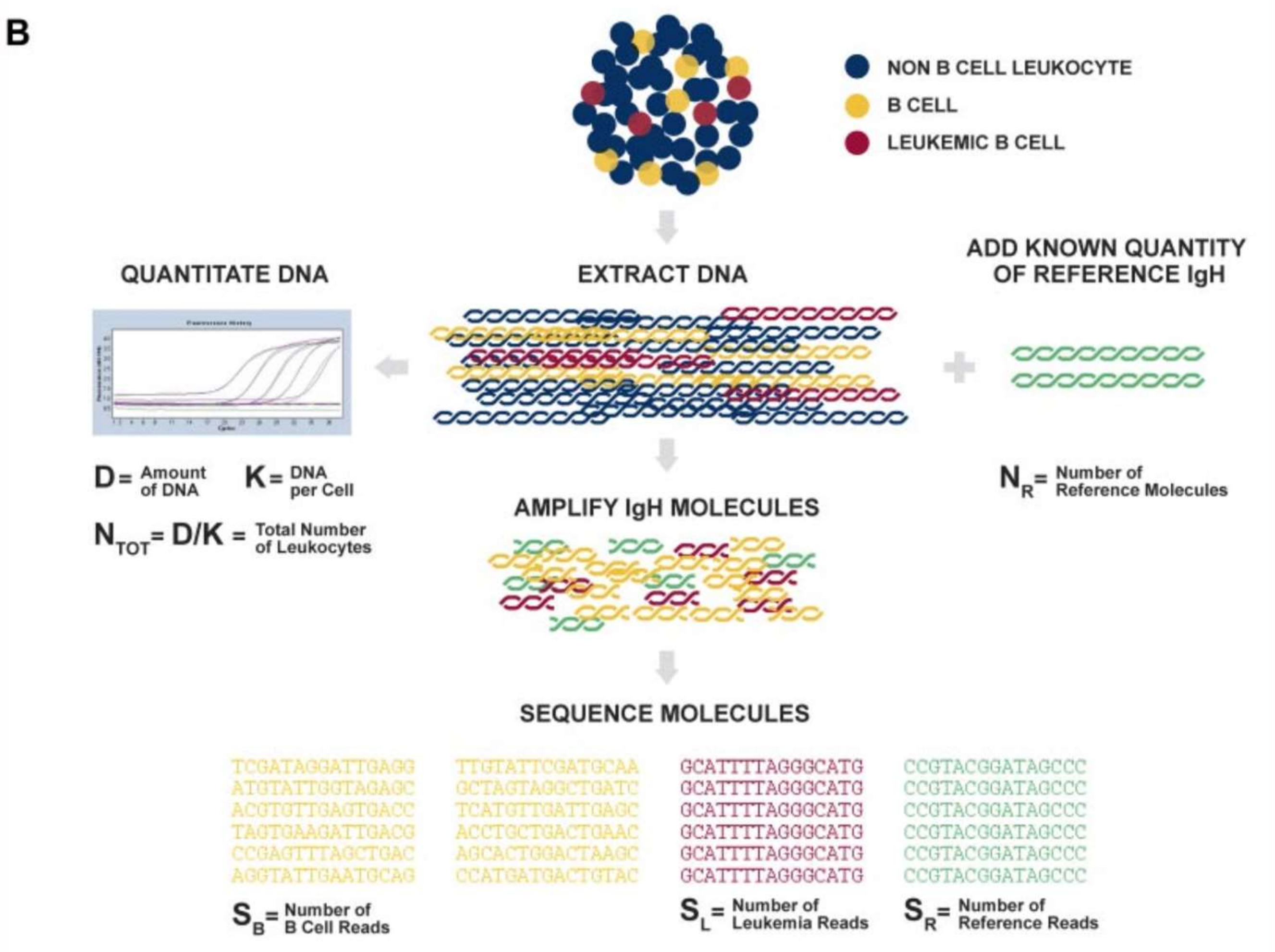
- Hematopathology Laboratory at UWMC
- Michael Borowitz MD PhD
- Entire COG ALL subcommittee
  - Steven Hunger, Bill Carroll, Mignon Loh
- Adaptive Biotechnologies
  - Harlan Robins (FHCRC)
- Steve Salipante, David Wu, Nikhil Patkar

Future

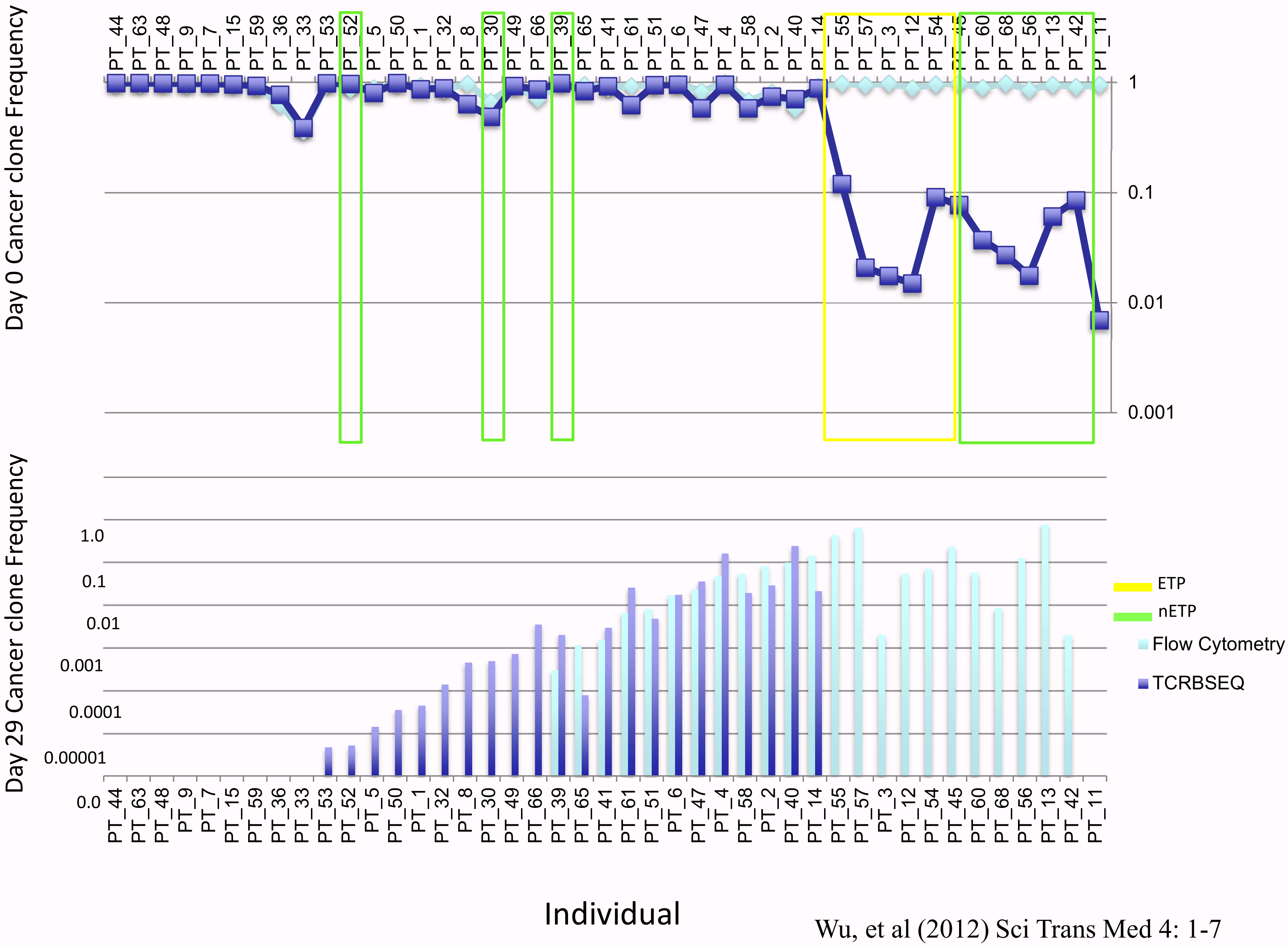
IgH and TCR Diversity



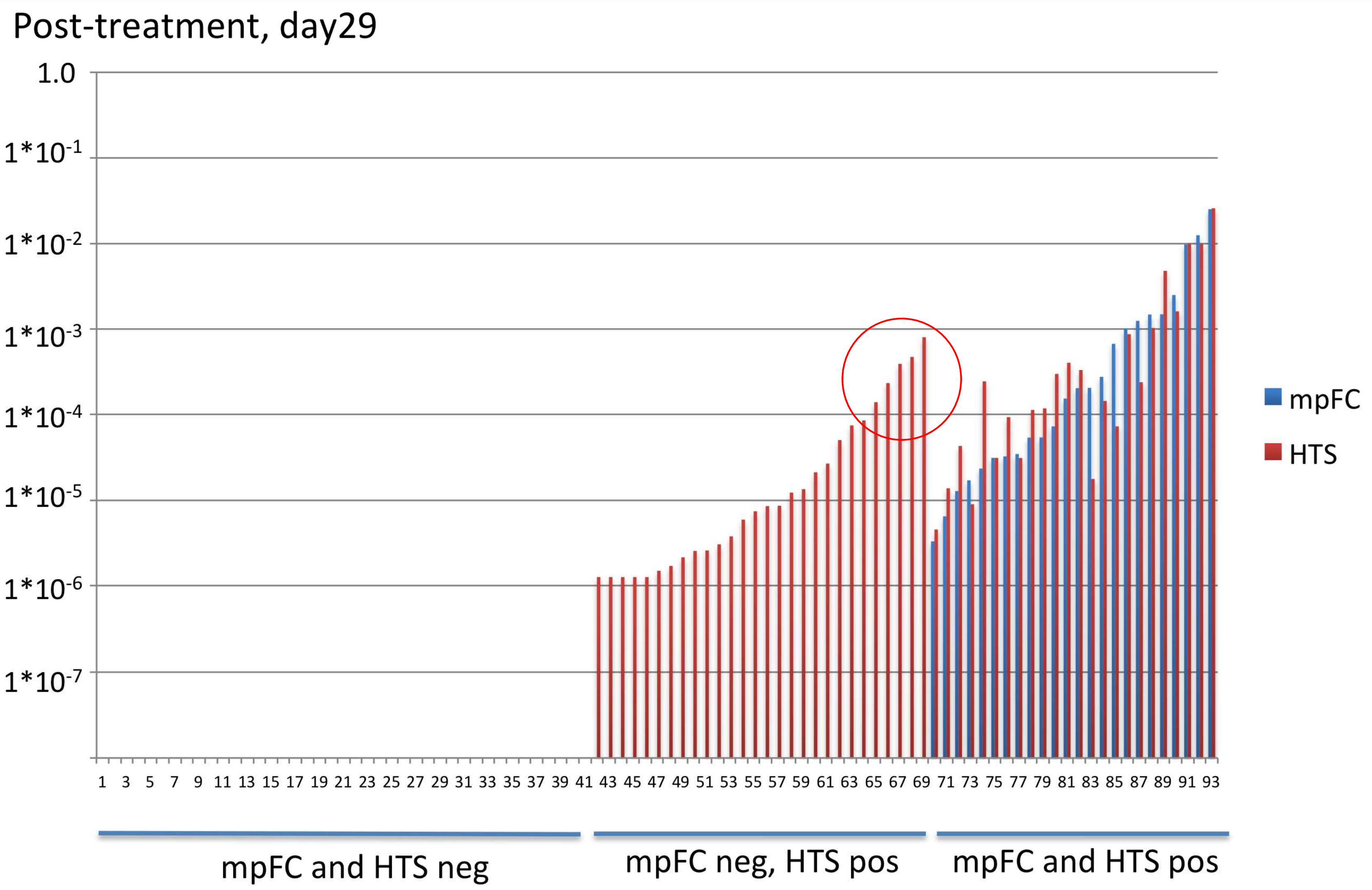
MRD by NGS



Faham et al (2012) Blood 120:5173-80



Wu, et al (2012) Sci Trans Med 4: 1-7



Residual COG AALL0932 samples

93 / 98 (95%) with IgH rearrangement

Remainder: 2 with D-J, 1 with clonal D-J + many VH, 2 none

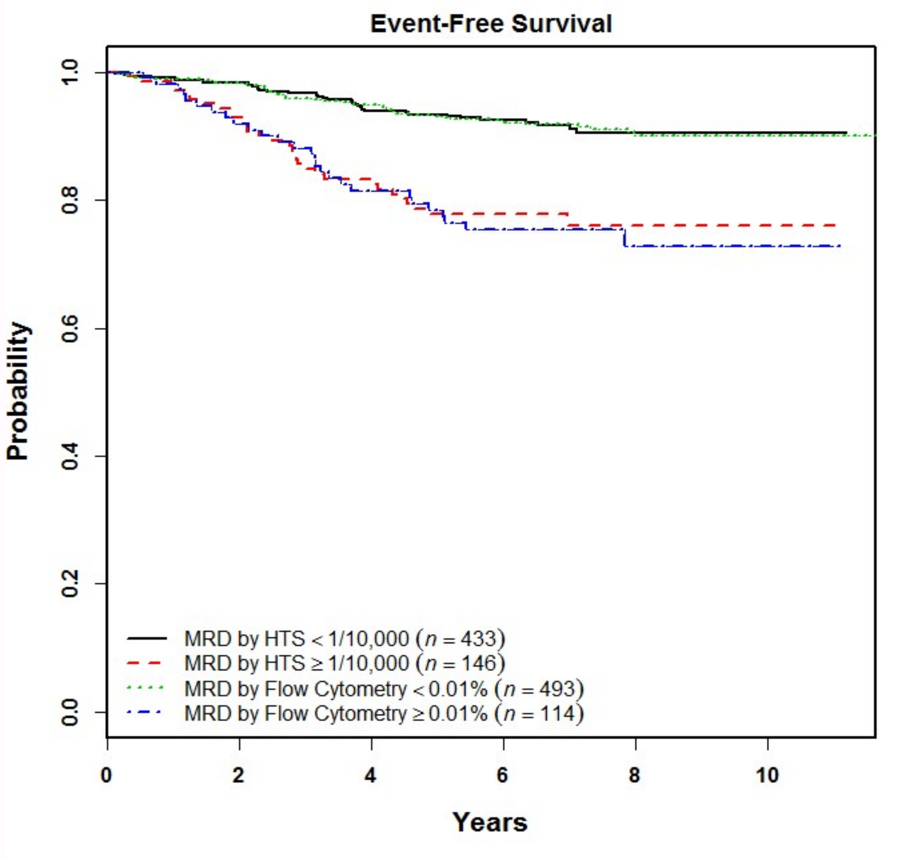
Wu, et al (2014) Clin Cancer Res 20:4540-8

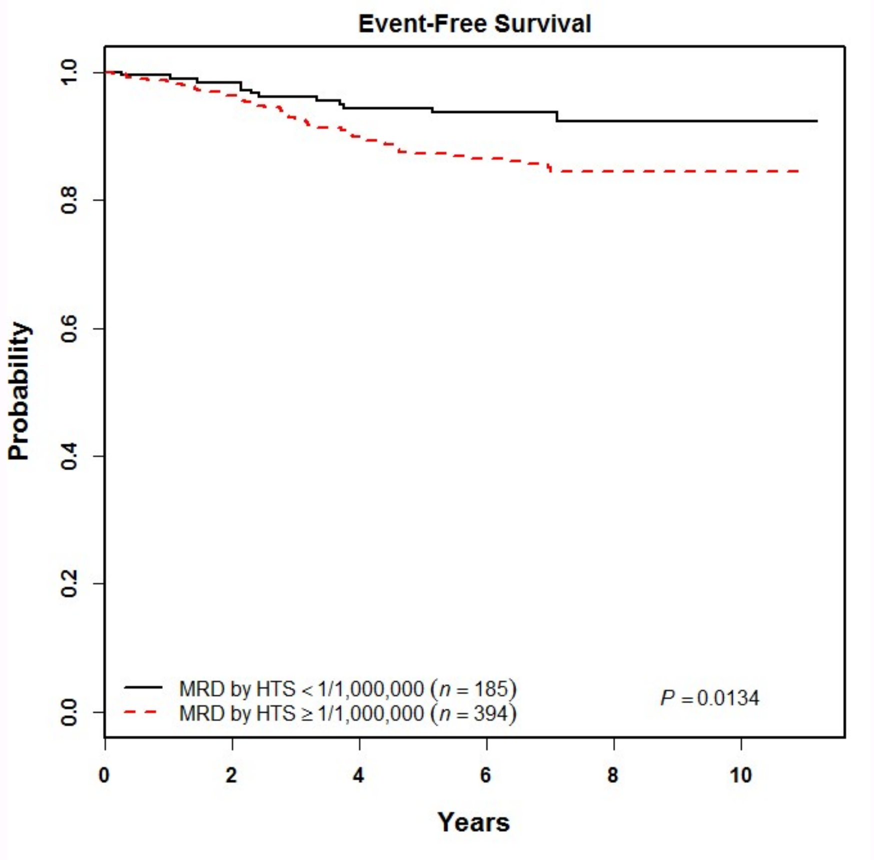
Methods

- 619 paired Pretreatment and Day 29 B-ALL samples
  - Patients with B-ALL enrolled on Children’s Oncology Group (COG) AALL0331 (standard-risk, N=282) and AALL0232 (high-risk, N=297)
  - Previously assayed for MRD by flow cytometry
  - MRD at Day 29 < 0.1% by flow cytometry
- Assayed by HTS of CDR3 regions of IGH and TCRG.
  - 93.2% informative by HTS for IgH in pretreatment sample
  - Dominant clonal CDR3 sequences in the pretreatment samples were quantitated in the paired Day 29 samples as residual disease of total nucleated cells
  - Blinded to FC results
- Relationship of MRD by HTS and FC to 5-year EFS and OS evaluated using Kaplan-Meier statistics

Wood, et al (2017) Blood DOI 10.1182/blood-2017-09-806521

B-LL End of Induction MRD

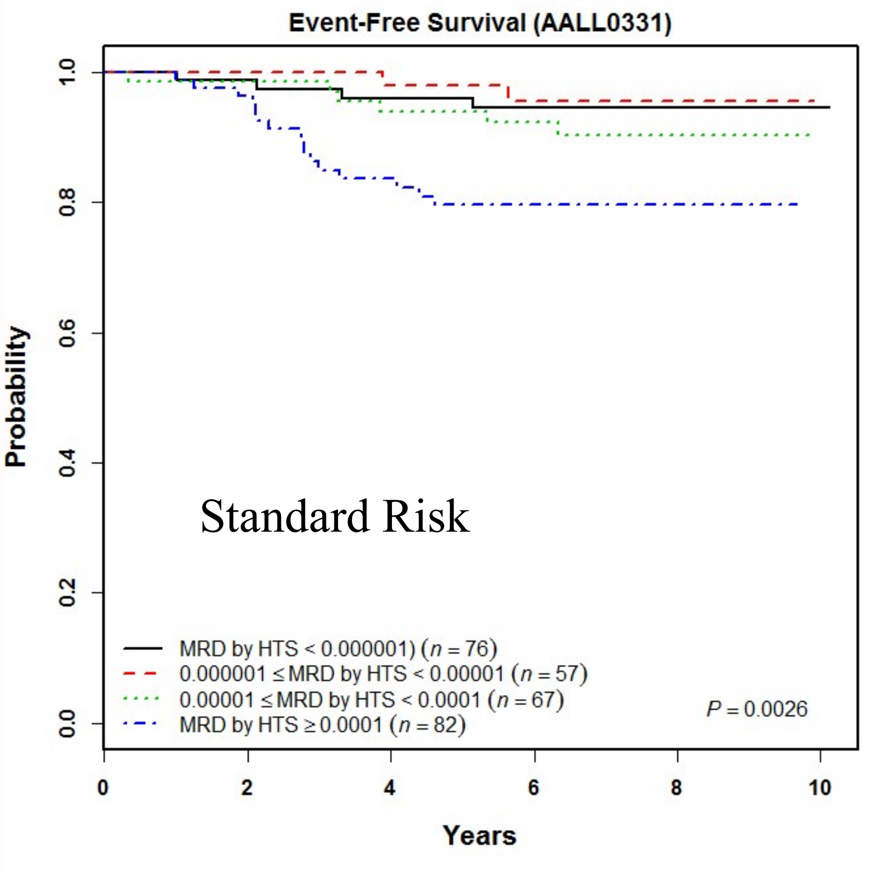


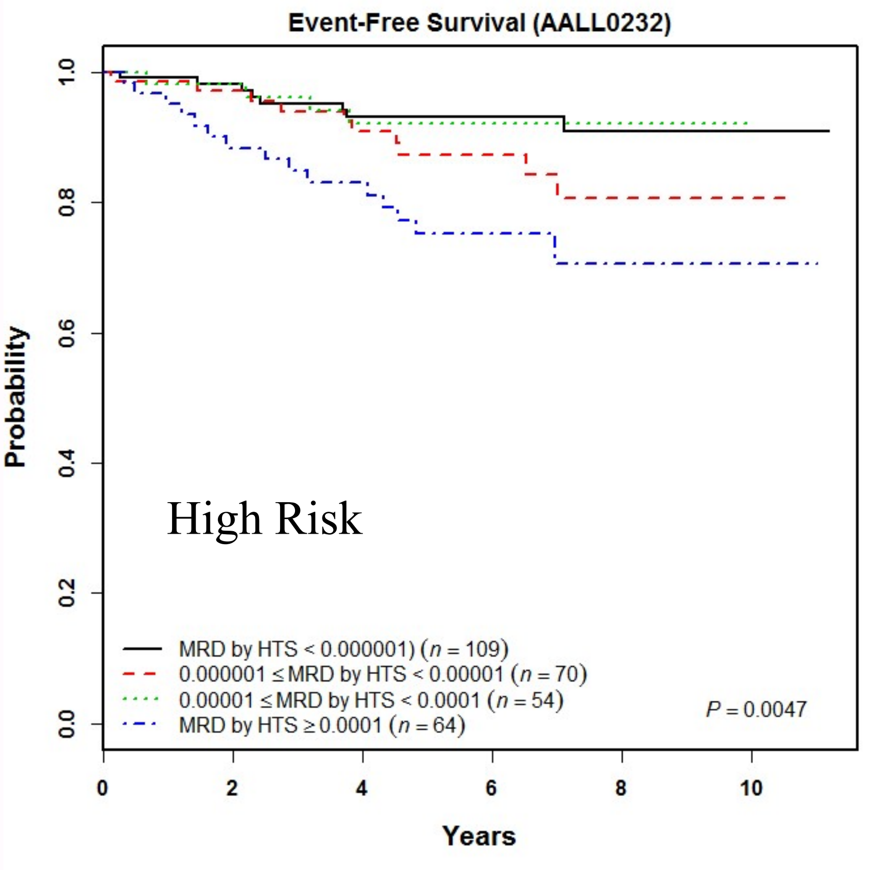


Flow and HTS are equivalent at 0.01%

Use of cutoff of 0.0001% does not improve risk stratification

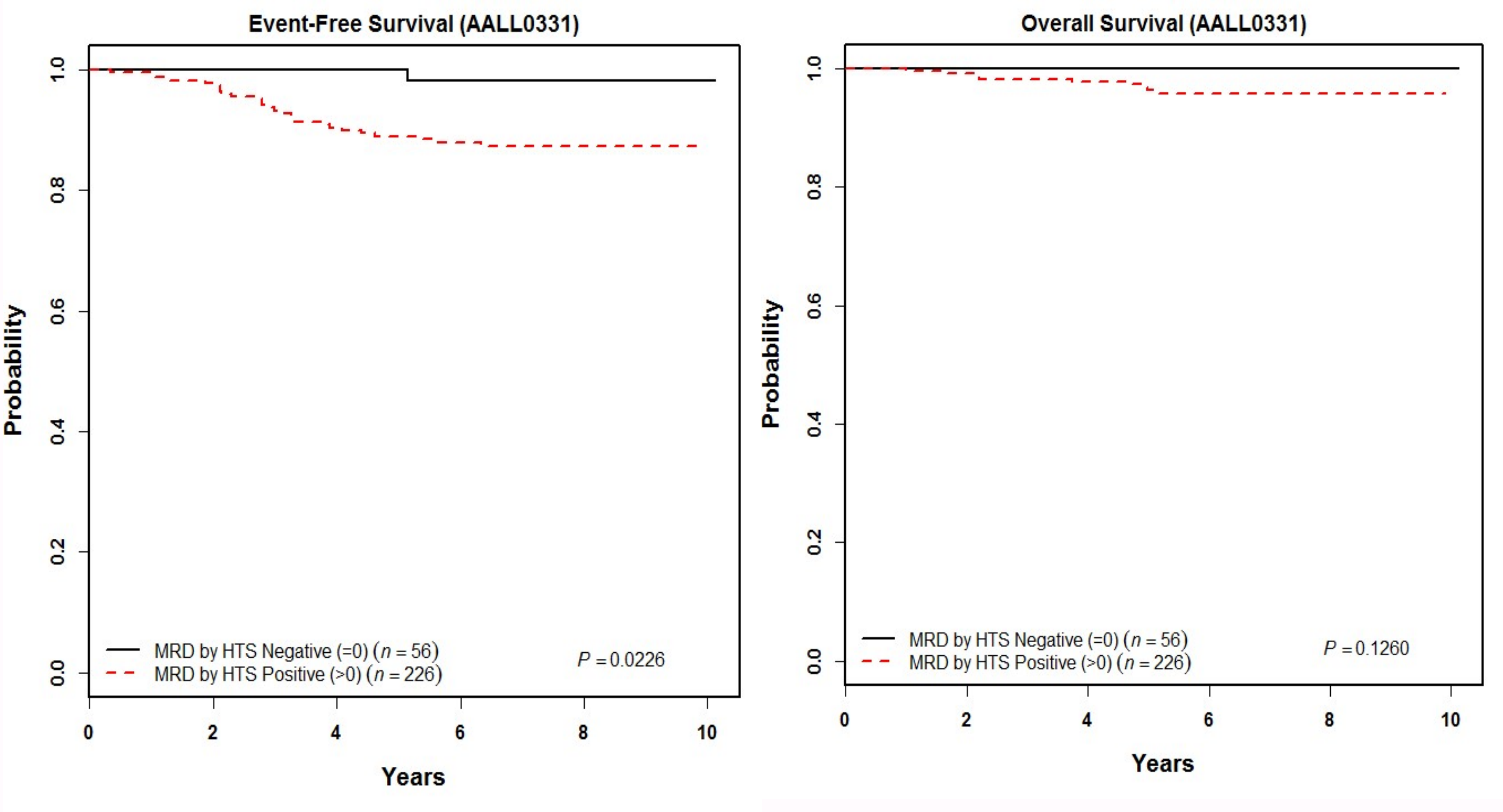
B-LL End of Induction MRD





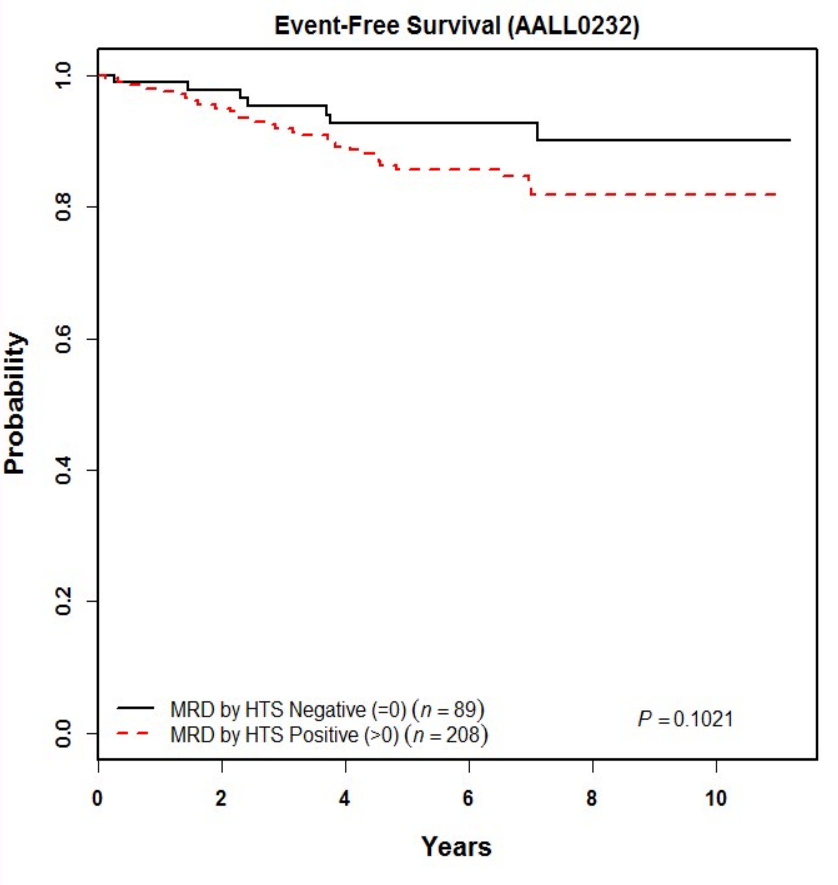
0.001% cutoff suggests poorer outcome for high risk

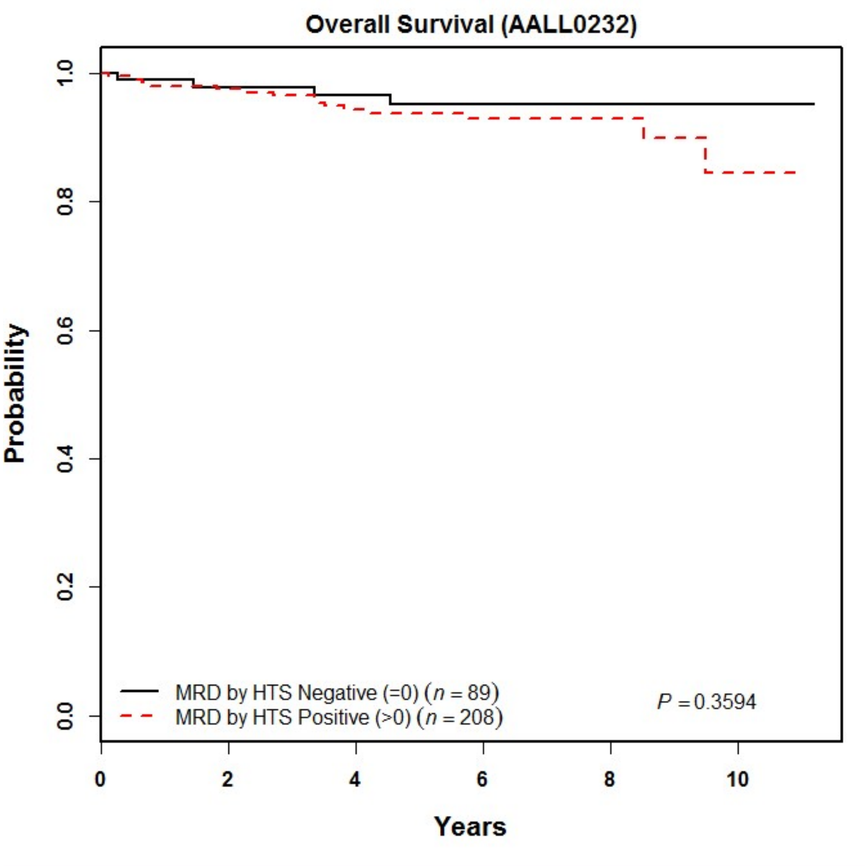
B-LL End of Induction MRD



Absence of detectable MRD by NGS = Excellent outcome
Standard risk patients

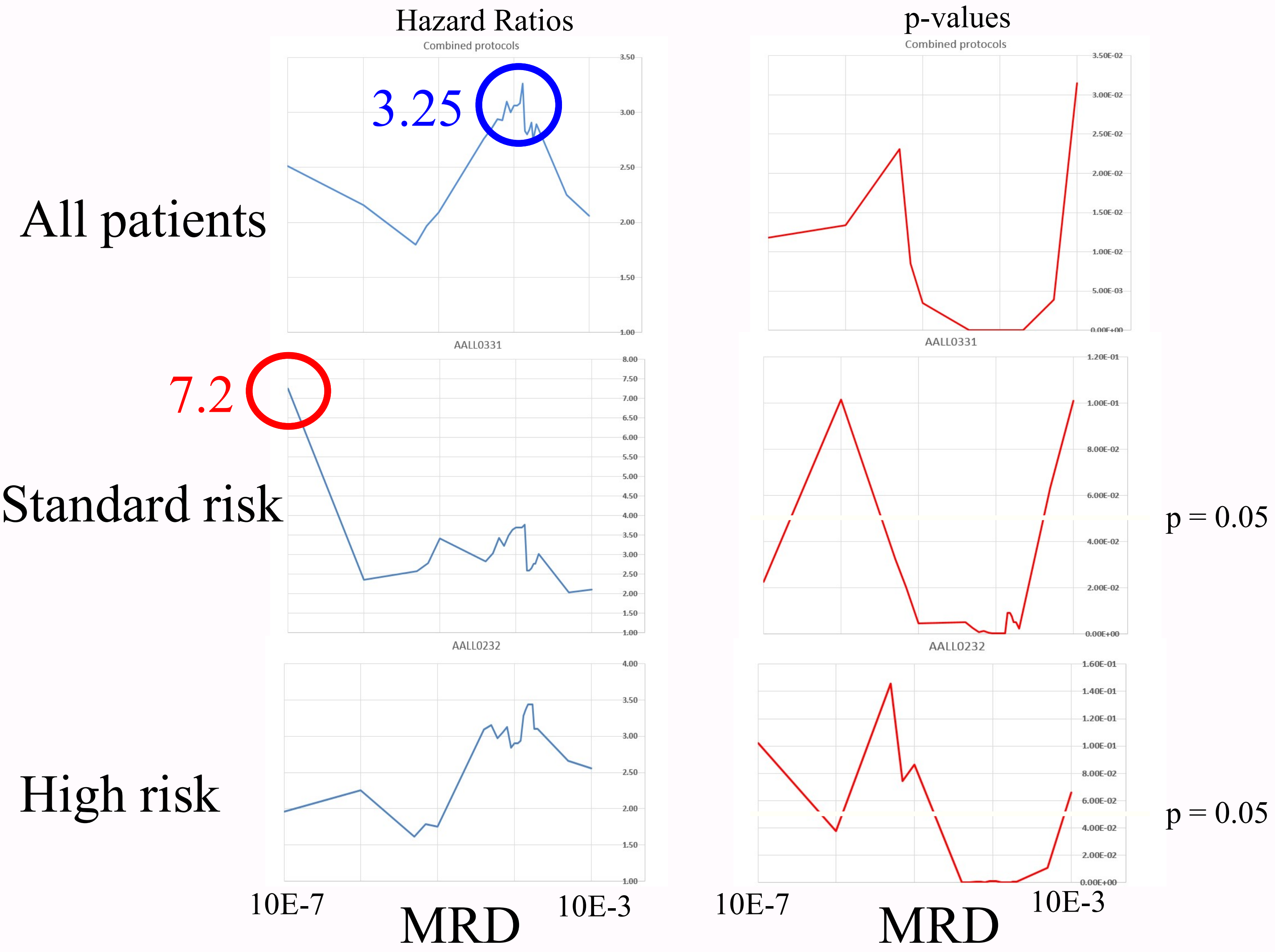
B-LL End of Induction MRD





Absence of detectable MRD by NGS for
High risk patients ≠ Standard risk patients

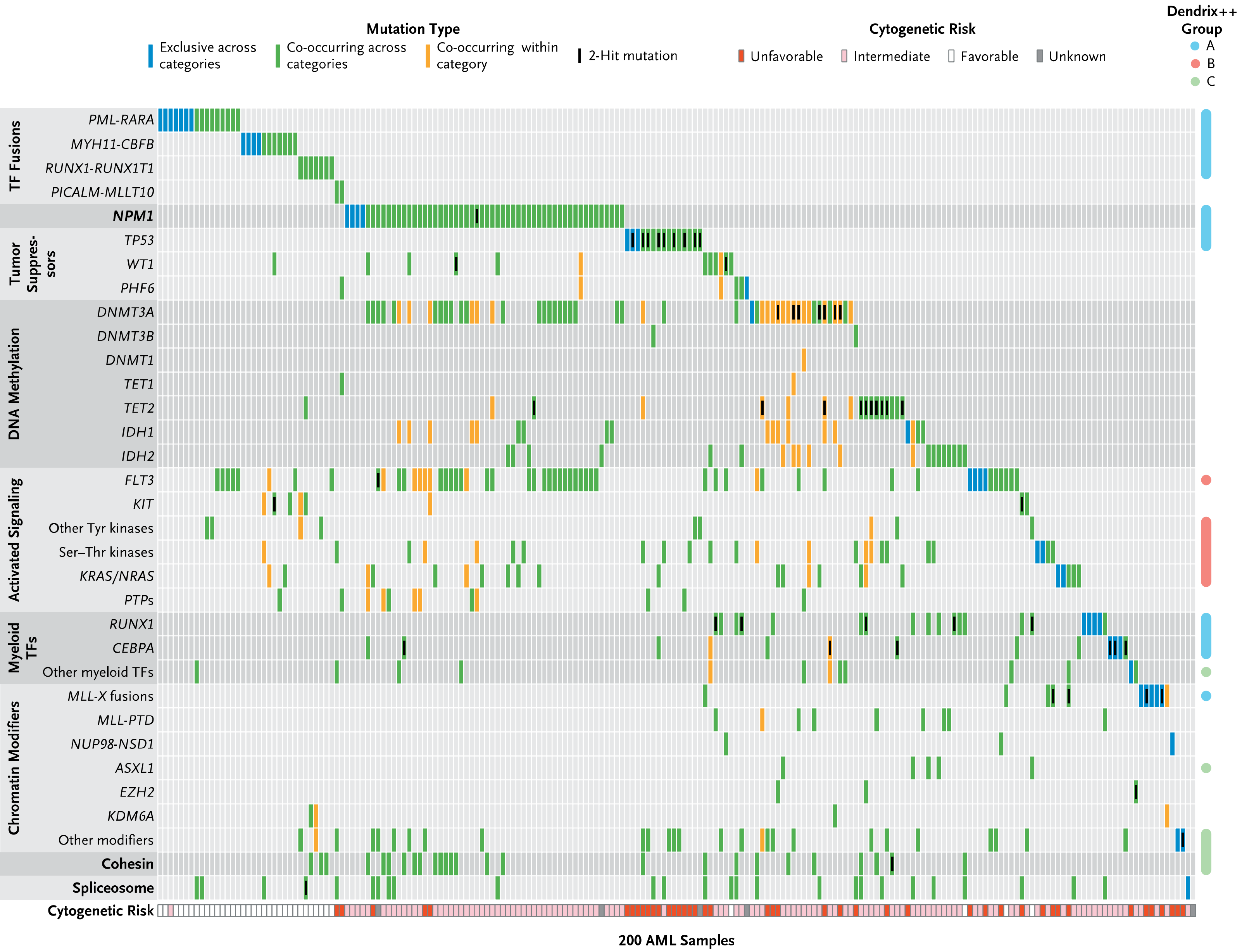
Cut Point Analysis



Optimal cut point for combined patients is ~0.01%

NGS Conclusions

- NGS is capable of MRD detection
  - Can reproduce risk groups
- Sensitivity = NGS > ASO-PCR > Flow
  - NGS sensitivity ~ 10^{-6}
- Uses
  - Standardize testing
  - Simplify sample requirements
  - Define MRD(-) low-risk group early
  - Define MRD(+) high-risk group late (EOC)
  - ? better for targeted immunotherapy



The Cancer Genome Atlas Research Network (2013) NEJM 368:2059-74

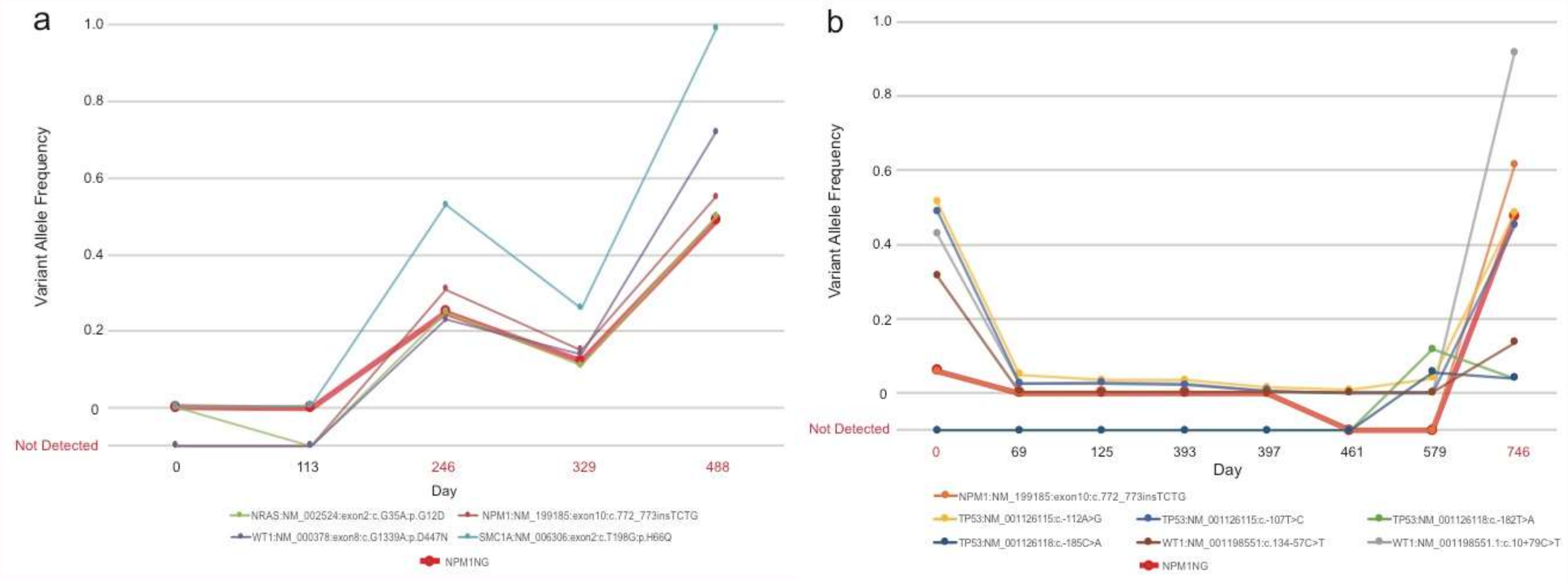
AML MRD NGS

Gene	Capture Design	Nucleotides sequenced (bp)	Number smMIP probes	Gene	Capture Design	Nucleotides sequenced (bp)	Number smMIP probes
ABL1	Hotspot	817	8	*NT5C2*	Hotspot	444	4
ALK	Hotspot	333	3	*PHF6*	Full Gene	927	9
BRAF	Hotspot	222	2	*PIK3CA*	Hotspot	333	3
CEBPA	Full Gene	203	2	*PPM1D*	Full Gene	1971	22
DNMT3A	Hotspot	111	1	*PTPN11*	Full Gene	2418	24
EZH2	Full Gene	3117	32	*RAD21*	Full Gene	2410	25
FAM5C	Full Gene	2583	28	*RET*	Hotspot	111	1
FLT3	Hotspot	873	8	*ROS1*	Hotspot	333	3
HNRNPK	Full Gene	2281	24	*RUNX1*	Full Gene	1894	19
IDH1	Hotspot	111	1	*SMC1A*	Full Gene	5060	53
IDH2	Full Gene	205	2	*SMC3*	Full Gene	5147	52
JAK2	Hotspot	120	1	*STAG2*	Full Gene	5163	53
KIT	Hotspot	1041	10	*TET2*	Full Gene	6326	66
KRAS	Hotspot	333	3	*TP53*	Full Gene	2069	22
NPM1	Hotspot	89	1	*U2AF1*	Hotspot	222	2
NRAS	Hotspot	222	2	*WT1*	Full Gene	1688	16
				Cell line variants	Hotspot	999	9
				Total	N/A	50176	511

32 gene panel of AML mutations
Coverage for ~85% of AML
Sensitivity of 0.011%

Salipante, et al (2017) Haematologica doi: 10.3324/haematol.2017.169136.

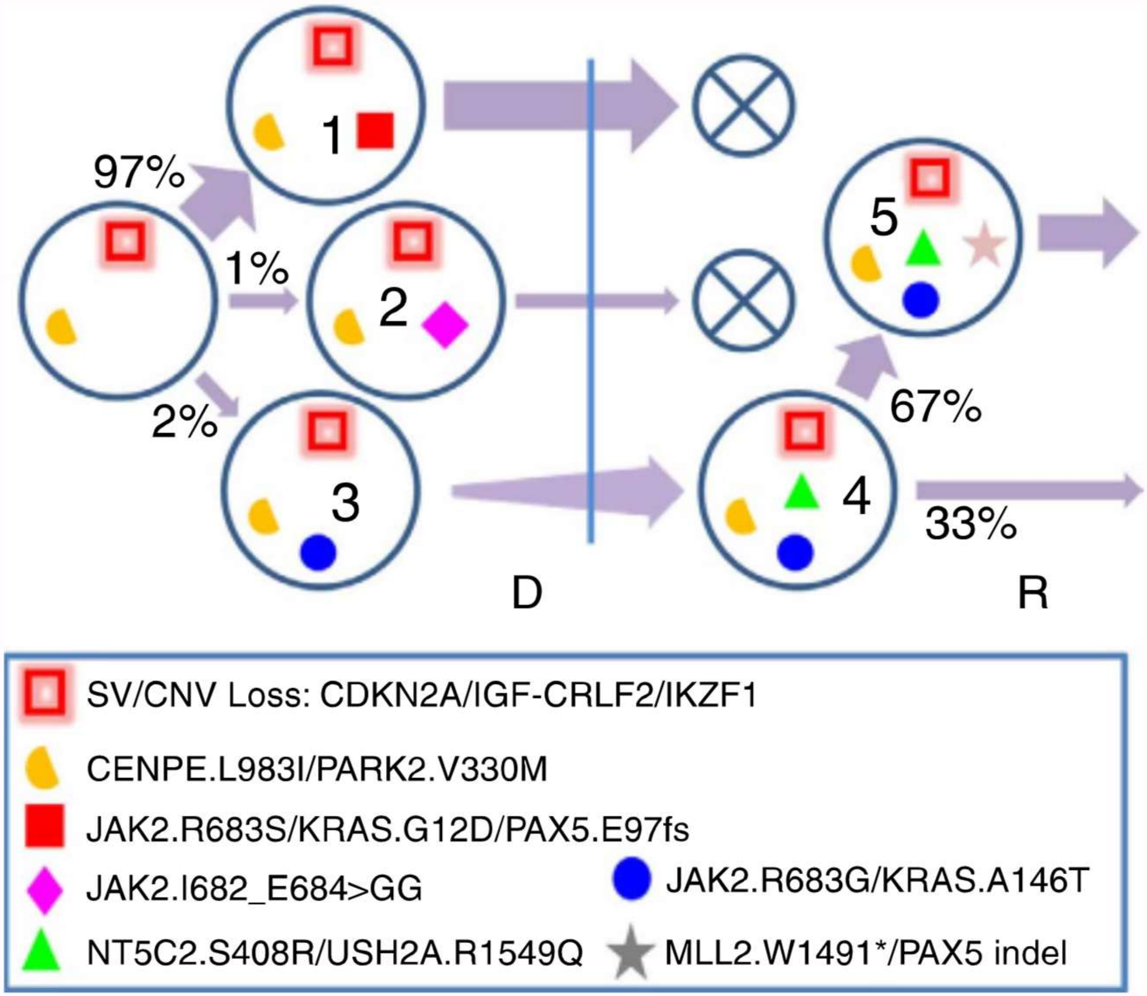
AML MRD NGS



Good correlation between multigene assay and standard NPM1

Salipante, et al (2017) Haematologica doi: 10.3324/haematol.2017.169136.

Tumor Heterogeneity



Ma, et al (2015) Nature Communications DOI:10.1038/ncomms7604