Time point-dependent concordance of flow cytometry and real-time quantitative polymerase chain reaction for minimal residual disease detection in childhood acute lymphoblastic leukemia

Giuseppe Gaipa,[1] Giovanni Cazzaniga,[1] Maria Grazia Valsecchi,[2] Renate Panzer-Grümayer,[3] Barbara Buldini,[4] Daniela Silvestri,[2] Leonid Karawajew,[5] Oscar Maglia,[1] Richard Ratei,[5] Alessandra Benetello,[4] Simona Sala,[1] Angela Schumich,[3] Andre Schrauder,[6] Tiziana Villa,[1] Marinella Veltroni,[7] Wolf-Dieter Ludwig,[5] Valentino Conter,[8] Martin Schrappe,[6] Andrea Biondi,[1] Michael N. Dworzak,[3] and Giuseppe Basso[4]

[1]M. Tettamanti Research Center, Pediatric Clinic University of Milano Bicocca, Monza, Italy; [2]Medical Statistics Unit, Department of Clinical and Preventive Medicine, University of Milano Bicocca, Monza, Italy; [3]Children's Cancer Research Institute and St. Anna Children's Hospital, Vienna, Austria; [4]Laboratorio di Oncoematologia Pediatrica, Department of Pediatrics, University of Padova, Padova Italy; [5]Hematology, Oncology and Tumor Immunology, Robert-Roessle-Clinic at the HELIOS Klinikum Berlin, Charité Medical School, Berlin, Germany; [6]Department of Pediatrics, University Medical Center Schleswig-Holstein, Campus Kiel, Kiel, Germany; [7]Department of Pediatric Hematology Oncology, A.O.U. Meyer, Firenze, Italy, and [8]Department of Pediatrics, Ospedali Riuniti di Bergamo, Bergamo, Italy

Citation: Gaipa G, Cazzaniga G, Valsecchi MG, Panzer-Grümayer R, Buldini B, Silvestri D, Karawajew L, Maglia O, Ratei R, Benetello A, Sala S, Schumich A, Schrauder A, Villa T, Veltroni M, Ludwig W-D, Conter V, Schrappe M, Biondi A, Dworzak MN, and Basso G. Time point-dependent concordance of flow cytometry and real-time quantitative polymerase chain reaction for minimal residual disease detection in childhood acute lymphoblastic leukemia. Haematologica 2012;97(10):1586-1593. doi:10.3324/haematol.2011.060426

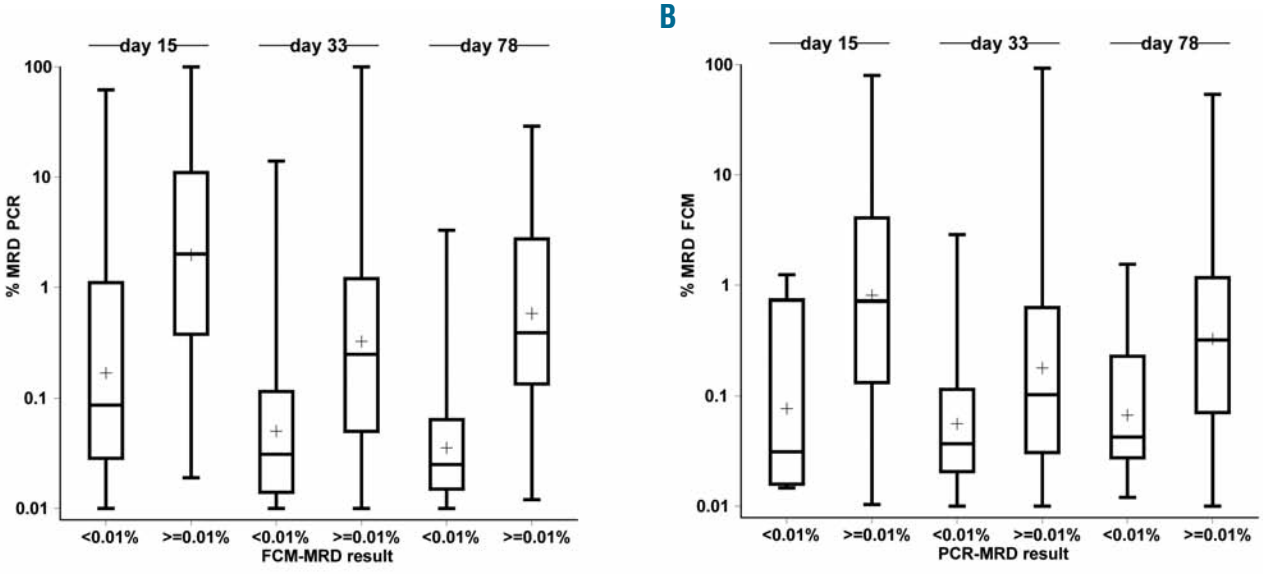
Online Supplementary Appendix

Minimal residual disease-derived risk group classification and final stratification

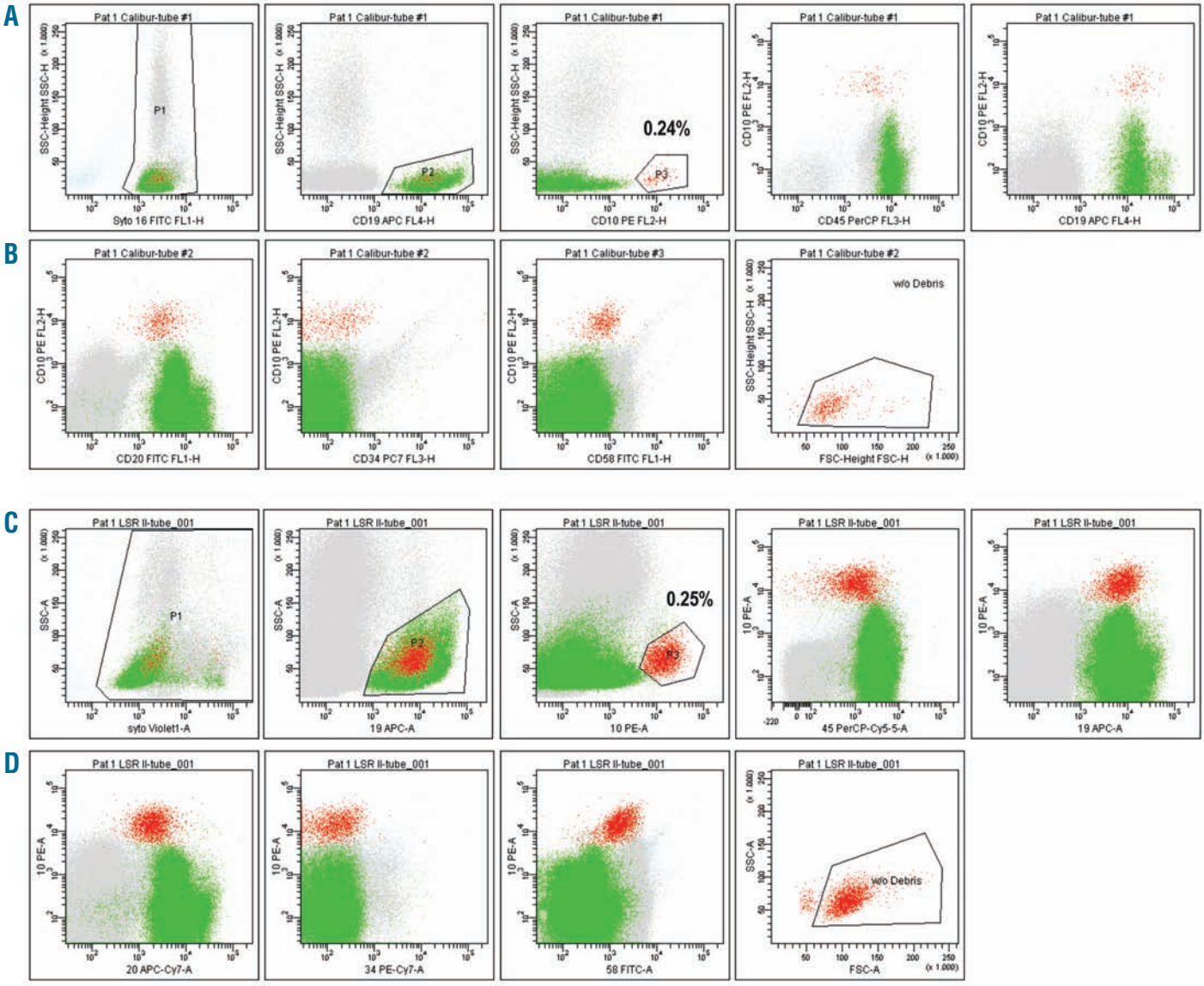
Patients were defined as having standard-risk minimal residual disease (MRD-SR) if no MRD was detected on both day 33 (TP1) and day 78 (TP2), using at least two molecular markers with sensitivity of $\leq 10^{-4}$.[1] If MRD levels differed between the two markers, the highest MRD level was chosen for the final MRD assessment. Patients were considered MRD intermediate risk (MRD-IR) when MRD was positive at one or both time points but at a level of $<10^{-3}$ at TP2 with at least two markers. Patients with MRD $\geq 10^{-3}$ at TP2 were defined MRD high risk (MRD-HR). Patients with a prednisone-poor response (i.e. with ≥1000 leukemic blasts/μL in the peripheral blood on day 8) or failure to achieve remission (i.e. with ≥5% leukemic blasts in the bone marrow on day 33, or persistent extramedullary disease) after induction phase IA (induction failure) or positivity for *MLL/AF4* fusion transcript were treated in the high-risk arm independently of their MRD results. If MRD evaluation was not available, patients were assigned to the intermediate-risk group or, based on clinical parameters, to the high-risk group; these patients are not including in this study.

Reference

1. van der Velden VH, Cazzaniga G, Schrauder, Hancock J, Bader P, Panzer-Grumayer ER, et al. Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. Leukemia. 2007;21(4):604-11.



Online Supplementary Figure S1. (A) Levels of PCR-MRD in patients with PCR ≥0.01% according to results of FCM-MRD, classified as discordant (FCM<0.01%) or concordant (FCM≥0.01%). (B) Levels of FCM-MRD in patients with FCM ≥0.01% according to results of PCR MRD, classified as discordant (PCR<0.01%) or concordant (PCR≥0.01%).



Online Supplementary Figure S2. Representative dot plots exemplifying the flow cytometric analysis and gating strategy. This day 15 bone marrow sample from a patient with BCP-ALL was divided, NC were prepared for four-color analysis (3 tubes; A and B), and MNC were prepared for seven-color assessment (1 tube; C and D). Events were acquired on a BD FACSCalibur™ (four-color assay) and on a BD LSRII™ (seven-color assay). Data sets were analyzed using FACSDiva™ software. First, gating was performed on cellular events positive with the cell-permeable nuclear dye SYTO®16 or -41 in order to include only relevant events in the quantitative assessment. Subsequently, B cells were identified in the data sets from the tubes containing the SYTO® dye (see *Online Supplementary Table S1*) by plotting CD19 against SSC, and potential leukemic CD19$^+$ cells (red) based on expression of the immaturity marker CD10 (normal B cells are painted green). In dual-color plots the supposedly leukemic cells were checked for leukemia-associated phenotypic aberrations in order to define MRD. In this case, asynchronous expression patterns distinct from regular differentiation as well as over-expression of CD58 were found. Finally, back-gating of MRD-cells in the FSC/SSC plot was used to exclude events from further calculations which appeared in the debris region. Note the good quantitative concordance of MRD estimates as well as the largely similar staining patterns between both set-ups, despite the use of different fluorochrome conjugates and different numbers of acquired cells (A and B: ≤300 000 cells; C and D: ≥500 000 cells per tube).

Online Supplementary Table S1. Antibody combinations used to detect leukemia-associated immunophenotypes at diagnosis and during follow-up in patients with either B-cell precursor (BCP)-ALL or T-ALL.

A. Four-color panels

Combination*	BCP-ALL			
1	SYTO 16	CD10 PE	CD45 PerCP	CD19 APC
2	CD58 FITC	CD10 PE	CD19 PE-CY7	CD45 APC
2a§	CD58 FITC	CD11a PE	CD10 PE-CY7	CD19 APC
3	CD20 FITC	CD10 PE	CD19 PE-CY7	CD34 APC
3a§	CD20 FITC	CD34 PE	CD10 PE-CY7	CD19 APC
4	CD10 FITC	CD11a PE	CD19 PE-CY7	CD34 APC
5	CD10 FITC	CD34 PE	CD19 PE-CY7	CD45 APC
6	CD10 + CD20 FITC	CD38 PE	CD19 PE-CY7	CD34 APC
6a§	CD20 FITC	CD38 PE	CD10 PE-CY7	CD19 APC

Combination	T-ALL			
1	SYTO 16	CD7 PE	CD45 PerCP	sCD3 APC
2	CD99 FITC	CD5 PE	CD7 PE-CY7	sCD3 APC
2a§	CD99 FITC	CD7 PE	CD5 PE-CY7	sCD3 APC
3	CD99 FITC	CD7 PE	iCD3 PE-CY7	sCD3 APC
4	TdT FITC	CD7 PE	iCD3 PE-CY7	sCD3 APC
5	TdT FITC	CD5 PE	iCD3 PE-CY7	sCD3 APC

B. Seven-color panels

Combination*	BCP-ALL						
1	CD58 FITC	CD10 PE	CD45 PerCP	CD34 PE-Cy7	CD19 APC	CD20 APC-Cy7	Syto 41
2	CD10 FITC	CD11a PE	CD45 PerCP	CD34 PE-Cy7	CD19 APC	CD20 APC-cy7	Syto 41

Combination	T-ALL						
1	TdT FITC	CD56 PE	sCD3 PerCP	iCD3 PE-Cy7	CD7 APC	CD45 APC-Cy7	Syto 41
2	CD2 FITC	CD99 PE	sCD3 PerCP	CD5 PE-Cy7	CD7 APC	CD45APC-CY7	Syto 41

*iCD means intra-cytoplasmic staining; sCD means surface staining. *Underlined markers in each BCP-ALL combination indicate recurrent triple back bone CD10/CD19/CD45 or CD10/CD19/CD34. §Combinations used by some groups in alternative to that indicated above with the same number, or introduced by all groups in a subsequent period of the study.*

Online Supplementary Table S2. Concordance in MRD detection and performance of FCM as compared to PCR at different time points in patients with B-cell precursor (BCP)-ALL (Table 2A) or T-ALL (Table 2B)

A.

	PCR-MRD Day 15 (n. of samples)			PCR-MRD Day 33 (n. of samples)			PCR-MRD Day 78 (n. of samples)		
	PCR ≥0.01%	PCR <0.01%	Total	PCR ≥0.01%	PCR <0.01%	Total	PCR ≥0.01%	PCR <0.01%	Total
FCM-MRD									
FCM ≥0.01%	341	5	346	176	76	252	35	12	47
FCM <0.01%	47	17	64	206	534	740	100	845	945
Total	388	22	410	382	610	992	135	857	992
FCM sensitivity		341/388 = 88%			176/382 = 46%			35/135 = 26%	
FCM specificity		17/22 = 77%			534/610 = 88%			845/857 = 99%	
Concordance rate		358/410 = 87%			710/992 = 72%			880/992 = 89%	
Overall concordance rate					1948/2394 = 81%				

B.

	PCR-MRD Day 15 (n. of samples)			PCR-MRD Day 33 (n. of samples)			PCR-MRD Day 78 (n. of samples)		
	PCR ≥0.01%	PCR <0.01%	Total	PCR ≥0.01%	PCR <0.01%	Total	PCR ≥0.01%	PCR <0.01%	Total
FCM-MRD									
FCM ≥0.01%	40	0	40	42	3	45	13	2	15
FCM <0.01%	11	0	11	39	24	63	30	63	93
Total	51	0	51	81	27	108	43	65	108
FCM sensitivity		40/51 = 78%			42/81 = 52%			13/43 = 30%	
FCM specificity		--			24/27 = 89%			63/65 = 97%	
Concordance rate		40/51 = 78%			66/108 = 61%			76/108 = 70%	
Overall concordance rate					182/267 = 68%				